

SYNTHESIS AND BIOLOGICAL EVALUATION OF THIENYLTHIAZOLE-ARYL-THIOUREA HYBRIDS AS ANTI-CANCER AGENTS OVER INTRINSIC APOPTOTIC PATHWAYS
TİYENİL-TİYAZOL-ARİL-TİYOÜRE HİBRİT MOLEKÜLLERİN SENTEZİ VE BİYOLOJİK AKTİVİTELERİNİN APOPTOTİK YOL ÜZERİNDEN ANTI-KANSER ETKİLERİNİN ARAŞTIRILMASI

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ABSTRACT

Thienyl-thiazole-aryl-thiourea derivatives (**4a-4e**) were synthesized from 2-amino-4-(2-thienyl) thiazole and phenylisothiocyanates. The newly synthesized compounds were characterized by IR, ¹H-NMR and mass spectral data. All the five thiourea derivatives were screened for anti-proliferative activity on A549 (non-small cell lung cancer NSCLC) cells by real-time cell analyser (RTCA) xCELLigence system. The effects of 2 of them, which have been shown to be cytotoxic in real time system were further investigated for the molecular action of mechanisms, including mitochondrial membrane potential $\Delta\psi_M$, cytochrome c, Bcl-2 on A549 by spectrophotometric, western blotting and qRT-PCR, respectively. **4e** was found to be more potent than the others. IC₅₀ of **4e** was 29.6 μ M and 22.3 μ M for 24 h and 48 h, respectively. **4e** molecule including a trifluoromethyl (-CF₃) moiety can thus be considered as an anticarcinogenic agent and deserves further research.

Keywords: Thiourea; Anticancer activity; Thiazole; Synthesis

ÖZ

Tiyenil-tiyazol-aril-tiyöüre türevleri (**4a-4e**) amino-4-(2-tiyenil) tiyazol ve fenilizotiyasiyanatların reaksiyonları sonucu sentezlenmiştir. Sentezlenen bileşikler IR, ¹H-NMR ve kütle spektrumları ile karakterize edildi. Beş tiyöüre türevinin anti-proliferatif etkileri A549 (KHDAK) hücrelerinde gerçek zamanlı hücre analizörü xCELLigence sistemi ile incelendi. Gerçek zamanlı sistemde sitotoksik olduğu gösterilmiş olan 2 tiyöüre türevinin moleküler etki mekanizmaları, mitokondriyal membran potansiyeli $\Delta\psi_M$ spektrofotometrik olarak, sitokrom c western blot methoduyla ve Bcl-2 ise qRT-PCR ile araştırılmıştır. **4e** diğerlerinden daha etkili bulunmuştur. **4e** IC₅₀ değerleri 24 ve 48 saat için sırasıyla 29,6 μ M ve 22,3 μ M olarak hesaplanmıştır. Triflorometil (-CF₃) grubunu içeren **4e** molekülü anti-kanserojen madde olarak düşünülebilir ve daha ileri araştırmalar yapılabilir.

Anahtar kelimeler: Tiyöüre; Antikanser activite; Tiyazol; Sentez

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1. INTRODUCTION

Cancer, which is seen almost every region and socio-economic level, is a tremendous global health issue. Comprehensive efforts are being made to explore new treatment approaches as well as improving the prevention and molecular diagnostic systems. Cytotoxicity and genotoxicity of the anticancer drugs to the normal cells are the major problems in cancer therapy. Although the dose of anticancer drug is sufficient to kill tumor cells, it is often toxic to the normal tissue and leads to many side effects, which in turn, limits its treatment efficiency. Thus, the development of novel selective anticancer therapeutic agents without the disagreeable side effects is the one of the most preliminary aims in current medicinal chemistry (1-7). In recent years, hybridization approaches are widely used for producing selective anticancer therapeutic agents. In this approach, hybrid molecules, exerting their activities at two different pharmacological target structures, are synthesized (8). Thiophenes are sulfur containing heterocyclic compounds and are found in both natural and synthetic products. They exhibit a wide spectrum of biological activities such as anti-tumor, anti-ulcer, anti-metabolite, anti-viral, anti-HIV-1, anti-proliferative, anti-inflammatory and analgesic effects (9-11). Thiazole derivatives have attracted a great deal of interest owing to their anti-fungal, anti-bacterial, anti-rheumatoid, anti-hypertensive, anti-cancer, anti-convulsing and anti-helminthic activities. In addition, thiazole structure is found in vitamin B1 which is very important for decarboxylation process of carbohydrate metabolism acting as coenzyme (12-14). Substituted thioureas and their derivatives have numerous important applications. They are used as plant growth regulators and agricultural herbicides in agricultural field, as starting materials in organic chemistry and in medicinal chemistry used as anti-cancer, anti-bacterial, anti-fungal, anti-tubercular and HIV-1 protease inhibitors (15-19). In the light of the above-mentioned considerations, and as ongoing efforts to identify new potent antitumor agents, a new series of thiophene-thiazole containing thiourea derivatives **4a-4e** were designed as hybrid molecules and their anti-cancer activities were investigated.

