An-Najah National University

Faculty of Graduate Studies

SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL SCREENING OF CURCUMIN BASED AZO DYES AND AZO-DYES METAL COMPLEXES

By

Ala'a Abed Al-Raheem Janem

Supervisor

Prof. Othman Hamed

This Dissertation is Submitted in Partial Fulfillment of the Requirements for the Degree of PhD of Chemistry, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.

2023

SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL SCREENING OF CURCUMIN BASED AZO DYES AND AZO-DYES METAL COMPLEXES

By

Ala'a Abed Al-Raheem Janem

This Thesis was Defended Successfully on 13/8/2022 and approved by

Prof. Othman Hamed	
Supervisor	Signature
Dr. Orwah Houshia	
External Examiner	Signature
Prof. Ismail Warad	
Internal Examiner	Signature
Prof. Mohammed Al-Nuri	
Internal Examiner	Signature

Dedication

I dedicate my thesis to my beloved, supportive family, my soulmate husband AmeedAmireh for his support and cooperation. Many thanks and love for my dear kids Zain & Yazan.

I also dedicate it to my loving and caring mother great supportive father. I wish them both long lives and good health. I also dedicatee this thesis to my beloved brothers Ashraf, Ameer, sultan and Hamode and my precious sisters Noura, Amera and Nidaa and for all their kids .

I also dedicate it to my Mother's soul in law (Eman), Which I will never forget her support and her help to take care of my kids.

I also dedicate it to my real friends EmanMakharzeh, Malak Daqqa, MalakNassr, Suha Hudud and Clara Yaish.

Finally, I dedicate it to all my teachers who never failed to support me with vital information during my studies. Finally, I would like to thank everyone who has helped, supported, and encouraged me throughout my studies,

Acknowledgment

First of all, I would like to thank our Great and Merciful Almighty Allah for letting me through all the difficulties.

I would like to express my sincere gratitude to my great advisor Prof. Othman Hamed for his continuous support, patience, motivation, enthusiasm, and immense knowledge. His guidance, Notes and directions helped me during my study. I'm so glad to have a scientist like him as my supervisor.

A special thanks and appreciation for Dr. Ghader Omar for her time, advice and knowledge and for her great work in cancer related material in this thesis.Special thank to Prof. Avni for the molecular docking work.

Finally, this Ph.D. degree was a dream to me, but I wouldn't have earned it without help, guidance, educations, and assistance from the Chemistry Department at

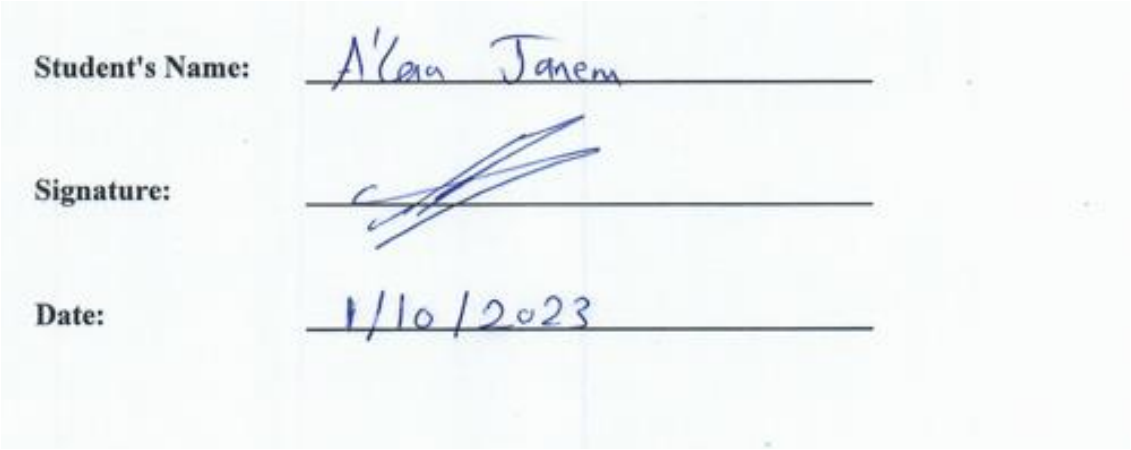
Al-Najah National University represented by chairman of the department, lecturers, professors, and laboratory technician.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL SCREENING OF CURCUMIN BASED AZO DYES AND AZO-DYES METAL COMPLEXES

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.



List of Contents

List of Tables

List of Figures

List of Appendices

SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL SCREENING OF CURCUMIN BASED AZO DYES AND AZO-DYES METAL COMPLEXES

By

Ala'a Abed Al-Raheem Janem

Supervisor

Prof. Othman Hamed

Abstract

Background: Curcumin is a natural active, vital, and important component derived from Curcuma longa. A significant attention was given to curcumin and its derivatives due to their natural origin, non-toxicity also to their bio functional properties including antioxidant, anti-inflammatory, antitumor, and anti-cancer.

Objectives: The main objective of this work is to develop a new set of curcumin derivatives with broad spectrum of bioactivities. Curcumin-based azo compounds and curcumin with alkyl sulfonate moiety were selected to achieve the target objective.

Methodology: The method synthesizing curcumin derivatives involves converting some aromatic amines with chlorine, sulfonyl, carboxyl, and ammonium groups to diazonium slat then coupling the diazonium salt with curcumin to produce curcumin-based azo compounds and direct condensation of curcumin alkali with sultone.

Results: The newly prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR and melting point. The azo compounds performance as dyes were evaluated. The color change of the azo as a factor pH were studied. The bioactivities of the azo compounds and complexes such as antimicrobial and anticancer wereevaluated (*Escherichiacoli, Staphylococcus aureus, klebsiella pneumonia*and Methicillin-resistant Staphylococcus aureus,MCF-7, HEPG2, B16-F110, Colon and HeLa cell lines viability MTT assay in Palestine).

Conclusion: A new novel set of curcumin azo dyes and curcumin with alkyl sulfonate moieties were successfully prepared. The antimicrobial and anticancer activities were very promising, which make the compound possible future drugs for treatment of various kinds of cancer.

Keywords: Curcumin; Azo; Anticancer; Curcumin Sulfonate; Sultone, Genotoxic.

Chapter One

Introduction

1.1 Preface

Colors have been used throughout history for many purposes, including artworks, fabrics, home items and others. A dye could be water soluble or insoluble substance, mainly of organic nature (1, 2). The dye material, usually stable to light, it penetrates the material to be colored and binds to it mainly by physical forces such as dipole-dipole and H-bonding, that makes it stable in cleaning and drying. Coloring substances with inorganic origins are known as pigments, but can also be of organic based, such as madder lake. They are bound onto the surface of material to be colored through a binding medium such as protein, oil, or gum(1). Dye materials are known to absorb light in the visible region, due to the presence of chromophores, that is an unsaturated functional group such as conjugated double bonds, carbonyl group, nitroso group. Afunctional groups that modify the light such as hydroxyl group, amino group, and the carboxyl group are called auxo chrome. Research in organic dyes constitutes a keystone in organic chemistry. The first dye to be synthesized was Mauveine which happened in1856, its chemical structure is shown in Figure A58 (appendix A)(3). Mauveine is also known as aniline purple that was first discovered in 1857.

