INVESTIGATION OF CYTOTOXIC PROPERTIES OF NEW ISOINDOL DERIVATIVES IN LUNG AND CERVICAL CANCERS

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

in Chemistry

by
Yasir ALMUSAWI

November 2019

İZMİR

We approve the thesis of **Yasir ALMUSAWI**

Examining Committee Members:

Prof. Dr. Gülşah ŞANLI MOHAMED
Department of Chemistry, İzmir Institute of Technology

Prof. Dr. Münire Nalan DEMİR
Department of Biochemistry, Dokuz Eylül University

Prof. Dr. Ali ÇAĞIR
Department of Chemistry, İzmir Institute of Technology

15 November 2019

Prof. Dr. Gülşah ŞANLI MOHAMED
Supervisor, Department of Chemistry, İzmir Institute of Technology

Prof. Dr. Ahmet Emin EROĞLU
Head of the Department of Chemistry

Prof.Dr. Mehtap EANES
Dean of the Graduate School of
Engineering and Sciences

ACKNOWLEGMENTS

First of all, I would like to express my warmest thanks to my supervisor **Prof. Dr. Gülşah ŞANLI-MOHAMED**, who fearlessly accepted me as MSc student for her project and was the main creator of the great ideas, techniques and whole background of this thesis. We experienced together all the ups and downs of routine work, the shared happiness of success and the depression of failure when (sometimes) everything went wrong. She managed to teach me how to work independently (which is very important), but at any time, her useful advice was available to me. In my opinion, this is how an ideal supervisor should be. It is a real pleasure to work and communicate with such a polite, honest and open-minded person. I would also like to acknowledge her language abilities which were extremely useful, particularly at the beginning of my work and life in Turkey.

I gratefully acknowledge expressing my appreciation to Biotechnology and Bioengineering Research Center specialists, especially **Özgür Yılmazer** and **Murat DELMAN** for their kindly help.

Also, I would like to thank all of my friends in IYTE especially my friend **Derya METE** to teach me and support me in everything joined in this thesis.

Besides, thanks from my deep heart to **IYTE** and my second home **Turkey** for good hospitality and I wish it will be more and more good.

I would also thank and there is no word to express my deep and endless thanks to my family, my father, and my mother. I am so grateful to them for their endless support, patience, limitless love and encouraging me to reach my dreams.

Above all, I would like to thank my wife for her love and constant support, for all the late nights and early morning, thank you for being my muse, editor, proofreader, and sounding board. But most of all, thanks for being my best friend. I owe you everything

ABSTRACT

INVESTIGATION OF CYTOTOXIC PROPERTIES OF NEW ISOINDOL DERIVATIVES IN LUNG AND CERVICAL CANCER

Cancer is one of the most common diseases in the world. Recently, there are many methods developed by researchers to treat this disease. One of these treatments is targeted for chemotherapy. It is preferred by researchers because it is less toxic and has fewer side effects than other cancer treatments.

This study emphasizes the anticancer properties of the newly synthesized Isoindole derivatives. Thus, it was hoped to be a significant improvement based on new generation anticancer compounds with high efficacy and fewer side effects.

The main objective of this study was to investigate the biological activity of seven newly synthesized Isoindole derivatives. The anticancer activity of these compounds was evaluated against HeLa (cervical carcinoma) and A549 (lung adenocarcinoma) cancer cell lines.

This study is divided into three parts. Firstly, the cytotoxic activity of these compounds was determined by measuring the cell viability of each compound on HeLa and A549 cell lines. The main objective of this analysis is to measure the IC_{50} value of each compound and determine which compound is best to kill at least half of the cells. Secondly, the effects of programmed cell death and cell cycle were investigated for compounds with the best IC_{50} for each cell line by using Annexin V-FITC in flow cytometry. Finally, a scratch assay was performed to investigate the effect of these new Isoindole derivatives on cell migration.

ÖZET

AKCİĞER VE RAHİM AĞZI KANSERLERİNDE YENİ İZOİNDOL TÜREVLERİNİN SİTOTOKSİK ÖZELLİKLERİNİN ARAŞTIRILMASI

Kanser, dünyadaki en yaygın hastalıklardan biridir. Son zamanlarda, bu hastalığı tedavi etmek için araştırmacıların geliştirdiği birçok yöntem mevcuttur. Bu tedavilerden biri olan hedeflenen kemoterapi; daha az toksik olması ve diğer kanser tedavilerinden daha az yan etkisi olması nedeniyle araştırmacılar tarafından tercih edilmektedir.

Bu çalışma, sentezlenen yeni Isoindole türevlerinin antikanser özelliklerini vurgulamaktadır. Böylece yüksek etkinliği ve daha az yan etkisi olan yeni nesil antikanser bileşiklere dayanan önemli bir gelişme olması umut edilmiştir.

Bu çalışmanın temel amacı, yeni sentezlenen yedi Isoindol türevinin biyolojik aktivitesini incelemektir. Bu bileşiklerin antikanser aktivitesi, HeLa (Yumurtalık kanseri) ve A549 kanser hücre hücresine (Akciğer kanseri) karşı değerlendirilmiştir.

Bu çalışma üç bölüme ayrılmıştır. İlk olarak, bu bileşiklerin sitotoksik aktivitesi, her bir bileşiğin HeLa ve A549 hücre hatları üzerindeki hücre canlılığı (MTT analizi) ölçülerek belirlenmiştir. Bu analizin temel amacı, her bileşiğin IC_{50} değerini ölçmek ve hücrelerin en az yarısını öldürmek için hangi bileşiğin en iyi olduğunu belirlemektir. İkincisi, akış hücre sitometrisinde Annexin V-FITC kullanarak her hücre hattı için en iyi IC_{50}'ye sahip bileşikler için programlanmış hücre ölümünün etkisi ve hücre döngüsü üzerindeki etkisi araştırılmıştır. Son olarak, bu yeni Isoindol türevlerinin hücre göçü üzerindeki etkisini incelemek üzere çizik deneyi yapılmıştır

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

Figure **Page**

CHAPTER 1

INTRODUCTION

1.1. Cancer

There is more than one definition for cancer and all these definitions indicate the same meaning and it could be formulated in a simple definition. The cancer is a type of disease that occurs when the cells going to abnormal growth. This operation has been done by changing in gene expression, which is leading to dysregulated cell proliferation and the cell will die. Consequently, the patient will favour and growth of a tumor. The tumor divide into two types the first one is called benign tumor, which is like a friend for human as it doesn't spread through the body, while the second one called malignant tumor, which is the danger than the first one as it spreads very fast through the body (Hanahan and Weinberg 2000, Pudata, Subrahmanyam, and Jhansi 2011).

There are three different types of cancer:

- **Carcinomas:** this type of cancer forms 85% in the world and develops in epithelial cell-like Lung cancer, Oral cancer, Skin cancer and Bladder cancer (Mehta 2011, Khan 2010, Farhadieh et al. 2011).
- **Lymphomas:** this type forms 7% in the world. It is cancer of lymph cells in the immune system. The structure of the lymph nodes is very small, therefore, it is along with blood in the human body (Bali et al. 2010).
- **Sarcomas:** this type of cancer formed in mesoderm tissue which is known as sarcomas (Lau, Chan, and Wei 2011). The example of this type of cancer is Bone, muscle, fat tumor of hematopoietic tissue.

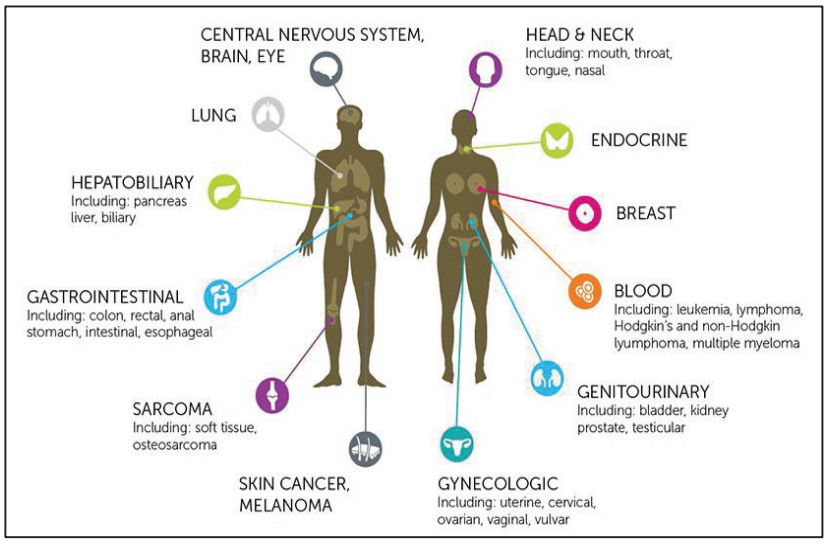


Figure 1. 1. Type of cancer

(Source: Mary Crowley Cancer Research)

There are many types of cancer treatments. These treatments, including surgery, radiation, immune therapy, targeted therapy and chemotherapy.

Surgery is a frequent and most common treatment, by removing the tumor during surgery. This type of treatment could minimize cancer, but it could not eliminate it definitively. The types of surgery are a biopsy, ultrasonography, endoscopy and open surgical exploration (Khan 2010).

Chemotherapy is one of the cancer treatment types by using anticancer drugs to kill cancer cells. Chemotherapy should use after or before surgery which gives a positive point to the patient because he does not need radiation therapy after this (Abdel Ghany et al. 2011). Chemotherapy has some side effects, these side effects will kill the normal cells that are close to cancer cells, thus, for that reason the scientist still working to develop some drugs with fewer side effects and without killing normal cells (Pöpperl et al. 2005).

Radiation therapy also one of the cancer treatments by using gamma rays, x-rays and charged particles (Wagner and Vorwerk 2011). This treatment almost using for endometrial cancer.

Targeted therapy is a small drug molecule that could enter inside the cancer cell and kill it, it also helps the immune system and stops the dividing of the cancer cells (Shaghayegh, Mahdi, and Ali 2010). The most common side effects for this treatment are hypertension, diarrhea, hyperpigmentation and headache (Nieder et al. 2010)

1.2. Lung Cancer and A549 Cell line

According to the statistic of the "American Cancer Society" lung cancer considered the most common cancer in each woman and man in the world. Where the percentage of human mortality in lung cancer rather other kinds of cancer is "154,050 deaths of 234,030 new cancer cases in 2018" (Mehta 2011)("A.C. Society, Key Statistics for Lung Cancer, (2018) (Accessed 31 May 2018), Https://Www.Cancer.Org/Cancer/Non-Small-Cell-Lung-Cancer/about/Key-Statistics. Html.No Title," n.d.). The standard treatment for lung cancer is chemotherapy, it is still good therapy for this disease because it gives significant improve symptoms for a patient that has lung cancer (Eisenberg et al. 1998, Group 1995, ten Bokkel Huinink et al. 1999). Depending on the histological science, lung cancer cells classified as small cell lung cancer and non-small cell lung cancer (NSCLS). NSCLC is the most common type of lung cancer cell, and it is about 85% of all lung cancer cases (Xia et al. 2017).

The A549 lung cancer cell line was discovered in 1972 by removing and culturing of lung tissue for 58-year-old Caucasian male and it is classified through pulmonary epithelium cell type (Lieber et al. 1976).

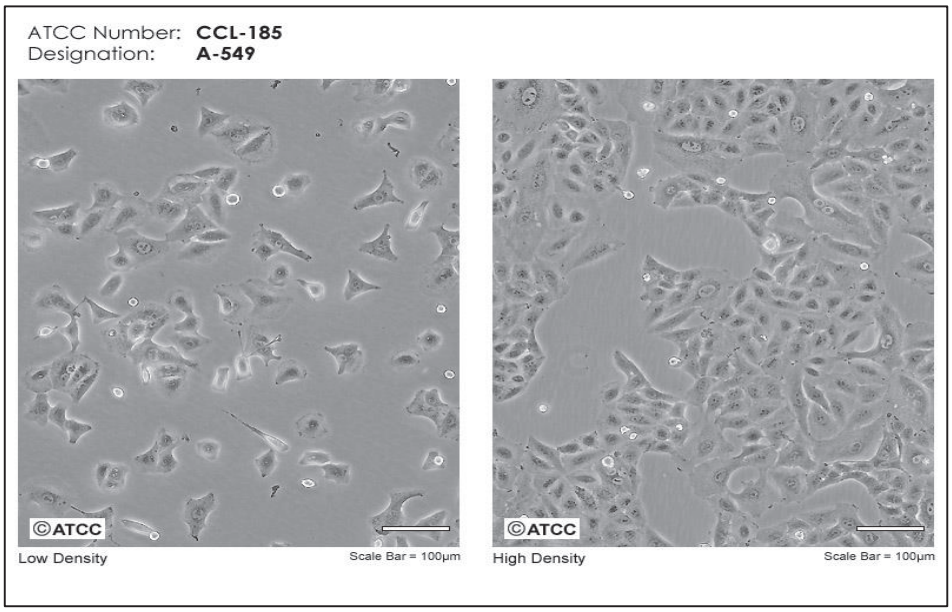


Figure 1. 2. Morphology of A549 cells monolayer
(Source: ATCC global biological materials resource)

1.3. Ovarian Cancer and HeLa Cell Line

Ovarian cancer classified as the sixth most common cancer in women worldwide and also the most common gynaecologic malignancy, it is caused around "125,000 deaths per year" (Armstrong et al. 2006, Ouyang et al. 2009, Yang et al. 2012, Gao et al. 2012). Ovarian cancer incidence has increased and it is continuing to grow to become the highest in Europe, Northern America and slightly in Japan (Rao and Pagidas 2010, Maciejczyk and Surowiak 2013). The procedure of early diagnosis for ovarian cancer is limited, therefore, the death rate of ovarian cancer did not change over decades (GERCEL-TAYLOR, Feitelson, and Taylor 2004, Luo et al. 2011). Additionally, around 80-90% of ovarian cancer is epithelial, while the nature of heterogenic for the tumor is conferring a weak prognosis and an increase in lethality (Chen et al. 2011, Mazumder et al. 2012, Solomon et al. 2008).

The HeLa cell was first found in 1951 and the name of these cells took from Henrietta Lacks, the woman that donated ovarian cancer cells, therefore, for this reason, it called by her name. She was born in Virginia 1920, and one day she felt a pain in her abdomen, after diagnosis, the result shows that she has cancer in her ovarian, and she died in 1951. The doctor took a sample from her cancer cells and do growing in the laboratory to do the research and develop a new drug for this type of cancer.

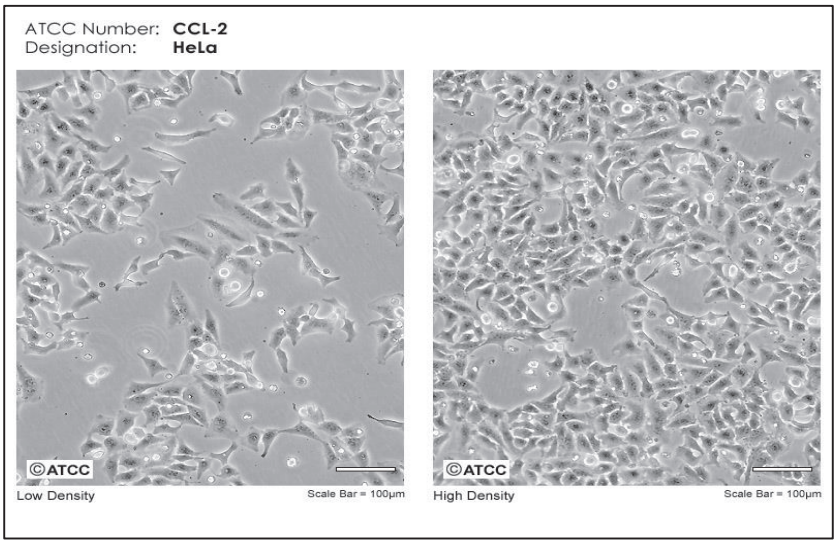


Figure 1. 3. Morphology of HeLa cells monolayer

(Source: ATCC global biological materials resource)

1.4. Cell Cycle

The first description of the cell cycle has been stated by Howard and Pele in 1951. The cell cycle has been defined that the interval between mitosis completion and the subsequent mitosis completion in one or both new cells (Gali-Muhtasib and Bakkar 2002). It is divided into four phases, mitosis (M) phase, G_1 phase, S phase and G_2 phase. The center of this process represents by the cyclin-dependent kinase (CDKs). This cyclin protein regulates cell progression during all the cycle stages.

Mitosis (M) phase contains four phases (prophase, metaphase, anaphase, and telophase). G1 and G2 phases describe the "gaps" between mitosis and DNA synthesis. The first stage is G1 phase, which is the duration between mitosis and the starting of DNA synthesis. The second stage is S phase represents the interval of which of DNA is replicated. The third stage is G2 phase represents the interval between consummation of DNA synthesis and mitosis (Alberts et al. 1986, Patt and Quastler 1963, Baserga 1968).

In addition, during the anti-cancer development for CDKs, two steps should be considered, the first one called direct strategy, which is done by inhibition the activity of CDKs, and the second one called the indirect strategy, which is done by targeting the majority of regulators activity for CDKs (Vermeulen, Bockstaele, and Berneman 2003).

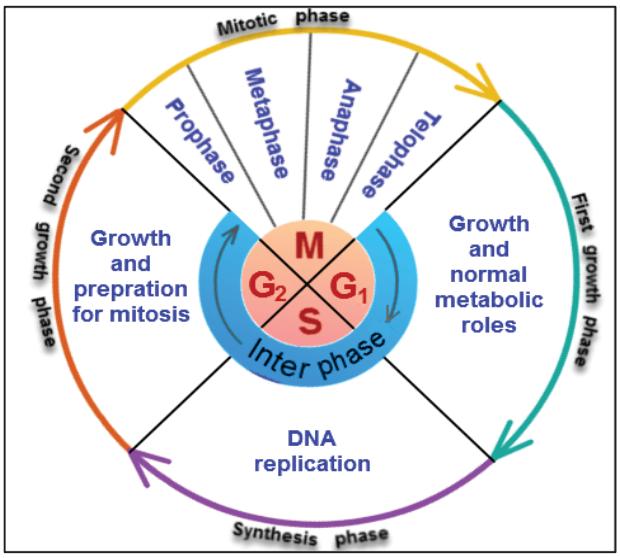


Figure 1. 4. The cell cycle stages

(Source: Tutor Vista, Biology)

1.5. Cell Viability Assay (MTT)

Any new drugs that have been produced by the scientist, the cell viability and cytotoxicity should be taken into account to check whether works for the cancer treatment or not. Thus, the MTT assay has been used for these examinations. MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) is the most common test for cell viability and cytotoxicity, which stands for Methyl-Thiazolyl-Tetrazolium (Mosmann 1983, Tully et al. 2000, Pagliacci et al. 1993).

The principle of (MTT) depends on reducing the tetrazolium salts in the formazan crystal. This process occurs by mitochondrial reductase, and the color will change from yellow to purple color, which means that all the viable cells reduce tetrazolium salts (Nikkhah et al. 1992, Van Meerloo, Kaspers, and Cloos 2011, Etxeberria, Mendarte, and Larregla 2011). Many authors prefer the (MTT) principle into two way (a) the reducing process of MTT occurs in mitochondria (b) use it as an indicator of mitochondria to evaluate the activity and functions (Saravanan et al. 2003, Van de Sandt, Rutten, and Koëter 1993, Du et al. 2010). The intensity of purple color depends on the number of viable cells, if it has a lot of viable cells, the color will be dark otherwise the color will be light, which means that the most of the cells are dead (Saravanan et al. 2003).