2. MATERIALS AND METHODS

2.1 Chemistry

THF, benzene, toluene was distilled from sodium-benzophenone just prior to use. All reagents were purchased and used as received. All volatiles were removed under the reduced pressure. All reaction mixtures and column eluents were monitored by TLC using commercial glass backed thin layer chromatography (TLC) plates. Melting points were measured using, open glass capillaries and are uncorrected. Infrared (IR) spectra were recorded in the range 4000-600 cm⁻¹ via ATR diamond. MS spectra were recorded on a LC/MS Triple Quadrupole Liquid Chromatograph Mass Spectrometer Shimadzu (8040) instrument at the Erciyes University. ¹H NMR spectra were recorded on a Bruker AM 400 spectrometer (at 400 MHz, respectively) in DMSO-d₆ or CDCl₃ solution. Coupling constants, J, are reported in hertz to the nearest 0-5 Hz. Deuterated solvents were used for the homo nuclear lock, and the signals are ref-

erenced to the deuterated solvents peaks.

2.1.1. Synthesis of 2-(bromoacetyl) thiophene (2) and 4-(2-thienyl)-2-thiazolamine (3)

2-(Bromoacetyl)thiophene (**2**) (20, 21) and 4-(2-thienyl)-2-thiazolamine (**3**) (13, 22) derivatives were prepared in accordance with previously reported methods.

2.1.2. General procedure for the synthesis of thienyl-thiazole-aryl-thiourea derivatives (4a-4e)

4-(2-thienyl)-2-thiazolamine (**3**) (1 mmol) was added to benzylisothiocyanate (1,1mmol) in 5 mL toluene. The mixture was stirred at 80 °C until complete, and then cooled to room temperature. The precipitate was filtered off and washed with toluene. The resulting residue was purified by crystallization from EtOH to afford thiourea **4a-4e**.

2.1.3. N-[4-(2-thienyl)-2-thiazolyl]-N'-phenyl-thiourea (4a)

Light yellow solid; Mp: 188-190 °C; IR (ATR) 3163, 2988, 1625, 1517, 1508, 1380, 1188. ¹H NMR (400 MHz, DMSO) δ 11.99 (s, 1H), 10.75 (s, 1H), 7.64 (d, J = 7.9 Hz, 2H), 7.57 – 7.49 (m, 2H), 7.45 – 7.34 (m, 3H), 7.26 – 7.17 (m, 1H), 7.15 – 7.07 (m, 1H). MS (EI): [M-H]⁻, found 316.05. C₁₄H₁₁N₃S₃ requires 316.01.

2.1.4. N-[4-(2-thienyl)-2-thiazolyl]-N'-4-bromophenyl-thiourea (4b)

Light brown solid; Mp: 224-226 °C; IR (ATR) 3155, 2988, 1589, 1571, 1510, 1374, 1193. ¹H NMR (400 MHz, DMSO) δ 12.07 (s, 1H), 10.61 (s, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.54 – 7.48 (m, 2H), 7.41 – 7.34 (m, 1H), 7.11 (dd, J = 4.9, 3.7 Hz, 1H). MS (EI): [M-H]⁻, found 394.00. C₁₄H₁₀BrN₃S₃ requires 393.91.

2.1.5. N-[4-(2-thienyl)-2-thiazolyl]-N'-4-iodinephenyl-thiourea (4c)

Light yellow solid; Mp: 232-234 °C; IR (ATR) 3148, 2915, 1626, 1563, 1508, 1374, 1187. ¹H NMR (400 MHz, DMSO) δ 12.09 (s, 1H), 10.40 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.59 – 7.46 (m, 4H), 7.38 – 7.28 (m, 1H), 7.10 (dd, J = 5.0, 3.6 Hz, 1H). MS (EI): [M-H]⁻, found 441.95. C₁₄H₁₀I N₃S₃ requires 441.90.

