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Research Article

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Cytotoxic Activity Studies of *p*-Chloro and Fluoro Substituted Formazan Derivatives on Lung and Prostate Cancer Cell Lines

Akciğer ve Prostat Kanser Hücre Dizilerinde *p*-Kloro ve Floro İçeren Formazan Türevlerinin Sitotoksik Aktivite Çalışmaları

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ABSTRACT

The cytotoxic activity properties of formazan derivatives (**CF** and **FF**) bearing halogen chlorine and fluorine groups placed at para position in 1-phenyl ring have been systematically investigated in this work. The synthesized two compounds were tested as anticancer agents against human cancerous (A-549 and PC-3) cell lines for 48 h. **FF** containing *p*-fluoro in 1-phenyl ring was demonstrated more antiproliferative activity in A-549 and PC-3 cell line as compared to the compound **CF** having *p*-chloro. Particularly, compound **FF** gave better result than **CF** and cisplatin, which is used as a positive control drug, against PC-3 cell line. The results obtained showed that these two compounds have cytotoxic effect towards screened cell lines.

Key Words

Formazan derivatives, cytotoxic activity, prostate cancer, lung cancer.

ÖZ

Bu çalışmada 1-fenil halkasında para konumuna yerleştirilmiş halojen klor ve flor grupları taşıyan formazan türevlerinin (**CF** ve **FF**) sitotoksik aktivite özellikleri sistematik olarak araştırılmıştır. Sentezlenen iki bileşik, 48 saat boyunca insan kanserli (A-549 ve PC-3) hücre dizilerine karşı antikanser maddeleri olarak test edildi. A-549 ve PC-3 hücrelerinde, 1-fenil halkasında *p*-floro içeren **FF**, *p*-kloro'ya sahip olan **CF** ile karşılaştırıldığında daha fazla antiproliferatif aktivite göstermiştir. Özellikle **FF** bileşiği, PC-3 hücre dizinine karşı, pozitif control ilaç olarak kullanılan cisplatin ve **CF**'den daha iyi sonuç vermiştir. Elde edilen sonuçlar, bu iki bileşiğin, taranan hücre soylarına karşı sitotoksik etkiye sahip olduğunu göstermiştir.

Anahtar Kelimeler

Formazan türevleri, sitotoksik aktivite, prostat kanser, akciğer kanser.

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INTRODUCTION

The formazans have the characteristic chain of atoms –N=N–C=N–NH–, which contain both azo and hydrazo groups. They are important class of organic compounds as colored materials due to π–π* transitions of the conjugated double bonds in structure. For formazan derivatives, the study of intramolecular hydrogen bonding, tautomer properties, infrared spectra, absorption spectra and the X-ray diffraction structures as well as DFT calculation were commonly reported in the literature [1-2,3,4]. At the same time, metal and boron complexes of formazans have been synthesized and their catalytic, optical and electrochemical properties have been explored broadly [5-6,7,8]. Moreover, in our previous study, bis-formazan was utilized for recognition of paracetamol to modify of pencil graphite electrode as electrochemical sensor [9]. Moreover, the tetrazolium/formazan couple providing oxidation of formazans or reduction of tetrazolium salts, is comprehensively employed in diverse branches of the biological sciences, for instance, immunology, pharmacology, medicine and botany [10]. Also, formazans analogues have been researched extensively owing to their varied chemical reactivity and wide range of biological activities [11,12], such as antimicrobial [13], antifungal [14], anti-tubercular [15], anticancer and anti-HIV [12,16,17]. Recently, formazan derivatives and their conjugates showed variant promising anticancer activity in photodynamic therapy (PDT) activity against cancer cells [18]. As well, the formazans are comparatively more practical in the recognition of efficiency of anti-cancer drugs [19].

Additionally, lung cancer is accepted to be the foremost a reason of death in European countries [20]. So, prostatic carcinoma, which is offtime diagnosed cancer in men, is the second widespread reason of deaths about cancer in western civilization [21,22] Up to the present, therapeutic options existent for cancer patients contain surgery, hormonal therapy, chemotherapy and radiation therapy. Nonetheless, all this methods are temporal and ensure palliative treatment risking the healthy nearby cells. Consequently, cancer patients may be required choose alternative treatment methods, which have no side effects and cheaper. In this regard, the studies about potential anticancer from the organic compounds as anticancer agent and drugs are extensively investigated by many researchers.