Then, the progress in dye chemistry started which led to the production and commercialization of various types of dyes (4-6). As a result, the strength and success of many chemical companies are based originally on dye chemistry. Though the traditional organic dyeis used for printer colors, hair, textiles, photography, cosmetics, food, etc. is still highly relevant, organic dyes also appear in highly sophisticated applications such as in photo polymerization (7 ,6), OLEDs(8), semiconductors (7),solar cells (8, 9), and organic NLO materials (10).

Synthetic dyes, prepared from small organic materials starts currently to play important roles in modern life with applications in both scientific laboratories (e.g., fluorescent tracers and photo redox catalysts) and industry (e.g., paint industry). In this regard, a various dye, such as azo dyes, aryl amines, anthraquinones, fluorescein's, carbazoles, cyanine's, thiophene, oxazines, phenothiazines, rhodamines, were developed.

1.2 Azo Dyes

The azo dyes are those containing an azo group (-N=N-). The first example of azo dye reported in the literature was in 1958(11), this type of dye was not very promising until Bottiger produced the Congo red in 1984 (12). John Peter showed that diazo compounds could be prepared from reacting nitrous acid with aromatic amine, then he showed that diazo compounds could be coupled with another aromatic amine to generate the azo compounds (12). Since then, this are of research has expanded to include triazo, tetrakisazo and polyazo dyes. Azo dyes now become the largest and the most versatile group of dyes. The Azo is derived from Azote originate form French that is derived from Greek a Zoe (to live). The N of the azo functional group has a sp^2 hybrid orbital, the most common azo are those with aryl-N=N-R type of group, R could be aryl, heterocyclic ring, or conjugated C=C bonded to carboxyl or another functional group.Due to the simple nature of azo dye many examples are available in the literature. As mentioned above the first example of promising azo dyes shown in Figure A59 (appendix A) was prepared by Bismarck Brown in 1958(13).

Today, azo dyes represent a huge industry, and the importance of this materials may increase in near future due to its unlimited number of applications. They play a crucial role in printing market and in the governance of the dyes, a simple method of preparation of dye consists of diazotization and coupling. Different modifications on the synthesis procedure and reagents are to obtain the desired color properties like particle size and yield to improve dispensability of the dye (10). Approximately 72% of all dyes consumed by the industrial sectors areazo based(14-16).

Moat azo compounds aresoluble in water (17) have shining and bright colors, especially orange, red, and yellow, and they are the most significant type of synthetic colorant and comprise most of the dyes now in use. They are categorized based on how many azo groups are present. Most azo dyes are monoazo, they only include one azo group. However, some azo dyes may also have two or four azo groups (tetrakisazo) (18). An example on azo dye is shown in Figure A60 (appendix A)

.

As shown in Figure A61 (appendix A). The azo dye chemical structure is a combination of several functionalities that are connected by covalent bonds. The main part is the backbone (skeleton) that attached to it the chromophoric groups, auxochrome groups, and ionic groups (19-21).

Azo dyescolordepends on number of azo groups, type ofauxochromes and chromophores. In general, the type of functional group affects it is ability to bind to the textile structure for instance carboxyl groups bind stronger to textile than ammonium (21, 22).

1.2.1 Methods of making Azo dyes

1. Direct diazotization

This method involves reacting primary aromatic amine with nitrous acid prepared form reacting $NaNO_2$ with HCl at 0-5 ^oC as shown in (Eq. 1). The diazonium salt then is coupled with aryl compound or with conjugated group containing various functionalities(12, 23).

$$R{-}NH_2 \xrightarrow[0\ ^oC]{HCl,\ NaNO_2} R{-}N_2^+ \qquad \text{Eq. 1}$$

2. Diazotization of a weak basic amine:

A weak basic amine is dissolved inconcentrated sulfuric acid andtreated with nitrosylsulphuric acid which is usually prepared by mixing sodium nitrite with concentrated sulfuric acid(24, 25).

3. Diazotization inorganic solvents

This method is used for amines that are hardly soluble or slightly soluble in water. First, it is treated with acid then diazotized with a solution of sodium nitrite(25, 26).

4. Coupling

Azo type of reaction involves an electro philicaromatic substitution of the diazonium compound with a nucleophile such as activated benzene ring (benzene with electro donating group) or any other conjugated system Figure A62 (appendix A). (25, 26).

1.2.2 Classification of azo dyes

Azo dyes are classified by chemical guideline such as the criteria: color, application of dye and number of azo groups.

The azo dyes are classified depending in the number of azo groups:

- Mono dyes: Contain one group of azo, examples on this type are shown in Figure A63 (appendix A) (25, 27).

- II. Diazo dyes: Dyes that contains two azo groupsas those shown in Figure A64 (appendix A)(25, 28).

Classification can be also based on acidic or basic functional groups present in the dyes and called acid or basic azo dyes Figure A65 (appendix A)**.** The acidic dyes contain an acidic group like carboxyl or sulfonyl, the presence of these groups enhance the solubility of azo dyes in water and makes it bon stronger to fiber (25, 28).

The basic azo dyes are characterized by the presence of basic groups such as primary, secondary or tertiary amine groups like those shown in Figure A66 (appendix A) (25, 29).

Also, dyes can be classified according to their way of application, Figure A67 (appendix A) shows an example on that direct or substantive azo dyes. This type of dye can be attached directly to the material to be colored(25, 29).

1.2.3 Azo dyes application

Azodyes are accessible of all synthetic dyes, they used inplastics,thefields of pharmacy, and living cells cancer hypnotic medicine.In addition of biological (30), and pharmacological activities (31). Many applications with high technology likelaser, nonlinear optical systems have been found (32). Alsoinphotodynamic therapy (33), sensitized to solar cells(34), dyeing of textiles (35), metal-lochromic indicators (25), leather, paper, food (35), medicines (36), and cosmetics (37).These dyes are also involved biological reactions for example protein and RNA synthesis, nitrogen fixation and carcinogenesis, DNA inhibition (38).

Drugs

Ehrlich(39)had observed that some dyes are selective to tissues. He related that to reaction between the tissue and the dye during the staining process. He studied this behavior tofindthatcell in dyesare selective to microorganisms and could be lethal to these microorganisms. Then laterin 1935 the daughter of Gerhard Domagk (40). German doctor worked for a dye factory suffered from asevere streptococcal infection contracted from a pinprick.Her father gave her an oral dose of the dyeProntosil,it which showed to inhibit the growth of streptococci in mice. The fever dropped and the girl recovered. The event laid the foundation of modern chemotherapy.Later,it was demonstrated that prontosil hydrolyzed to sulphanil amid, which is the active agent against streptococci. Figure A68 (appendix A)(25).

The dye was prepared as shown in the figure below by diazotizing sulphanilamide followed by coupling with m-phenylenediammine Figure A69 (appendix A) (25).

The drug then gained popularity and was used in the treatment sore throat, streptococcal, child bed streptococcal fever, acute endoarditicandvariouserysipelatous. Due to its low solubility in water, it was generally used as hydrochloride slat. The it was replaced by Prontosil water soluble, which his superior antibacterial drug Figure A70 (appendix A)(25).