2.1.6. N-[4-(2-thienyl)-2-thiazolyl]-N'-2,4-dichlorophenyl-thiourea (4d)

Light yellow solid; Mp: 218-220 °C; IR (ATR) 3142, 2973, 1633, 1585, 1509, 1194, 1060. ¹H NMR (400 MHz, DMSO) δ 12.38 (s, 1H), 10.41 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.74 (bs, 1H), 7.62 – 7.32 (m, 4H), 7.17 – 7.03 (m, 1H). MS (EI): [M-H]⁻, found 384.00. C₁₄H₉Cl₂N₃S₃ requires 383.93.

2.1.7. N-[4-(2-thienyl)-2-thiazolyl]-N'-3,5-bis(trifluoromethyl) phenyl-thiourea (4e)

Light yellow solid; Mp: 195-197 °C; IR (ATR) 3313, 2924, 1613, 1473, 1542, 1195, 1119. ¹H NMR (400 MHz, DMSO) δ 12.04 (s, 1H), 10.71 (s, 1H), 7.78 – 7.47 (m, 5H), 7.45 – 7.33 (m, 1H), 7.17 – 7.07 (m, 1H). MS (EI): [M-H]⁻, found 452.05. C₁₆H₉F₆N₃S₃ requires 451.99.

2.2 Biological activity

2.2.1. Cell Cultures and Reagents

A549 human lung adenocarcinoma cells were obtained from the American Type Culture Collection (CCL-185, ATCC) and cultured in Kaighn's modification Ham with F12 nutrient mixture supplemented with 10% heat inactivated fetal bovine serum (FBS, Biochrome), 100 U/mL penicillin, and 100 µg/mL streptomycin (Biological Industries). Cells were maintained at 37 °C in a humidified

fied atmosphere of 95% air and 5% CO₂.

2.2.2. xCELLigence Real-Time Cell Analysis (RTCA)

Optimal seeding concentration of A549 cells were determined and then the cells (12,500 cells/well) were seeded in 96-well E-plate (ACEA). Cell proliferation, attachment and spreading were monitored every 15 minutes via the impedance of E-plate wells. Approximately 24 h post-seeding when the cells were in the log growth phase, we treated cells logarithmic concentrations with **4a**, **4b**, **4c**, **4d** and **4e** at 10 nM, 100 nM, 1 µM, 10 µM and 100 µM. After that A549 cells was treated with **4e** again with six different concentrations as 1, 5, 25, 50 100 and 150 µM. The experiments were run for about 96 h. Cytotoxic effect of these molecules was monitored with xCELLigence RTCA system as described by the manufacturer's instructions (Roche Applied Science and ACEA Biosciences, San Diego, CA, USA) with slight modifications. IC₅₀ (half maximal inhibitory concentration) values were calculated via RTCA-integrated software of the xCELLigence system at 24 h.

2.3.3. JC-1 Mitochondrial Membrane Potential Assay Kit

ΔψM, is an important parameter of mitochondrial function used as an indicator of cell health. JC-1 is a lipophilic, cationic dye that can selectively enter mitochondria and reversibly change color from green to red as the membrane potential increases. In healthy cells with high ΔψM, JC-1 spontaneously forms complexes known as J-aggregates with intense red fluorescence. On the other hand, in apoptotic or unhealthy cells with low ΔψM, JC-1 remains in the monomeric form, which shows only green fluorescence. The assay was carried out using a specific commercial kit (Cayman Chemical) in line with the manufacturer's protocols. In healthy cells JC-1 forms J-aggregates which display strong fluorescent intensity with excitation and emission at 535 nm and 595 nm respectively. In apoptotic or unhealthy cells JC-1 exists as monomers which display strong fluorescent intensity with excitation and emission at 485 nm and 535 nm respectively. Changes in ΔψM determined as a ratio of healthy: unhealthy using a fluorescence plate reader.