Moreover, halogen atoms having electron withdrawing properties have excellent chemical properties utilized to modify the electronic properties of molecules. As well-leaving groups, they are important subgroups in the synthesis of organic intermediates as well as biological systems. Also, halogens are playing fundamental roles in natural systems. Already, the halogenated compounds have commonly utilized as designing protein inhibitors and drugs towards biomedically considerable targets [23]. Particularly the fluorine and chlorine atoms are commonly used subunits in medicinal chemistry like drug design [24]. With modulating physical and biological properties of the molecules, fluorine atom has been moved a fundamental role in new drug exploration [25,26]. Many drugs are inclusive of fluorine atom such as fluphenazine, halothane, tipranavir and Ofloxacin [27,28]. Furthermore, formazan derivatives having halogen subunits were commonly researched biological activities [12] and recently, fluorine substituted formazan derivatives were studied detailed antimicrobial activities [13]. In our previous study, we reported that formazan derivatives were showed the high cytotoxic activity against human colon cancer (DLD-1) cell line according to antiproliferative activity [29].

Herein, as an attempt to study the antiproliferative activity properties with potential applications in biological fields, we had designed and synthesized two formazan derivatives bearing of *para*-chloro and -fluoro for halogen atom subunits, (*2Z,4E*)-3-(4-(benzyloxy) phenyl)-1-(4-chlorophenyl)-5-(4-methoxyphenyl)formazan (**CF**) and (*2Z,4E*)-3-(4-(benzyloxy)phenyl)-1-(4-fluorophenyl)-5-(4-methoxyphenyl)formazan (**FF**) (Scheme 1) [29,30]. The antiproliferative activity studies of these compounds were tested against human prostate adenocarcinoma cell line (PC-3) and human lung carcinoma cell line (A549) using MTT assay methods. According to data obtained, two compounds demonstrated cytotoxic activity in both cell lines.

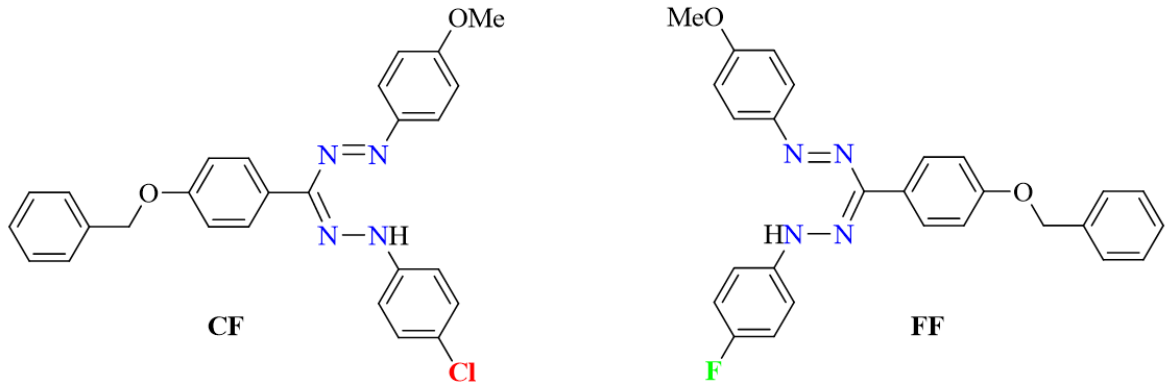
MATERIALS and METHODS

Chemistry

The synthesis and characterization of formazan derivatives **CF** and **FF** were described in our previous work [29,30].

Cell culture studies

Human lung carcinoma cell line (A549) (ATCC® CCL-185™) and human prostate adenocarcinoma cell line (PC-3) (ATCC® CRL-1435™) were purchased from ATCC



Scheme 1. Molecular structures of formazan derivatives **CF** and **FF**.