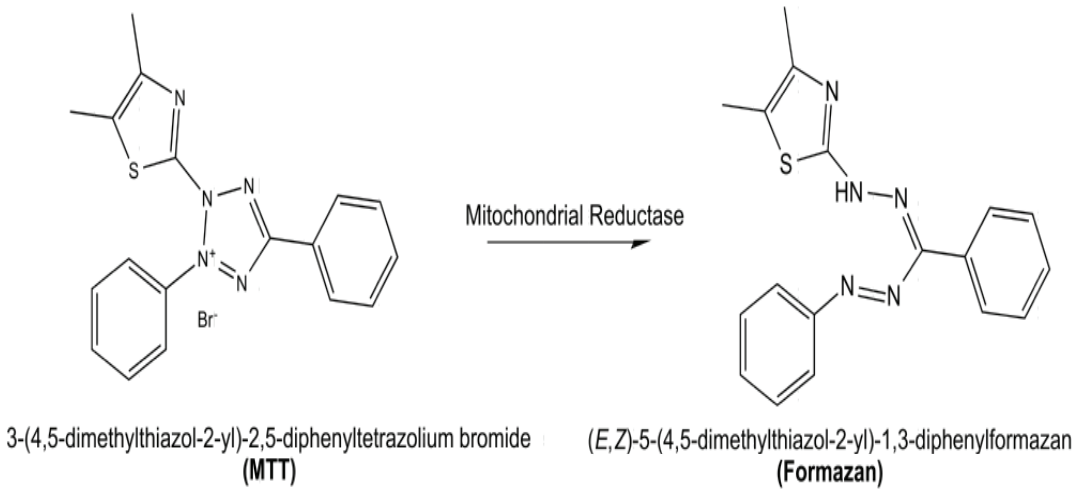


Figure 1. 5. Chemical structure of MTT and reduced to formazan

(Source: MyBioSource)

1.6. Apoptosis

Apoptosis is a process that the cells kill themselves by programmed pathways (Horvitz 1994, Vaux, Haecker, and Strasser 1994). Apoptosis has been used for the first time by Kerr, Wyllie, and Currie in 1972 for characterizing the morphological distinct of cell death (Kerr, Wyllie, and Currie 1972, Kerr 2002). One question could be raised, why the cells try to kill itself? two reasons answer this question, firstly, the cells may get the infection by Bactria or viruses, secondly, it may be because morphological shape like a hand for mammals to have good hand shape as shown in Figure 1.6.

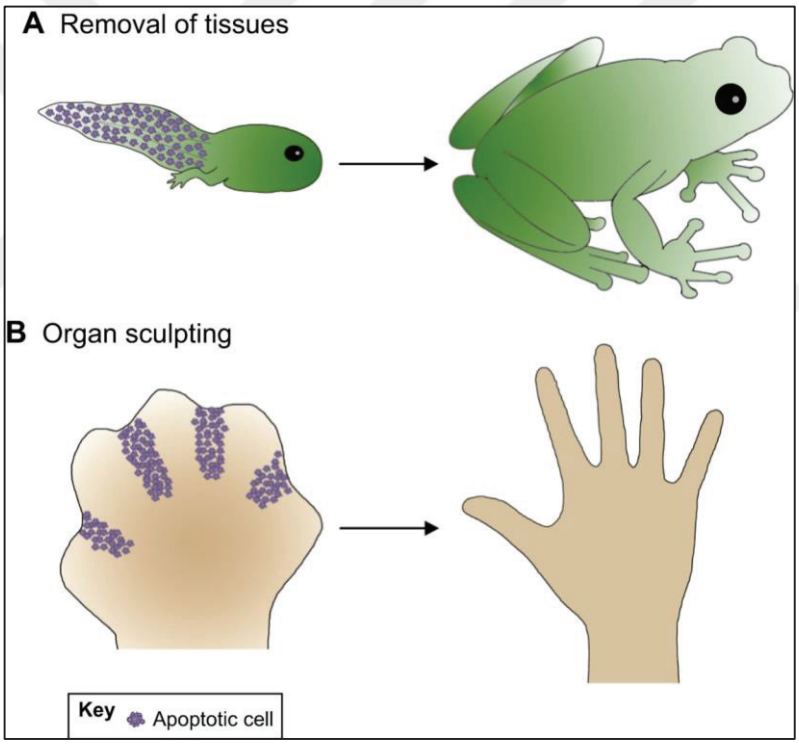


Figure 1. 6. Role of apoptosis in tissue and sculpting
(Source: The Company of Biologists)

As it is known the cancer cells grow very quickly, which may lead to a tumor and then cancer, therefore, there is a need for apoptosis to stop this spreading.

1.6.1. Mechanism of Apoptosis

First of all, all the cells contain a protein that is responsible for cell expiry (death) called Caspase-3 (cysteine-aspartic acid protease), if this protein has appeared in the cells, it will die. When this protein activated, the cell will acquire the proteases or nucleases, which means that the DNA and RNA will be destroyed (Martinvalet, Zhu, and Lieberman 2005, Hengartner 2000, McIlwain, Berger, and Mak 2015, Lakhani et al. 2006).

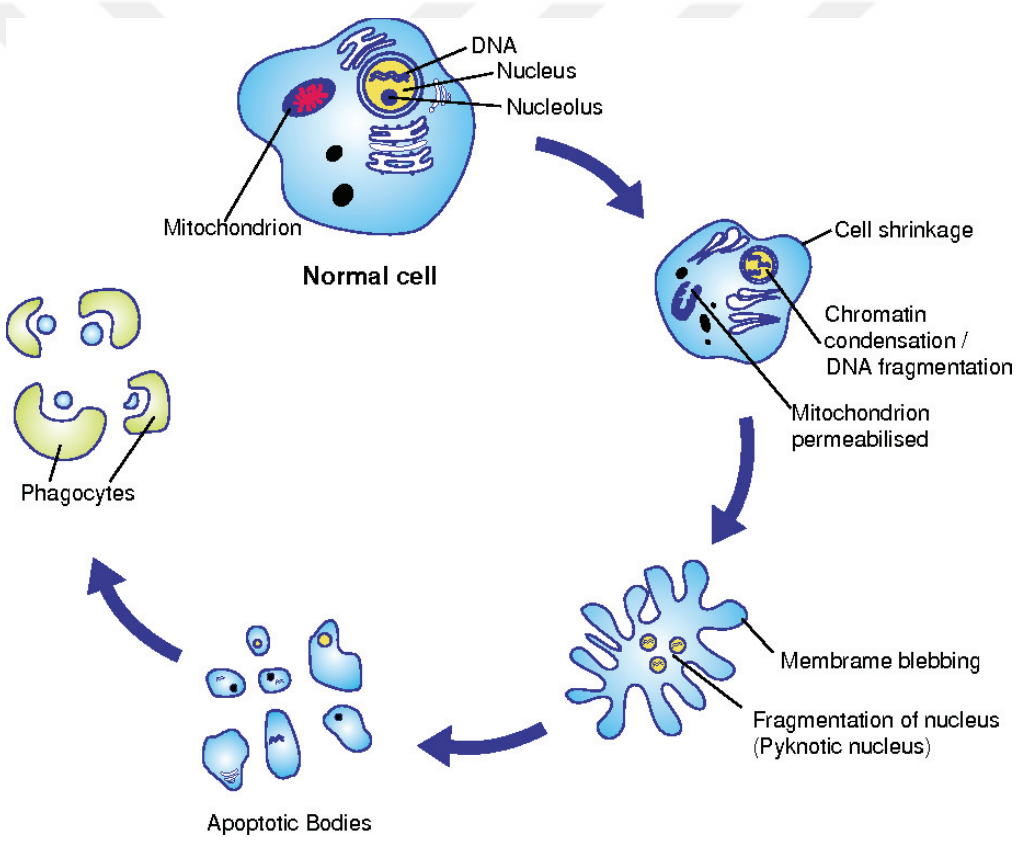


Figure 1. 7. Morphological feature of apoptosis cell

(Source: Marc, Declan, Colm and Thomas 2006)

The mechanism of apoptosis is divided into two ways:

1- **Extrinsic Pathway:** this pathway is happened by outside signal effect to the cell, when any weird body, for example, Bactria or virus touches the cell receptors. The cell has two receptors and these respecters are very close to each other, thus this process indicates the death-inducing signaling complex (DISC) (Wong 2011, Schneider and Tschopp 2000). DISC will activate the pro-Caspase-8 and this protein going to activate Caspase-8, Caspase-8 responsible for activating the Caspase-3 protein. Consequently, when the Caspase-3 is activated it will be deactivated of nuclease inhibitor, in this case, nuclease becomes active and has the ability to degradable the DNA of the cell (Ashkenazi and Dixit 1998,Hengartner 2000,Walczak and Krammer 2000).

2- **Intrinsic Pathway:** this pathway takes place inside the cell, the reason for this pathway is genetic damage. The target of this pathway is mitochondria and cytosol. As stated earlier the main reason for cell death is because of the DNA damage (Wong 2011). The DNA contains a protein like ATM and CHK, if these proteins are damaged, they will activate the P53, Thus, the cells will be in a very dangerous situation because it could give multiple proteins. After that, the P53 will activate (BAX) protein and this protein will create pores in mitochondria (Decaudin et al. 1998,Green and Kroemer 2004), Therefore, this pores will release cytochrome from mitochondria to the cytosol, which gives a direct signal that the cell will die, this is because cytochrome should present inside mitochondria. Cytochrome will bind with Apaf-1 protein and this protein will be converted to Caspase-9, when the Caspase-9 is activated the Caspase-3 will be active, and this protein will kill the cell(Tsujimoto et al. 1984, Reed 1997, Kroemer, Galluzzi, and Brenner 2007, Wong 2011).

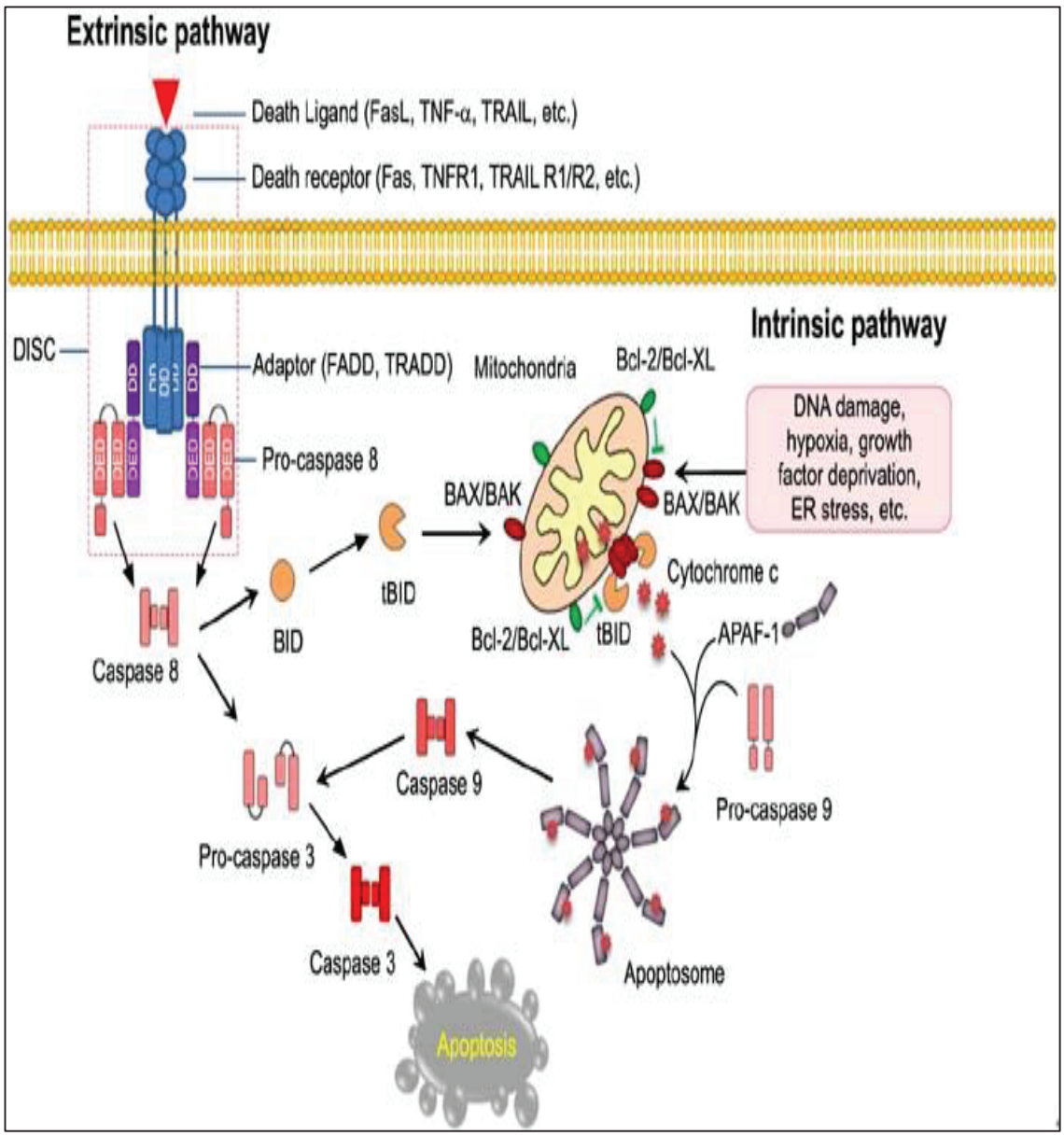


Figure 1. 8. Extrinsic and Intrinsic Apoptosis pathway

(Source: Lorenzo, Catherine, Eugenia, Zahia and Guido 2008)

1.6.2. Difference between Apoptosis and Necrosis

Necrosis is a process for cellular death, which happens when the cells exposed to an external condition different from natural conditions (Hirsch et al. 1997). This leads to damage the internal cellular and rapid cellular tissue damage (Majno and Joris 2004). Thus, the main job of the cell is to save the cell balance and detect the hard conditions that are leading the cell to die.

The differences between Apoptosis and Necrosis based on three important points have been explained below:

1. **The cause and presentation:** Necrosis is a pathological process; it is also harmful to the body. It happens when the cells exposed to the toxicant or hard conditions, which may increase the temperature or decrease the oxygen level (Kroemer and Dallaporta 1998). In addition, Necrosis may lead to damage to the wall or membrane of the cell and reduce its ability to maintain the normal function, which also leads to having imbalance cell (Kerr 1971). Apoptosis is a normal process which necessary and maybe a pathological or physiological process for the body. (Thompson 1995).
2. **Structural Changes:** In necrosis, there are many integrity, which may lead to the swelling and disintegration for the small cells. However, in apoptosis, there are no disintegration or membrane but there is shrinkage, which may lead to the formation of bodies called apoptotic bodies. These bodies have been recognized by the immune system and destroyed effectually by the immune cells, thus is a well-coordinated process (Farber 1994).
3. **Biochemical Reactions:** Necrosis is a bad process because it requires energy and it could occur randomly at any time. In Apoptosis it is an active process requires energy, but it occurs in a controllable way that makes it needs many proteins and enzymes in its operation (Ciechanover and Schwartz 1994).

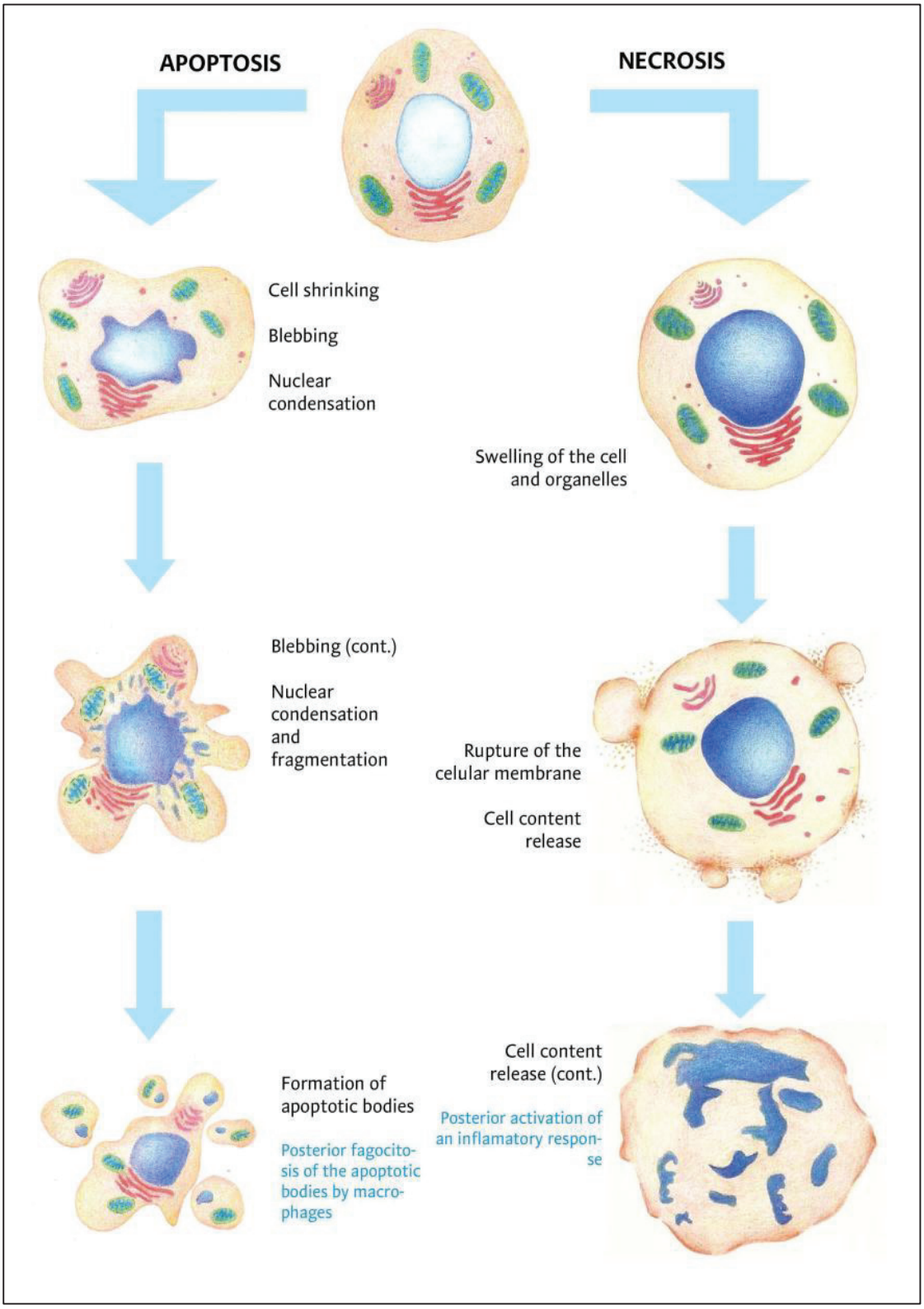


Figure 1. 9. Apoptosis and Necrosis

1.7. Wound Healing Assay (Scratch Assay)

Cell migration is a very important process for developing cells in many physiological disorders like cancer invasion, angiogenesis, metastasis and inflammatory (Klein-Soyer et al. 1994, Fischer, Stingl, and Kirkpatrick 1990). Also in the physiological repair process (Saga et al. 2003, Eccles, Box, and Court 2005, Lauffenburger and Horwitz 1996).

Furthermore, this migration is very important because it explains the parities of molecular mechanisms. Additionally, it also shows the screening of pharmaceutical compounds that are editing the migration of the cells. Thus, this process is considered as a positive chance to treat pathological cases (Decaestecker et al. 2007, Friedl, Hegerfeldt, and Tusch 2004).

Previously, people have used the plants and their preparations to accelerate the healing of the wound (Reuter et al. 2009). At that time they couldn't have any experience in medical treatments and have less knowledge about these compounds (Schmidt et al. 2009). Recently, there are many processes with the development of the new sciences to accelerate wound healing. The most common technique that has been used for this issue is wound healing assay or "scratch assay" for monolayer wound (Guan 2005). Wound healing assay is used in the studies of drug development for anticancer during a test the ability of drugs against cell migration, cell polarization, matrix remodeling and another process (Lu et al. 2004, Mc Henry et al. 2002).