2.3.4. Western Blot Analysis

The cytochrome c protein expression level was estimated by Western blot assay. 1 × 10⁶ cells were seeded in 6 well plates for overnight and incubated with test compounds for 24 h. Cell scraper was used to harvest the cells with phosphate-buffered saline (PBS) and proteins were isolated with ripa lysis buffer which contains protease inhibitors. After sonication, total protein concentration was measured by using BCA protein assay kit (Cell Signaling). The cell lysates were boiled at 95 °C for 5 min. The protein extracts were analysed in a 10% separating gel by an electrophoresis method and then the gels were transferred onto polyvinylidene difluoride membranes. After the transfer procedure, membranes were blocked with 5% milk solution, prepared in a Tris-buffered saline containing 0.1% Tween-20 (TBST), for 1 h at room temperature before incubation at 4 °C with the primary antibody (Cell Signaling Technology) for overnight. The antibody was typically diluted at a ratio of 1:1000. Blots were rinsed with TBST for three times and incubated for 2 h at room temperature with horseradish peroxidase-conjugated secondary antibody (Cell

Signaling Technology) anti-rabbit at a 1:2000 dilution. After secondary antibody incubation, blots were rinsed with TBST for another three times. ECL was used for reactive bands visualization under the Imaging System (New ImageQuant 350). The intensities of the bands were calculated with Image J (ImageJ 1.48, ABD). For equal protein loading, β-actin antibody (Cell Signaling Technology) was used as a control.

2.3.5. Quantitative Real-Time Reverse Transcriptase -Polymerase Chain Reaction

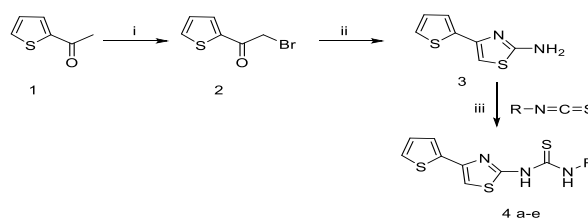
The Bcl-2 mRNA expression level was estimated by qRT-PCR 1 × 10⁶ cells were seeded in 6 well plates for overnight and incubated with test compounds for 6 h. Total RNA was extracted from the cells using a RNAzol (Sigma) according to the manufacturer's instructions. The concentration and purity of the RNA were determined by measuring the absorbance at 260 nm and determining the ratio of the readings at 260 nm and 280 nm. cDNA was synthesized from 1 µg of total RNA using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche) and RT-PCR was performed with LightCycler 480 Probes Master Kit (Roche) according to the manufacturer's instructions. The template cDNA thus obtained was incubated with gene-specific primers. The sequences of the primers were: forward—GCACCTGCACACCTGGAT reverse—AGCCAGGAGAAATCAAACAGAG. The sequences of the β-actin primers were: forward—TCCTCCCTGGAGAAGAGCTA and reverse—CGTGGATGCCACAGACT.

2.3.6. Statistical Analyses

The IC₅₀ value was obtained using the RTCA-integrated software of the xCELLigence system. The CI was calculated from repeated experiments (n = 4) with the xCELLigence system. Statistical analysis was performed GraphPad Prism Software Version 7.01 (La Jolla, CA, USA) using to compare differences in values between the control and experimental group. The results are expressed as the mean ± SD. Statistically significant values were compared using one-way ANOVA and Dunnett's post-hoc test, p < 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

Thienylthiazole-aryl-thiourea derivatives (**4a-4e**) were prepared via a facile synthetic approach [Scheme 1, steps (i)-(iii)]. α-bromo ketone **2** was obtained from commercially available 2-acetyl-thiophene (**1**) bromination using molecular bromine in dichloromethane. The starting material 2-amino-4-(2-thienyl) thiazole (**3**) was obtained by condensation of α-bromo ketone **2** with thiourea. Compound **3** was reacted with arylthioisocyanates in toluene to get the final products (**4a-4e**). The newly synthesized compounds were characterized by IR, ¹H-NMR and mass spectral data.



Scheme 1. Reagents and conditions: (i) bromination (ii) NH₂CSNH₂, EtOH, rt, 2h. (iii) Toluene, 80 °C, 24h.

All synthesized compounds (Figure 1) were evaluated anti-tumor activity against human non-small lung carcinoma (NSCLC) cell line A549 by real-time cell analyzing xCELLigence system *in vitro*.

