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Synthesis of New Anthraquinone Derivatives and Anticancer Effects on Breast Cancer Cell Lines

Ebru HACIOSMANOGLU
Bezmialem Vakif University

Funda OZKOK
Istanbul University

Alper Kemal ONSU
Istanbul University

Muhammet BEKTAS
Istanbul University

Basak VAROL
Istanbul University

Sacide PEHLIVAN
Istanbul University

Abstract: Antraquinone and their analogues are among the important compounds investigated to develop novel bioactive and biocompatible molecules with potential for medical applications. The most important quinone class as anthraquinones occur widely in plants such as aloe vera and tipton's weed. Anthraquinone derivatives have aroused special interest since they have demonstrated potential therapeutic uses as antibacterial, antiviral, antifungal agents and other biological activities. Mitoxantrone, an anthraquinone analogue, is known as a synthetic anticancer analog of anthracycline antibiotics. Mitoxantrone as anticancer drug is a powerful inhibitor of the enzyme that is in charge with the repair of damaged DNA. In this study, new anthraquinone derivatives([1-(4-Chlorothiophenyl)-9,10-dioxoanthraquinone],[1-(4-Aminothiophenyl)-9,10 dioxoanthraquinone]) were characterized by spectroscopic methods (1H-NMR, 13C-NMR, FT-IR, UV-Vis analyzes). Breast cancer cell lines (MDA- MB-231 and MCF-7) and human umbilical vein endothelial cells (HUVECs) were proliferated in standard culture conditions. Cells were incubated with these derivatives for 24 and 48 h with in different concentrations. Cell proliferation assays, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) and CCK-8 (Cell Counting Kit-8), were performed to reveal anticancer effect of new anthraquinone derivatives. As a result, Cell viability of MDA- MB-231 and MCF-7 cells were decreased significantly ($p<0.05$) by new anthraquinone derivatives treatment whereas cytotoxic effect was not observed in HUVECs. In this study new synthesized anthraquinone derivatives were tested and comparable results were observed both in vitro cytotoxicity assay and statistical analysis. This anthraquinone derivatives are promising for its further development as an anticancer drug.

Keywords: Anthraquinone derivatives, Thiol, breast cancer, Cell lines

Introduction

Anthraquinone derivatives have aroused special interest since they have demonstrated potential therapeutic uses as antibacterial, antiviral, antifungal agents, anticancer and other biological activities (Ferrazzi, Palumbo, Valisena, Antonello, & Palu, 1986; Huang et al., 2002; Locatelli et al., 2011; Palumbo et al., 1987; Pickhardt et al., 2005; Rassu et al., 1991). Anthraquinones are widely used in pharmaceutical, dye and material chemistry

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research due to high biological potential activities. 1,4 and 1,5-disubstituted anthraquinone molecules as fluorescent were shown to be in EJ138 bladder cancer cells after CYP1A2 bioactivation (Errington et al., 2018). Mitoxantrone, an amino antraquinone analogue, is known as a synthetic anticancer analog of anthracycline antibiotics. Mitoxantrone as anticancer drug is a powerful inhibitor of the enzyme that is in charge with the repair of damaged DNA. Emodin extract from *Aloe vera* which compound demonstrates apoptosis in cancer cells (Wang et al., 2012). The present study aims to evaluate the in vitro cytotoxic activity of the newly synthesis anthraquinone derivatives, ([1-(4-Chlorothiophenyl)-anthracene-9,10-dione] and [1-(4-Aminothiophenyl)-anthracene-9,10-dione] were characterized by different spectroscopic methods and test against two human breast cancer cell lines MCF-7, MDA-MB-231 and also endothelial cell line, Human umbilical vein endothelial cells (HUVECs).

Methods

Synthesis of 1-(4-Chlorothiophenyl)-anthracene-9,10-dione (3) *(TR2016/19610)*

1-Amino anthraquinone compound (1) (1 g, 4.12 mmol) and 25 mL ethylene glycol were stirred in the reaction flask then 1-(4-Chlorothio) phenol (2) (0.59 g, 4.07 mmol) was added in the reaction flask. A yellowish reaction mixture was obtained at the end. 10 ml of aqueous potassium hydroxide solution was added to this yellowish mixture and the reaction temperature was raised to 120-130°C. After reflux (36h) orange thio anthraquinone compound (3) was obtained. *(TR2016/19610)* The new product was extracted with chloroform (30 mL). Organic layer was washed with water and was dried with calcium sulphate. Synthesized novel analogue was purified by column chromatography. The chemical structure of new anthraquinone derivative was identified by spectroscopic methods such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, MS.