The principle of wound healing assay based on a scratch on the mono cell layer in the flask piece. After this step, the open area that did will return to comeback by the migration of cells after sometimes, and the evaluation of this migration it doses by using bright-field imaging (Brooks and Schumacher 2001).

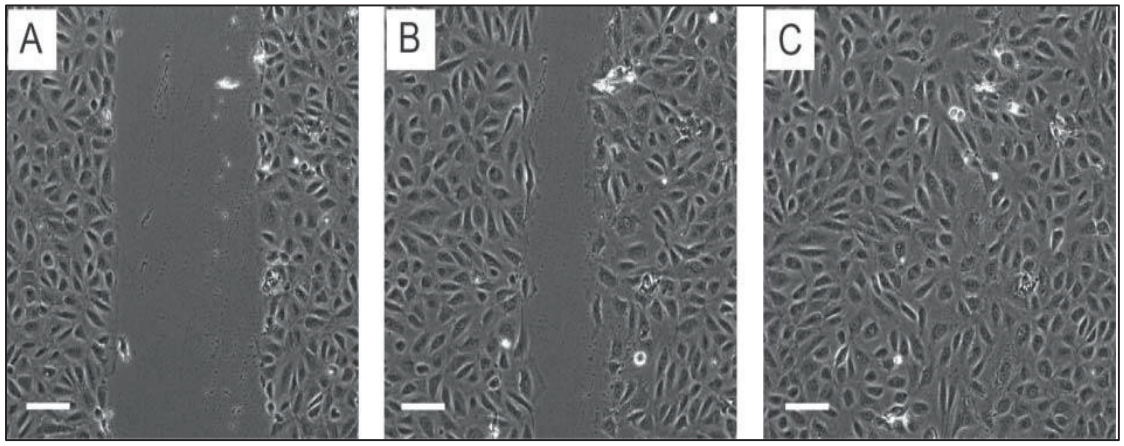


Figure 1. 10. Images of scratch assay under microscope

(Source: James, Judith, Fen, Miria, Jennifer,Katarzyna and Pina 2014)

1.8. Isoindole and Derivatives

Due to the development in the cancer treatment by chemotherapy and the malignancies. However, the anticancer agents need more studies to fill the gap of any new cancer (Bhatia 2017). Therefore, the chemist is always looking for the best compounds to have good efficacy and less toxicity to treat the cancer, and the heterocyclic compounds have proved that are good molecule for cancer (Cairns 1985, Demirbaş, Ugurluoglu, and Demirbaş 2002).

Isoindole structure has been founded by the scientists last decades and it could be found easily in pharmaceutical and natural compounds (Liu et al. 2017). The Isoindoles is important because it has a 10π aromatic ring so it is hard to find it. Isoindoles are unstable because of the ortho-quinoid, so, for that reason, its main job is to limit the electrophilic aromatic substitution reactions. On the other hand, they have little examples of nucleophilic aromatic substitution reactions (Diana et al. 2011). Thus the uses of Isoindoles are very wide because of their efficient. Lenalidomide and pomalidomide are anticancer drugs which have effect on multiple myeloma, and contains isoindole structure.

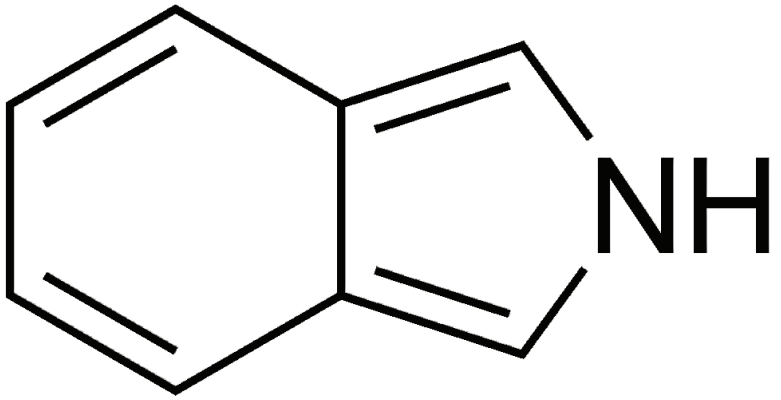


Figure 1. 11. Isoindole structure

(Source: ChemSpider Search and share chemistry)

Isoindole is one of the good heterocyclic compounds for cancer treatment (Joule and Mills 2008). The complete member of the Isoindole called isoindoline, while isoindoline and phthalimide was the oxidized form of Isoindole as shown in Figure 1.11 (Heugebaert, Roman, and Stevens 2012).

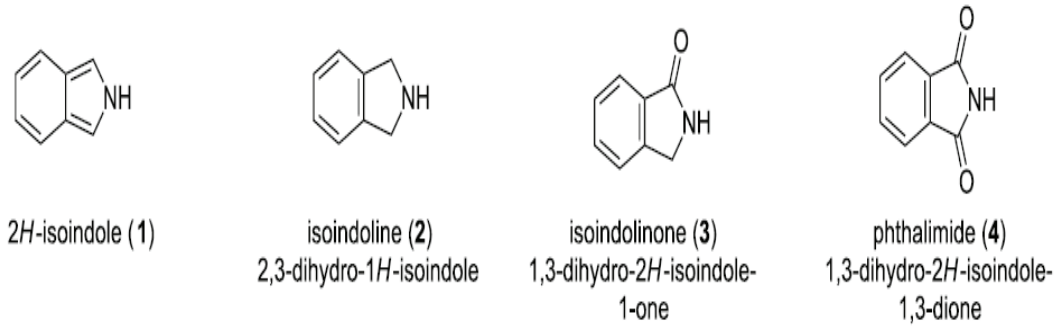


Figure 1. 12. Isoindole and related structures

(Source: Richa Kaur Bhatia 2017)

According to all features that have been stated above, the Isoindoles and derivatives have more applications especially in science (Hsu and Lin 2009). For example, they have been used in various fields of science because they contain BODIPY dyes highly fluorescent materials (Karolin et al. 1994). Also used for the biochemical process, laser dyes (Shah et al. 1990) and fluorescent sensors (Rurack et al. 2000).

The approval of Isoindoles derivatives by U.S (FDA) food and drug administration for treatment myeloma (Latif et al. 2012) makes it very interesting for the researchers to develop these derivatives for cancer treatment.

1.9. Isoindoles Derivatives as Anticancer Agents

Many studies in the literature have used the Isoindoles derivatives as anticancer because of its efficacy and less toxicity. Some of these studies have been explained below.

Diana et al. synthesized 1-acetyl-carboaldehyde-3-substituted Isoindole derivatives and the pLC_{50} value, pTGI value and pGI_{50} value have been measured to determine the efficacy of these compounds for antitumor. These compounds show that cytotoxic activity from micromolar to submicromolar concentration obtain good result against the cell line that investigated (Diana et al. 2011).

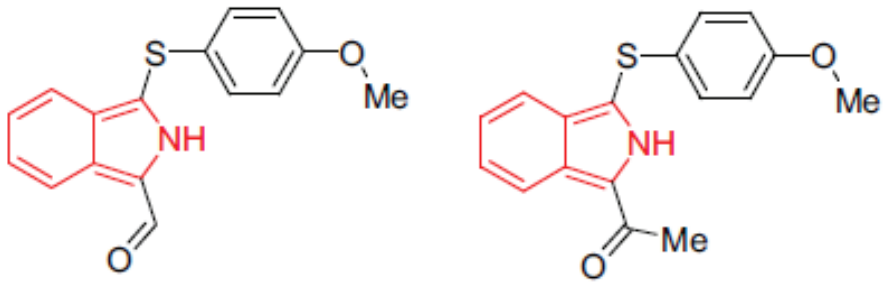


Figure 1. 13. 1-acetyl-carboaldehyde-3-substituted Isoindole derivatives

(Source: Diana et al, 2011)

Berger et al. prepared more than one compound from Isoindole alkanenitrile, α-aryl-α-thioether-alkyl and alkanecarboxylic acid methyl esters of tetrahydroisoquinoline and isoindoline. Then, these compounds have been evaluated during multidrug resistance (MDR) for human colon cancer cells in vitro. This compound has been evaluated in vivo against vincristine resistance murine P388 leukemia and also investigated for skin cancer in mice. After that, this investigation shows that these compounds produce good results in vitro and vivo (Berger et al. 1999). On the other hand, some of these compounds were very active when treated with Hela cell line while the IC_{50} was very low between 3.16 µM and 2.56 µM (Muñoz et al. 2013).

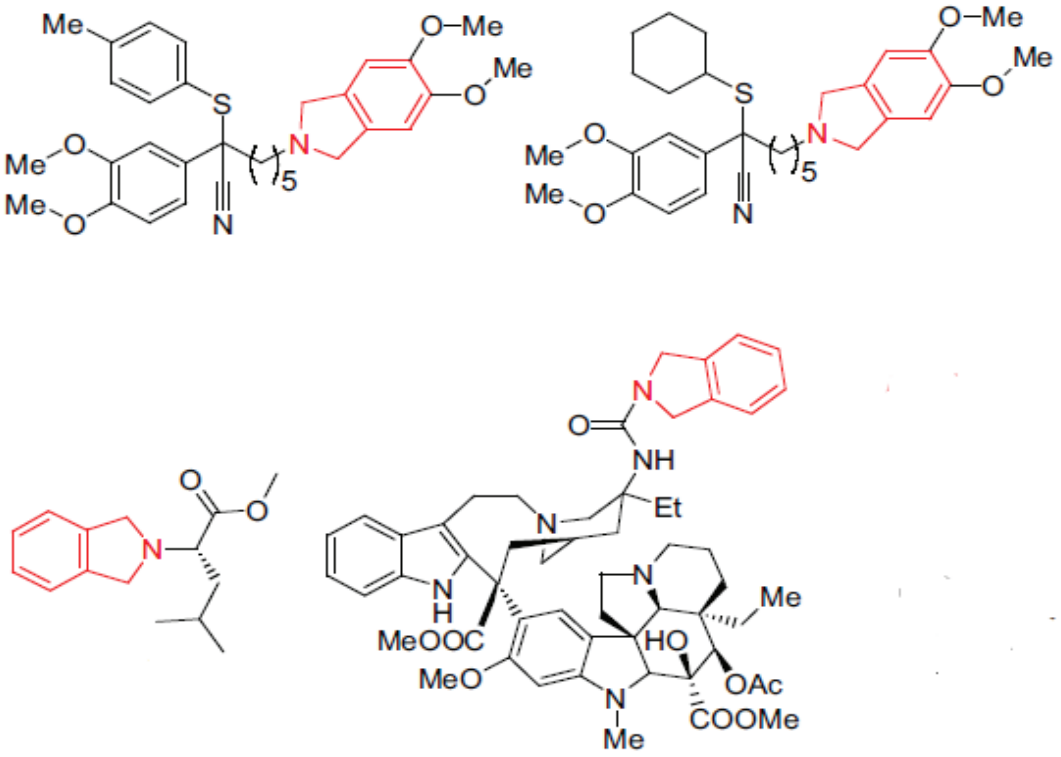


Figure 1. 14. Berger et al. Isoindoles derivatives
(Source: Berger et al. 1999)

Bhatia et al. prepared compounds from Isoindole and used them as anticancer. They synthesized "N-[4-[[2- (azepan-1-yl)ethyl]carbamoyl]phenyl]-1,3-dihydro-2Hisoindole- 2-carboxamide" and "N-[3-(isoindolin-2-yl)- 2-hydroxypropyl]aryl(alkyl) carboxamide derivatives" these two compounds have investigated for Hela cell during the measurement of IC_{50} and EC_{50} where the IC_{50} was 0.100µM and EC_{50} between 0.101 µM and 10.0 µM (Bhatia 2017).

In another research in 1998, Huang and Bobek synthesized some amino-deoxy 7-hexofuranosylpyrrolo[2,3-d] pyrimidines and evaluate their antitumour activity on four different cell lines, human ovarian A121, human lung NSCLC A549, human colon HT-29, and human breast MCF7. They reported as compound 22 and 23 more active than others in terms of growth inhibition, IC_{50} ranging from 3.9 to 11.5 µM (Huang and Bobek 1998).

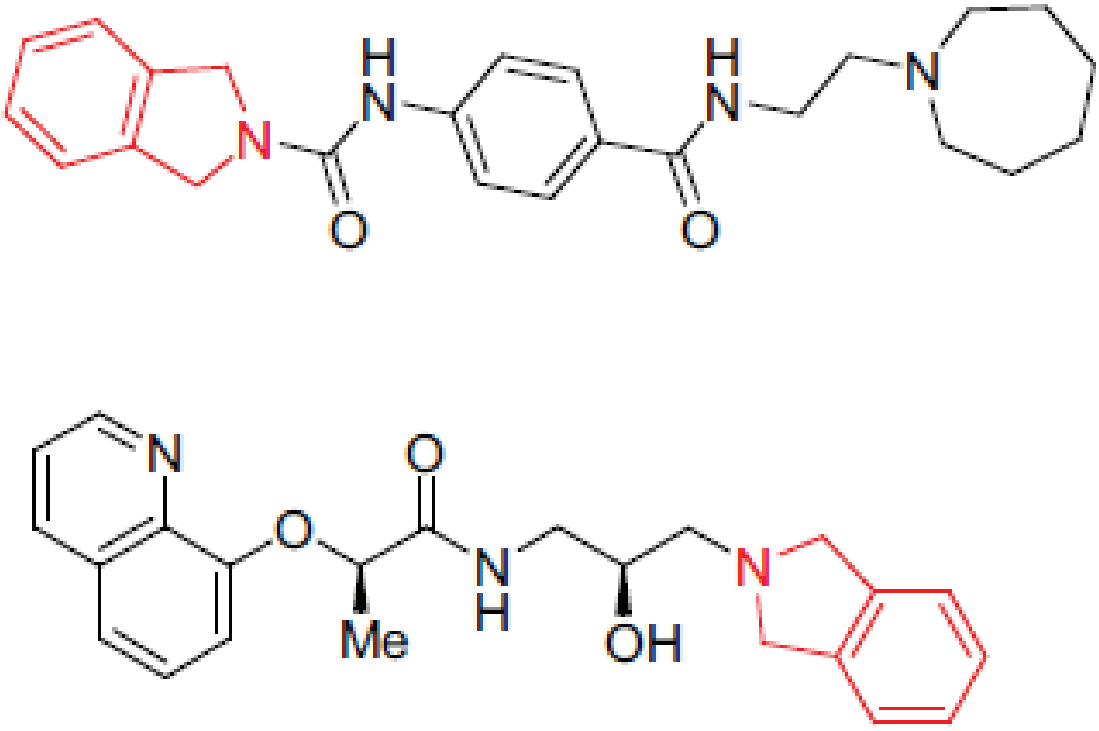


Figure 1. 15. Isoindoles derivatives

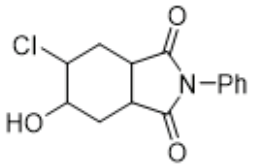
(Source: Duncan et al.2014)

Davidson et al. synthesized compounds from isoindoline and isoquinoline as an activator for apoptosis and caspase to treat the neoplasm and they obtained good results (Davidson et al. 2018). In addition, Kung and his team get a patent by using Isoindoles derivatives as PDK inhibitors and they have done several modifications to convert the Hsp90 inhibitor to all PDK isoforms with high specific (Kung et al. 2009).

In 2014, Khayyat and Amr Ael synthesized a series of macrocyclic peptides which were based on pyridine structure and evaluated their biological activities on breast cancer cell lines and female athymic pathogen-free nude mice. Some of the molecules from the series showed both in vitro and in vivo anti-tumour activity (Khayyat and Amr Ael 2014).

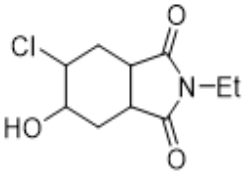
1.10. Chemical Structure of Drugs

Drugs were supplied in a powder form from Prof. Dr. Yunus Kara in Ataturk University Department of Chemistry, Organic Chemistry Laboratory.



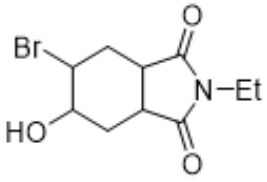
Molecular Weight: 279,72

61(2-8)



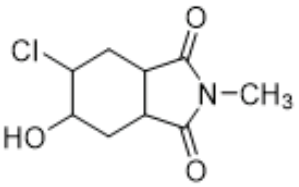
Molecular Weight: 231,68

62



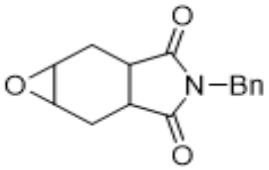
Molecular Weight: 276,13

63(4-12)



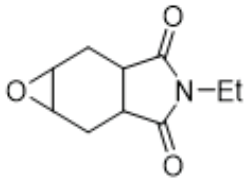
Molecular Weight: 217,65

40



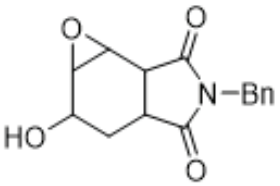
Molecular Weight: 257,29

70(23-33)



Molecular Weight: 195,22

71



Molecular Weight: 273,29

78(63-40)

1.11. Aim of the Study

The main aim of this study was to evaluate the biological activity of seven newly synthesized Isoindole derivatives. The anticancer activity of these compounds has been evaluated against HeLa (cervical carcinoma) and A549 (lung adenocarcinoma) cell lines.

The cytotoxic activity of these compounds has been determined by measuring cell viability via MTT assay on HeLa and A549 cell lines. Then, the apoptotic effect for the compounds that have the best IC_{50} on the indicated cells was investigated by using Annexin V-FITC. The cell cycle analysis of the cell lines in the presence of the most effective compounds has been performed using flow cytometry. In addition, in order to understand the effects of the compounds that have the best cytotoxicity on the migration of HeLa and A549 cells, a scratch assay was performed.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Cell Lines

The following human cancer cell lines were used for cervical carcinoma (HeLa) and lung adenocarcinoma (A549). The cell lines have been provided by IZTECH Biotechnology and Bioengineering Research and Application Center (BIYOMER).

2.1.2. Drugs

Drugs were supplied in a powder form from Prof. Dr. Yunus Kara in Ataturk University, Department of Chemistry, Organic Chemistry Laboratory. Stock solutions and fresh solutions were prepared with DMSO (Dimethyl sulfoxide).

2.1.3. Culture Medias

The medium that is used for Hela and A549 cell lines is RPMI 1640 (sigma) with 4500 mg Glucose/L, pyridoxine, $NaHCO_3$, and HCl, without L-glutamine. This medium used by adding 1% Penicillin-Streptomycin solution (Sigma), 10% Fetal Bovine Serum (Sigma) and 1% L-glutamine Solution (Sigma).

2.2. Methods

2.2.1. Cell Culture

Hela and A549 cell lines were cultured by using RPMI 1640 medium with 10% Fetal Bovine Serum (FBS), 1% Penicillin streptomycin (P/S) and 1% L-glutamine by using atmosphere condition in 5% CO_2 incubator at 37C° (Audus et al. 2002).

2.2.2. Thawing Out Cells

The frozen cells were taken from -80 C° refrigerator. Then, the cells were put in a water bath 37C° and wait until all frozen cells thawed. After thawing, all the cells have been taken carefully to the cabin and put them in a 75cm Flask, which contains 12-13 mL fresh RPMI 1640 that is already prepared during the thawing process. The cells have been placed in 5% CO_2 incubator at 37 C° for one night to grow and get more.

2.2.3. Passaging Cells

After the thawing process and waiting for one night, the cells are taken from the incubator and removed from the medium. 2-5 ml from PBS has been added to the flask in order to wash the cells. After that, 1-3 ml from sterile Trypsin is added to the flask and the cells have placed in 5% CO_2 incubator at 37 C° for 2-3 minutes to separate the cells from the flask. Later, 6-9 ml fresh RPMI 1640 has been added to the medium to inhibit Trypsin and taken all solution in 15 ml falcon and place them in the centrifuge at 800 rpm for 5 minutes. After 5 minutes the cells are taken from the centrifuge. By using mechanical pipet supernatant, the cells are removed and 2 ml fresh RPMI 1640 has been added to the falcon, after good re-homogenize, the cells with medium transported to the 75 cm flask contain fresh medium and put in 5% CO_2 incubator at 37 C°. All solutions and medium that used was put in a water bath at 37 C° and then used, the passage should do every 2-3 day.