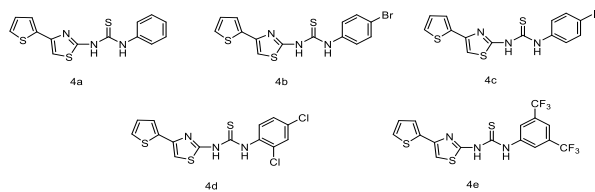


Figure 1. Structure of synthesized thienylthiazole-aryl-thiourea derivatives (4a-4e)

The cell viability and cytotoxicity were determined by xCELLigence system. Firstly, a general dosage screening of these molecules was performed via using this real time cell analyzer. **4a**, **4b** and **4d** were observed to be cytostatic (Figure 2a, 2b and 2d) whereas **4e** was observed to be cytotoxic at 10 and 100 μM concentration (Figure 2e). Further evaluation with **4e** using 1, 5, 25, 50, 100 and 150 μM has revealed that it was cytotoxic at 25, 50, 100 and 150 μM concentrations (Figure 2f). To reflect the cytostatic effect **4d** was chosen whereas to reflect the cytotoxic effect **4e** was chosen according to the cell index profiles from the RTCA system for further action of mechanism studies.

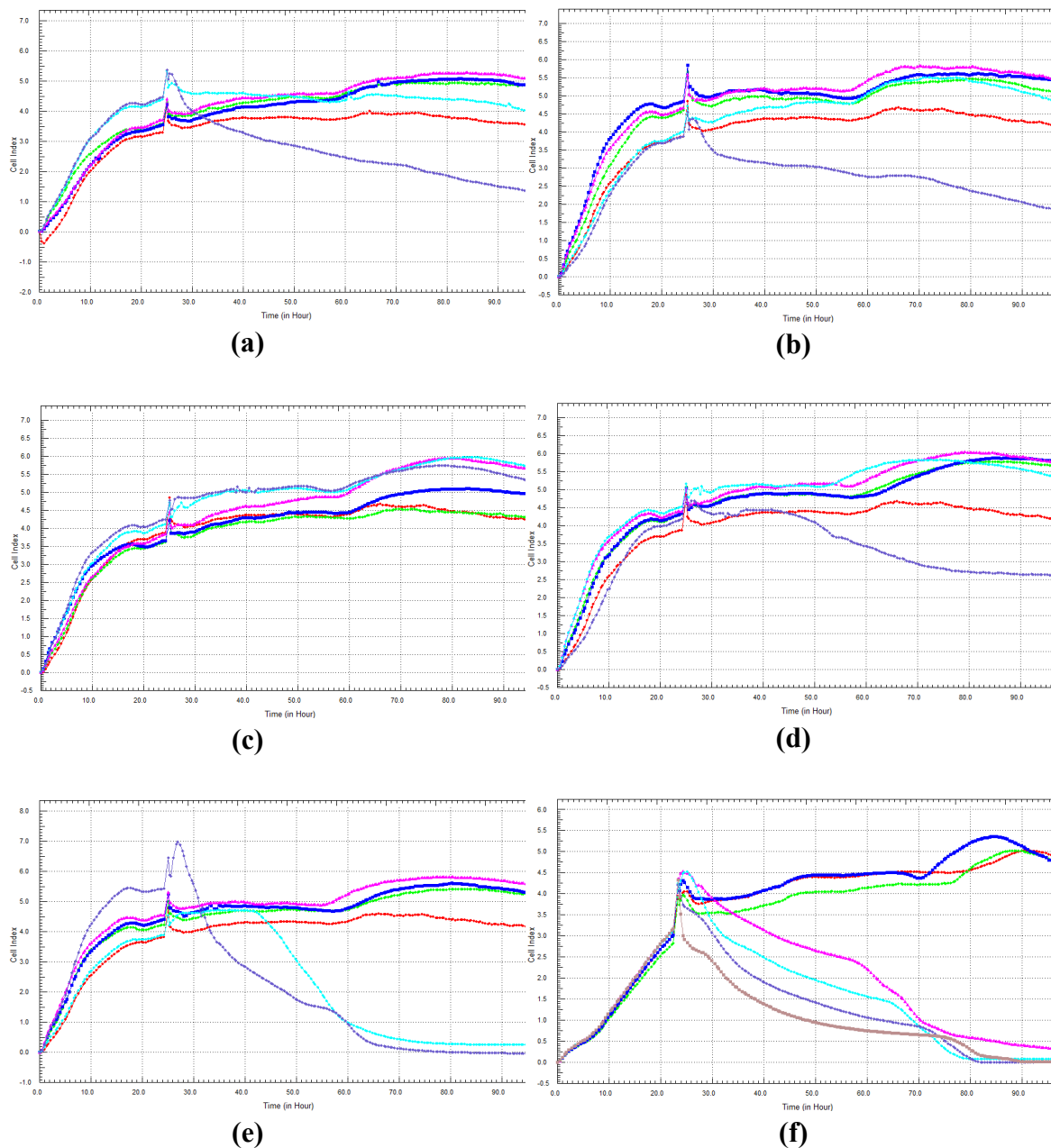


Figure 2. Real-time monitoring the effects of general dosage screening of the compounds using the xCELLigence system on A549 cells: (a) 4a; (b) 4b; (c) 4c; (d) 4d; (e) 4e at 10 nM (green line), 100 nM (dark blue line), 1 μM (pink line), 10 μM (blue line), 100 μM (purple line); (f) Real-time monitoring the effects of 4e at 1, 5, 25, 50, 100 and 150 μM

Table 1. IC₅₀ values of A549 cells for 24 h and 48 h*

Compound	24 h	48 h
4e	29.6 µM	22.3 µM

* The IC₅₀ values of **4e** were obtained based on the dose-response curves of CI during 24 h and 48 h exposure in and calculated from repeated experiments (n = 4) with the real-time xCELLigence system.

Literature suggests that most of the anti-tumor therapies induce apoptosis of cancer cells (23). There are two pathways of apoptosis, one of them is the intrinsic pathway is mediated by mitochondria and the other one is extrinsic pathway is mediated by the death receptors. Mitochondria mediated apoptotic signaling pathway can be activated over the modulation of different anti-apoptotic and pro-apoptotic proteins of the Bcl-2 family (24) and is