The substituted sulphanilamideis used in the treatment of diseases like gonorrhea, pneumonia and some type of sinus infection, meningitis blood poisoning, and scarlet fever tonsillitis(41).

Now a days, the needfor a novel antimicrobial drug has increased due to the increasing resistance of certain microbes. The applications of transition metal azo dyein biological field have been reported some time ago(42, 43). A wide range of biological and pharmacological activities have been demonstrated by various azo dyes after complex formation with transition metal(44).These activities attracted a number ofscientists and researchers to prepared more novel azodyes-basedtransition metal complexes with enhanced activities against antimicrobial (45). Theazo-dyes ligand as a chelating agent with several coordination sites providing more options for metal binding(46).

The azo dye metal complexes showed various bioactivities that include antimicrobial (46), anticancer(47), antituberculosis(48), antitumor (49), anti-HIV (50), anti-inflammatory(50), antioxidant (51), antiviral (52), antileishmanial activities (53), many more activities (54, 55)and antiproliferative (56). In this work we are concerned about developing azo dye with antimicrobial and anticancer activities.

1.3 Understanding cancer

Cancer is an abnormal cell in a part of the human body, that leads to uncontrolled manner of cell growth and division(57-59). Causing normal cells to do uncontrolled cell division or separate into another cell or die. Usually, cancer cells undergo uncontrollable growth and spread as shown in Figure A71 (appendix A), the spreading of the cancer cells can be fatal. Several studies showed that 90% of deaths related to cancer are caused due to the spreading of the cancer cells in a process known as metastasis(60).

Different types of cancers are known, and many of them can be mobile through the blood circulation or lymph vessels (metastasis), like breast cancer can spread through the blood circulation to liver. Most of cancer cells are develop due to a damage in the DNA. Awkwardly, some time the damaging of DNA can be inherit to people from their parents, which explain the inherited cancer or could happened due to different environmental factors like smoking (56). No matter where cancer proliferates, metastatic cancer retains the original cancer (the primary one). Such as, lung cancer that spreads to the bones known as (metastatic lung cancer), although the patient may be suffering from bones pains or problems. Furthermore, breast cancer when spread to the liver isn't called liver cancer, instead it's called breast cancer. Each type of cancer behaves in special way from other types; such as, lung and breast cancer are completely different diseases. They are growing with different rates also they can be treated in different ways. Not all tumors are cancerous. Tumors that are confined to one area are called benign, benign tumors don't spread to other parts or tissue of the body, unlike malignant tumors. In sometimes benign tumors grow to be as a large solid mass, and t don't grow back again after removing, but sometimes malignant tumors in some cases do. Benign brain tumors may be life threatening sometimes, unlike most benign tumors in the other parts of the body.

1.3.1 Normal Cell and cancer cells

Maturation is one of the differences between abnormal cells and normal, in the case of normal cells they more specialized , also mature into limited distinct cell type, however the cancer cells continue to multiplicate and proliferate without stopping before it becomes mature(58, 59, 61, 62).Communication is the second between difference between normal and cancer cells, normal cells interact with each other whereas the cancer cells do not. In addition, the normal cells are programmed to response to the nearby signals to stop growing and dividing, the case is the opposite for the cancer cells, they do not response to the cell's physiological division rules, and they ignore signals from adjacent cells warning(58, 59, 61, 62).

1.3.2 Communication

Normal cells interact with other while cancer cells do not interact. Also, normal one respond to the signals of near cells that tell other cells to stop dividing and growing or begin the programmed cell death or apoptosis that the body uses to dispose the worn-out, damaged, and unneeded cells, while cancer cells disregard the physiological rules of the cell division and ignore signals from nearby cells warning overgrowth, and uncontrollably grow (58, 59, 61, 62).

1.3.3 How Cancer Arises

Cancer is a genetic disease that results because of the changes in a gene control the cell functions, especially the growth and division of the cell. The reasons for this are sometime from patents or appears throughout person’s lifetime due to some errors may happen through cell division or the damage of DNA as a result to change in many factors such as mutations, hormones, and immune conditions inside the cell. Moreover, external factors also cause affect and causes damage of the DNA including exposures to smoking, chemicals and UV radiations from the sun. Mutations in tumor oncogenes and suppressor is the main causes for development of cancer. As well as the mutation in a master gene controlling cells division may shepherd normal cells to abnormal chromosomal replication, leading to deletion or duplication of the entire chromosomes’ sections figure 1.6. The abnormal amount of a particular protein is produced regardless of the actual need, due to this genetic change. When the protein that plays a critical role

in the cell cycle is affected by any chromosomal aberration qualitatively or quantitatively a cancer may arise (58, 59, 61, 62).

1.3.4 Types of cancers

Cancers are named for the organ and tissue where they originate. Moreover, cancers also described according to the kind of cell initially altered, like epithelial cell a squamous cell (58).

- **Carcinomas:** Is the most famous type which formed by altered epithelial cells.
- **Lymphoma:** This type of cancer arises in lymphatic system, (In lymphoma) with abnormal lymphocytes are formed in lymph nodes, lymph vessels and in other organs of body. (58).
- **Leukemia:** is cancerous white blood cells originated in the bone marrow and characterized by formation of a huge amount of abnormal white blood cells in the blood and bone marrow instead of solid mass tumors formed in other types of cancer. (67).
- **Sarcomas:** are abnormalities in the fats, bone, blood, muscle, connective tissue, and lymph vessels (67).
- **Myelomas:** are cancers in the white blood cells that produce antibodies (67,76).
- **Hela:** This type of cancer was taken from Henrietta Lacks, who died of cervical cancer in 1951. Her cells, were taken without her consent, and cultivated for research, becoming the first known immortal human cell line. This means they can continuously divide and grow in the lab (68)
- **Colon cell**: A colon cancer cell is abnormal copy of a healthy cell inside the lining of colon or the rectum. This appears from a uncontrolled division and growth, the mature colon epithelium has goblet cell and enterocyte one (67).
- **Skin cancers:** This type arise from the skin, due to the development of abnormal cells that are capable to spread to other parts. Skin cancer is the most commonly one in humans, and the main types are BCC, SCC and melanoma(67).

- **Liver cancer:** It's known also as **primary hepatic malignancy** this type can starts from the liver(primary) or the cancer spread from somewhere else to the liver(secondary) (67).

- Breast cancer: this type caused to different reasons such as: obesity, alcohol consumption, hormone therapy, and there is different signs like breast shape, dimpling of the skin, fluid coming from the nipple, milk rejection, a newly inverted nipple, or a red or scaly patch of skin(68).

1.3.5 Treatment methods of cancers

So far, all research are trying to discover novel cancer treatments. Treatments of cancer depends on cancer progress, type and its locality. Chemotherapy, radiotherapy, surgery, and radiation-based surgical knives are the common and more used for cancer treatments.

1. Surgery and modern technology

Cancer can return after being removed by surgery as researches said, so many people until now think many types of cancers can't be removed completely. After inventing anesthesia in 1846, the surgeons Handle, Bilroth, and Halsted led an operation to remove all of the entire tumor using lymph nodes. Then it was noticed that the cancer cell is spreading to other parts of the body by the bloods stream(56).