(USA). Dulbecco's Modified Eagle's Medium-high glucose (DMEM) and penicillin-streptomycin were purchased from Sigma-Aldrich Chemie GmbH. F-12K medium (Kaighn's Modification of Ham's F-12 Medium) was purchased from TermoFisher Scientific. Fetal Bovine Serum (FBS) was taken from PAN Biotech (South America). GlutamaxTM-1 (100X) was purchased from Gibco by Life Technologies. Trypsin-EDTA was bought from Wisent Inc. Phosphate Buffered Saline (PBS) was bought from VWR life science. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye were purchased from BioFrox (Germany).

Cytotoxic activity studies of compounds

The cytotoxic activity procedures were performed according to the method in the literature [31]. The A549 cells were cultured in DMEM supplemented with 10% FBS, 1% GlutaMAX, and 1% penicillin-streptomycin. F-12K instead of DMEM was used as medium for growing PC-3 cells. The cells were seeded into sterile 96-well plates at a density of 5 x 10^3 cells/well. Plates were incubated for 24 h. Following this, medium in each well was removed, and the cells were treated with the compounds at six different concentrations (5, 10, 20, 50, 100 and 200 µM). The 96-well plates were incubated for 48 h. After the incubation completed, the medium was carefully aspirated and the MTT stock solution (5 mg/mL, 50 µL) was added to each well. The plates were incubated for 2 h. Then, 200 µL DMSO was added into each well and the plates were mixed on a rocker for 30 minutes. Promega reader device was used for measuring the absorbance values at 560 nm. The GraphPad Prism 5 software was used for calculating IC_{50} values.

Table 1. IC_{50}, K_i values and inhibition types for AR and SDH enzymes.

Compounds	IC_{50} (µM)	
	A549	PC3
CF	97.06	76.25
FF	30.05	22.58
Cisplatin	25.47	46.47

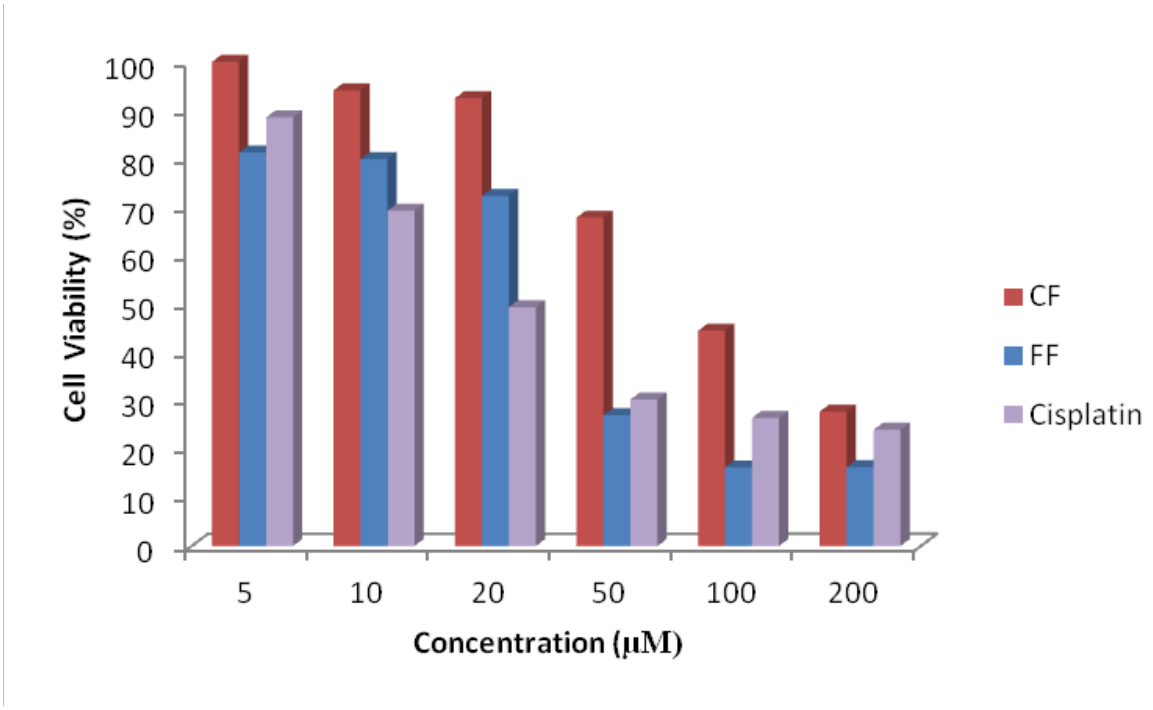


Figure 1. Cell viability ratio of A549 cells exposed to compounds for 48 h.

