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Research Article

Investigation of cytotoxic effects of some novel synthesized iminothiazolidinone derivatives on HeLa cell line (CCL2)

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Abstract

In medicinal chemistry, the thiazolidinones are a practical framework which can be leaned as a pharmacophore in a large diversity of biologically active compounds. Furthermore, they build up the base of antibacterials, anti-convulsant, anti-tumorals, antivirals, anti-diabetic, anti-inflammatory, anti-HIV compounds in many other therapeutic agents. In this study, the cytotoxic effects of some novel synthesized iminothiazolidinone derivatives (Compound A-E) on HeLa (3) cell line (CCL2) which arising from human cervical carcinoma were studied. Accordingly, kinetics parameters of proliferation rate, mitotic and the labeling index were determined upon the application of the iminothiazolidinone derivatives. 1 x 10^{-6}, 5 x 10^{-6}, 10 x 10^{-6} M concentrations of the derivatives were implemented for 72 hours to find out the optimum concentrations, and the result was explicated by reproduction rate analysis. Parameters of the mitotic and labeling index of the cells which were treated with the optimum concentrations of the uniquely synthesized iminothiazolidinone derivatives for 0-72 hours were calculated. The results indicated that the tested compounds caused a remarkable decrease in the propagation of HeLa cell cultures and the 10 x 10^{-6} M concentration was found to be the most effective concentration of the iminothiazolidinone derivatives regarding reducing the reproduction rate. Drugs can be obtained from these derivatives will offer a promising treatment modality in cervix carcinoma in the future.

Key words: Iminothiazolidinone, HeLa cells, viability, mitotic index, labelling index.
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Bazı yeni sentezlenmiş iminotiazolidinon türevlerinin HeLa hücre hattı üzerindeki sitotoksik etkilerinin araştırılması

Özet

Tıbbi kimyada, tiazolidinonlar, büyük bir çeşitlilik barındıran biyolojik olarak aktif bileşikler içinde farmokofor olarak kullanılabilecek kapasiteye sahiptir. Ayrıca, birçok terapötik ajanlarda antibakteriyel, anti-konvülsan, anti-tümöral, antiviral, anti-diyabetik, anti-inflamatuar ve anti-HIV bileşiklerinin tabanını oluştururlar. Bu çalışmada, bazı yeni sentezlenmiş iminotiazolidinon türevlerinin (A-E Bileşikleri) insan servikal karsinomu kaynaklı HeLa (3) hücre hattı (CCL2) üzerine sitotoksik etkileri araştırılmıştır. Buna göre iminotiazolidinon türevlerinin uygulanması üzerine yayılma hızı, mitotik ve işaretlenme indeksinin kinetik parametreleri belirlenmiştir. Optimum konsantrasyonların bulunması için $1x10^{-6}$, $5x10^{-6}$, $10x10^{-6}$ M konsantrasyonlarında tiazolidinon türevleri 72 saat süreyle uygulanmış ve sonuç, üreme hızı analizi ile açıklanmıştır. 0-72 saat boyunca benzersiz şekilde sentezlenmiş iminotiazolidinon türevlerinin optimum konsantrasyonları ile muamele edilen hücrelerin mitotik ve işaret indeks parametreleri hesaplanmıştır. Sonuç olarak, test edilen bileşiklerin HeLa hücre kültürlerinin çoğalmasında, kayda değer bir düşüşe neden olduğu ve $10x10_{-6}$ M konsantrasyondaki iminotiazolidinon türevlerinin çoğalma hızının düşüşüne ilişkin en etkili konsantrasyon olduğu bulunmuştur. Bu türevlerden elde edilecek ilaçların gelecekte serviks karsinoması tedavisinde umut verici bir model sunması ümit edilmektedir.

Anahtar kelimeler: İminotiazolidinon, HeLa hücreleri, canlılık, mitotik indeks, işaretlenme indeksi