Recognizing the limitations of cancer surgery requires an understanding of the cancer spreading mechanism(s). Most exploratory surgeries were supplanted by advances in computed tomography, ultrasound, magnetic resonance imaging, and positron emission tomography in the 1970s. Colon, esophageal, and bladder tumors can all be removed through tubes by surgeons utilizing endoscopy and tiny video cameras. Cryosurgery, which involves spraying liquid nitrogen on a tumor to freeze and kill cancer cells, is one of the less intrusive methods of removing malignancies that are currently being researched. Lasers can also be used to remove tumors from the skin, rectum, liver, cervix, larynx, and other organs(57). Moreover, the tumor can be removed with no risk of tissue damage. Several types of surgeries, either minimally invasive or open, can be performed depending on different factors;

- The surgery reasons

- The preference of the patients
- The type of solid mass or tumor that will be removed
- The region where the surgery will be performed

According to the level of cancer there are various types of surgery

- Debulk a tumor when its removal may damage a specific organ.
- Remove the entire tumor from a specific part of body.
- Relieve the symptoms of cancer when the large tumor causes intense pressure or pain to a specific part of the body (68).

2. Chemotherapy

During the latter half of the 20th century, surgeons developed anew method for treatment of cancer by combining chemotherapy with surgery or radiation. After 50 years X-rays was discovered by Roentgen. After that doctors found that nitrogen mustard has the ability to destroy lymphoma cancer cells. Through the years, cancer cells were treated successfully using chemotherapy. Now a continuous studies are performed to decrease the side effects of chemotherapy including: A combinations of drugs therapy, monoclonal and Liposomal antibody used to target specifically cancer cells, chemo protective agents was used to reduce the side effect of the chemotherapy.

3. Hormonal therapy

Thomas Beatson discovered in 1878 that producing milk by rabbit's breasts was stopped after he removing the ovaries. Later, after the removing of the testes researchers notes that prostate cancer has dramatically regressed. Now a new kinds of drugs are being used to treat breast and prostate cancers(59)

4. Radiation therapy

The X-ray process which was discovered in 1896 by Roentgen(63)was used for diagnosis and cancer treatment. Early in the 20th century, researchers found that radiation causes cancer. Nowadays different radiation are used including, the first is conformal proton beam therapy depends on using proton beam for killing tumor cells, the second one is stereotactic and therapy which using gamma knife for brain tumor,

finally therapy includes intraoperative radiation was used to remove cancer surgically followed by radiation treatment of the adjacent tissues(56).

5. Adjuvant therapy

Chemotherapy is used to destroy the rest of cancer cells after surgery cells in the body. This method is common in testis and colon cancers (56).

6. Immunotherapy

Immunotherapy is a biological agent used by the body to control the growth of tumor. Now these natural agents can be produced including cytokines, interleukins, interferon's antigens. Scientists are currently working on vaccines to strengthen the immune system's defenses against cancer cells(56).

7. Treatments targeted Cells

Until l990 the effect of the most therapy drugs worked by cytotoxic effect that means killing cancer cells(64) in addition tonormal cells.

8. Growth inhibitors

Cells are informed when to divide and grow by growth factors. During 1980’s scientists documented those changes in the singling of the growth factors can leads to an abnormal performance of cancer cells. Among the therapies that block growth factor signals are gefitinib cetuximab, trastuzumab and imatinib(56).

9. Apoptosis induction

Cellular DNA can be damaged by apoptosis process. Apoptosis caused by drugs whichforces cancer cells to die without repairing DNA(56, 64).

10. Antiangiogenic chemotherapy

Angio inhibitors were used recently in several clinical trials along with established chemotherapy. The clinical trial usually involves a combination of a small dose of chemotherapy thenfollowed by administration of Angio inhibitor therapy. Angio inhibitorsneed to be tested for approval for use in cancer treatment. Preventive Angio inhibitory therapy is important since it showed low toxicity and with no induction todrug resistance(56).

11. Nanotechnology

This method includes using a nanoparticle for diagnostic imaging to the precise location of tumors for effective drug delivery to cancer cells(65).

Despite the several attempts and various means to treat cancer, the statistics confirm that this dreaded disease has continued to spread, causing high-rate death among patients. Unfortunately, the old traditional tools to treat cancer have a low success rate and are still associated with high mortality. Therefore, it seems necessary to identify new strategies to prevent or treat this dangerous disease (66). As a result, recent research in synthetic chemistry announced the natural compounds have a good effect that create better methods of curing and blocking a lot of cancer types(67).

Hence medicinal uses of some plant species are referred to the presence of phytochemicals. Alkaloids, phenylpropanoids, terpenoids and other phytochemicals were notified to have antitumor activity (68). Upon that the production of vincristine, vinblastine, camptothecin, and taxol which are plant-derived anticancer drugs was known from several years ago (69). This is explained by the fact that more than 60% of cancer patients use vitamins or herbs as therapy (70).Therefore, diets rich in phytochemicals can minimize cancer danger by 20% (71).

The global burden of cancer is huge and growing. In 2018, there will be > 18 million new cancer cases and 9.6 million deaths (72, 73). Death certificates collected by the Palestinian Ministry of Health for Palestinians living in the West Bank. Cancer is the second leading cause of death in Palestine at 14%, exceeded only by heart disease at 30%. The cancer issue in Palestinian territories is expected to reacha level that further challenge the infrastructural and the financial resources of the Palestinian health-care regime(75). All 2016 reported fatalities were attributed to cancer mortality at a rate of 14%. In contrast, cancer fatalities made up 14.7% of all reported deaths in 2017, with men accounting for 53.2% of cancer deaths and women for 46.8%. Lung cancer accounted for 17.3% of all cancer-related deaths in 2017, followed by colon cancer (11.8%), breast cancer (10.0%), leukemia (8.4%), and brain cancer (8.2%) in that order. Lung cancer was the first leading cause of mortality from cancer among males, followed by colon cancer. The top cause of mortality in women was breast cancer, which was followed by colon cancer(74). Therefore, in order to treatment of cancer

patients more effective and to reduce drugs side effect, researchers currently started to focus on finding an alternative natural based or adjuvant that confer cancer full treatment with less side effect on patients bodies(75).

In addition to synthesized anticancer drugs, manynatural based anti-cancer reagents are active against various tumor cells have been isolated from plants and other natural sources, as Betula alba, Catharanthus roseus, and Cephalotaxus species, and several others (76). Among these, curcumin is the most important reagent that is extracted in pure crystalline form from rhizomes turmeric (77, 78). Curcumin and its derivatives have received enormous attention in the past 10 years due to their multifunctional bioactivity such as anti-inflammatory, anti-tumor activities and antioxidant (79). These bioactivities are related to its unique structure (80). So, several published scientific studied the structure activity relationship of curcumin trying to improve its biological and physiochemical properties(81, 82).

1.4 Medicinal, Natural plants and cancer

The chemotherapeutic drugs mentioned as mentioned that have been used exhibited high toxicity to the tumor and to the normal cells of the body. Moreover, the increase in the incidence of different cancer types creates a need for continuous novel chemotherapeutic drugs from natural sources. (44).