RESULTS and DISCUSSION

Formazan derivatives (*2Z,4E*)-3-(4-(benzyloxy) phenyl)-1-(4-chlorophenyl)-5-(4-methoxyphenyl)formazan (**CF**) and (*2Z,4E*)-3-(4-(benzyloxy)phenyl)-1-(4-fluorophenyl)-5-(4-methoxyphenyl)formazan (**FF**) were synthesized via the coupling of the (*E*)-1-(4-(benzyloxy) benzylidene)-2-(4-chlorophenyl)hydrazine and (*E*)-1-(4-(benzyloxy)benzylidene)-2-(4-fluorophenyl)hydrazine with diazonium salt of 4-methoxyaniline according to our previous work (Scheme 1) [29,30].

Cytotoxic activity evaluation of compounds

The cytotoxic activity studies of these compounds were done against A549 and PC-3 human cell lines. Cisplatin was also tested as a positive control drug under the same experimental conditions. The results are given in Table 1.

Compounds **CF** and **FF** were screened against A549 cell line at concentrations ranging from 200 µM to 5 µM for 48 h. The results clearly showed that compound **FF** as an anticancer drug candidate demonstrated more cytotoxic activity with 30.05 µM value than compound **CF** with IC_{50} value of 97.06 µM against A549 cell line. The antiproliferative activity result of compound **FF** were found to be close to effect of cisplatin in lung cell line and IC_{50} value were obtained as 25.47 µM for cisplatin, which is well known an anticancer drug, in A549 cell line. The effects of compounds **CF** and **FF** on PC-3 were also investigated. Both molecules (**CF** and **FF**) were found to be effective in prostate cancer cell line with IC_{50} values of 76.25 and 22.58 µM, respectively. Especially, compound **FF** including fluoro group demonstrated more antiproliferative activity than positive control drug cisplatin with IC_{50} value of 22.58 µM. IC_{50} value of standard drug was calculated as 46.47 µM in the same cell line (Table 1). Compound **CF** including chloro group were be less effective compare to molecule **FF** against both cell lines. Therefore, substituents on the structure of molecule play a significant role on activity. The cell viability ratios dependent on concentrations are given in Figures 1 and 2.

vThe cytotoxic effects of the **CF** and **FF** on the screened cell lines changed depending on the tested drug concentrations (Figs. 1 and 2). The percentages of live cells are seen to decrease with increasing concentrations of tested compounds **CF** and **FF** (Figs. 1 and 2). The highest cell viability ratio was observed at 5 mM concentration of compounds in both cell lines. For example, the cell viability of A549, which applied with **CF** and **FF**, were obtained as 100% and 81.28%, respectively (Figure 1). Compound **CF** did not show a very toxic effect on the