Introduction

Nowadays, 4-thiazolidinones are one of the most praised compounds in medicinal chemistry. Its small heterocyclic molecular structure and medicinal chemists properties, as well as its structural and therapeutic diversity, have encouraged the researches (Hamama et al. 2011). 4-thiazolidinone is a promising scaffold for the search of new potential antibacterial, antiviral, antidiabetic and anticancer agents (Oleh et al. 2012). Thiazolidinone ring system has been participated in a wide range of biologically effective compounds, either as a substituent group or as a revision of other ring, which has excited the researchers to synthesize various compounds including this moiety (Chavan and Pai 2007; Jain et al. 2012). In addition, thiazolidinones and their derivative products have a momentous position in the area of heterocyclic compounds based on their presence in the structures of macrocyclic complex medications. They also have some applications in industry and pharmaceutical area because of their biological particulars (Verma and Saraf 2008; Hamama et al. 2008; Jain et al. 2012). Due to their reaction capabilities both as N-nucleophiles and S-nucleophiles, thioureas are successfully used in the synthesis of biologically active compounds. As a result of comprehensive research made in recent years, new thiourea compounds having antiarthiritic, anti-inflammatory, antiviral, antidiabetic, antithyroidal, fungicidal, bactericidal, insecticidal and pesticidal have been discovered (Rahman et al. 2005; Jain et al. 2012; Liu et al. 2012). Against imino analogues of thiazolidinones are less widespread, even the accurate possibility to infer an additional manage for chemical variety and hereby allow of examination of unconjugated regions of a thiazolidinone-based pharmacophore (Laurent et al. 2004). On the other hand, anticancer action, antiproliferative effect, antimicrobial, antituberculosis and antibacterial activities of iminothiazolidinone compounds have been observed in their biological and pharmacological activity studies (Küçükgüzel et al. 2008; Kumar et al. 2014; Kaminskiyy et al. 2016). In addition, the existence of anti-inflammatory (Ottana et al. 2005) and anti-fungal (Liu et al. 2000) effects of these compounds have also been shown.

In this study, the antiproliferative properties and cytotoxic effects of the newly synthesized iminothiazolidinone derivatives was evaluated on HeLa cell line (CCL2) arising from human cervix carcinoma. In order to achieve these purposes, cell kinetic parameters such as propagation assay, mitotic and labeling index analysis were determined. 1 x 10^{-6}, 5 x 10^{-6}, 10 x 10^{-6} M concentrations of iminothiazolidinone derivatives were adopted to cells for 24, 48 and 72 hours. It was shown that all applications in all experiential series ($p<0.05$) showed a perceptible decrease in mitotic index, labeling index and cell proliferation.

Materials and methods

Chemistry

Iminothiazolidinones were synthesized in two steps in this study. In the first step, substituted thioureas which were planned to use as substrates in the principal reactions, were prepared by the reaction of substituted phenyl isothiocyanate with substituted hetaryl amines. In the second step which is the main part of the study, the cyclocondensation of each of the previously prepared thioureas with chloroacetic acid and substitute thiophene-2-carboxaldehyde were achieved by the technique of one-pot multicomponent reaction; and newly synthesized 5-substitued-2-imino-4-thiazolidinone compounds (A-E) were obtained (Tuğcu 2009). The confirmation of the structures of newly synthesized compounds were carried out by the use of (1) H NMR, (13) C NMR and HR-MS data.

Cell culture

In this study, the employed HeLa cell line (CCL-2) was obtained from American Animal Cell Culture Collection. Cells were grown in Medium-199 (M-199, Sigma, USA) including 0.1 mg/ml streptomycin (Streptomycin sulphate, I. E. Ulugay), 10% foetal bovine serum (FBS, Gibco Lab), 100 IU/ml penicillin (Pronapen, Pfizer), amphotericin B (Sigma, USA) and 0,002 M glutamine, pH 7.2, at 37ºC in 5% CO_2 moisturized air.

Compound concentrations

Compound concentrations that used in the present study were determined according to previous *in vitro* and clinical studies. At first, a 1000 µM stock solution was prepared with M-199 supplemented with 10% FBS. Three different concentrations were prepared by dilution of the stock solution. These compounds were called as concentration 1 (D1= 1 x 10^{-6} M), concentration 2 (D2= 5 x 10^{-6} M), concentration 3 (D3=10 x 10^{-6} M). The experiments were carried out by using these three compound concentrations. HeLa cells were treated with the above compound concentrations for the time periods of 24, 48 and 72 hours.

Preparing of ^{3}H-Thymidine

Nine ml deionized water was added to a vial containing 1 mCi/ml ^{3}H-Thymidine (TRA-120, Amersham, England) and stock solution was prepared. Then 1 mCi/ml solution was diluted to 1 µCi/ml with cell culture medium. The cells will be labeled with this solution.

Cell proliferation assay

The effects of iminothiazolidinone derivates (Compounds A-E) on cell proliferation of HeLa cells were measured by MTT assay. Method is based on the competency of live cells to transform tetrazolium salt into purple formazan. HeLa cells were emplaced into 96-well plates at a density of $2x10^{4}$ cells per well and incubated overnight. Then the cells were cultivated with the concentrations of 1 µM, 5 µM and 10 µM iminothiazolidinone derivates (Compounds A-E). The medium in each well was removed and 40 µl fresh MTT solution (5 mg/ml in PBS) were added into each well, and the content was incubated at 37 ^{o}C for 4 h at the end of the experimental period. Then, 160 µl DMSO (Dimethyl Sulfoxide, Sigma) was added into each well and cells were shaken thoroughly for 1 h on a shaker. Then, the absorbance of the samples was measured against a blank using an Elisa reader (µQuant, Bio-Tek Instruments Inc) at 450-690 nm.