So far, many studies have been focused to find and develop new derived -drugs using the terrestrial plants and marine environments as well. As known many types of plants used to treat different diseases. As traditional medicine different types of plants are consumed in huge amounts all over the worldwide due to their medical benefits (44).

There are four main classes of natural product-derived anticancer drugs including;

1. Taxanes (paclitaxel, cabazitaxel, and docetaxel)
2. The camptothecin derivatives (camptotecin, topotecan and irinotecan)
3. The vinca alkaloids (vinblastine, vinorelbine, vindesine, vincristine and vinflunine)
4. The epipodophyllotoxins (podophyllotoxin, etoposide and teniposide)

1.5 Understanding types of bacteria

The resistance of bacterial against antibacterial agents is increased day per day and this make it worldwide problem. Accordingly, so there is a continuous and growing interest and studies to develop a new antibacterial agents with many functionalities and novel mechanisms of action. In this study a group of new curcumin-based azo dyes compounds were synthesize and a report of their antibacterial activities against various Gram-positive and Gram-negative bacteria was done (44, 45).

1.5.1 Gram positive and gram-negative Bacteria

According to the reaction and function of the bacteria toward the Gram strain and the composition of the cell wall it was divided into to types the first called Gram positive and the other one is gram-negative bacteria. In the 1800s, Hans Gram developed the Gram staining test, identifies bacteria based on the reaction of their cell walls to certain chemicals and dyes (crystal violet dye) (44).

Gram-positive bacteria has a purple-color of stain, figure A75(appendix A). This color due to the their cell walls are consist of thick layer of especial substance called peptidoglycan. In contrast, Gram-negative bacteria the cell walls has a very thin layer of peptidoglycan and has outside membrane consist of a lipopolysaccharide which isn't exist in Gram positive one, so they appear pinkish or red after Gram staining, figure A75(appendix A) (44,45).

The main difference between gram positive and negative one is the components present in their wall. The positive bacteria have thick layers from 15 to 80 nanometers of peptidoglycan as shown in figure A76 (appendix A) (44).

Peptidoglycan is a macro-molecule composed of glycan and chains of peptide which are linked together forming many different types of peptidoglycan. The benefits of Peptidoglycan is to protect bacteria and gives their shape. While the cell walls of Gram positive also have a molecules called teichoic acids that extend from the plasma membrane and running perpendicular to the peptidoglycan sheets. These acids are special to the cell walls of Gram-positive bacteria and help them to infect cell and cause disease (44, 45).

The complexity of the cell wall of gram negative type is more than positive type as shown in figure A77(appendix A) It consists of only one and thin layer 10nm of peptidoglycan surrounded by outer membrane. This membrane of Gram-negative type has a lipopolysaccharide (LPS). LPS due to endotoxin is a large complex of glycolipid has three parts; lipid A (hydrophobic component), O-specific oligosaccharide chain. And a hydrophilic core oligosaccharide. LPS work as a shield to protect bacteria from unlikely substances (44).

Gram positive bacteria are not dangerous as Gram-negative ones, 90 to 95% of Gram-negative bacteria due to the presence of LPS in their outer membrane are pathogenic. While many of Gram-positive bacteria are not pathogenic because they have contain peptidoglycan layer can be dissolved easily by lysozyme (44,46).Below a lost of gram negative and gram-positive bacteria.

1.5.1.1 Staphylococcus aureus

Staphylococcus aureus is Gram-positive type pathogenic one has a diameters of (0.5–1.5 μm) also characterized by individual cocci, which contains many planes forming microscopic-grape like clusters, figure A78(appendix A). The staphylococcus is grown by aerobic respiration or by fermentation facultative anaerobic, also its non-motile, and non-spore. Also it can grow over a temperature between 7 and 48°C, while the optimum temperature about 37 °C [15-17]. The Staphylococcus cells can live in high salinities up reach to 20%. This type of bacteria found on human nose, groin, armpit, and carriage via skin, coughing and sneezing. It can also cause infections such as; endocarditis, joint and bone infections, pneumonia and bloodstream infections (44-46).

1.5.1.2 Methicillin resistant Staphylococcus aureus

Methicillin resistant Staphylococcus aureus- (MRSA) is a pathogen often carried asymptomatically on the body of human. These type of strain as seen in figure A79 (appendix A) have acquired a gene that makes them resistant to almost all beta-lactam antibiotics. MRSA is one of the most known causes of significant and nosocomial infections, inclusive pneumonia, severe sepsis endocarditis, soft and skin tissue infections, necrotizing fasciitis and toxinoses. (44-46).

The infections of MRSA occur among in-patients who have been in hospitals or who have weakened immune systems it's known as health care-associated MRSA (HA-MRSA). While these strains are easier to treat with it, some may move into hospitals and become increasingly resistant to drugs other than beta-lactams (45).

1.5.1.3 Klebsiella

Klebsiella is a type of gram-negative anaerobic, encapsulate bacterium and non-motile, lives in the gut, skin and mouth, Klebsiella usually associated with liver abscess, pneumonia, urinary tract infections (UTIs), meningitis, bloodstream infections, respiratory infections, and wounds Commonly, K. pneumoniae is an opportunistic pathogen mostly affects patients who has an immunodeficiency, and people who are taking long courses of particular antibiotics, and tends to cause hospital-acquired (HA) infections. K. pneumoniae is provided by two factors make them chronic and antibiotic resistance; the biofilms that formed in vivo protect the pathogen from being attacked by the antibiotics and host immune responses. And the extended-spectrum β-lactamases that is commonly make them effective against beta-lactam antibiotics and show multidrug-resistance Figure A80(appendix) (44-47).

1.5.1.4 Escherichia coli

Escherichia coli, known as E. coli, a type of gram-negative bacteria with rod-shaped. Commensal of warm-blooded animals and humans. They are facultative anaerobes, nonsporulating, non-encapsulated, and they could be either motile or non-motile figure A81(appendix A). The toxic of E. coli strains cause chronic illness, including diarrhea and in some times cause kidney damage and it is cause of meningitis during the neonatal period (44-47).

1.6 Curcumin

1.6.1 Curcumin Chemical Structure

1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin) is a naturally occurring substance that serves as the primary coloring agent in the plant Curcuma longa's rhizomes. This natural pigment reportedly has several pharmacological applications in addition to being widely utilized as a coloring additive in cosmetics and foods. In addition of being an antioxidant, an anti-inflammatory, and a bile-secretion-stimulating substance. Additionally, it is said to be a powerful inhibitor of several internal and skin cancer growths (83-86).

1.6.2 Curcumin bioactivities

Numerous research has examined the curcumin therapeutic effects, including its impact on cancer, infections, inflammation, antioxidants, hepatotoxicity, hyperlipidemia, viruses, and Alzheimer's disease (83-86). Despite having a wide range of reported pharmacological effects, curcumin showed insufficient biological stability, poor absorption and bio distribution, poor aqueous solubility, low bioavailability, rapid systemic elimination and rapid metabolism in clinical trials and pharmacokinetic data (87-90). The hydrophobic curcumin (91), which underwent biotransformation into curcumin glucuronides in the liver and colon (92, 93), is quickly degraded (94) and eliminated through feces(90, 91, 95). This made its clinical application more limited. To circumvent this drawback, several techniques were created, including nanoparticles and formulations in liposomal, micellar, or phospholipid complex. The discovery and synthesis of novel curcumin derivatives and analogues with improved medicinal qualities and bioavailability is one of the most popular tactics, and scientists in this field have made considerable efforts in this direction. Furthermore, a fascinating class of curcumin hybrids and the expected synergy based on their chemical structures are also investigated.