(3): Orange crystal, mp: 227-228ºC. Yield: 0.76 g (52%). R_f [Petrolium Ether/Chloroform (1:1)]: 0.43. IR (KBr, cm^{-1}): υ= 3021, 2913 (C-H_{arom}), 1594 (C=C), 1647 (C=O). UV-vis($CHCl_3$): λ_{max} (logε)= 3.79 (427 nm), 4 (302 nm), 4.63 (247 nm). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ= 7.29-8.17 (m, 4H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ= 123.27, 125.95, 126.47, 127.21, 129.33, 129.53, 130.69, 131.64, 131.92, 132.87, 133.38, 134.14, 135.30, 136.10, 136.34, 144.69 (C_{arom} and CH_{arom}), 181.93 (C=O). $C_{20}H_{11}O_2SCl$, (M, 350.82 g/mol) (Figure 1).

Synthesis of 1-(4-Aminothiophenyl)- anthracene-9,10-dione (5) (*TR2016/19610)*

1-Amino anthraquinone compound (1) (1g) and 25 mL ethylene glycol were stirred in the reaction flask then 1-(4-Aminothio) phenol (4) was added in the reaction flask. A yellowish reaction mixture was obtained at the end. 10 ml of aqueous potassium hydroxide solution was added to this yellowish mixture and the reaction temperature was raised to 120-130°C. After reflux (36h) red thio anthraquinone compound (5) was obtained (*TR2016/19610).* The new product was extracted with chloroform (30 mL). Organic layer was washed with water and was dried with calcium sulphate. Synthesized novel analogue was purified by column chromatography. The chemical structure of new anthraquinone derivative was identified by spectroscopic methods such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, MS.

(5): Red solid, mp: 208-209ºC. Yield: 0.78 g (57%). R_f [Petrolium Ether/Chloroform (1:1)]: 0.48. IR (KBr, cm^{-1}): υ= 2923, 2852 (C-H_{arom}), (C=C), (C=O). UV-vis($CHCl_3$): λ_{max} (logε)= 3.77 (430 nm), 4.04 (303 nm), 4.71 (249 nm). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ= 6.69-6.71 (m, 4H, H_{arom}), 7.09-8.30 (m, 7H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ= 125.83, 126.46, 127.79, 130.76, 131.62, 131.67, 132.64, 133.07, 133.27, 134.09, 136.11, 136.59, 147.10, 147.32 (C_{arom} and CH_{arom}), 182.23 (C=O). $C_{20}H_{13}NO_2S$, (M, 331.39 g/mol) (Figure 1).