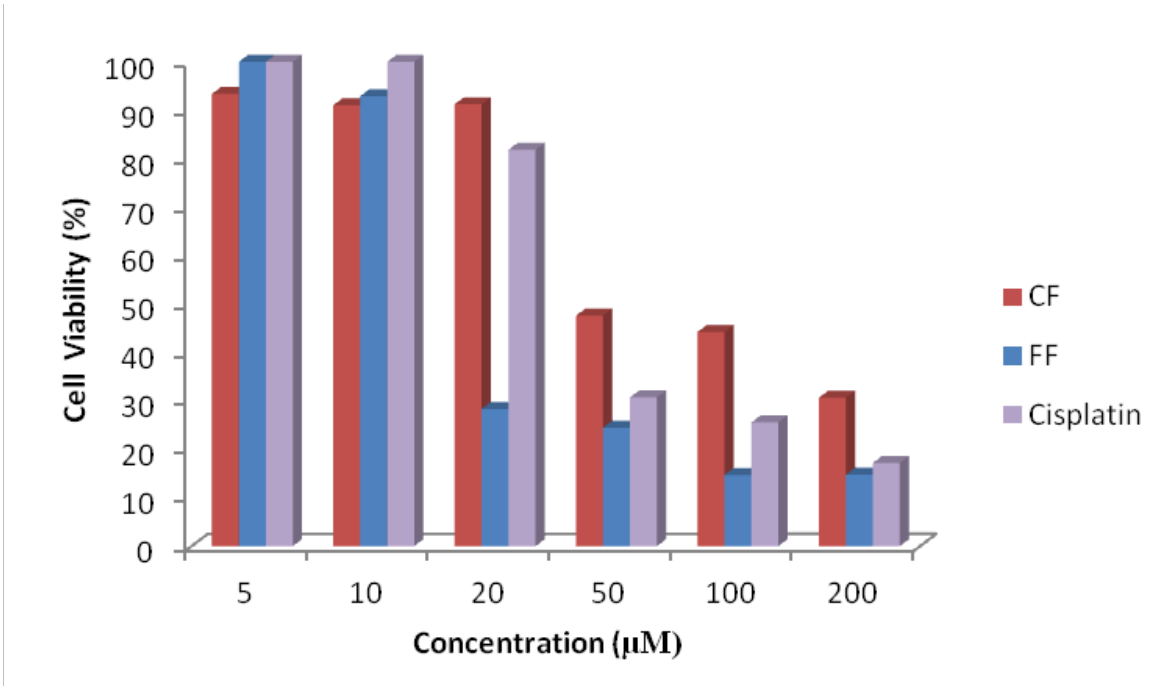


Figure 2. Cell viability ratio of PC3 cells exposed to compounds for 48 h.

A549 cell line up to a concentration of 50 µM, while the molecule **FF** showed a dose-dependent trend between 5 µM and 200 µM. On the other hand, when Figure 2 is examined, it appears that a sharp decrease in the cell viability ratio of PC-3 cells at 20 µM concentration of molecule **FF**. This decrease is from 92.92% to 28.32% when the concentration is increased from 10 µM to 20 µM. The Leica inverted microcopy images of PC-3 cells are given in Figure 3.

The morphological studies of PC-3 cells demonstrate that the growths of cells are affected by compound **FF** and cisplatin (Figure 3). In negative control group, PC-3 cells had fluently grows. The growth of cells interacted with 200 µM of the compound **FF** and cisplatin slowed down a lot compared to negative control. In addition, the above images show that compound **FF** and cisplatin induced distinctive morphological changes on PC-3 cells.

CONCLUSIONS

In this study, two formazan derivatives **CF** and **FF** containing chloro and fluoro subunits at para position in 1-phenyl ring were tested for determined their antiproliferative activity properties. The results showed that the relative compounds have cytotoxic effect against both lung and prostate cancerous cell lines. Formazan **FF** containing terminal fluoro in 1-phenyl ring has indicate more antiproliferative activity in A-549 and PC-3 cell line as compared to the compound **CF** having p-chloro. Especially, compound **FF** gave better result than **CF** and cisplatin, which is used as a positive control drug, against PC-3 cell line. Moreover, these results demonstrate that formazan derivatives could present promising strategy for development of new anticancer agents.

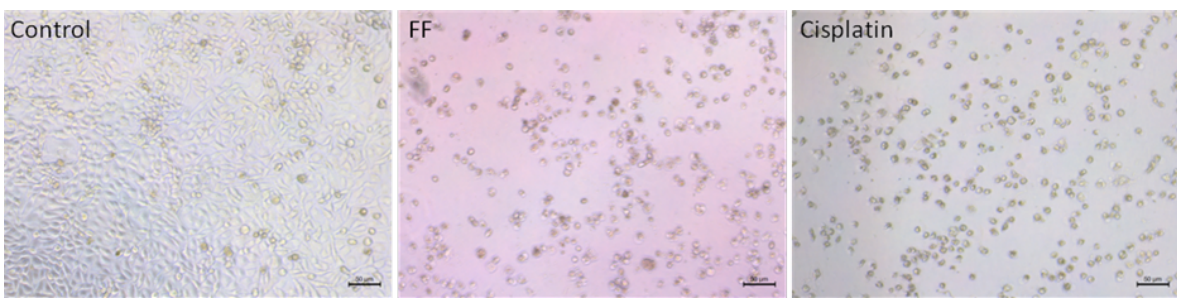


Figure 3. The images of cytotoxic effect of **FF** and cisplatin towards PC-3 cells.

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