2.2.4. Cells Counting

A 100 µL from the cells were taken and put it in a 1.5 ml microfuge tube, 900 µL of 0.4 % trypan blue solution (Sigma) was added to the cell suspension. After well mixed, a small amount from trypan blue-cell suspension was taken and put in a chamber on the hemocytometer. After this step hemocytometer was put under the microscope to count cells. The cells with blue color are non-viable cells and the viable cells remain opaque. The plate show under the microscope on four square in each corner and just the cells inside these squares will be counting. The following equation using for calculation the number of cells.

$$\text{Cells per mL} = \text{Average count of cells per square} * \text{Dilution factor} * 10^4$$

2.2.5. Freezing Cells

When a passage is doing some of the cells were taken for freezing. DMSO (Sigma) was added by 5 % concentration for these cells and re-homogenize well and transported 1 mL of cells to cryogenic vials and stored at – 80 C°.

2.2.6. Cell Viability Assay (MTT Assay)

Hela and A549 cell line 95 µL were cultured in 96 well plates flat bottom (SPL Life Sciences) at 10-4 cell density per well with initial 5000 cell/ 100 µL complete RPMI 1640 medium and incubated in 5% CO_2 at 37 C° for 24 h to detached cells. All seven compounds were dissolved in sterile dimethyl sulfoxide DMSO and prepared 7 concentrations for each compound 20, 50, 100, 200, 500, 1000, 2000 µM. After adding all compounds cells put in the incubator in 5 % CO_2 at 37 C° for 24h,48h, and 72h to determine the cytotoxicity of this compound. MTT (Sigma) was dissolved in phosphate-buffered saline (PBS) 5mg/mL and filtered. 10 µL from MTT was added to each well and

cells were put in the incubator for 3.5 h. After incubation cells took from incubator and centrifuge for 10 min at 1800 rpm. The supernatant was removed and 100µL from DMSO was added to each well and cells were put on a plate shaker for 15 min to dissolve all formazan crystals. Absorbance was determined at 540 nm (Fogh and Trempe 1975, T. R. Chen et al. 1987).

2.2.7. Apoptosis Analysis

Apoptosis was detected by using compounds that give good IC_{50} for HeLa and A549 cell lines by using an Annexin V-FITC detection kit (Bio Legend). A 6- well plate (SPL Sciences) used in this analysis and 1980 µL from cell suspension with density 5×10^5 have been placed in each well and incubated in 5% CO_2 at 37 C° for 24 h to attachment cells. After 24 h, 20 µL from compounds that had to choose and prepare four concentrations for each compound 20,100,250,500 µM, after adding compounds cells incubated in 5% CO_2 at 37 C° for 48 h. Next, the incubation trypsin was added to the separated cells and centrifuge for 5 minutes at 800 rpm. Afterward, centrifuge supernatant was ignored and cells were washed with 3 mL from phosphate-buffered saline (PBS) and centrifuge again for 5 min at 800 rpm. After 5 min of centrifuging supernatant was ignored and 100 µL from the binding buffer, 1 µL from Annexin V-FITC, 2.5 µL from PI were added to each tube and tubes were incubated for 15 min at room temperature in the dark. After incubation 400 µL from binding buffer was added to each tube and all tubes analyzed by Flow Cytometry (BD FACSCanto).

2.2.8. Scratch Assay

Cell migration has been detected by using Scratch assay, glass lamellas were washed with ethanol (Sigma) and put in the autoclave for one night. After 24 h, it has been placed in 6 well plates, after putting one glass lamellae in each well HeLa cells and A549 cells were seeded 3×10^5 cells/well with a volume 1980 µl and incubated in 5% CO_2 at 37 C° for 24 h. Next, the period of incubation is finished, the cells were scratched on each lamella with parallel lines by using a sterile 100 µl plastic pipette tip. The old

medium was removed and changed by fresh one after cells washed by phosphate-buffered saline (PBS). Drugs were added to each well containing 1 % DMSO as a final concentration and one well leaved without drug as a control. Cells were incubated in 5% CO_2 at 37 C° for 24h and 48h. the experiments were performed at least one duplicate (Fronza et al. 2009, (Gough et al. 2011).

2.2.9. Cell Cycle Analysis

The cell cycle was detected in HeLa and A549 cell lines of compounds that gave good IC_{50} by using propidium iodide staining (PI). The A549 and HeLa were seeded in 6 well plates at density 5×10^5 in 1980 µl medium, and incubated in 5% CO_2 at 37 C° for 24 h. after 24 h compounds selected prepared by dissolved in DMSO and diluted for different concentrations 500,250,100,20 µM and 20 µl from each compound was added to cells and cells were incubated in 5% CO_2 at 37 C° for 48 h. Next, the period of incubation, the cells were washed by PBS 1 ml and suspension collected, 250 µl from trypsin was added to cells and centrifuged for 5 minutes at 800 rpm. After centrifuge suspension was collected. After the collection process, 1 ml from cold PBS and 4 ml from -20 C° ethanol (Merck) were added by slowly to suspension. After these additions cells were incubated at – 20 C° for at least 24 h. after 24 h cells were centrifuged for 10 minutes at 1200 rpm 4 C°. cells were washed with PBS by adding 5 ml PBS to cells and centrifuged for 10 min at 1200 rpm. After centrifuge supernatant ignored and 200 µl from phosphate buffer including 0.1% Triton X-100 was added. After this addition 20 µl from RNase A (200 µg/ml) was added to the cells suspension and cells incubated in 5% CO_2 at 37 C° for 30 minutes. After 30 minutes 20 µl from propidium iodide (PI) was added to the cells and incubated at room temperature for 15 minutes. After the time of incubation is finished cell cycle was determined by Flow Cytometry (BD FACSCanto).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Cell Viability

The cytotoxic effect of the newly synthesized isoindole compounds was determined on HeLa (cervical carcinoma) and A549 (lung adenocarcinoma) cell lines, IC_{50} was calculated for each compound by using Graphed prism 6 software program for at least three triplicate experiment.

The results show that the best cytotoxic one of these seven compounds is 78 because it has good IC_{50} value compared to others during all the time periods of 24, 48 and 72 h. The IC_{50} value for compound 78 was 251.4 μM for A549 cell line and 206.9 μM for HeLa cell line for 48 as shown in Figures 3.3 and 3.6. Compound 71 was also a reasonably effective compound after 78 on the cell lines compared to other compounds. The IC_{50} for compound 71 was 289 μM for A549 cell line and 296.4 μM for HeLa cell line for 48 h incubation period of time as shown in Figure 3.3 and 3.6. The compound 70 had a minimal cytotoxic effect on both cell lines. On the other hand, the other compounds did not show any significant cytotoxic effect on both cell lines. The effect of compound 78 might be related to its structure and therefore play an important role in the cytotoxicity of the cell lines.

The structure of the compounds 70, 71 and 78 were investigated and the similarities and differences were analyzed. The similarities were the benzene group between compound 78 and 71 and the hydroxyl group between compound 78 and 70. These results indicate that the benzene group and hydroxyl group together may show better effect on cytotoxicity on the cell lines among Isoindole derivatives.

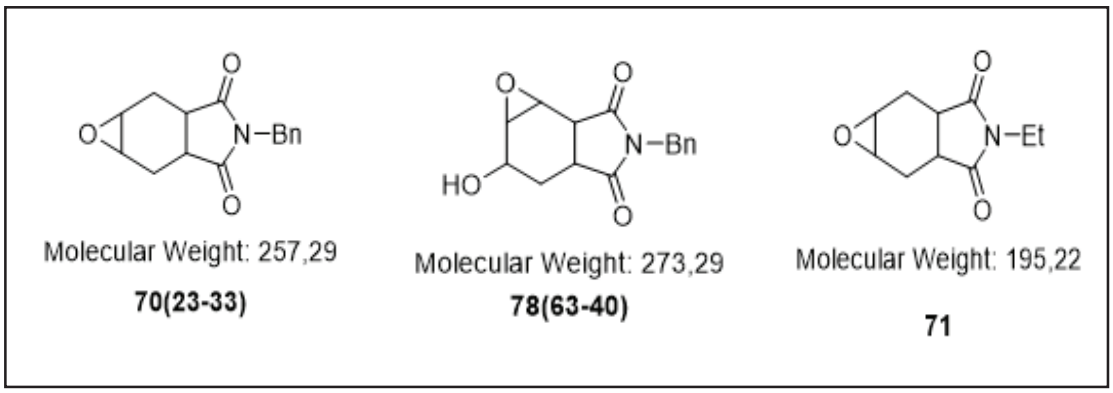


Figure 3. 1. Chemical structure of compounds 70,71 and 78.

Köse and his group worker synthesized new Isoindole derivatives 8a and 8b. They evaluated the biological activity of those compounds on A549 and MCF-7 cell lines. They found a metabolic activity as 39% for A549 cell line and 55% for MCF-7 using 50 µM concentration of 8a compound after 24 h incubation. Using the same concentration for compound 8b, the observed metabolic activity was 53% for A549 and 64% for MCF-7 cell lines after 24 h. The cytotoxicity of both compounds increased as a time-dependent manner up 72 h (Köse et al. 2017).

H.El-Diwan et al synthesized 2-arylbenzimidazole-4-carboxamides and tested this compound with 60 cell lines including HeLa and A549 in different five concentrations at 48 h incubation period-time. The results show that the best concentration was 100 µM and this compound was very potent. This effect was attributed to the structure of the compound due to the substitution of position 5 by a diazo group connected to the five-membered ring which resulted in an enhanced cytotoxic activity (El-Naem et al. 2003).

Bhatia et al. prepared compounds from Isoindole and used them as anticancer. They synthesized "N-[4-[[2- (azepan-1-yl)ethyl]carbamoyl]phenyl]-1,3-dihydro-2Hisoindole- 2-carboxamide" and "N-[3-(isoindolin-2-yl)- 2-hydroxypropyl]aryl(alkyl) carboxamide derivatives" these two compounds have investigated for Hela cell during the measurement of IC_{50} and EC_{50} where the IC_{50} was 0.100µM and EC_{50} between 0.101 µM and 10.0 µM (Bhatia 2017).

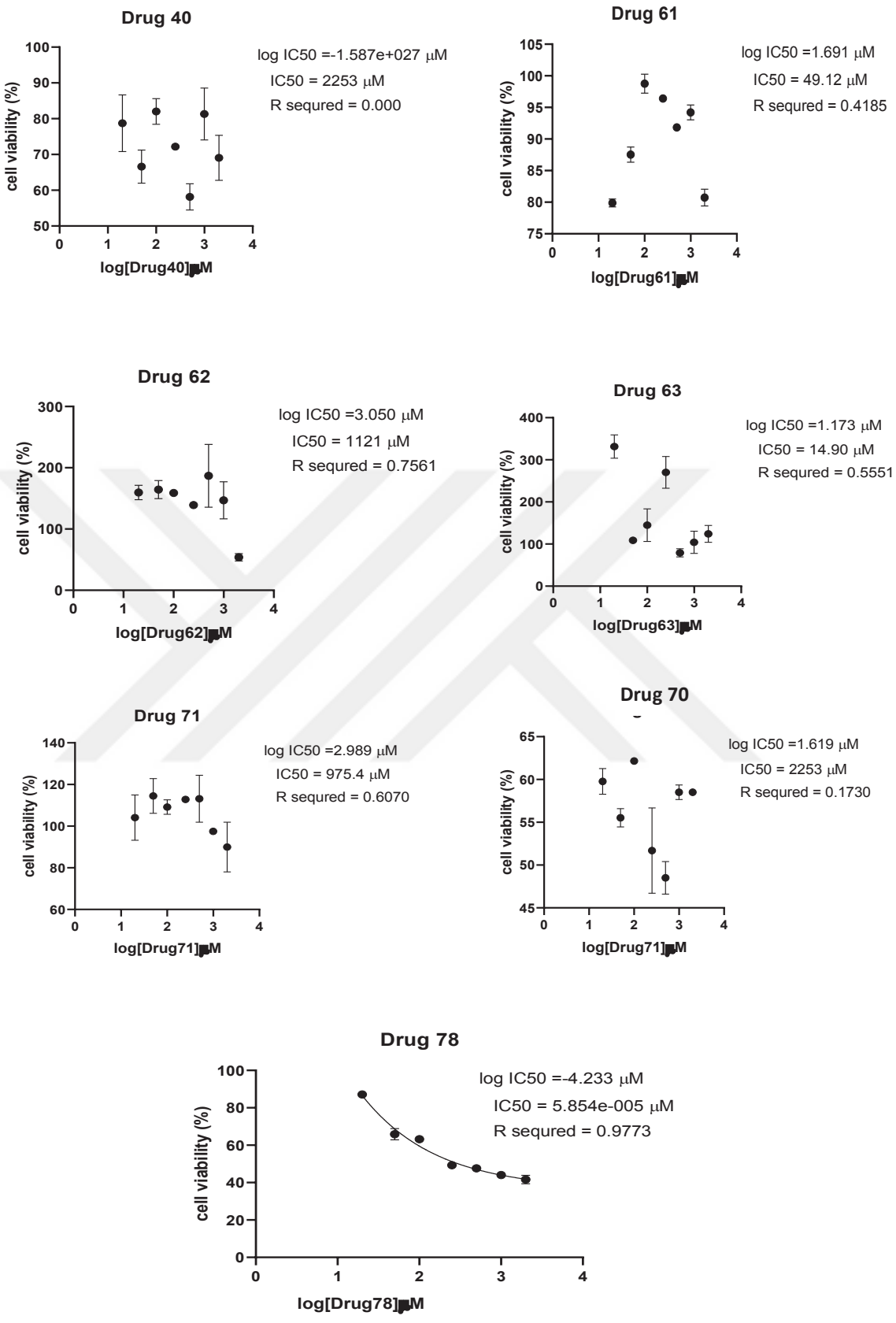


Figure 3. 2. Cell viability results on A549 cells for 24 h incubation period.

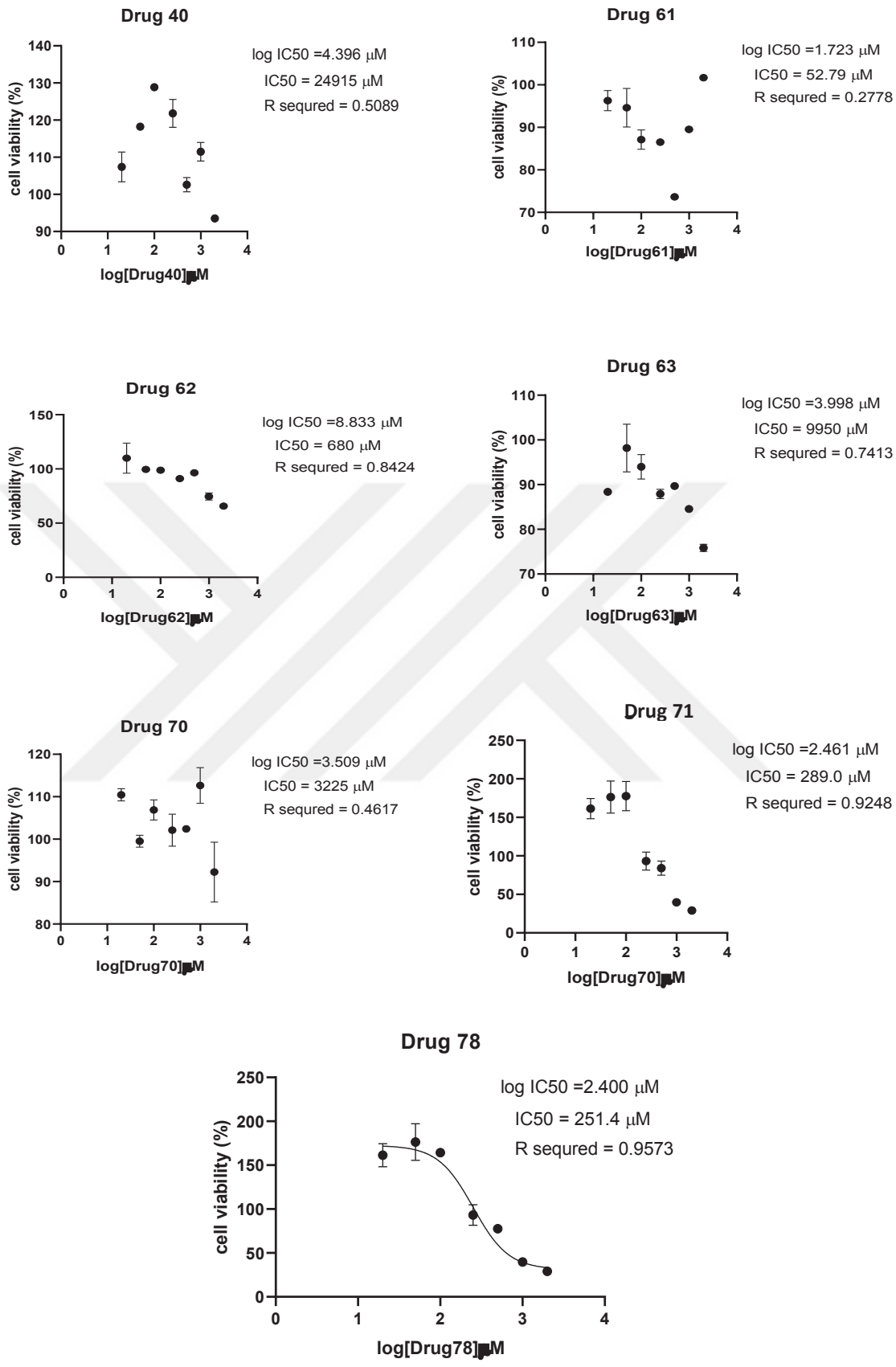


Figure 3. 3. Cell viability results on A549 cells for 48 h incubation period.