regulated by keeping the balance between the expression of anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins. A decreased Bcl-2/Bax ratio indicates an enhanced pro-apoptotic effect (25). Literature suggest that ΔψM (26) and apoptosis pathway proteins (27) are critical for the progression of cancer. To evaluate the anti-cancer potential of this novel thiourea derivatives (**4d** and **4e**), the role of intrinsic pathway of apoptosis (via cytochrome c protein and Bcl-2 mRNA expressions) and mitochondrial membrane potential (ΔψM) assay were studied.

The effects of **4d** and **4e** on ΔψM was demonstrated by JC-1 Mitochondrial Membrane Potential Assay Kit (Cayman Chemical). **4d** at 50 and 100 µM, **4e** at 50 µM were significantly decreased ΔψM. The results showed that **4d** (50 µM and 100 µM) and **4e** (50 µM) might trigger apoptosis due to the decrease in mitochondrial membrane potential. Healthy/unhealthy ratio in control group was 15,53±0,53. Treatment with **4d** and **4e** significantly decreased healthy/unhealthy ratio (Table 2).

Table 2. The effects of 4d and 4e on ΔψM. Changes in ΔψM determined as a ratio of healthy: unhealthy.

Compound	25	50	100
4d	-	3,28±1,10*	6,50±3,12*
4e	15,50±2,12	8,28±1,10*	6,42±2,94*

*p<0.05 vs control, ANOVA/Dunnett's test

To determine the induction of intrinsic pathway of apoptosis in relation with the mitochondrial membrane potential alterations by **4d** and **4e** (Table 3), we treated A549 cells with 50 and 100 µM to **4d** and 25, 50 and 100 µM **4e** for 24 hours, **4d** and **4e** induced apoptosis in A549 cells by increasing cytochrome-c protein level at all concentrations.

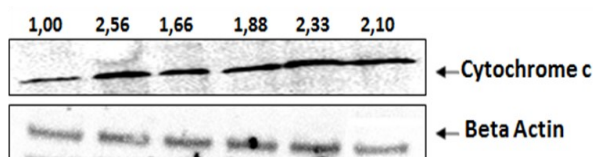


Figure 3. 4d and 4e induced apoptosis in A549 cells by increasing cytochrome-c protein level.

Table 3. Fold change in the relative cytochrome c release in A549 cells with 4d and 4e treatments. The relative intensity of each band was determined as a ratio to its corresponding β-actin band.