Determination of optimal concentration with cell viability analysis

To determine the most effective concentrations of iminothiazolidinone derivates on HeLa cells, three different concentrations (D1= 1 μM, D2= 5μM, D3= 10μM) were applied to cell culture for 72 h. The cytotoxic effects of the different concentrations were evaluated with MTT assay. At the end of this application, the most effective concentration which was determined according to the absorbance values was used for the following parameters.

Mitotic index analysis

For mitotic index analysis, HeLa cells were seeded into round coverslips which were in 24-well plates at a density of $2x10^4$ cells per well and incubated overnight. Then the cells were treated with 1 μM, 5 μM and 10 μM iminothiazolidinone derivates (Compounds A-E) concentrations. At the end of the experimental period cells were fixed with Carnoy's fixative (3:1 methanol-acetic acid). Mitotic index were determined by the methods of Feulgen. The cells were processed with 1 N HCl at room temperature for 1 minute and then hydrolyzed with 1 N HCl for 10.5 minutes at 60°C after treated with Feulgen. After lamellas were treated with Feulgen, they were washed in distilled water and for 3 minutes stained with 10% Giemsa stain solution pH 6.8, and then washed twice in phosphate buffer. After dyeing, the coverslips were rinsed in distilled water. And then they were air dried. Mitotic index was calculated for each drug concentration and control group by counting metaphases, anaphases and telophases. Finally, the mitotic index was calculated by counting metaphases, anaphases and telophases for each tested drug concentration and control. At least 3000 cells were examined from each slide for determination of the mitotic index by the same scorer. At least three thousand cells were viewed from each cover glass for the mitotic index.

^{3}H-thymidine labeling index analysis

For ^{3}H-thymidine labeling index analysis which determine cells in the synthesis phase, HeLa cells were seeded into round coverslips which were in 24-well plates at a density of $2x10^4$ cells per well and incubated overnight. Then the cells were treated with 1μM, 5 μM and 10 μM iminothiazolidinone derivates (Compounds A-E) concentrations. At the end of the experimental period, cells were treated with medium containing 1 μCi/mL ^{3}H-thymidine for 20 min to evaluate the labeling index and the labeled cells.

Autoradiography

After labeling, the cells were fixed with Carnoy's fixative (3:1 methanol-acetic acid) and the remaining radioactive material was washed twice with 2% perchloric acid at 4°C for 30 min. The slides were prepared and were coated with K.2 gel emulsion (Ilford) prepared with distilled water at 40°C to determine thymidine labeling index. After 3 days exposure at 4°C, autoradiograms were washed with D-19 b developer (Kodak) and fixed with Fixaj B (Kodak). The slides were evaluated after being stained with Giemsa for 3 min. The same person evaluated all the slides by counting at least 3000 cells from each slide. The labeling index was calculated by examining 100 areas on each slide at a magnification of ·12.5. At least three thousand cells were examined from each coverslip.

Statistics

Values of proliferation rate, MI and AI were evaluated relative to controls and to each other in order to find how significant they are. For this reason, values obtained from all experimental groups were analyzed using one-way ANOVA test. The significance between control and experimental groups was determined by DUNNETT's test, and the significance between experimental groups was found by Student's t-test.

Results

Synthesis of iminothiazolidinone derivatives (Compounds A-E)

A blend of the appropriate amine (1 mmol) and substitute phenylisothiocyanate (1.2 mmol) was ruffled in CH_2Cl_2 at room temperature for 24 h. The crude product was concentrated under vacuum and recrystallized from ethanol. The

appropriate thiourea (1 mmol), chloroacetic acid (1.2 mmol) and substitute thiophene-2-carbaldehyde (1 mmol) were stirred with a magnetic stirrer at 40 ºC for 24 h. The crude product was purified by column chromatography ($CHCl_3$ or CH_2Cl_2) as explained by Kasmi-Mir et al. (2006). At the end of the process, five iminothiazolidinone derivatives were obtained.

Compound A

2-[(5-chloropyridin-2-yl)imino]-3-phenyl-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one: Yellow solid, m.p. 284-5 ºC;

FTIR (near) $\gamma_{max/cm}^{-1}$**:** 3082, 3017, 2961, 1704, 1585, 1562, 1544, 1458, 1364, 1353, 1157, 862, 832, 794; ^{1}H NMR (500MHz, $CDCl_3$)**:** δ **(**ppm) 7.01 (1H, d, J=8.4 Hz), 7.20 (1H, dd, J=4.8; 4.0 Hz), 7.40 (2H, brd, J=7.3 Hz), 7.44 (1H, brd, J=3.7 Hz), 7.53 (1H, d, J=7.7 Hz), 7.54 (2H, dd, J=7.7; 7.3 Hz), 7.55 (1H, dd, J=8.4; 2.7 Hz), 7.60 (1H, brd, J=5.1 Hz), 8.03 (1H, s), 8.50 (1H, brs); ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 121.81, 122.71, 125.59, 128.05, 128.50, 128.72, 129.02, 129.37, 131.53, 132.99, 135.55, 137.97, 138.56, 145.75, 154.23, 156.23, 166.24; MS (m/z)**:** 399 M^{+2}, 398 M^{+1}, 397 M^+, 396 M^{-1}, 230, 140, 112, 96, 76.