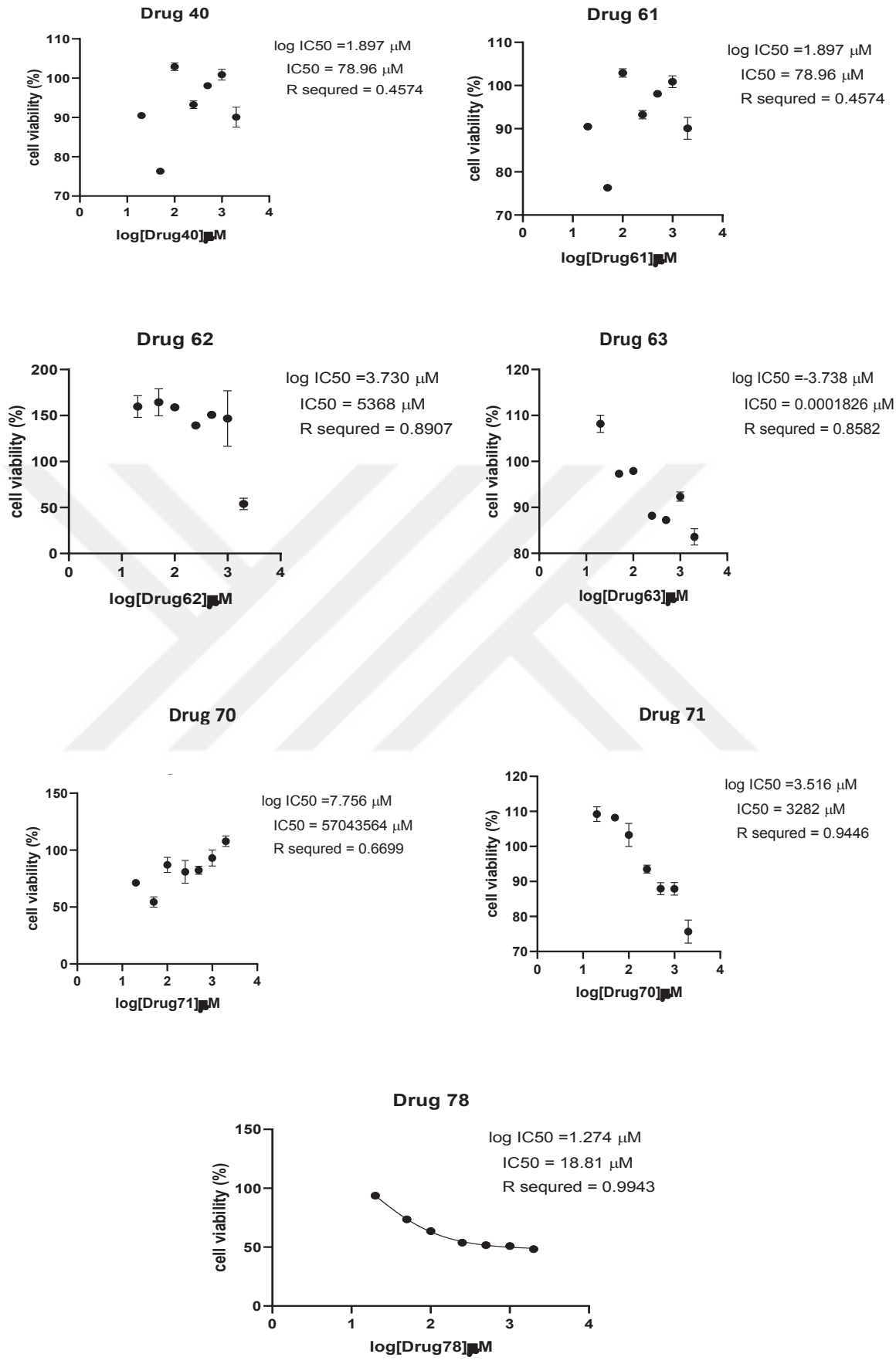


Figure 3. 4. Cell viability results on A549 cells for 72 h incubation period.

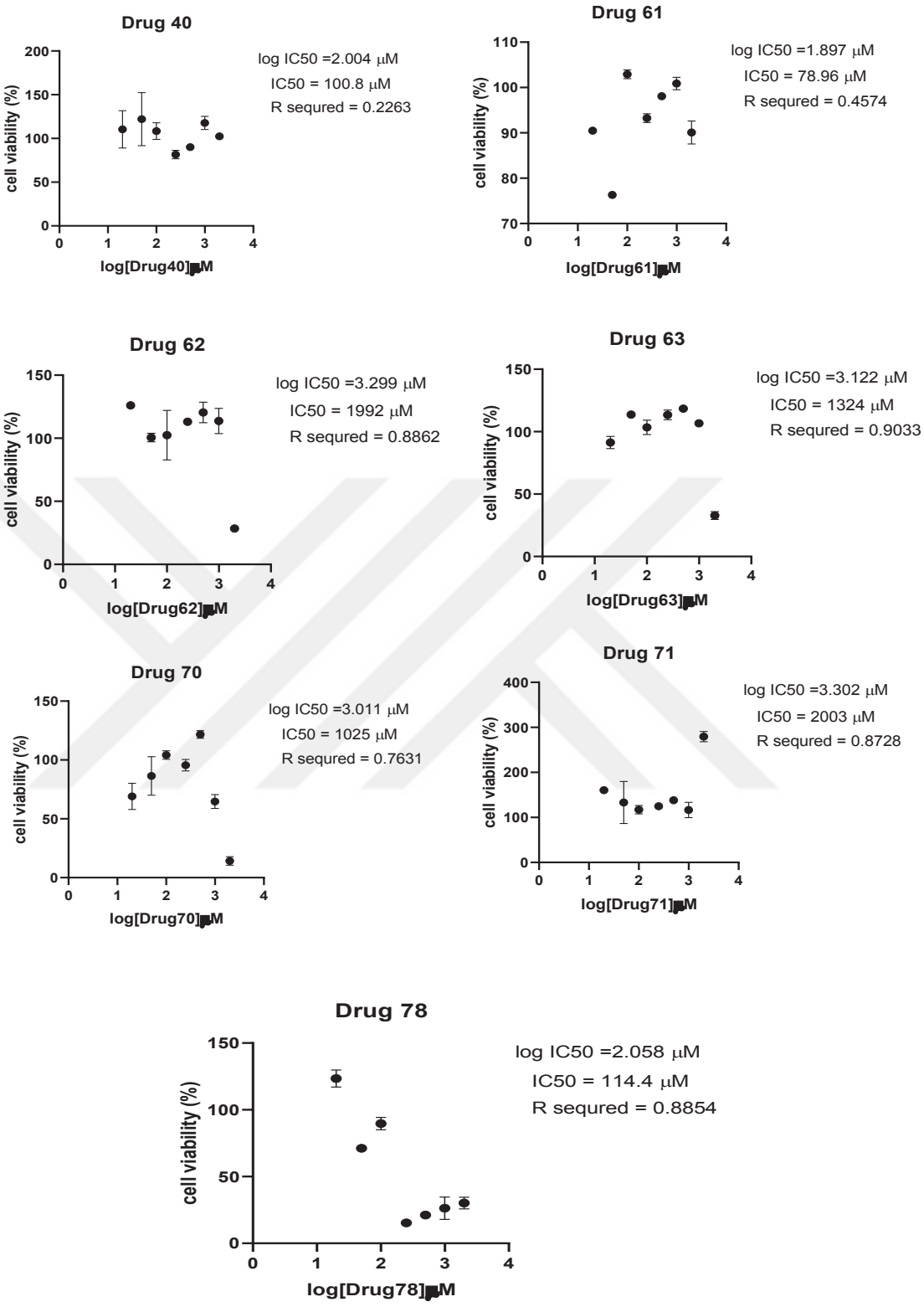


Figure 3. 5. Cell viability results on HeLa cells for 24 h incubation period.

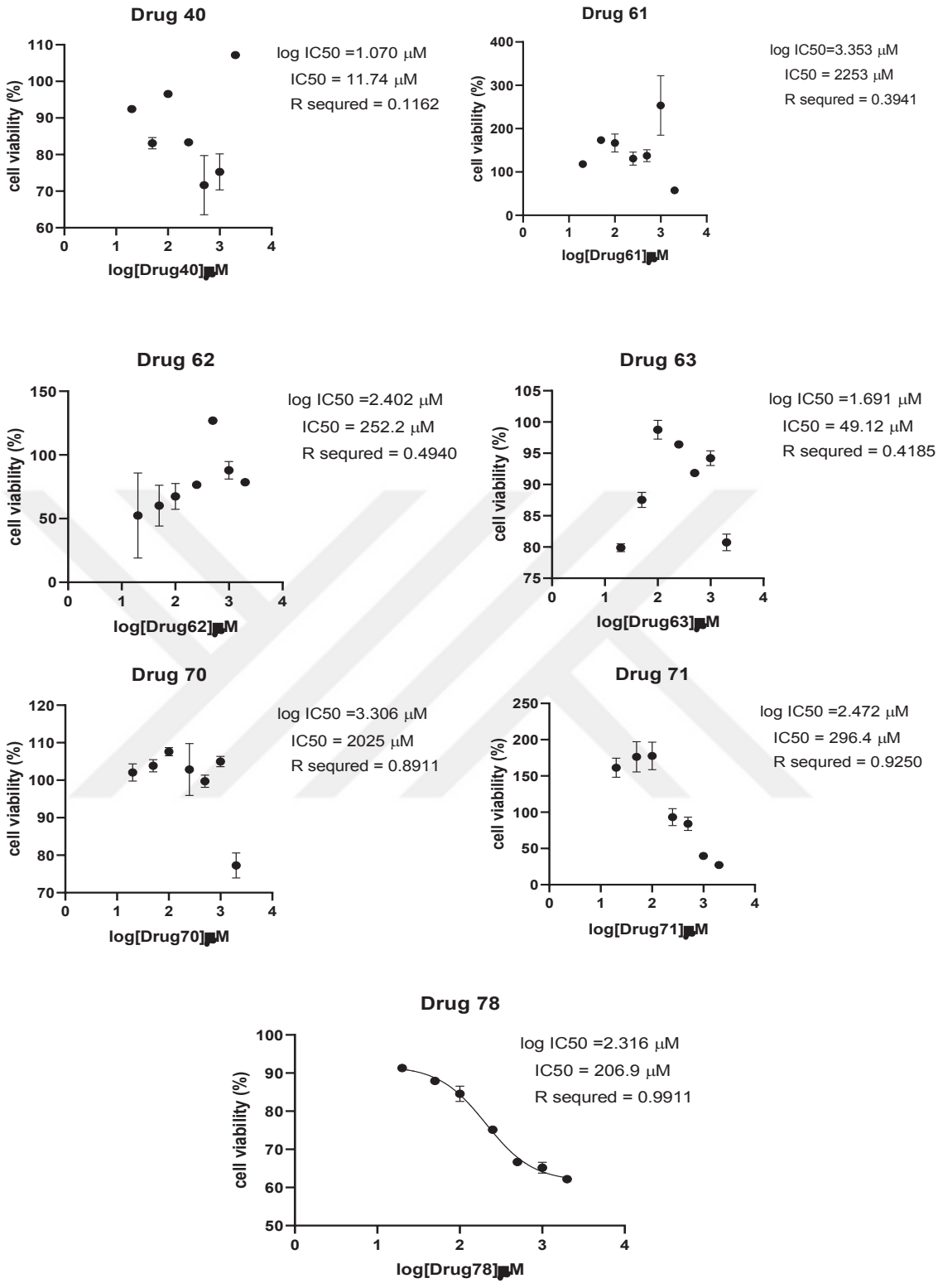


Figure 3. 6. Cell viability results on HeLa cells for 48 h incubation period.

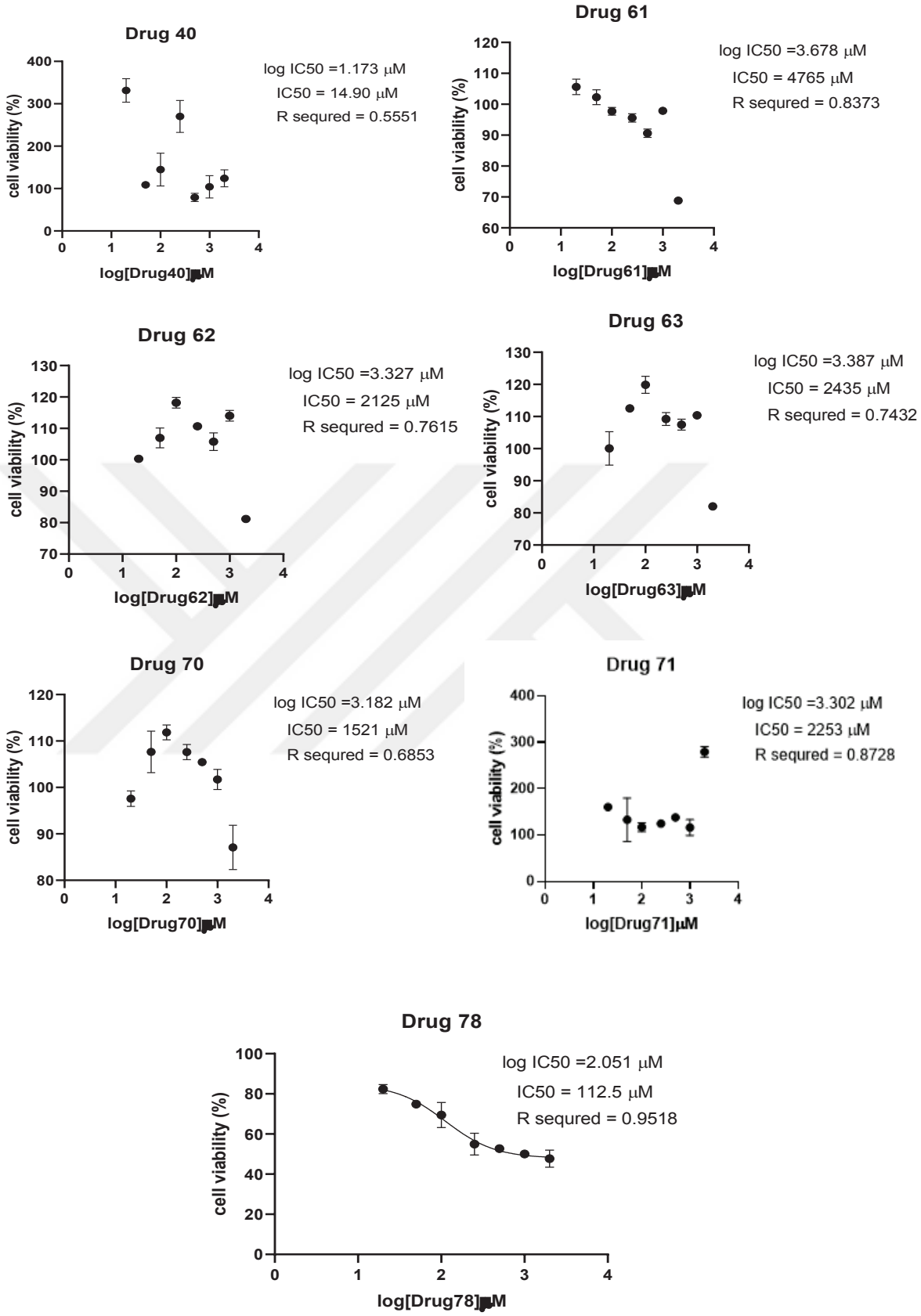


Figure 3. 7. Cell viability results on HeLa cells for 72 h incubation period.

3.2. Apoptosis Analysis

Apoptosis, which is the process of programmed cell death in a normal cell growth, is characterized by exact morphologic properties. One of the earliest features is the loss of plasma membrane asymmetry. As in normal cells the membrane phospholipid phosphatidylserine (PS) is located in the inner side of the plasma membrane, in apoptotic cells it is translocated to the outer side of the plasma membrane.

Annexin V, which is a Ca^{2+} dependent phospholipid-binding protein, has a great affinity for phospholipid phosphatidylserine (PS). When Annexin V is conjugated to fluorescein is thiocyanate (FITC), which is a fluorochrome, and Annexin V-FITC serves as a sensitive probe for flow cytometry analysis of apoptotic cells.

Apoptosis, programmed cell death effect has been investigated for the compound that gave good IC_{50} in the cell viability assay and these compounds were 78 and 71. Annexin V-FITC was used to investigate the effect of these compounds on HeLa (cervical carcinoma) and A549 (lung adenocarcinoma) cell lines.

For the A549 cell line, the results show that the compound 78 gave the best results and the cells going to death when increasing the concentration of this compound as shown in Figure 3.8. The rate was 23.8% for necrosis, 27.17% for late apoptosis, 0.4% for early apoptosis and 40.7% for live cells. All these numbers goes to decrease when decreasing the concentration of the compound, except live cells. This indicates that the compound 78 was very effective and gave significant results for A549 cancer cell line.

The second compound that chosen to investigate the apoptotic effect for the A549 cell line is compound 71. The results of compound 71 was not significant like compound 78. The rate was 2.13% for necrosis, 10.53% for late apoptosis, 4.83% for early apoptosis and 82.57% for live cells as shown in the Figure 3.9.

On the other hand, when investigating the apoptotic effect of these two compounds on the Hela cell line, the results of compound 78 were very significant according to these numbers as shown in Figure 3.10. The rate was 20.27% for necrosis, 14.63% for late apoptosis, 1.23% for early apoptosis and 64.37% for live cells. Also, these numbers decrease when decreasing the concentration of compound except for live cells. Compound 71 results was not significant with the HeLa cell line as shown in Figure 3.11. The results were 6.27% for necrosis, 6.8% for late apoptosis, 4.63% for early apoptosis and 83% for live cells.

Diana at al investigated the Caspase-3 activation programmed cell death for compound (3-Methoxy-5H-isoindolo[2,1-a] quinoxalin-6-one) on Jurkat cell line. The results show that the compound has had the ability to induce the activation of Caspase-3 and it activates Caspase-9. This activation leads the cell to the apoptosis (Diana et al. 2008).

In another study, two new compounds of Isoindole 5a and 5g have been synthesized and evaluated based on the apoptotic effect on HepG2 cell line (human liver) and MCF7 cell line (breast cancer). The percentage of apoptotic cells have increased with HepG2 cell line when treated with compound 5a, while the percentage was at 1.65% with untreated cells, and 21.25% with compound 5a. MCF7 cell line treated with compound 5g and also noted that it has an increase in apoptotic rate from 1.92% untreated cells to 18.26% treated cells. This indicates that these two compounds are very potent for apoptotic programmed cell death (Philoppes and Lamie 2019).

Zhao et al. 2012 found that the effect of compound 2-(3-(2-Oxo-2-p-tolylethylthio)-5-(3,4,5-trimethoxyph-enyl)-4H-1,2,4-triazol-4-yl) isoindoline-1,3-dione for apoptotic effect of HepG2 cell line was dose-dependent (Zhao et al. 2012).

Davidson et al. synthesized compounds from isoindoline and isoquinoline as an activator for apoptosis and caspase to treat the neoplasm and they obtained good results (Davidson et al. 2018). In addition, Kung and his team get a patent by using Isoindoles derivatives as PDK inhibitors and they have done several modifications to convert the Hsp90 inhibitor to all PDK isoforms with high specific (Kung et al. 2009).

In 2012, 34 compounds which have diindolylmethanes were synthesized and their biological activites were investigated for HeLa, A549 and MCF-7 cancer cell lines. It was reported that HeLa and A549 cells were more sensitive than MCF-7 to these indole derivatives. Compound 7d was reported as the most promising compound for inhibiton of HeLa cells and also 7d caused G1 phase arrest on HeLa cells (Sharma et al. 2012).