Curcumin, as mentioned before, is distinguished by a unique chemical structure and a variety of biological and pharmacological effects (83-86). The curcumin structure includes various active sites such as aryl ring, olefinic chain, the diketo, and the active methylene, these sites make up the favored scaffold of curcumin, as shown in Figure A72 (appendix A)To study the associated change in stability and bioactivity curcumin,

several curcumin derivatives and analogues have been developed to target each of the above-mentioned active sites. According to numerous articles, the diketo moiety is responsible for curcumin's instability and rapid in vivo metabolism, and it must be addressed by including scaffolds that can increase the compound's effectiveness. Reddy et al. hypothesized in a study of the structure-activity relationship of curcumin's diketone chain that adding hydrazine results in derivatives with more rigid structure, which enhances the anticancer activity (98). Furthermore, Jankun et al. postulated that cyclizing the diketo site of curcumin could lead to improved bioactivity of curcumin (99). Curcumin and derivatives have a wide range of therapeutic effects, ranging from chemo-preventive, anti-inflammatory, anti-metastatic and anti-proliferative. A novel set curcumin- based heterocyclics were screened for their antitumor activity against HeLa cells. The *in vitro* cytostatic and cytotoxic effect of the prepared hetero-cyclic was done using MTT reagent test. The in vitro anticancer performance of synthesized curcumin-based heterocycles derivatives against HeLa cancer cells was evaluated by the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphen-yltetrazolium bromide (MTT) assay. The study showed that the derivatives exhibited a promising anticancer activity against HeLa cancer cells at a nontoxic concentration (96).Herein a simple and convenient method for the synthesis of curcumin based azo compounds is presented. The azo curcumin-based compounds performance as dyes will also be evaluated. Some of the bioactivities of the azo compounds and complexes such as antimicrobial, genotoxicity and anticancer will also be evaluated.

1.6.3 Curcumin derivatives and analogues

Heterocyclic molecules like isoxazole and pyrazoles, which function as suitable scaffolding from natural products in medicinal chemistry have significantly improved the therapeutic potential of natural products (100).Based on previously ordered investigations, it is hypothesized that the diketone site is the perfect scaffold for the integration of the heterocyclic moiety. The most often produced heterocyclic curcuminoids that have been studied for a variety of actions include isoxazole, pyrazole, N-phenyl pyrazole, and N-amido-pyrazoles. As compared to curcumin, these alterations increase stability and bioavailability, increasing the possibility of retaining curcumin-like efficacy (101). Connecting by five carbon chains to create cucrcumin analogues can inhibit the growth of cancer through regulating and modulating NFKB (102, 103),

STAT3 (104, 105), AKT-PTEN (106), MAPK-ERK (94, 103), cell cycle arrest, and apoptosis (106). DAPs' therapeutic effects against cancer have been investigated in a variety of cancer cell lines, including colorectal (GO-Y035, GO-Y030, FLLL-11, FLLL-12, HO-3867, and EF24), breast (GO-Y035, GO-Y030, HO-3867, EF24, and EF31), lung (GO-Y035, HO-3867, and EF24), liver bile duct (GO-Y030), pancreatic (GO-Y035, GO-Y030, FLLL-11, FLLL-12, and EF31), prostate (HO-3867, EF24, and ca27)(107), thyroid gland (GO-Y035 and GO-Y030), stomach (GO-Y035) ovarian (HO-3867, EF24, and EF31) and cervical (EF24) cancers as summarized by Paulraj et al., 2019 (108). Figure A73 (appendix A).

Accumulating results demonstrated that,the DAP compounds shown in Figure A73 (appendix A), and specially compoundsthat contain 3-OCH_3 and 4-OH groups had stronger cytotoxicity compared to curcumin against the lung cancer-NCI-460, melanoma-UACC-62, ovarian cancer-OVCAR-3, renal cancer-786-0, prostate cancer-PC3 (107, 109, 110),LNCap and DU145 (111), breast cancer-MDA-MB231 (107, 110)and MCF-7 (107, 109), cervical cancer-HeLa and CaSki(110), nasopharyngeal cancer-CNE (112, 113), colon cancer HT-29 and leukemia-K-562 (109), HCT116 and SW480 (114). Studies employing DAPs on colon cancer SW480 with chromosomal instability (CIN), p53 mutations andKRAS, however, were restricted to the cytotoxicity effect and did not examine the effects on anti-proliferative or apoptotic processes.

The new curcumin mimic (1E,4E)-1,7-bis(4-hydroxyphenyl) (hepta-1,4-dien-3-one) ((MDA Figure A74(appendix A).) obtained from mistletoe was synthesized and the results of anti-cancer investigations were reported by Hong et al. It was initially examined for in vitro cytotoxicity, where it shown micromolar activity. Additionally, four human breast cancer cell lines exposed to MDA demonstrated greater potency than cis-platinum (SKBR3, MCF-7, MDA-MB231, and MDA-MB453). MDA considerably reduced the IC50 values for the breast cancer cell lines when compared to cis-platinum. Human LO2 liver cells, human GES-1 gastric epithelial cells, and human BEAS-2B lung epithelial cells were used to test the cytotoxicity of MDA on normal cells. The outcomes showed that MDA had a minimal inhibitory effect on normal cells, with each group having a lower than 5.0% rate of inhibition, that is significantly lower than the rate on cancer cells at same concentration. The results indicate that MDA compounds are selective in regards to the toxic effects on cancer cells but on normal cells (94).

In this study, several functionalized curcumin with azo and alkyl sulfate groups are synthesized by electrophilic aromatic substitution on phenol ring and by Biomolecular Nucleophilic substitution reactions (S_N2). The compounds will be synthesized via a simple and cost-effective one-pot method. The target compounds were evaluated for various bioactivities such as anticancer and antimicrobial in a addition for use as coloring compounds.

1.7 Scope of This Study

The primary objective of this research is to synthesize curcumin based azo and curcumin with alkyl sulfonate moieties and evaluate their bioactivities as anticancer and antimicrobial agents.

The specific objectives of this proposal include to:

1. Developing a method for adding the azo functionality to curcumin structure.
2. Using the developed method to synthesize various curcumin based azo compounds.
3. Evaluate the performance of the azo compound as dyes for coloring fabrics and others.
4. Determine the azo dye color change as a function of pH.
5. Characterize the new the curcumin based azo and sulfonateusing various spectroscopic and analytical techniques.
6. Study the bioactivities of the curcumin based azo and sulfonate (antimicrobial, genotoxicity and anticancer).