Compound B

3-(2,4-dimethylphenyl)-5-[(3-methylthiophen-2-yl)methylidene]-2-(phenylimino)-1,3-thiazolidin-4-one **:** Yellow solid, m.p. 155-6 ^{0}C; FTIR (near) $\gamma_{max/cm}^{-1}$**:** 3083, 3068, 3032, 2977, 2952, 2922, 1711, 1633, 1592, 1495, 1356, 1272, 1172, 1155, 870, 820, 770; ^{1}H NMR (500MHz, $CDCl_3$)**:** δ **(**ppm) 2.27 (3H, s), 2.37 (3H, s), 2.42 (3H, s), 6.96 (2H, brd, J=8.6 Hz), 6.97 (1H, d, J=7.8 Hz), 7.18 (1H, d, J=4.7 Hz), 7.19 (1H, m), 7.20 (1H, s), 7.21 (1H, d, J=7.8 Hz), 7.35 (2H, dd, J=8.2; 7.4 Hz), 7.48 (1H, d, J=4.7 Hz), 8.06 (1H, s); ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 14.70, 17.93, 21.49, 118.79, 121.40, 122.98, 124.99, 128.17, 128.56, 129.40, 129.62, 131.27, 131.76, 132.25, 132.66, 135.98, 139.80, 142.77, 148.67, 150.55, 166.54; MS (m/z)**:** 406 M^{+2}, 405 M^{+1}, 404 M^+, 403 M^{-1}, 389, 371, 312, 222, 202, 154, 121, 118, 91, 77.

Compound C

3-(4-butylphenyl)-5-[(3-methylthiophen-2-yl)methylidene]-2-(phenylimino)-1,3-thiazolidin-4-one**:** Yellow solid, m.p. 284-5 ^{0}C;

FTIR (near) $\gamma_{max/cm}^{-1}$**:** 3061, 3032, 2957, 2922, 2854, 1702, 1632, 1590, 1510, 1359, 1297, 1269, 1167, 1146, 874, 835, 775 ; ^{1}H NMR (500MHz, $CDCl_3$)**:** δ **(**ppm) 0.95 (3H, t, J=7.4 Hz), 1.41 (2H, m), 1.65 (2H, m), 2.42 (3H, s), 2.67 (2H, t, J=7.8 Hz), 6.97 (1H, d, J=5.1 Hz), 6.99 (2H, brd, J=8.6 Hz), 7.17 (2H, brd, J=8.6 Hz), 7.36 (5H, m), 7.47 (1H, d, J=5.1 Hz), 8.06 (1H, s); ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 14.15, 14.68, 22.68, 33.49, 35.66, 118.78, 121.39,122.92, 124.36, 125.02, 127.91, 129.46, 129.66, 131.26, 132.65, 132.73, 134.23, 142.68, 143.87, 148.59, 151.11, 166.76; MS (m/z)**:** 434 M^{+2}, 433 M^{+1}, 432 M^+, 431 M^{-1}, 389, 250, 207, 154, 121, 77.

Compound D

3-(4-butylphenyl)-5-[(5-methylthiophen-2-yl)methylidene]-2-(phenylimino)-1,3-thiazolidin-4-one **:** Yellow solid, m.p. 160-1 ^{0}C;

FTIR (near) $\gamma_{max/cm}^{-1}$**:** 3066, 3027, 2955, 2926, 2852, 1710, 1638, 1591, 1512, 1359, 1266, 1171, 1146, 830, 805, 773; ^{1}H NMR (500MHz, $CDCl_3$)**:** δ **(**ppm) 0.95 (3H, t, J=7.4; 7.0 Hz), 1.41 (2H, m), 1.65 (2H, m), 2.52 (3H, s), 2.67 (2H, t, J=7.8 Hz), 6.80 (1H, d, J=4.7 Hz), 6.99 (2H, brd, J=7.4 Hz), 7.17 (2H, brd, J=7.4 Hz), 7.35 (5H, m), 7.38 (1H, d, J=5.5 Hz), 7.91 (1H, s); ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 14.18, 16.10, 22.70, 33.52, 35.67, 118.11, 121.39, 124.74, 124.99, 127.24, 127.91, 129.49, 129.52, 132.58, 133.05, 136.35, 143.91, 147.15, 148.66, 151.20, 166.71; MS (m/z)**:** 434 M^{+2}, 433 M^{+1}, 432 M^+, 431M^{-1}, 389, 250, 207, 154, 121, 77.