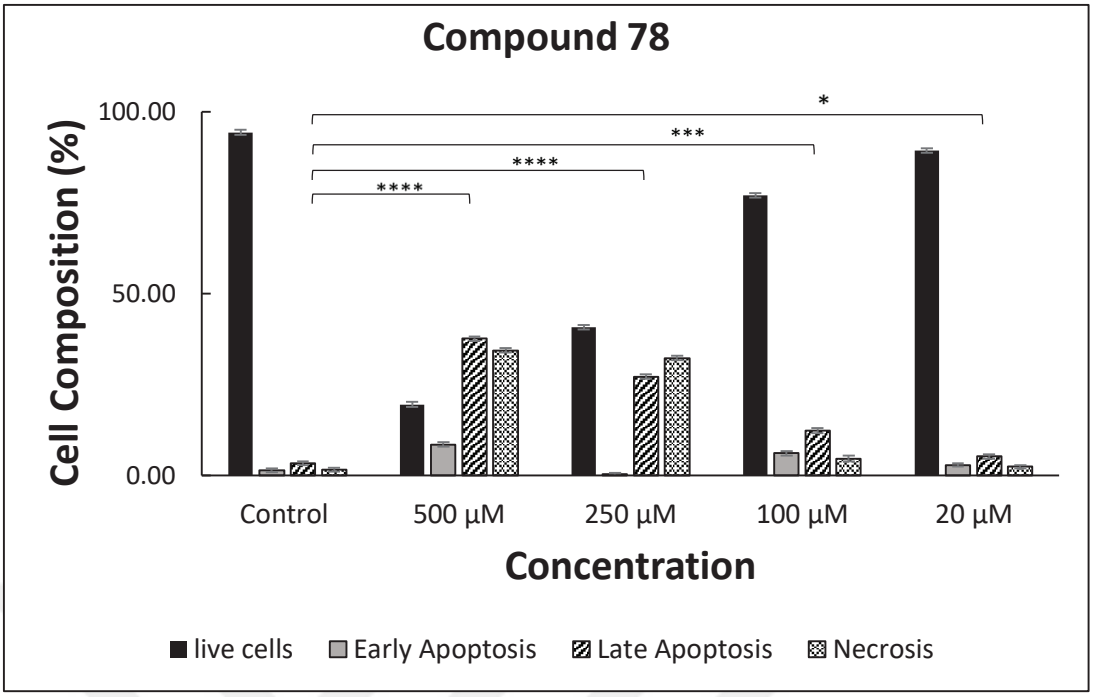


Figure 3. 8. Apoptosis analysis results for compound 78 at IC_{50} dose in 48 h incubation period on A549 cells. Bars indicate SD, ****p<0.0001, ***p<0.001, *p<0.05

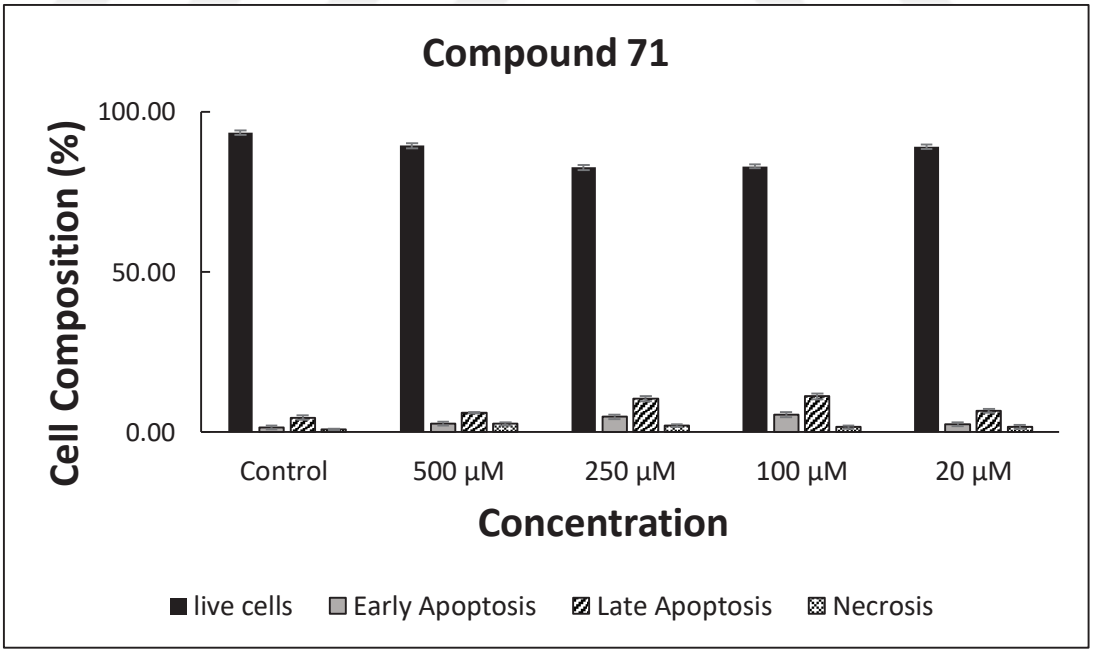


Figure 3. 9. Apoptosis analysis results for compound 71 at IC_{50} dose in 48 h incubation period on A549 cells.

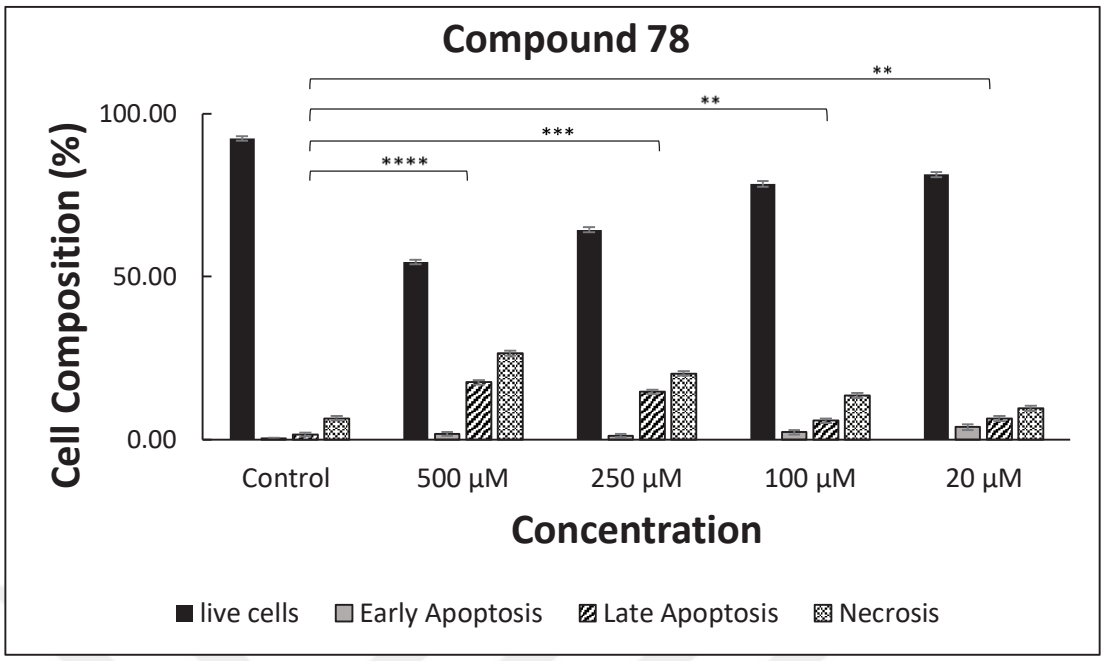


Figure 3.10. Apoptosis analysis results for compound 78 at IC_{50} dose in 48 h incubation period on HeLa cells. Bars indicate SD, ****p<0.0001, ***p<0.001, **p<0.01.

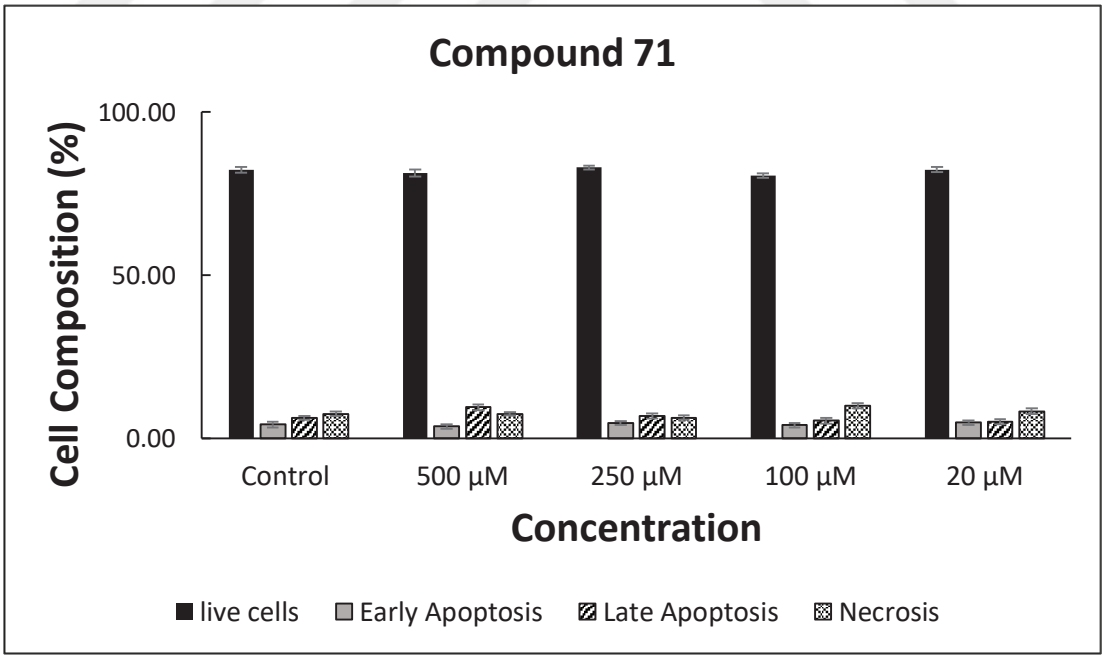


Figure 3. 11. Apoptosis analysis results for compound 71 at IC_{50} dose in 48 h incubation period on HeLa cells.

3.3. Scratch Assay

In this study, the effect of compounds 78 and 71 on cell migration has been evaluated by using a scratch assay on A549 (lung adenocarcinoma) and HeLa (cervical carcinoma) cell lines. The effect has been evaluated for two-time periods 24 h and 48 h at IC_{50} dose. For the A549 cell line and according to the results shown in Figure 3.12, the compounds were very effective to prevent the cell migration and compound 78 was better than compound 71, the difference is obvious comparing to the control state.

On the other hand, the effect of compounds 78 and 71 was very good to prevent the cell migration on the HeLa cell line in both time period of the test as shown in Figure 3.13.

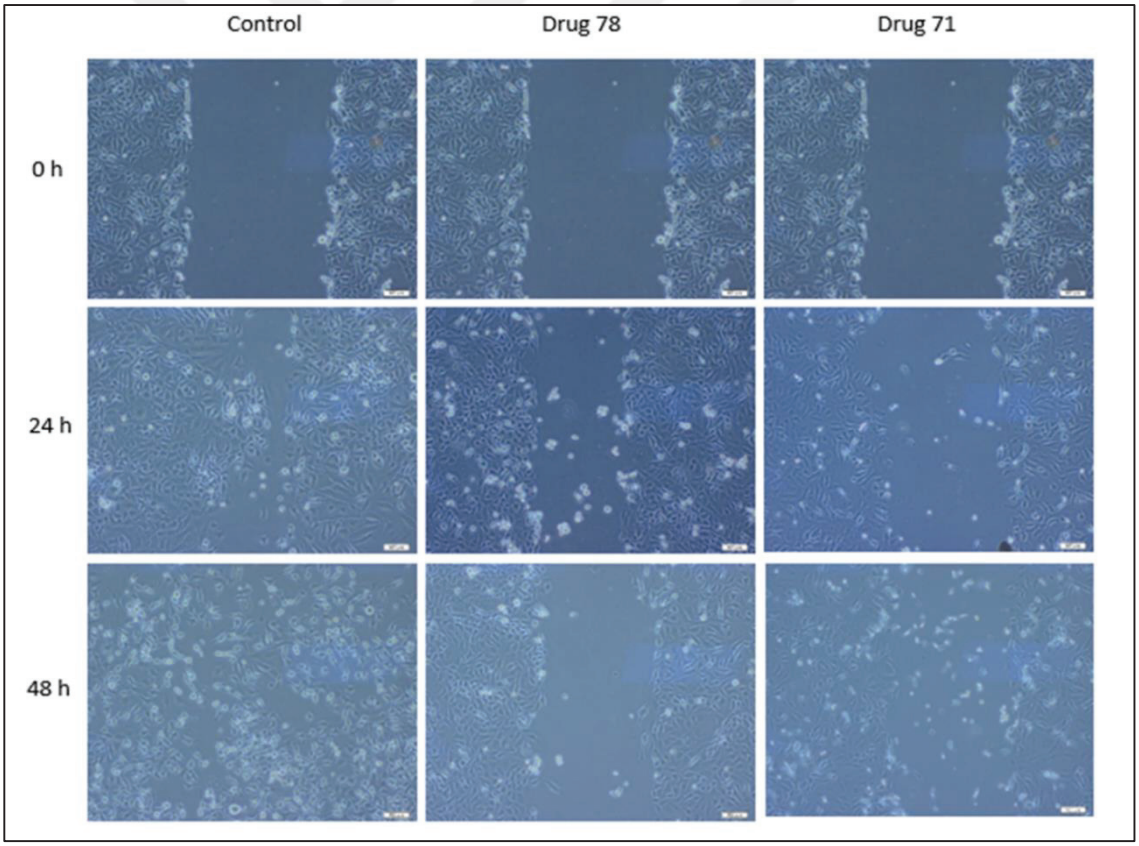


Figure 3. 12. Cell migration (Scratch assay) results on A549 cells for 0 h, 24 h and 48 h period incubation at IC_{50} dose.

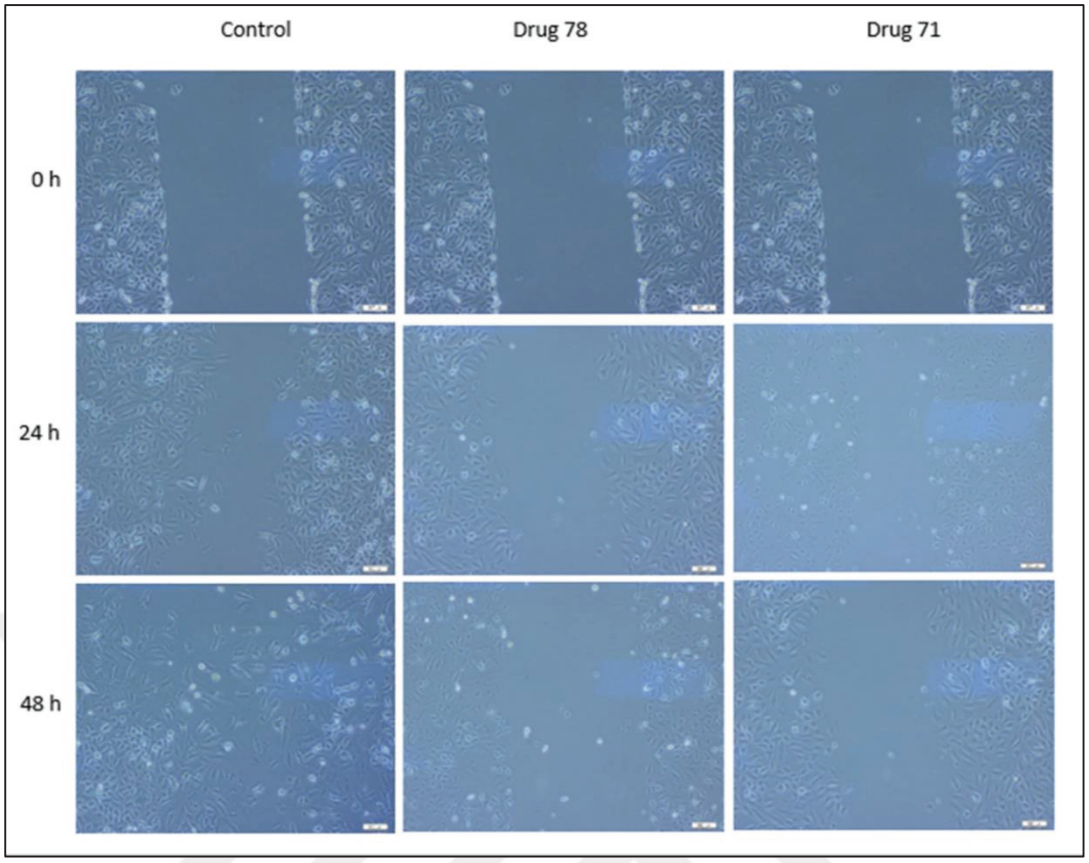


Figure 3. 13. Cell migration (Scratch assay) results on HeLa cells for 0 h, 24 h and 48 h period incubation at IC_{50} dose.

In 2010, Wang et al. synthesized pyrrole-imidazole (PI) polyamide to target MMP-9 which is related with metastasis. They used HeLa cells and MDA-MB-231 cells for in vitro studies and six-week-old male athymic nude mice were used for in vivo studies. The study demosntrated that the PI polyamide has inhibitory effect on HeLa and MDA-MB-231 cell invasion and cell migration for 48h incubation. Besides this, they observed almost no effect on proliferation of these two cell lines, and they concluded that PI polyamide might inhibit metastasis. The results of in vivo studies suggested that PI polyamide suppressed liver metastasis. They explained theese strong effects of pyrrole-imidazole polyamide with penetration into nuclei and staying there for a long time (Wang et al. 2010).

3.4. Cell Cycle Analysis

During the division of the cell, it will go through four stages as is mentioned in section 1.4. These stages are G1 (growth phase), S (Synthesis phase) DNA replication, G2 the phase that cell prepares for mitosis stage and the last phase is a mitotic phase, this phase includes four phases (Prophase, Metaphase, anaphase, and Telophase).

In this study, the main aim of cell cycle analysis is to have full information about the suggested compounds to prevent the cell from dividing. In addition, the dose that has been chosen for this assay is IC_{50} for two compounds 78 and 71 at 48 h incubation period.

Figure 3.14 shows the effect of compound 78 on A549 cell line, it is very clear that is arrested the cells in S phase and the results were very significant. Also, cells could not pass to the G2 phase, which means that the cells cannot duplicate their DNA. For compound 71, Figure 3.15 shows that most of the cells arrested in G1 phase and it was not significant.

On the other hand, the effect of compound 78 on Hela cell line gave significant results and most of the cells are arrested in the S phase as shown in Figure 3.16. As for the compound 71 Figure 3.17 shows that almost the cells are staying in G1 phase, which means that the result was not significant.

Philoppes and Lamie in 2019 investigated two newly synthesized 5a and 5g for cell cycle analysis using two cell lines HepG2 and MCF7. It was found that the compounds were very potent for both cell lines and the percentage of cells in G1 phase was 55.37% for 5a and 48.22% for 5g. Also, the percentage of cells in G2/M phase was 31.22% and 33.54% for 5a and 5g, respectively (Philoppes and Lamie 2019).

Diana at al found compound (3-Methoxy-5H-isoindolo[2,1-a] quinoxalin-6-one) stop most of the cells in G2/M phase at 35% by using tubulin polymerization assay on Jurkat cell line. It was found that this compound inhibited tubulin polymerization in a concentration-dependent manner. For that reason, it could be concluded that the cells arrested in phase G2/M (Diana et al. 2008).

In 2014, 14 compounds of Isoindole derivative were investigated against HepG2 and SW620 cell lines. It has been founded that the percentage of cells is 37.3% and 41.7% of compounds 3a and 8c, respectively after 24 h and 48 h incubation. Thus, the suggested compounds are very robust and good to arrest the cells in the G2/M phase for both cell lines (1995 2014).

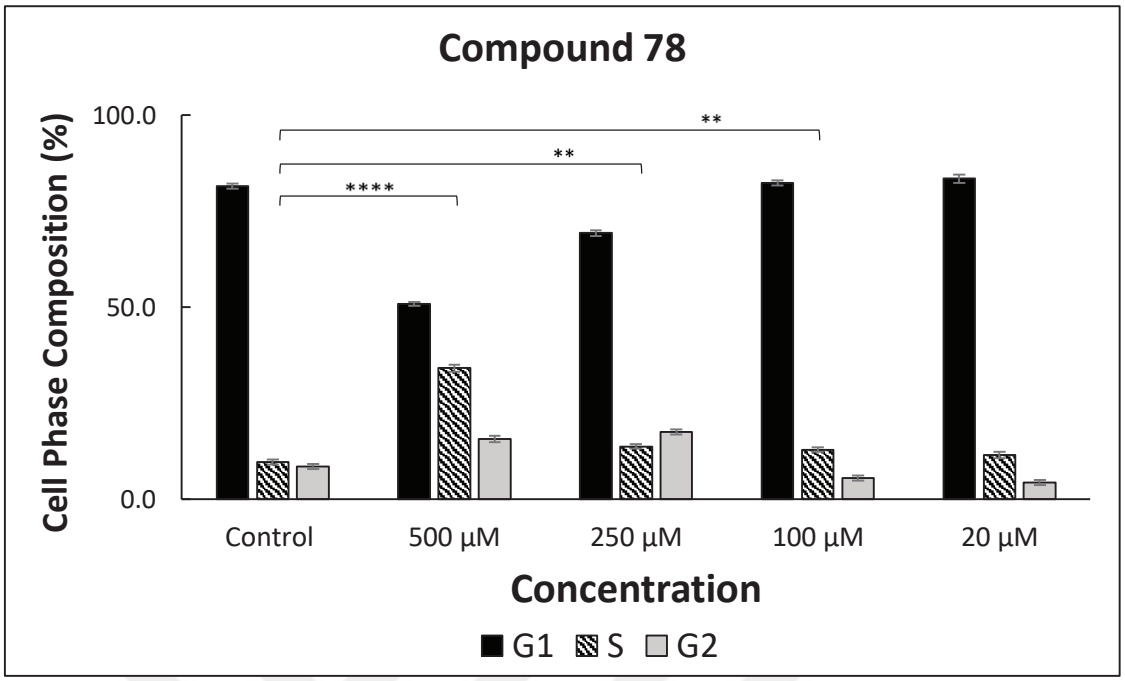


Figure 3. 14. Cell cycle analysis for compound 78 at IC_{50} dose on A549 cells incubated for 48 h period. Bars indicate SD, ****p<0.0001, **p<0.01.