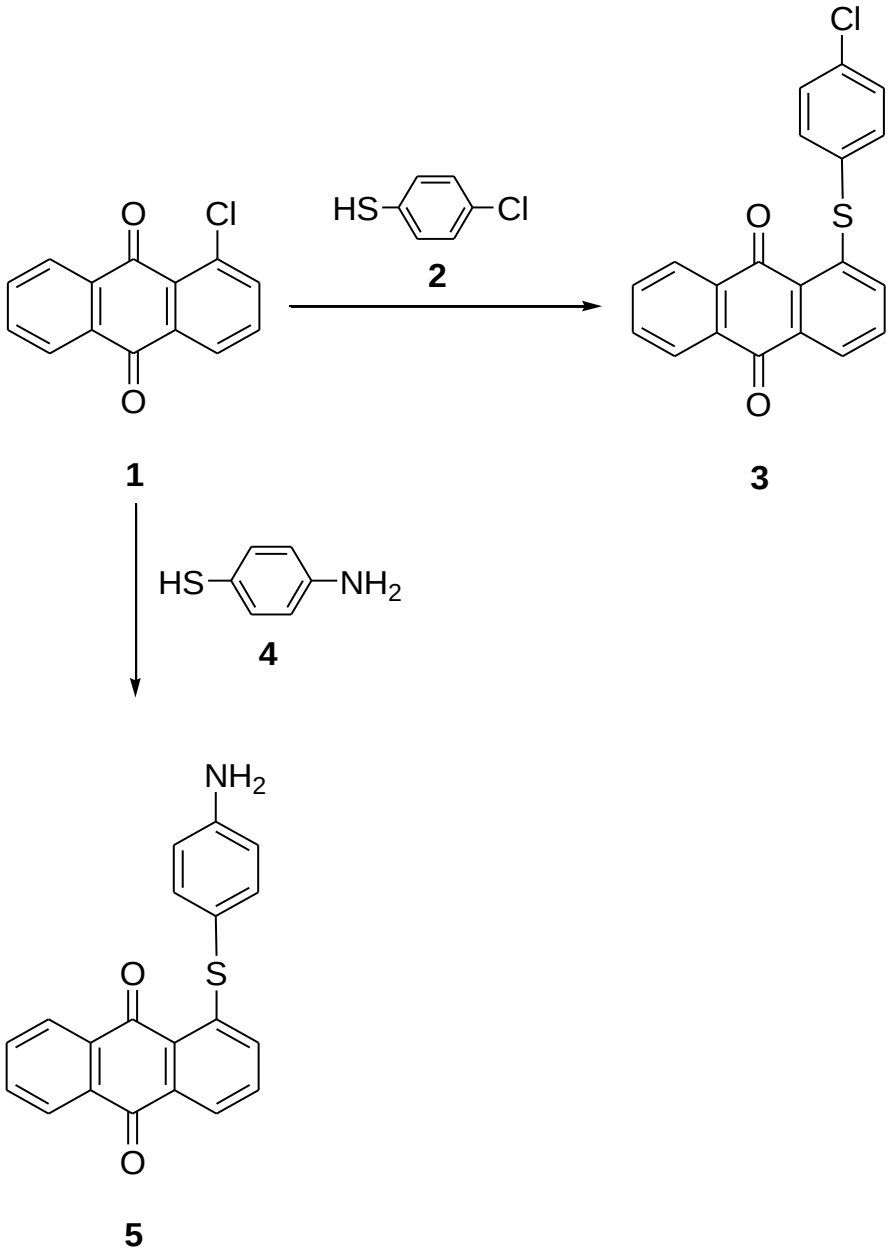


Figure 1. Synthesis of novel anthraquinone analogues

Cell Culture

Breast cancer cell lines, MCF-7, MDA-MB-231 and also HUVECs as healthy cell line were obtained from American Type Culture Collection (ATCC), Minnesota, USA. All cells were cultured with Dulbecco's Modified Eagle Medium: Nutrient Mixture-F-12 (DMEM/F-12, Gibco, USA) supplied 10% Fetal Bovine Serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in a humidified atmosphere incubator of 5% CO_2 and 95% air at 37 °C.

Cell Viability Determination by MTT and CCK-8 Assay

MCF-7, MDA-MB-231 and HUVECs were seeded in 96 well plate as $4x10^3$ cells per well a day before the experiment. Newly synthesis two anthraquinone compounds were applied separately with increasing concentrations (1, 5, 10 µg/ml) and incubated for 24 and 48 h in standard culture conditions. After incubation, 3-

(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT, Sigma-Aldrich) and Cell Counting Kit-8 (CCK-8, Dojindo) assays were realized to measure cell viability. Cells were incubated with 0.1 µg/ml MTT (5 mg/ml) for 4h. Dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added to solubilize the produced product and absorbance was measured at wavelengths of 570 nm with micro plate reader (Meran et al., 2018). Another viability test, CCK-8 was performed to support results of MTT assay . After incubation of anthraquinone treatment, [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)- 5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) was added onto cells and incubated for 3h. Absorbance of the cells were measured at 450 nm using a micro plate reader.

Statistical Analysis

The significance of the differences between control to treatment was analyzed by student-t test with GraphPad Prism 7 and p value less than 0.05 was considered significant.