Compound E

2-[(4-butylphenyl)imino]-5-[(5-methylthiophen-2-yl)methylidene]-3-phenyl-1,3-thiazolidin-4-one**:** Yellow solid, m.p. 137-8 ^{0}C;

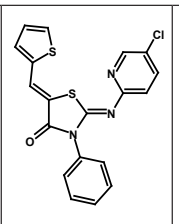
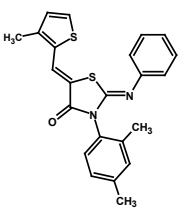
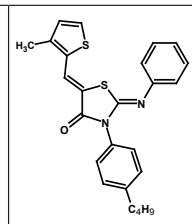
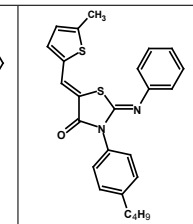
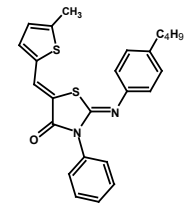
FTIR (near) $\gamma_{max/cm}^{-1}$**:** 3065**,** 3029, 2957, 2886, 2867, 2851, 1712, 1641, 1599, 1496, 1364, 1270, 1183, 1145, 854, 831, 802; ^{1}H NMR (500MHz, $CDCl_3$)**:** δ **(**ppm) 0.95 (3H, t, J=7.4 Hz), 1.37 (2H, m), 1.62 (2H, m), 2.53 (3H, s),

2.62 (2H, t, J=7.8 Hz), 6.81 (1H, d, J=4.7 Hz), 6.90 (2H, brd, J=8.6 Hz), 7.16 (2H, brd, J=8.2 Hz), 7.17 (1H, d, J=4.7 Hz), 7.44 (1H, m), 7.47 (2H, m), 7.52 (2H, brd, J=7.0 Hz), 7.92 (1H, s); ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 14.18, 16.08, 22.58, 33.80, 35.38, 118.26, 121.13, 124.61, 127.24, 128.30, 128.95, 129.40, 132.96, 135.25, 136.40, 139.65, 145.98, 147.05, 150.34, 166.58; MS (m/z)**:** 434 M^{+2}, 433 M^{+1}, 432 M^{+}, 431M^{-1}, 389, 250, 207, 154, 121, 77.

Determination of optimal concentrations with mitochondrial dehydrogenase enzyme activity analysis

In this study, three different concentrations (D1=1μM, D2=5μM, D3=10μM) of iminothiazolidinone derivatives (Compound A-E) were applied to HeLa cell culture for the period of 24, 48 and 72 h. The absorbance values of each concentration for 72 h are shown in Table 1. The absorbance differences between control and experimental groups were found statistically significant ($p<0.05$). In addition, significant differences among the experimental groups were also noted ($p<0.05$). The results revealed that 10 μM concentration is the optimum concentration for the tested compounds (Table 1). The results from all parameters shown that the tested compounds caused a significant decrease in the proliferation of HeLa cell cultures in a concentration-dependent manner. Viability values of HeLa cells treated with iminothiazolidinone derivatives (Compound A-E) were shown in Figure 1 a-e. The results showed that compound E (2-[(4-butylphenyl) imino]-5-[(5-methylthiophen-2-yl) methylidene]-3-phenyl-1,3-thiazolidin-4 one) is the most effective compound among the tested compounds.

Table 1. Absorbance values of mitochondrial dehydrogenase enzyme activity of HeLa cells treated with different concentrations of iminothiazolidinones for 72 h (±SD, $p<0.05$).

Experimental Groups					
	Compound A	**Compound B**	**Compound C**	**Compound D**	**Compound E**
Control	**457,67×10^{-3*} ±0.09**	**462,12×10^{-3*} ±0.05**	**464,12×10^{-3*} ±0.08**	**447,28×10^{-3*} ±0.06**	**464,58×10^{-3*} ±0.02**
D_1	**445,33×10^{-3}±0.07**	**385,04×10^{-3}±0.02**	**417,29×10^{-3}±0.09**	**395,34×10^{-3}±0.03**	**479,29×10^{-3}±0.05**
D_2	**403,57×10^{-3}±0.09**	**447,29×10^{-3}±0.02**	**453,54×10^{-3}±0.11**	**302,44×10^{-3}±0.07**	**345,32×10^{-3}±0.04**
D_3	**345,00×10^{-3}±0.04**	**326,52×10^{-3}±0.04**	**411,33×10^{-3}±0.08**	**284,22×10^{-3}±0.04**	**285,08×10^{-3}±0.01**

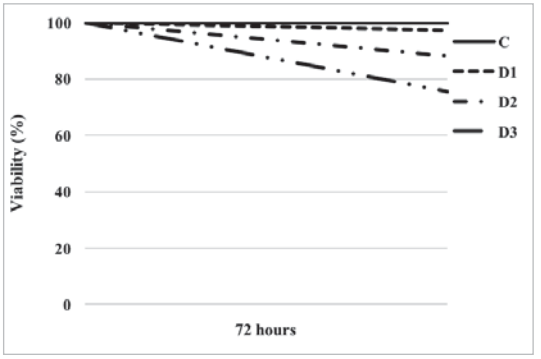


Figure 1a. Viability (%) values of HeLa cells treated with 1 μM, 5 μM and 10 μM concentrations of compound A for 72 h (450- 690 nm) ($p<0.05$).