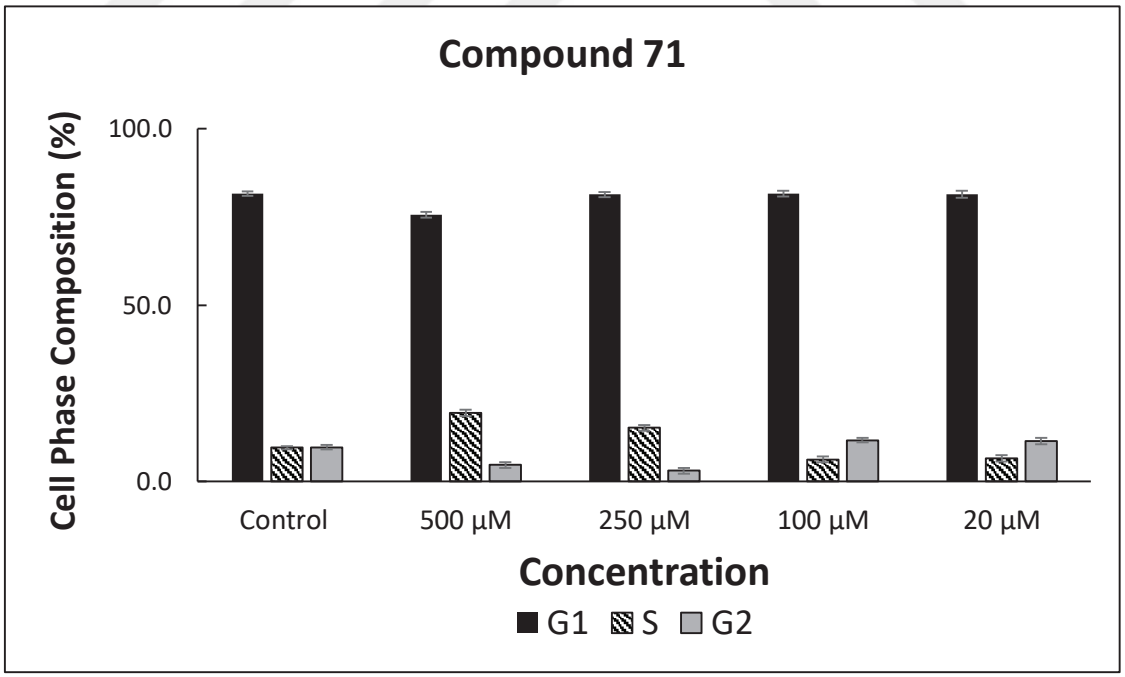


Figure 3. 15. Cell cycle analysis for compound 71 at IC_{50} on A549 cells incubated for 48 h period.

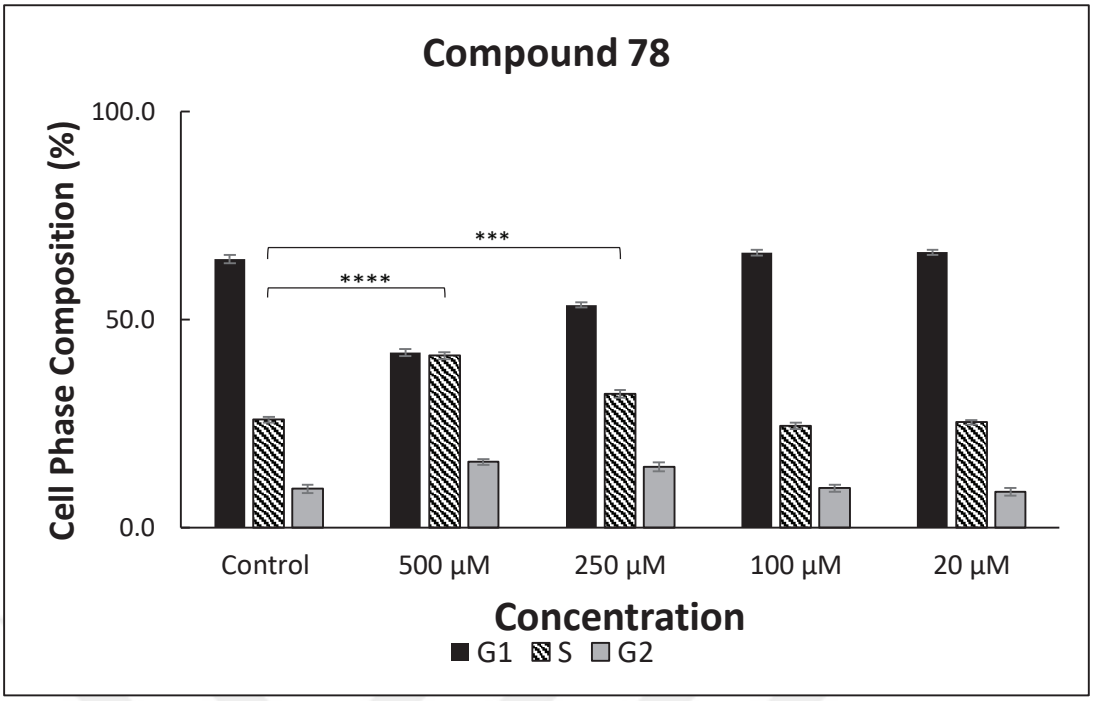


Figure 3. 16. Cell cycle analysis for compound 78 at IC_{50} on HeLa cells incubated for 48 h period. Bars indicate SD, ****p<0.0001, ***p<0.001.

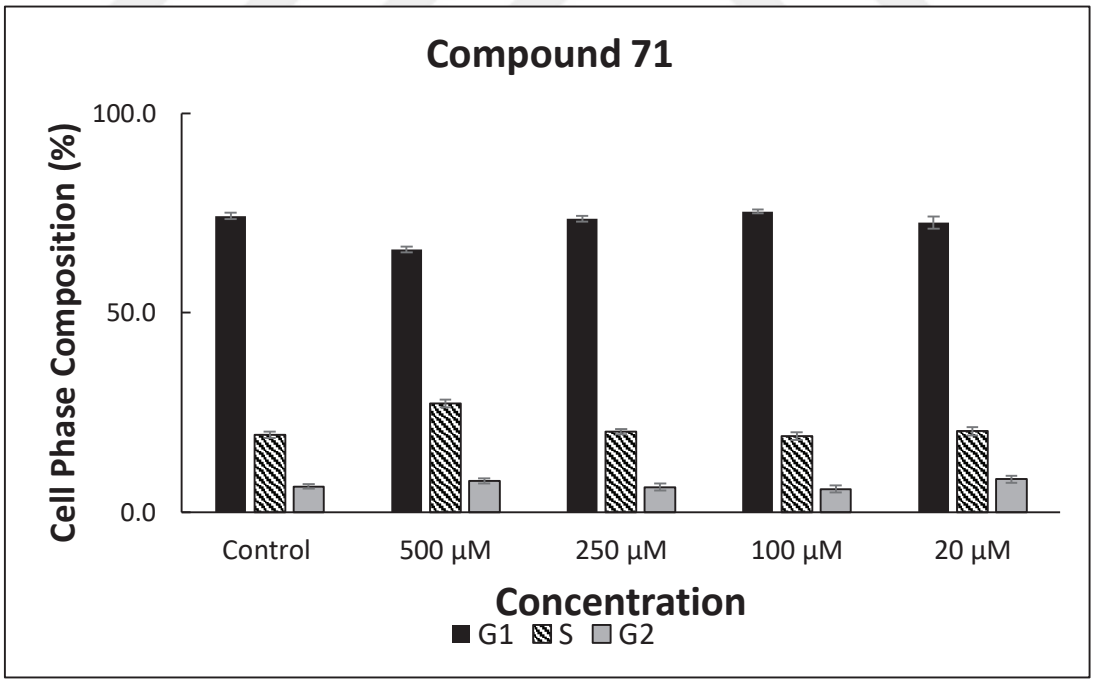


Figure 3. 17. Cell cycle analysis for compound 71 at IC_{50} on HeLa cells incubated for 48 h period.

CHAPTER 4

CONCLUSION

Isoindole and its derivatives are one of the important biological active heterocyclic compounds. It is continued to attract the attention of many researchers because of its diversity, effectiveness as anticancer with high efficacy and fewer side effects compared to other treatments. Tyrosine kinases are known to be good target for cancer treatment because of their roles in cell growth signalling. Isoindole and its derivatives, are potential tyrosine kinase inhibitors which means they might be used in targeted therapy.

In this study, new seven compounds from Isoindole derivatives were investigated in the biological activity in order to evaluate the efficacy of these compounds.

HeLa (cervical carcinoma) and A549 (lung adenocarcinoma) cell lines have been chosen to investigate the suggested compounds for their biological activity. The cell viability and apoptosis effect have been investigated using cell cycle analysis and the effect of the best compound on cell migration assay.

Generally, the results have proved that the best compounds have good IC_{50} which were compounds 78 and 71 in the 48 h period time. IC_{50} was 251.4 µM for compound 78 and 289 µM for compound 71 on A549 cell line. For Hela cell line, the IC_{50} was 206.9 µM for compound 78 and 296.4 µM for compound 71. Other compounds were less effective based on IC_{50} values compared to compound 78 and 71.

For the apoptosis effect, compound 78 was better than compound 71 with A549 cell line for 48 h incubation. The apoptosis rate for compound 78 was 20.4 % and 17.2 % for compound 71. For the HeLa cell line, the apoptosis rate for compound 78 was 21.2 % and 13.8 % for compound 71, which means that compound 78 was very effective with each of cell line, and compound 71 was more effective with A549 than HeLa cell line.

On the other hand, cell cycle effects on the results of the compound 78 arrest the cells in G1 phase with A549, and in G1/S phase with HeLa cell line. The compound 71 arrest the cells in G1 phase for each of cell line.

The last test in this study is the evaluation of the effect of two compounds on cell migration. The results show that each compound 78 and 71 inhibit the cell migration, and compound 78 had a significant inhibition on both cell lines. The results exhibited that two compounds could prevent cell proliferation in biological systems.

REFERENCES

1995, Thompson. 2014. "Novel Phenyl and Pyridyl Substituted Derivatives of Isoindolines: Synthesis, Antitumor Activity and DNA Binding Features." *European Journal of Medicinal Chemistry* 87: 372–85.

"A.C. Society, Key Statistics for Lung Cancer, (2018) (Accessed 31 May 2018), Https://Www.Cancer.Org/Cancer/Non-Small-Cell-Lung-Cancer/about/Key-Statistics. Html.No Title." n.d.

Abdel Ghany, S M, N T El Melegy, A A R Mohamed-Hussein, and R S Hana. 2011. "Emerging Prognostic Biomarkers in Non Small Cell Lung Cancer Patients: Impact of Treatment with Nimesulide (COX-2 Inhibitor) Combined with Chemotherapy." *J Pulmonar Respirat Med S* 5: 2.

Alberts, B, D Bray, J Lewis, M Raff, K Roberts, and J D Watson. 1986. "Molecular Biology of the Cell Garland, New York, 1983. 9 J. Darnell, H. Lodish, and D. Baltimore." *Molecular Cell Biology*.

Armstrong, Deborah K, Brian Bundy, Lari Wenzel, Helen Q Huang, Rebecca Baergen, Shashikant Lele, Larry J Copeland, Joan L Walker, and Robert A Burger. 2006. "Intraperitoneal Cisplatin and Paclitaxel in Ovarian Cancer." *New England Journal of Medicine* 354 (1): 34–43.

Ashkenazi, Avi, and Vishva M Dixit. 1998. "Death Receptors: Signaling and Modulation." *Science* 281 (5381): 1305–8.

Audus, Kenneth L., Michael L. Avery, Christine G. Oster, Mary M. Mayer, and Kimberly A. Foster. 2002. "Characterization of the A549 Cell Line as a Type II Pulmonary Epithelial Cell Model for Drug Metabolism." *Experimental Cell Research* 243 (2): 359–66. https://doi.org/10.1006/excr.1998.4172.

Bali, A, M P Singh, Khorate M Padmavathi, and J Ahmed. 2010. "Malignant Fibrous Histiocytoma: An Unusual Transformation from Benign to Malignant." *J Cancer Sci Ther* 2: 53–57.

Baserga, Renato. 1968. "Biochemistry of the cell cycle : a review," 167–91.

Berger, Dan, Ron Citarella, Minu Dutia, Lee Greenberger, William Hallett, Rolf Paul, and Dennis Powell. 1999. "Novel Multidrug Resistance Reversal Agents." *Journal of Medicinal Chemistry* 42 (12): 2145–61.

Bhatia, Richa Kaur. 2017. "Isoindole Derivatives: Propitious Anticancer Structural Motifs," 189–207. https://doi.org/10.2174/1568026616666161605301.

Bokkel Huinink, Willem W ten, Bengt Bergman, Assad Chemaissani, Wolfgang Dornoff, Peter Drings, Piikko-Liisa Kellokumpu-Lehtinen, K Liippo, Karin Mattson, Joachim von Pawel, and Sergio Ricci. 1999. "Single-Agent Gemcitabine: An Active and Better Tolerated Alternative to Standard Cisplatin-Based Chemotherapy in Locally Advanced or Metastatic Non-Small Cell Lung Cancer." *Lung Cancer* 26 (2): 85–94.

Brooks, Susan A, and Udo Schumacher. 2001. *Metastasis Research Protocols*. Vol. 1. Springer.

Cairns, John. 1985. “The Treatment of Diseases and the War against Cancer.” *Scientific American* 253 (5): 51–59.

Chen, Sophie, Matt Cooper, Matt Jones, Thumuluru Kavitha Madhuri, Julie Wade, Ashleigh Bachelor, and Simon Butler-Manuel. 2011. “Combined Activity of Oridonin and Wogonin in Advanced-Stage Ovarian Cancer Cells.” *Cell Biology and Toxicology* 27 (2): 133–47.

Ciechanover, A, and A L Schwartz. 1994. “The Ubiquitin-Mediated Proteolytic Pathway: Mechanisms of Recognition of the Proteolytic Substrate and Involvement in the Degradation of Native Cellular Proteins.” *The FASEB Journal* 8 (2): 182–91.

Davidson, James Edward Paul, James Brooke Murray, I-Jen Chen, Claire Walmsley, Mark Dodsworth, Johannes W G Meissner, Paul Brough, Imre Fejes, János Tatai, and Miklós Nyerges. 2018. “Isoindoline or Isoquinoline Compounds, a Process for Their Preparation and Pharmaceutical Compositions Containing Them.” Google Patents.

Decaestecker, Christine, Olivier Debeir, Philippe Van Ham, and Robert Kiss. 2007. “Can Anti-migratory Drugs Be Screened in Vitro? A Review of 2D and 3D Assays for the Quantitative Analysis of Cell Migration.” *Medicinal Research Reviews* 27 (2): 149–76.

Decaudin, Didier, Isabel Marzo, Catherine Brenner, and Guido Kroemer. 1998. “Mitochondria in Chemotherapy-Induced Apoptosis: A Prospective Novel Target of Cancer Therapy.” *International Journal of Oncology* 12 (1): 141–93.

Demirbaş, Neslihan, Reyhan Ugurluoglu, and Ahmet Demirbaş. 2002. “Synthesis of 3-Alkyl (Aryl)-4-Alkylidenamino-4, 5-Dihydro-1H-1, 2, 4-Triazol-5-Ones and 3-Alkyl-4-Alkylamino-4, 5-Dihydro-1H-1, 2, 4-Triazol-5-Ones as Antitumor Agents.” *Bioorganic & Medicinal Chemistry* 10 (12): 3717–23.

Diana, Patrizia, Annamaria Martorana, Paola Barraja, Alessandra Montalbano, Anna Carbone, and Girolamo Cirrincione. 2011. “Nucleophilic Substitutions in the Isoindole Series as a Valuable Tool to Synthesize Derivatives with Antitumor Activity.” *Tetrahedron* 67 (11): 2072–80.

Diana, Patrizia, Annamaria Martorana, Paola Barraja, Alessandra Montalbano, Gaetano Dattolo, Girolamo Cirrincione, Francesco Dall’Acqua, Alessia Salvador, Daniela Vedaldi, and Giuseppe Basso. 2008. “Isoindolo [2, 1-a] Quinoxaline Derivatives, Novel Potent Antitumor Agents with Dual Inhibition of Tubulin Polymerization and Topoisomerase I.” *Journal of Medicinal Chemistry* 51 (8): 2387–99.

Du, X M, Y Yan, Z L Bai, J P Zhang, Z Wang, L L Liu, and L J Feng. 2010. “Evaluation of the Cytotoxicity of a Two Photon Absorbing Fluorescence Compound on Human HepG2 Cells and Its Application to Tracking Human Hepatic Cancer Cells in Mice.” *Biotechnic & Histochemistry* 85 (2): 107–13.

Eccles, Suzanne A, Carol Box, and William Court. 2005. “Cell Migration/Invasion Assays and Their Application in Cancer Drug Discovery.” *Biotechnology Annual Review* 11: 391–421.

Eisenberg, David M, Roger B Davis, Susan L Ettner, Scott Appel, Sonja Wilkey, Maria Van Rompay, and Ronald C Kessler. 1998. “Trends in Alternative Medicine Use in the United States, 1990-1997: Results of a Follow-up National Survey.” *Jama* 280

(18): 1569–75.

El-Naem, Sh I, A O El-Nzhawy, H I El-Diwani, and A O Abdel Hamid. 2003. "Synthesis of 5-Substituted 2-Methylbenzimidazoles with Anticancer Activity." *Archiv Der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry* 336 (1): 7–17.

Etxeberria, Aitzol, Sorkunde Mendarte, and Santiago Larregla. 2011. "Determination of Viability of Phytophthora Capsici Oospores with the Tetrazolium Bromide Staining Test versus a Plasmolysis Method." *Revista Iberoamericana de Micología* 28 (1): 43–49.

Farber, Emmanuel. 1994. "Programmed Cell Death: Necrosis versus Apoptosis." *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc* 7 (5): 605–9.

Farhadieh, Rostam Dariush, Petr Otahal, Kiarash Taghavi, Arash Salardini, Pamela Russell, and Robert Smee. 2011. "Second Primary Tumours of the Head and Neck Are Not Associated with Adverse Overall Survival in Oral Sccs." *Journal of Cancer Science and Therapy* 3 (2): 30–34.

Fischer, Eike G, Alexandra Stingl, and C James Kirkpatrick. 1990. "Migration Assay for Endothelial Cells in Multiwells: Applications to Studies on the Effect of Opioids." *Journal of Immunological Methods* 128 (2): 235–39.

Fogh, Jørgen, and Germain Trempe. 1975. "New Human Tumor Cell Lines." In *Human Tumor Cells in Vitro*, 115–59. Springer.

Friedl, Peter, Yael Hegerfeldt, and Miriam Tusch. 2004. "Collective Cell Migration in Morphogenesis and Cancer." *International Journal of Developmental Biology* 48 (5–6): 441–49.

Fronza, M, B Heinzmann, M Hamburger, S Laufer, and I Merfort. 2009. "Determination of the Wound Healing Effect of Calendula Extracts Using the Scratch Assay with 3T3 Fibroblasts" 126: 463–67. https://doi.org/10.1016/j.jep.2009.09.014.