Considering all the research done in this regard, the ongoing research project issue has emerged. Therefore, this project aimed to investigate the antitumor effect of different Curcuminazo derived compounds which are; 1: 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis (diazene-2,1-diyl))dibenzenesulfonic acid , 2: (1E,4Z,6E)-1,7-bis(3-((E)-(4-chlorophenyl) diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one, 3: (1E,4Z,6E)-1,7-bis(3-((E)-(2,4-dichlorophenyl)-4-hydroxy-5-methoxyphenyl)- 5-hydroxyhepta-1,4,6-trien-3-one, 4:4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene)) bis (diazene-2,1-diyl)) dibenzoic acid, and5: (1E,4Z,6E)-1,7-bis(3-((E)-(4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one, and curcumin derived

sulfonate compounds6: 3,3'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(propane-1-sulfonic acid) and 7: 4,4'-(((1E,3Z,6E) -3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(butane-1-sulfonic acid on B16-F110 skin melanoma, HEPG2 liver carcinoma, MCF-7 breast carcinoma, Colon human cancer and HeLa cervical carcinoma cell lines in Palestine, as no previous studies were conducted.

Chapter Two

Experimental Part

2.1 Materials and procedures

The chemicals used in this study, including solvents and reagents were obtained from Aldrich Company, they were used as received. The chemicals include $NaNO_2$, NaOH, DMSO, Acetone, Ethyl Acetate, HCl, Curcumin, Ethanol,tetrahydrofuran, Sulfanilicacid, 4-Chloroaniline, 2,4-Dichloroaniline, 4-Aminobenzoic acid and N,N-Dimethyl-p-phenylenediamine.

All ^1H NMR experiments are measured in parts per milliondownfield from tetra methyl silanewhich was used as an internal standard. DMSO-d6 (2.5ppm) was used as a solvent in the NMR analysis which was performed on a S400 NMR spectrometer (Varian VXR). The IR spectra were recorded using spectrophotometer by Shimadzu (820 PC FT-IR).

2.2 Curcumin coupling with diazo compounds

2.2.1 General procedure

2.2.1.1 Curcumin solution

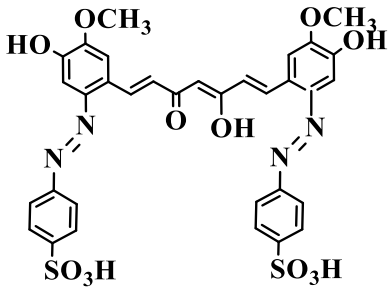
A 10% solution of sodium hydroxide in water was prepared in a 100-mL Erlenmeyer flask, by dissolving 3.0 g of sodium hydroxide in 27 ml water. A 5.4 ml of the NaOHsolution was added to another flask (150 ml), to it was added curcumin (0.368 g, 0.01mol), followed with a 10.0 ml of THF to increase the solubility of cucrcumin. The mixture was mixeduntil a clear solution was obtained, then the produced solution wascooled to -5 ^oC in an ice-salt water bath

2.2.1.2 Diazonium salt preparation

In a 100 mL beaker a solution of $NaNO_2$ (0.35 g, 0.005 mol) in water (2.5 mL) was prepared. In another beaker a solution of an aromatic aminewas prepared by dissolving a 0.002mol of the amine in$HCl_{(aq)}$(6.0 mL)diluted with 10 mLwater. The aminesolution was cooled in an ice-salt bath (-5 to -10 °C). While keeping the solution at -5 °C, it was treated with sodium nitrite solution by dropwise addition. The mixture was stirred to ensure the formation of diazonium salt in about 20 min.

2.2.1.3 Preparation of 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (5-hydroxy-4-methoxy-2,1-phenylene)) bis (diazene-2,1-diyl)) dibenzenesulfonic acid. (1)

The curcumin solution previously prepared was added dropwise to the sulfanilicaciddiazonium salt solution. In about 5 minutes after the addition was completed abrown solidstarted to appear. The reaction was kept at about-5^{o}C for 20 minutes,thenit was warmed slowly toroom temperature in about 30 minutes and kept at this temperature for another 60 minutes to ensure complete reaction. The reaction mixture was then treated with ice- cold water (50 mL) to precipitate the product. Theproduced brown precipitate was collected by vacuum filtration, washed with a small amount of waterand recrystallized from ethyl acetate/hexane mixture (2:3 ratio by volume). The precipitate was dried at 40 ^{o}C under vacuum. A 0.853g of brown solid was obtained (yield 81.31%). The melting point 138-140 °C Figure 2.1.IR: ν_{max} cm^{-1}1170(C-O alcohol),1513(C=C benzene),1692(C=O carbonyl),2974(O-H carboxylic acid),3385(O-H alcohol), Figure A1 (appendix A).^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.83(s, 6H, 2 -OCH3 methyl), 6.64(d, 1H, ethylene), 6.66(s, 1H, ethylene),6.68 (d, 1H, ethylene), 7.10(d, 1H, ethylene), 7.13(d, 4H, benzene), 7.21(d, 4H, benzene),7.60(d, 1H, ethylene),8.5(s, 2H, 2-OH, sulfonic acid),9.98 (s, H, enol) Figure A8 (appendix A).

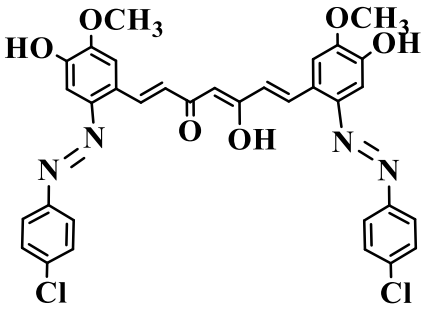


4,4'-((1*E*,1'*E*)-(((1*E*,3*Z*,6*E*)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(5-hydroxy-4-methoxy-2,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid

2.2.1.4Preparation of (1E,4Z,6E)-1,7- bis (2-((E)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (2)

The curcumin solutionpreviously prepared was added dropwise to the 4-chloroanilinediazonium salt solution. In about 5 minutes after the addition was completed a brown solid startedto appear. The reaction mixturewaskept at about-5 ^{o}C for 20 minutes,thenitwaswarmed slowly to room temperature in about 30 minutes and

kept at this temperature for another 60 minutes to ensure complete reaction. The reaction mixture was then treated with ice- cold water (50 mL) to ensure complete precipitation of the product. The orange precipitate was collected by suction filtration. washed with a small amount of cold water and recrystallized from ethyl acetate/hexane mixture (2:3 ratioby volume). The precipitate was dried at 40 ^{o}C under vacuum. The product weight was0.737g (Yield, 76.31%),the melting point 121-123^{o}C Figure 2.2.IRν_{max} cm^{-1}:833(C-Cl), 1244(C-N stretching),1169(C-O alcohol) 1585 (C=C, conjugated), 1732(C=O, carbonyl), 2225(C-N stretching)3326(O-H stretching)Figure A2 (appendix A). ^{1}H-NMR (400 MHz, DMSO-d6) δ:3.83(s, 6H, methyl)6.77(d, 1H, ethylene),6.78(s, 1H, ethylene),6.81(d, 1H, ethylene), 6.84(d, 1H, ethylene), 6.95(d, 1H, ethylene), 7.38(s, 2H, benzene), 7.41(s, 2H, benzene), 7.44(d, 4H, benzene), 7.49(d, 4H, benzene), 9.76(s, 2H, alcohol), 10.45(s, 1H, enol)Figure A9 (appendix A).