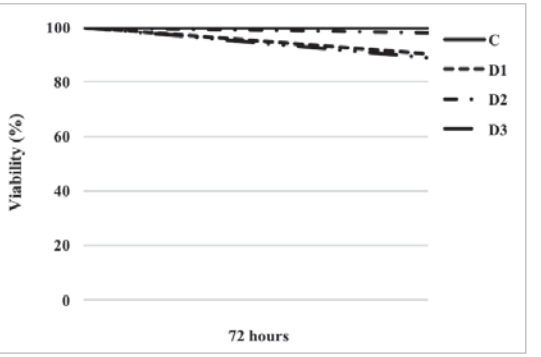


Figure 1b. Viability (%) values of HeLa cells treated with 1 μM, 5 μM and 10 μM concentrations of compound B for 72 h (450- 690 nm) ($p<0.05$).

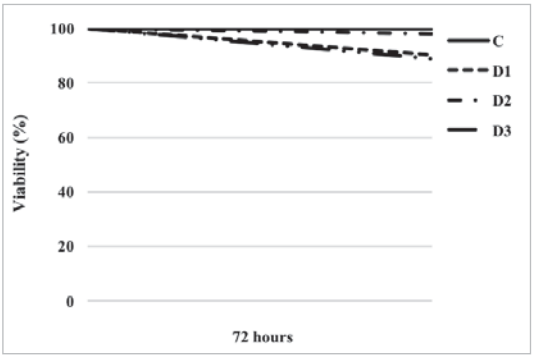


Figure 1c. Viability (%) values of HeLa cells treated with 1 µM, 5 µM and 10 µM concentrations of compound C for 72 h (450- 690 nm) ($p<0.05$).

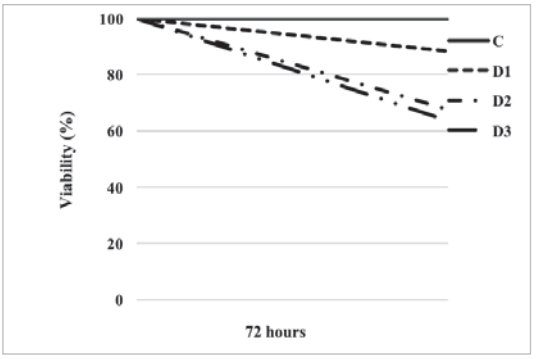


Figure 1d. Viability (%) values of HeLa cells treated with 1 µM, 5 µM and 10 µM concentrations of compound D for 72 h (450- 690 nm) ($p<0.05$).

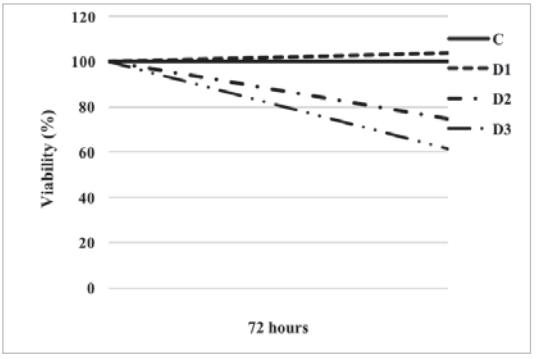
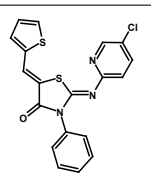
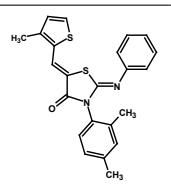
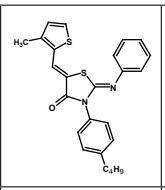
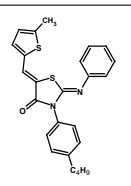
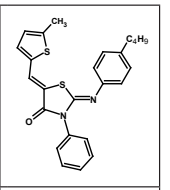


Figure 1e. Viability (%) values of HeLa cells treated with 1 µM, 5 µM and 10 µM concentrations of compound E for 72 h (450- 690 nm) ($p<0.05$).