Compound	25	50	100
4d	-	2,56±0,46*	1,66±0,17*
4e	1,88±0,27*	2,33±0,36*	2,1±0,42*

*p<0.05 vs control, Student t test

4d and **4e** inhibited the mRNA expression of Bcl-2 all the concentrations in A549 cells (Table 4). There was a significant reduction in all concentrations of **4d** and **4e** except 25 and 100 µM, respectively.

Table 4. Fold change in the expression of Bcl-2/β-actin mRNA expression in A549.

Compound	25	50	100
4d	0,78±0,19	0,26±0,01*	0,46±0,10*
4e	0,44±0,23*	0,34±0,14*	0,78±0,03

*p<0.05, ANOVA/Dunnett's test

In this study, **4e**-exposure significantly inhibited the proliferation of A549 cells in a dose-dependent manner and is found to be most effective on this cell line. The -CI derivative, **4d**, at only 100 µM induced cytotoxicity in A549 cells in this study. Apart from the xCELLigence real time cell analyzing assay, we have detailed the cytotoxic nature of **4e** and **4d** on intrinsic apoptotic pathways related to mitochondrial function by performing JC-1, western blot and qRT-PCR assays. It has been reviewed and well-studied that mitochondria might play a crucial role in the progression of apoptotic pathway (23-28). And furthermore some novel thiourea derivatives have been also shown to have antitumoral activity influencing the apoptotic pathways (19, 29-34). Taking into account all these findings cytotoxic effects of novel thiourea derivatives have been synthesized and investigated in a real time manner in addition to the identification of molecular action of mechanisms over intrinsic apoptotic pathway. According to our findings, by RTCA system **4d** was found to be cytostatic and **4e** was found to be cytotoxic and the IC₅₀ values of **4e** were 29.6 µM and 22.3 µM concentration at 24 h and 48 h, respectively. To observe the mitochondrial health and the role of mitochondria in inducing apoptosis, mitochondrial membrane potential was determined by using JC-1 staining. Interestingly, **4d** was selectively toxic to A549 cancer cells at 100 µM however, changes in ΔψM showed that there was loss of mitochondrial membrane potential at both 50 µM and 100 µM of concentrations. Evaluating the effects of **4e** on ΔψM, **4e** was found to reduce this potential at 50 µM but not at 25 µM although it was cytotoxic at this concentration. While exploring the molecular mechanism behind the cytostatic and cytotoxic effects of **4d** and **4e** derivatives, respectively; cytochrome c protein expressions and Bcl-2 mRNA expressions were measured to determine intrinsic apoptotic pathway in A549 cells. It was observed that **4d** (50 and 100 µM) and **4e** (25, 50 and 100 µM) also increased of cytochrome c protein level in A549 cells. The apoptosis mediated effects of **4d** and **4e** at that concentrations were further confirmed by Bcl-2 mRNA expressions. It

was found that both of the derivatives have reduced the Bcl-2 mRNA expression however these effects were not dose dependent. However, for the both compounds tested, 50 μ M was found to be most effective concentration for both increasing the cytochrome c level and reducing the Bcl-2 expressions in addition to their reduction in $\Delta\psi$ M at these concentrations. From these results we can infer that **4d** and **4e** may induce cell death according to the Cl and CF₃ substituents and CF₃ is more toxic than the Cl substituent.

From our experimental data, it is concluded that thiourea derivatives significantly reduce $\Delta\psi$ M and activate intrinsic apoptosis pathway over cytochrome c and Bcl-2. In this context, it could be thought that the outcome of our experimental results is in accordance to the literature indicating pharmacological potential of a thiourea structural moiety present in numerous bioactive compounds (29-34).

4. CONCLUSION

In conclusion, since the communication between mitochondria and the cell coordinates a diverse array of functions that are critical for cell metabolism, growth and survival, it can be search of new thiophene-thiazole containing thiourea derivatives that influence this pathway. Especially thiourea trifluoromethyl (-CF₃) derivative has a time- and dose-dependent effect on A549 cell line via mitochondrial function. With all these properties, **4e** molecule including a -CF₃ moiety can thus be considered as an anti-carcinogenic agent and deserves further research. Combining all these results, we conclude that this thiourea derivative **4e** is inducing both the cytotoxicity and mitochondrial pathways of apoptosis in A549 cells. According to the results, this novel molecule deserves to be taken into account for treatment of NSCLC.

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