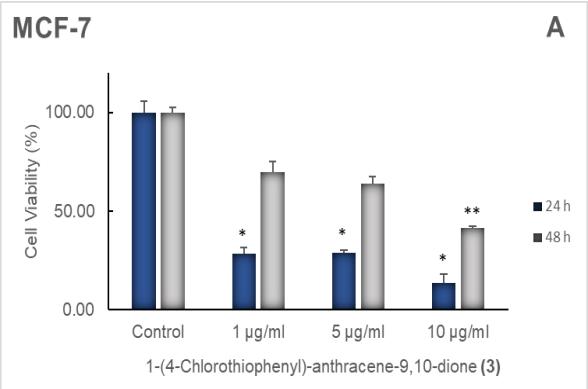
Results and Discussion

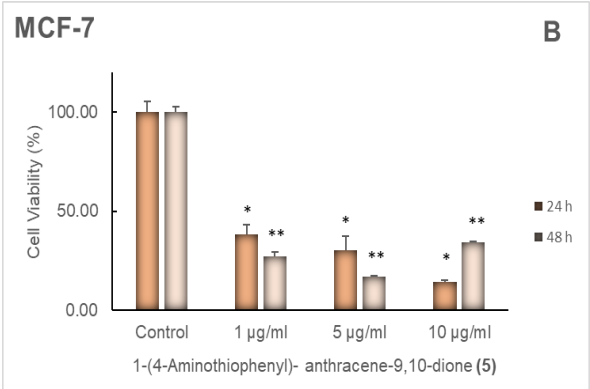
Chemistry

The compounds were synthesized, starting from 1-Amino anthraquinone compound (Ozkok F, 2016). However, under these reaction conditions as indicated in Methods section, significant amounts of the reaction products were isolated along with the desired reaction products. The molecular formulas of compound 3 and compound 5 were all determined by spectroscopic methods (Proton Nuclear Magnetic Resonance (^{1}H-NMR, ^{13}C-NMR), Fourier-transform infrared spectroscopy (FT-IR), Ultraviolet–visible spectroscopy (UV-Vis) analyzes.

In vitro Cytotoxic Activity

The *in vitro* cytotoxicity of these two newly synthesis anthraquinone compounds was tested with MTT and CCK-8. It's shown that these derivatives reveal antitumor activity significantly against the breast cancer cell lines MCF-7 and MDA-MB-231 while they do not effect on endothelial cell lines (Figure 1). The results suggested that compound 3 exhibited significant ($p<0.05$) antiproliferative activity in all concentrations for 24 h incubation. However, cell viability was decreased with just only highest concentration of compound 3 with 48 h incubation (Figure 2A, C) and it is observed that compound 5 has antitumor activity with all concentrations with 24 h as well as 48 h incubation (Figure 2B, D). Besides, cell viability of MCF-7, MDA-MB-231 and HUVECs were tested with CCK-8 to support MTT data and similar results were obtained (results not shown). There is no significant cytotoxic effect on HUVECs with both incubation of anthraquinone derivatives. It suggests that these compounds may be exhibit anticancer activity with 24 h and longer incubation period could be cause degradation of molecules or give time to cells for recovery.