Mitotic index

After application of five different imino-thiazolidinone derivatives (Compound A-E) to HeLa cell line for 24, 48 and 72 h, antimitotic effects of all treatments were evaluated with mitotic index analysis. As a result of this analysis, cells which were in mitosis or not were counted. Mitotic index (MI) values of the cells in a time-dependent manner were decreased significantly in all experimental groups (Table 2). In addition, statistically significant difference was noted among the all experimental groups ($p<0.05$). In a time-dependent manner, MI (%) values of HeLa cells treated with compound E (2-[(4-butylphenyl)imino]-5-[(5-methylthiophen-2-yl)methylidene]-3-phenyl-1,3-thiazolidin-4-one) at a concentration of 10 µM were shown at Fig. 2. The differences between the control and the each of experimental group were also found significant ($p<0.05$). As seen in Fig. 2, MI of compound E compared with the control group decreased from 7.67% to 3.78% at 24 h; 7.27% to 3.16% at 48 h and 6.98% to 2.27% at 72 h for a concentration of 10µM. It is obvious that the proliferation of HeLa cell lines was effectively inhibited by compound E.

Table 2. Mitotic index (%) values of HeLa cells treated with 10 µM concentrations of the tested compounds for 0-72 h (±SD, $p<0.05$).

Experimental Groups						
		Compound A	**Compound B**	**Compound C**	**Compound D**	**Compound E**
Mitotic Index (%)						
	Control	**D_3**	**D_3**	**D_3**	**D_3**	**D_3**
24 Hour	**7.67±0.18**	**6.73±0.33**	**6.07±0.34**	**7.08±0.43**	**3.89±0.17**	**3.78±0.36**
48 Hour	**7.27±0.28**	**5.98±0.17**	**5.82±0.32**	**6.59±0.21**	**3.08±0.18**	**3.16±0.17**
72 Hour	**6.98±0.37**	**5.06±0.23**	**4.75±0.25**	**5.97±0.13**	**2.89±0.14**	**2.27±0.41**

Labeling index

Labeling index (LI) values of HeLa cell line for 24, 48 and 72 h time period were shown in Table 3. It was observed that the iminothiazolidinone derivatives exhibited toxicity against HeLa cell lines compared to control group and especially compound E (2-[(4-butylphenyl)imino]-5-[(5-methylthiophen-2-yl)methylidene]-3-phenyl-1,3-thiazolidin-4-one) among the all treatments was found to have the most effective IC_{50} at 10 µM. LI values of the cells were increased significantly while decreasing from 24 h to 72 h for the control group. As seen in the Fig. 3, LI decreased from 6.24% to 3.03% at 24 h; 7.68% to 1.54% at 48 h and 8.11% to 0.82% at 72 h for 10µM concentration of compound E. The differences between the control and the experimental groups were found to be significant ($p<0.05$).

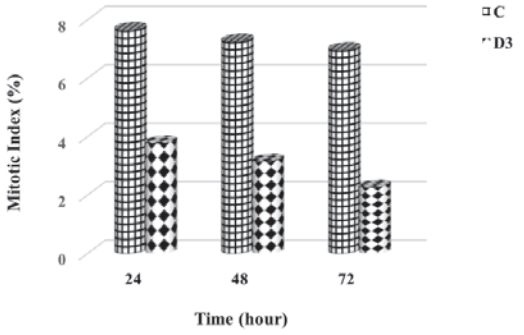


Figure 2. Mitotic index (%) values of HeLa cells treated with 10 µM of the compound E (2-[(4-butylphenyl)imino]-5-[(5-methylthiophen-2-yl)methylidene]-3-phenyl-1,3-thiazolidin-4-one) for 0-72 h ($p<0.05$).

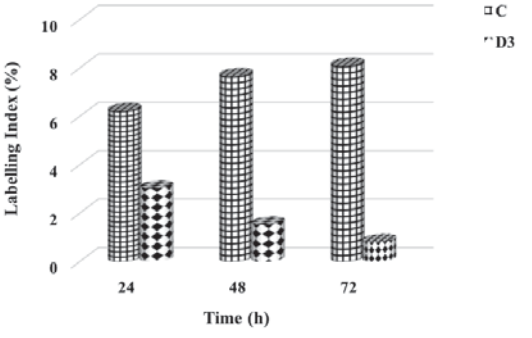
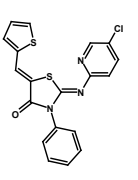
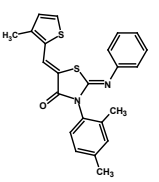
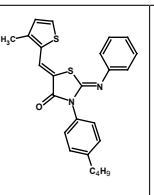
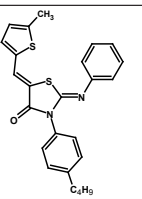
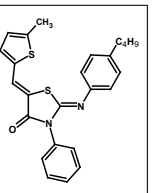


Figure 3. Labelling Index (%) values of HeLa cells treated with 10 µM of compound E (2-[(4-butylphenyl)imino]-5-[(5-methylthiophen-2-yl)methylidene]-3-phenyl-1,3-thiazolidin-4-one) for 0-72 h ($p<0.05$).