Gali-Muhtasib, Hala, and Nadine Bakkar. 2002. "Modulating Cell Cycle: Current Applications and Prospects for Future Drug Development." *Current Cancer Drug Targets* 2 (4): 309–36.

Gao, Xiang, BiLan Wang, XiaWei Wei, Ke Men, Fengjin Zheng, Yingfeng Zhou, Yu Zheng, MaLing Gou, Meijuan Huang, and Gang Guo. 2012. "Anticancer Effect and Mechanism of Polymer Micelle-Encapsulated Quercetin on Ovarian Cancer." *Nanoscale* 4 (22): 7021–30.

GERCEL-TAYLOR, CICEK, Anna K Feitelson, and Douglas D Taylor. 2004. "Inhibitory Effect of Genistein and Daidzein on Ovarian Cancer Cell Growth." *Anticancer Research* 24 (2B): 795–800.

Gough, Wendy, Keren I Hulkower, Renee Lynch, M Patrick, Mark Uhlik, Lei Yan, and Jonathan A Lee. 2011. "Alternative to the Scratch Assay," 155–63. https://doi.org/10.1177/1087057110393340.

Green, Douglas R, and Guido Kroemer. 2004. "The Pathophysiology of Mitochondrial Cell Death." *Science* 305 (5684): 626–29.

Group, Non-small Cell Lung Cancer Collaborative. 1995. “Chemotherapy in Non-Small Cell Lung Cancer: A Meta-Analysis Using Updated Data on Individual Patients from 52 Randomised Clinical Trials.” *BMJ: British Medical Journal*, 899–909.

Guan, Jun-Lin. 2005. *Cell Migration: Developmental Methods and Protocols*. Vol. 294. Springer Science & Business Media.

Hanahan, Douglas, and Robert A Weinberg. 2000. “The Hallmarks of Cancer.” *Cell* 100 (1): 57–70.

Hengartner, Michael O. 2000. “The Biochemistry of Apoptosis.” *Nature* 407 (6805): 770.

Heugebaert, Thomas S A, Bart I Roman, and Christian V Stevens. 2012. “Synthesis of Isoindoles and Related Iso-Condensed Heteroaromatic Pyrroles.” *Chemical Society Reviews* 41 (17): 5626–40.

Hirsch, Tamara, Philippe Marchetti, Santos A Susin, Bruno Dallaporta, Naoufal Zamzami, Isabel Marzo, Maurice Geuskens, and Guido Kroemer. 1997. “The Apoptosis-Necrosis Paradox. Apoptogenic Proteases Activated after Mitochondrial Permeability Transition Determine the Mode of Cell Death.” *Oncogene* 15 (13): 1573.

Horvitz, H Robert. 1994. “Genetic Control of Programmed Cell Death in the Nematode Caenorhabditis Elegans.” In *Apoptosis*, 1–13. Springer.

Hsu, Dong-Tsou, and Chih-Hsiu Lin. 2009. “Synthesis of Benzo [c] and Naphtho [c] Heterocycle Diesters and Dinitriles via Homoelongation.” *The Journal of Organic Chemistry* 74 (23): 9180–87.

Joule, John Arthur, and Keith Mills. 2008. *Heterocyclic Chemistry*. John Wiley & Sons.

Karolin, Jan, Lennart B-A Johansson, Leif Strandberg, and Tor Ny. 1994. “Fluorescence and Absorption Spectroscopic Properties of Dipyrrometheneboron Difluoride (BODIPY) Derivatives in Liquids, Lipid Membranes, and Proteins.” *Journal of the American Chemical Society* 116 (17): 7801–6.

Kerr, John F R. 1971. “Shrinkage Necrosis: A Distinct Mode of Cellular Death.” *The Journal of Pathology* 105 (1): 13–20.

———. 2002. “History of the Events Leading to the Formulation of the Apoptosis Concept.” *Toxicology* 181: 471–74.

Kerr, John F R, Andrew H Wyllie, and Alastair R Currie. 1972. “Apoptosis: A Basic Biological Phenomenon with Wideranging Implications in Tissue Kinetics.” *British Journal of Cancer* 26 (4): 239.

Khan, David R. 2010. “The Use of Nanocarriers for Drug Delivery in Cancer Therapy.” *J Cancer Sci Ther* 2 (3): 58–62.

Klein-Soyer, C, J-P Cazenave, J-M Herbert, and J-P Maffrand. 1994. “SR 25989 Inhibits Healing of a Mechanical Wound of Confluent Human Saphenous Vein Endothelial Cells Which Is Modulated by Standard Heparin and Growth Factors.” *Journal of Cellular Physiology* 160 (2): 316–22.

Köse, Aytekin, Yıldız Bal, Nurhan H Kishalı, Gülşah Şanlı-Mohamed, and Yunus Kara. 2017. “Synthesis and Anticancer Activity Evaluation of New Isoindole Analogues.” *Medicinal Chemistry Research* 26 (4): 779–86.

Kroemer, Guido, and Bruno Dallaporta. 1998. “The mitochondrial death / life regulator in apoptosis and necrosis.”

Kroemer, Guido, Lorenzo Galluzzi, and Catherine Brenner. 2007. “Mitochondrial Membrane Permeabilization in Cell Death.” *Physiological Reviews* 87 (1): 99–163.

Kung, Pei-Pei, Buwen Huang, Gang Zhang, Joe Zhongxiang Zhou, Jeff Wang, Jennifer A Digits, Judith Skaptason, Shinji Yamazaki, David Neul, and Michael Zientek. 2009. “Dihydroxyphenylisoindoline Amides as Orally Bioavailable Inhibitors of the Heat Shock Protein 90 (Hsp90) Molecular Chaperone.” *Journal of Medicinal Chemistry* 53 (1): 499–503.

Lakhani, Saquib A, Ali Masud, Keisuke Kuida, George A Porter, Carmen J Booth, Wajahat Z Mehal, Irteza Inayat, and Richard A Flavell. 2006. “Caspases 3 and 7: Key Mediators of Mitochondrial Events of Apoptosis.” *Science* 311 (5762): 847–51.

Latif, Tahir, Nabeel Chauhan, Rashid Khan, Andrea Moran, and Saad Z Usmani. 2012. “Thalidomide and Its Analogues in the Treatment of Multiple Myeloma.” *Experimental Hematology & Oncology* 1 (1): 27.

Lau, Gregory S K, Jimny Y W Chan, and W I Wei. 2011. “Role of Surgery in the Treatment of Radiation-Induced Sarcomas of the Head and Neck.” *Journal of Cell Science & Therapy*.

Lauffenburger, Douglas A, and Alan F Horwitz. 1996. “Cell Migration: A Physically Integrated Molecular Process.” *Cell* 84 (3): 359–69.

Lieber, Michael, George Todaro, Barry Smith, Andras Szakal, and Walter Nelson-Rees. 1976. “A Continuous Tumor-cell Line from a Human Lung Carcinoma with Properties of Type II Alveolar Epithelial Cells.” *International Journal of Cancer* 17 (1): 62–70.

Liu, Jian-Quan, Yong-Gang Ma, Mei-Mei Zhang, and Xiang-Shan Wang. 2017. “Consecutive Sonogashira Coupling and Hydroamination Cyclization for the Synthesis of Isoindolo [1, 2-b] Quinazolin-10 (12 H)-Ones Catalyzed by CuI/l-Proline.” *The Journal of Organic Chemistry* 82 (9): 4918–23.

Lu, Kan V, Kimberly A Jong, Ayyappan K Rajasekaran, Timothy F Cloughesy, and Paul S Mischel. 2004. “Upregulation of Tissue Inhibitor of Metalloproteinases (TIMP)-2 Promotes Matrix Metalloproteinase (MMP)-2 Activation and Cell Invasion in a Human Glioblastoma Cell Line.” *Laboratory Investigation* 84 (1): 8.

Luo, Haitao, Gary O Rankin, Zhaoliang Li, Laura DePriest, and Yi Charlie Chen. 2011. “Kaempferol Induces Apoptosis in Ovarian Cancer Cells through Activating P53 in the Intrinsic Pathway.” *Food Chemistry* 128 (2): 513–19.

Maciejczyk, Adam, and Paweł Surowiak. 2013. “Quercetin Inhibits Proliferation and Increases Sensitivity of Ovarian Cancer Cells to Cisplatin and Paclitaxel.” *Ginekologia Polska* 84 (7).

Majno, Guido, and Isabelle Joris. 2004. *Cells, Tissues, and Disease: Principles of General Pathology*. Oxford University Press.

Martinvalet, Denis, Pengcheng Zhu, and Judy Lieberman. 2005. “Granzyme A Induces Caspase-Independent Mitochondrial Damage, a Required First Step for Apoptosis.” *Immunity* 22 (3): 355–70.

Mazumder, Mohammed Ehsanul Hoque, Philip Beale, Charles Chan, Jun Q Yu, and Fazlul Huq. 2012. "Epigallocatechin Gallate Acts Synergistically in Combination with Cisplatin and Designed Trans-Palladiums in Ovarian Cancer Cells." *Anticancer Research* 32 (11): 4851–60.

Mc Henry, Kevin T, Sudha V Ankala, Arun K Ghosh, and Gabriel Fenteany. 2002. "A Non-antibacterial Oxazolidinone Derivative That Inhibits Epithelial Cell Sheet Migration." *ChemBioChem* 3 (11): 1105–11.

McIlwain, David R, Thorsten Berger, and Tak W Mak. 2015. "Caspase Functions in Cell Death and Disease." *Cold Spring Harbor Perspectives in Biology* 7 (4).

Meerloo, Johan Van, Gertjan J L Kaspers, and Jacqueline Cloos. 2011. "Cell Sensitivity Assays: The MTT Assay." In *Cancer Cell Culture*, 237–45. Springer.

Mehta, Rohtesh S. 2011. "Role of Race in Survival among Patients Who Refuse the Recommended Surgery for Early Stage Non-Small Cell Lung Cancer: A Seer Cohort Study."

Mosmann, Tim. 1983. "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays." *Journal of Immunological Methods* 65 (1–2): 55–63.

Muñoz, Cynthia R Trejo, Teresa Mancilla Percino, Elvia Mera Jiménez, José Correa-Basurto, and José G Trujillo Ferrara. 2013. "Partition Coefficient Determination of a Series of Isoindolines-2-Substituted and Its Correlation with Their Antiproliferative Activity on HeLa Cells." *Medicinal Chemistry Research* 22 (8): 4039–45.

Nieder, C, O Spanne, I Bilberg, and A Dalhaug. 2010. "Primary Tumour Characteristic s as Potential Prognostic Factors in Brain Metastases from Breast Cancer." *J Cancer Sci Ther* 2 (4).

Nikkhah, G, J C Tonn, O Hoffmann, H-P Kraemer, J L Darling, W Schachenmayr, and R Schönmayr. 1992. "The MTT Assay for Chemosensitivity Testing of Human Tumors of the Central Nervous System." *Journal of Neuro-Oncology* 13 (1): 13–24.

Ouyang, Gaoliang, Luming Yao, Kai Ruan, Gang Song, Yubin Mao, and Shideng Bao. 2009. "Genistein Induces G2/M Cell Cycle Arrest and Apoptosis of Human Ovarian Cancer Cells via Activation of DNA Damage Checkpoint Pathways." *Cell Biology International* 33 (12): 1237–44.

Pagliacci, M C, F Spinozzi, G Migliorati, G Fumi, M Smacchia, F Grignani, C Riccardi, and I Nicoletti. 1993. "Genistein Inhibits Tumour Cell Growth in Vitro but Enhances Mitochondrial Reduction of Tetrazolium Salts: A Further Pitfall in the Use of the MTT Assay for Evaluating Cell Growth and Survival." *European Journal of Cancer* 29 (11): 1573–77.

Patt, Harvey M, and Henry Quastler. 1963. "Radiation Effects on Cell Renewal and Related Systems." *Physiological Reviews* 43 (3): 357–96.

Philoppes, John N, and Phoebe F Lamie. 2019. "Design and Synthesis of New Benzoxazole/Benzothiazole-Phthalimide Hybrids as Antitumor-Apoptotic Agents." *Bioorganic Chemistry* 89: 102978.

Pöpperl, Gabriele, Thomas Helmberger, Wolfgang Münzing, Rupert Schmid, Tobias

Franz Jacobs, and Klaus Tatsch. 2005. “Selective Internal Radiation Therapy with SIR-Spheres® in Patients with Nonresectable Liver Tumors.” *Cancer Biotherapy & Radiopharmaceuticals* 20 (2): 200–208.

Pudata, Vanita, V Subrahmanyam, and K Jhansi. 2011. “Carcinogenesis & Mutagenesis A Short Note on Cancer” 2 (4): 2–7. https://doi.org/10.4172/2157-2518.1000128.

Rao, Sunita D, and Kelly Pagidas. 2010. “Epigallocatechin-3-Gallate, a Natural Polyphenol, Inhibits Cell Proliferation and Induces Apoptosis in Human Ovarian Cancer Cells.” *Anticancer Research* 30 (7): 2519–23.

Reed, John C. 1997. “Bcl-2 Family Proteins: Regulators of Apoptosis and Chemoresistance in Hematologic Malignancies.” In *Seminars in Hematology*, 34:9–19.

Reuter, Juliane, Irmgard Merfort, Ute Wölfle, Günter Seelinger, and Christoph M Schempp. 2009. “Botanicals in Dermatology and Skin Health.” In .

Rurack, Knut, Matthias Kollmannsberger, Ute Resch-Genger, and Jörg Daub. 2000. “A Selective and Sensitive Fluoroionophore for HgII, AgI, and CuII with Virtually Decoupled Fluorophore and Receptor Units.” *Journal of the American Chemical Society* 122 (5): 968–69.

Saga, Yasushi, Hiroaki Mizukami, Yuji Takei, Keiya Ozawa, and Mitsuaki Suzuki. 2003. “Suppression of Cell Migration in Ovarian Cancer Cells Mediated by PTEN Overexpression.” *International Journal of Oncology* 23 (4): 1109–13.

Sandt, J J M Van de, AAJJL Rutten, and HBWM Koëter. 1993. “Cutaneous Toxicity Testing in Organ Culture: Neutral Red Uptake and Reduction of Tetrazolium Salt (MTT).” *Toxicology in Vitro* 7 (1): 81–86.

Saravanan, B C, C Sreekumar, G C Bansal, D Ray, J R Rao, and A K Mishra. 2003. “A Rapid MTT Colorimetric Assay to Assess the Proliferative Index of Two Indian Strains of Theileria Annulata.” *Veterinary Parasitology* 113 (3–4): 211–16.

Schmidt, C, M Fronza, M Goettert, F Geller, S Luik, E M M Flores, C F Bittencourt, G D Zanetti, B M Heinzmann, and S Laufer. 2009. “Biological Studies on Brazilian Plants Used in Wound Healing.” *Journal of Ethnopharmacology* 122 (3): 523–32.

Schneider, Pascal, and Jürg Tschopp. 2000. “Apoptosis Induced by Death Receptors.” In *Pharmacochemistry Library*, 31:281–86. Elsevier.

Shaghayegh, K, A Mahdi, and K Ali. 2010. “Larynx Preserving Treatments in the Early and Advanced Laryngeal Cancers; a Retrospective Analysis.” *J Cancer Sci Ther* 1: 8–10.

Shah, Mayur, Kannappan Thangaraj, Mou-Ling Soong, Lionel T Wolford, Joseph H Boyer, Ieva R Politzer, and Theodore G Pavlopoulos. 1990. “Pyrromethene–BF2 Complexes as Laser Dyes: 1.” *Heteroatom Chemistry* 1 (5): 389–99.

Solomon, Leigh A, Shadan Ali, Sanjeev Banerjee, Adnan R Munkarah, Robert T Morris, and Fazlul H Sarkar. 2008. “Sensitization of Ovarian Cancer Cells to Cisplatin by Genistein: The Role of NF-KappaB.” *Journal of Ovarian Research* 1 (1): 9.

Thompson 1995. 1987. “WiDr Is a Derivative of Another Colon Adenocarcinoma Cell Line, HT-29.” *Cancer Genetics and Cytogenetics* 27 (1): 125–34.

Thompson, Craig B. 1995. “Apoptosis in the Pathogenesis and Treatment of Disease.” *Science* 267 (5203): 1456–62.

Tsujimoto, Yoshihide, Lawrence R Finger, Jorge Yunis, Peter C Nowell, and Carlo M Croce. 1984. “Cloning of the Chromosome Breakpoint of Neoplastic B Cells with the t (14; 18) Chromosome Translocation.” *Science* 226 (4678): 1097–99.

Tully, Douglas B, Bradley J Collins, J Diane Overstreet, Cynthia S Smith, Gregg E Dinse, Moiz M Mumtaz, and Robert E Chapin. 2000. “Effects of Arsenic, Cadmium, Chromium, and Lead on Gene Expression Regulated by a Battery of 13 Different Promoters in Recombinant HepG2 Cells.” *Toxicology and Applied Pharmacology* 168 (2): 79–90.

Vaux, David L, Georg Haecker, and Andreas Strasser. 1994. “An Evolutionary Perspective on Apoptosis.” *Cell* 76 (5): 777–79.

Vermeulen, Katrien, Dirk R Van Bockstaele, and Zwi N Berneman. 2003. “The Cell Cycle : A Review of Regulation , Deregulation and Therapeutic Targets in Cancer,” 131–49.

Wagner, Daniela, and Hilke Vorwerk. 2011. “Treatment Couch Modeling in the Treatment Planning System Eclipse.” *J Cancer Sci Ther* 3 (1): 188–93.

Walczak, Henning, and Peter H Krammer. 2000. “The CD95 (APO-1/Fas) and the TRAIL (APO-2L) Apoptosis Systems.” *Experimental Cell Research* 256 (1): 58–66.

Wong, Rebecca S Y. 2011. “Apoptosis in Cancer: From Pathogenesis to Treatment.” *Journal of Experimental & Clinical Cancer Research* 30 (1): 87.

Xia, Wenjie, Xinnian Yu, Qixing Mao, Wenying Xia, Anpeng Wang, Gaochao Dong, Bing Chen, Weidong Ma, Lin Xu, and Feng Jiang. 2017. “Improvement of Survival for Non-Small Cell Lung Cancer over Time.” *OncoTargets and Therapy* 10: 4295.

Yang, Yeong-In, Kyung-Tae Lee, Hee-Juhn Park, Tae Jin Kim, Youn Seok Choi, Ie-Ming Shih, and Jung-Hye Choi. 2012. “Tectorigenin Sensitizes Paclitaxel-Resistant Human Ovarian Cancer Cells through Downregulation of the Akt and NFκB Pathway.” *Carcinogenesis* 33 (12): 2488–98.

Zhao, Pei-Liang, Wei-Feng Ma, An-Na Duan, Min Zou, Yi-Chen Yan, Wen-Wei You, and Shu-Guang Wu. 2012. “One-Pot Synthesis of Novel Isoindoline-1, 3-Dione Derivatives Bearing 1, 2, 4-Triazole Moiety and Their Preliminary Biological Evaluation.” *European Journal of Medicinal Chemistry* 54: 813–22.

APPENDIX A

CHEMICALS, SOLUTIONS AND REAGENTS

NSCLC	Non-small cell lung cancer
CDKs	Cyclin dependent kinase
DNA	Deoxyribonucleic acid
MTT	Methyl-thiazolyl-tetrazolium
Caspase-3	Cysteine-aspartic acid protease
RNA	Ribonucleic acid
DISC	Death-inducing signaling complex
ATM	Ataxia telangiectasia mutated
CHK	Checkpoint kinase
BAX	Bcl-2-associated x protein
APAF1	Apoptotic protease activating factor 1
MDR	Multidrug resistance
DMSO	Dimethyl sulfoxide
FBS	Fetal Bovine Serum
P/S	Penicillin streptomycin
PBS	Phosphate- buffered saline
FITC	Fluorescein isothiocyanate
PI	Propidium iodide