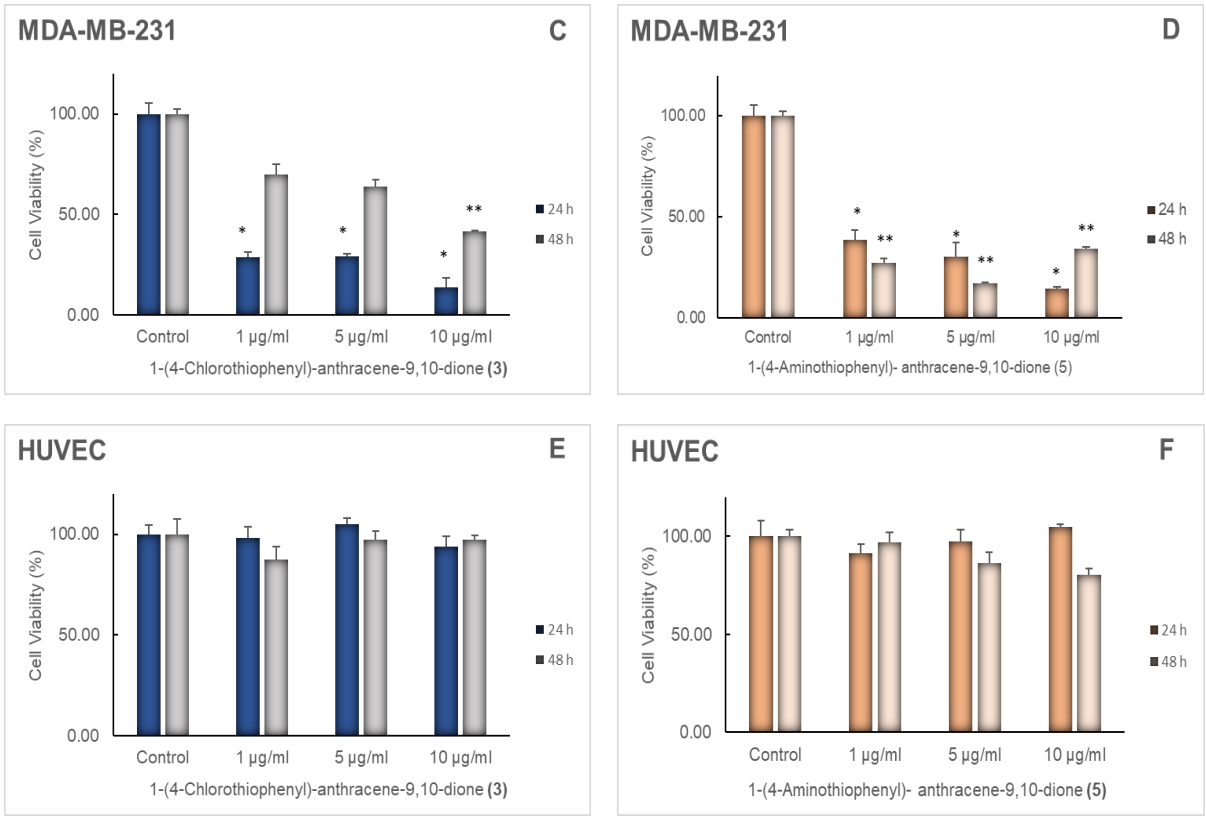


Figure 2. Cytotoxic activity of anthraquinone derivatives 3, 5.
(* $p<0.05$ for 24h incubation, ** $p<0.05$ for 48h incubation)

Conclusion

Amino and thio anthraquinone analogues are important material due to remarkable biological activities such as antimicrobial, antifungal, antioxidane anti-inflammatory and antitumor activity. These compounds are used medical research, drug design, drug delivery, anticancer treatment and biocompitable materials. The physical, chemical and biological specifications of anthraquinone-base derivatives are affected by its various substituents. And also it's known that sulphide compounds have strong anticancer effect. In this study, we described a method of synthesizing 1-(4-Chlorothiophenyl)-anthracene-9,10-dione (3) and 1-(4-Aminothiophenyl)- anthracene-9,10-dione (5) and comparing to their cytotoxicity on breast cancer cell lines. Both two derivaties have sulphide side group that attached amino group in compound 5 and chlorine in compound 3. Compound 5 has amino and sulphide groups and they are both effective on cancer cells (Iqbal et al., 2013; Nobili et al., 2009). Cell viability was decreased with compound 5 treatment with all concentrations for 24 and 48h instead of compound 3. It could be synergetic effect of amino and sulphide groups on breast cancer cell lines. Otherwise, cancer therapy could cause a toxic cell environment which destroy surrounding healthy cells, so it is important to minimize the side effects of these drugs (Almutairi et al., 2014). Therefore, the fact that these two compounds do not show toxic effects on HUVECs may be important for cancer treatment. Although the inhibition mechanism of compounds is still unclear to us, they might have the potential to be improved into a drug for anti-cancer therapies.

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,Author Information

Ebru Haciosmanoglu
Bezmialem Vakıf University, Faculty of Medicine, Department of Biophysics, Adnan Menderes Bulvarı (Vatan Cad.) P.K. 34093 Fatih / İstanbul,TURKEY
Contact E-mail: *ehaciosmanoglu@bezmialem.edu.tr*

Funda Ozkok
Istanbul University-Cerrahpasa, Faculty of Engineering, Department of Chemistry
Üniversite Mahallesi, İstanbul Ünv. Avcılar Kampüsü No:2, 34320 Avcılar/İstanbul,TURKEY

Alper Kemal Onsu
Istanbul University, Istanbul Faculty of Medicine, Department of Biophysics, Topkapı Mahallesi, Turgut Özal Millet Cd, 34093 Fatih/İstanbul,TURKEY

Muhammet Bektas
Istanbul University, Istanbul Faculty of Medicine, Department of Biophysics, Topkapı Mahallesi, Turgut Özal Millet Cd, 34093 Fatih/İstanbul,TURKEY

Basak Varol
Istanbul University, Istanbul Faculty of Medicine, Department of Biophysics, Topkapı Mahallesi, Turgut Özal Millet Cd, 34093 Fatih/İstanbul,TURKEY

Sacide Pehlivan
Istanbul University, Istanbul Faculty of Medicine, Department of Medical Biology, Topkapı Mahallesi, Turgut Özal Millet Cd, 34093 Fatih/İstanbul,TURKEY