Table 3. Labeling index (%) values of HeLa cells treated with 10 µM concentrations of iminothiazolidinones for 0-72 h (±SD, $p<0.05$).

Experimental Groups						
		Compound A	**Compound B**	**Compound C**	**Compound D**	**Compound E**
Labelling Index (%)						
	Control	**D_3**	**D_3**	**D_3**	**D_3**	**D_3**
24 Hour	**6.24±0.52**	**6.02±0.22**	**5.21±0.43**	**6.19±0.34**	**2.97±0.24**	**3.03±0.39**
48 Hour	**7.68±0.32**	**5.24±0.31**	**4.72±0.14**	**6.08±0.32**	**1.66±0.33**	**1.54±0.12**
72 Hour	**8.11±0.41**	**4.86±0.15**	**3.33±0.23**	**5.75±0.53**	**0.94±0.11**	**0.82.±0.30**

Discussion

Thiazolidinones are thiazolidine analogues due to having a sulfur atom at position 1, a nitrogen atom at position 3 and a carbonyl group at position 2, 4, or 5. Its analogues are applicable for the mostly studied moiety and its existence in penicillin was the first diagnosis of its availability in nature. Recently, 4-thiazolidones and the related heterocyclic compounds have been demonstrated to be a perspective source of innovative anticancer agents (Havrylyuk et al. 2009, 2010; Panchuk et al. 2012). These compounds are known for their broad biological activity, including antimicrobial, fungicidal, antiviral and anti-inflammatory activities (Lesyk et al. 2003; Lesyk and Zimenkovsky 2004). Novel pharmacological effects of 4-thiazolidones have been also found, such as antidiabetic and anticancer.

The aim of this study was to evaluate the effects of five newly synthesized 4-thiazolidinone derivatives on HeLa cell line (CCL2) originated from human cervical carcinoma by comparing the parameters of cell kinetic, labeling index and mitotic index. At the first stage of this work, the MTT-1 assay was applied to identify the cytotoxicity of iminothiazolidinones on the growth rates of HeLa cells after 24, 48 and 72 h of exposure. For this, the IC_{50} index was employed, calculated as a lethal concentration of compounds, which kills 50% cells in comparison with a control culture. All drugs, extracts, inhibitor applications carry out based on IC_{50} in experiments. This is optimum dose. It was found that 10 µM concentrations was the optimum concentration and compound E (2-[(4-butylphenyl) imino]-5-[(5-methylthiophen-2-yl) methylidene]-3-phenyl-1,3-thiazolidin-4 one) was the most effective compound among the tested compounds. The most effective compound and effective inhibiting concentration were evaluated. In Fig. 1a-e viability % values of HeLa cells treated with D1, D2 and D3 concentrations of five different compounds were shown. It was found that all compounds under study inhibited proliferation of CCL2 in a dose-dependent mode in 72 h after their addition to the culture medium.

Chandrappa et al. (2009) reported the synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl) methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid derivatives and evaluation of their cytotoxic activity. The compounds with electron donating groups at C-terminal of the phenyl ring concluded an increase in activity by urging cell death while compounds with electron with drawing groups (CN, F, CF3) showed decreased activity (Chandrappa et al. 2009).

According to the results of our study, the decrease in mitotic index (MI) and proliferation rate of cells were achieved at the dose level of 10 µM, especially at 72 h. It was observed that percentage of synthesis phase of HeLa cells treated with 10 µM concentration of tested compounds significantly reduced, and almost none of the cells experienced the synthesis phase (Table 2). Also, MI was 2.74% for the

experimental group and 6.98% for the control group after administration of compound E at D3 for 72 h. MI was significantly different between the control and experimental group ($p<0.001$). These data suggested that compound E had an antitumoral effect on HeLa cell line and arrested cell division G2/M phase in vitro.

In the current study, the application of 10μM of compound E for 72 h decreased the LI to 0.82% vs. 8.11% in the control group ($p<0.001$). This decrease was regarded as a significant result for the inhibition of tumor growth. The results revealed that treatments of compound E lower the percentage of the cells at S phase.

In the present study, changes in the cell cycle of HeLa cells caused by five newly synthesized iminothizolidones were investigated and, to our knowledge, this is the first investigation on this topic. The five newly synthesized iminothiazolidinone compounds had cytotoxic effect according to MTT test. The results showed that decrease in viability, mitotic index (MI) and labeling index (LI) of cells and the decline in proliferation rate were achieved at the dose level of 10 μM, especially at 72 h. According to the results, all of the tested compounds showed good antitumoral activity on HeLa cell line comparing to controls. We found that the compound E had the highest activity in reducing the reproduction rate of the cells. As a result, compound E effected cell kinetic parameters on HeLa cell line significantly ($p<0.001$). The possible anticancer effect of the compound E found in the current study may give us inspiration for the further research in the experimental animal models.

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