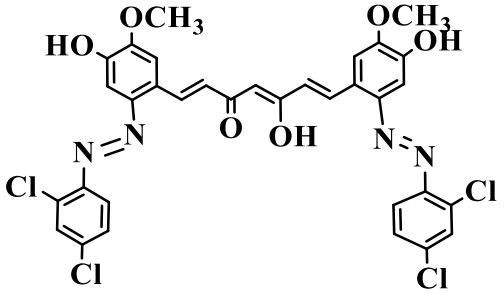


(1*E*,4*Z*,6*E*)-1,7-bis(2-((*E*)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl) -5-hydroxyhepta-1,4,6-trien-3-one

2.2.1.5Preparation of 1E,4Z,6E)-1,7-bis(2-((E)-(2,4-dichlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (3)

The curcumin solution prepared above was added dropwise to the 2,4-dichloroanilinediazonium salt solution. In about 5 minutes after the addition was completed a brown solidstarted to appear. The reaction mixture was kept at about-5 ^{o}C for 20 minutes,thenit was warmed slowly to room temperature in about 30 minutes and kept at this temperature for another 60 minutes to ensure complete reaction. The reaction mixture was then treated with ice- cold water (50 mL) to ensure complete precipitation of the product. The orange precipitate was collected by suction filtration. washed with a small amount of cold water and recrystallized from ethyl acetate/hexane mixture (2:3 ration by volume). The precipitate was dried at 40 ^{o}C under vacuum.The product weight 0.773g (Yield, 72.19%), the melting point 102-104 ^{o}C Figure 2.3.IR:

ν_{max} cm^{-1} 819(C-Cl),1127(C-Ostretching),1271(C-N stretching), 1512(C=Conjugated), 1732(C=O Carbonyl), 2372(C-N stretching), 3369(O-H stretching), Figure A3 (appendix A). ^{1}H-NMR (400 MHz, DMSO-d6) *δ:*3.83(s, 6H, methyl),6.78(d, 1H, ethylene), 6.79 (s, 1H, ethylene), 6.80(d, 1H, ethylene),6.81(d, 1H, ethylene), 6.82(s, 1H, benzene), 6.83(d, 1H, benzene), 6.84(d, 1H, benzene),7.42(d, 1H, benzene) 7.44(d, 1H, ethylene),6.78(s, 1H, benzene) 9.58(s, 2H, alcohol), 10.35(s, 1H, enol), Figure A10 (appendix A).

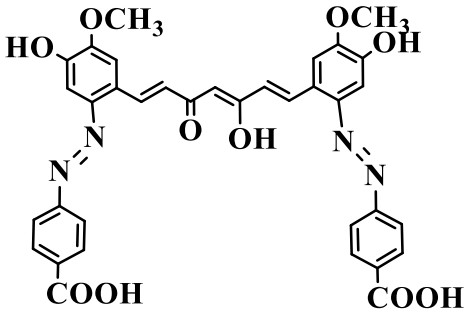


(1*E*,4*Z*,6*E*)-1,7-bis(2-((*E*)-(2,4-dichlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)
-5-hydroxyhepta-1,4,6-trien-3-one

2.2.1.6 Preparation of 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (5-hydroxy-4-methoxy-2,1-phenylene)) bis (diazene-2,1-diyl)) dibenzoic acid. (4)

The curcumin solution prepared above was added drop wise to the 4-aminobenzoic aciddiazonium salt solution. In about 5 minutes after the addition was completed a Brown solid started to appear. The reaction mixture was kept at about-5 ^{o}C for 20 minutes, then it was warmed slowly to room temperature in about 30 minutes and kept at this temperature for another 60 minutes to ensure complete reaction. The reaction mixture was then treated with ice- cold water (50 mL) to ensure complete precipitation of the product. The yellow precipitate was collected by suction filtration. washed with a small amount of cold water and recrystallized from ethyl acetate/hexane mixture (2:3 ration by volume). The precipitate was dried at 40 ^{o}C under vacuum.The productweightwas about 0.468g (yield 70.48%)ofyellowsolid. The melting point 132-134^{o}C Figure 2.4.IR: ν_{max} cm^{-1}1022(C-O alcohol), 1275(C-N), 1411 (C=C, conjugated),1656(C=C aromatic), 1713(C=O carbonyl) 2372 (C-N stretching), 2947 (=C-H), 3375(O-H stretching), Figure A4 (appendix A). ^{1}H-NMR (400 MHz, DMSO-

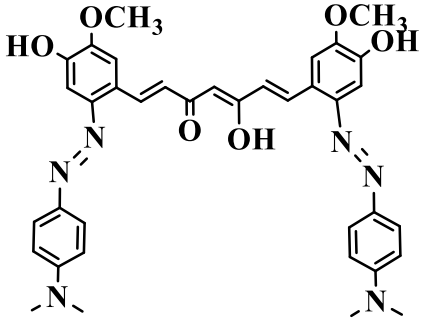
d6) *δ:* 3.83(s, 6H, methyl), 6.79(d, 1H, 1-ethylene), 6.81(s, 1H, 1-ethylene), 6.82(d, 1H, 1-ethylene), 7.03(d, 1H, 1-ethylene),7.11(s, 1H, benzene), 7.48(s, 1H, benzene),7.78(d, 1H, 1-ethylene), 8.46(s, 1H, alcohol),7.94(d, 4H, benzene), 7.95(d, 4H, benzene), 10.35(s, 1H, enol), 12.34(s, 1H carboxylic) Figure A11 (appendix A).



4,4'-((1*E*,1'*E*)-(((1*E*,3*Z*,6*E*)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(5-hydroxy-4-methoxy-2,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid

2.2.1.7Preparation of (1E,4Z,6E)-1,7-bis(2-((E)-(4-(dimethylamino) phenyl) diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (5)

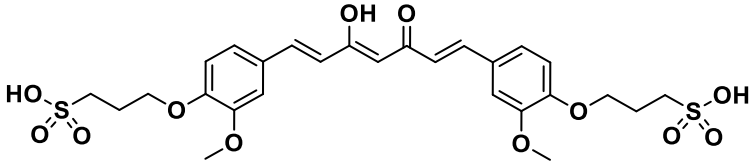
The curcumin solution prepared above was added dropwise to the N,N-dimethyl-p-phenylene diaminediazonium salt solution. In about 5 minutes after the addition was completed a brown solidstarted to appear. The reaction mixture was kept at about-5 ^{o}C for 20 minutes,thenit was warmed slowly to room temperature in about 30 minutes and kept at this temperature for another 60 minutes to ensure complete reaction. The reaction mixture was then treated with ice-cold water (50 mL) to ensure complete precipitation of the product. The brown precipitate was collected by suction filtration. washed with a small amount of cold water and recrystallized from ethyl acetate/hexane mixture (2:3 ratio by volume). The precipitate was dried at 40 ^{o}C under vacuum,0.269g (yield 81.26%).The melting point 217-219 ^{o}C Figure 2.5. IR: ν_{max} cm^{-1} 1270(N-C), 1519(=C-N), 1603(C=C, benzene), 1733(C=O, carbonyl), 2913& 2844(C-H, stretching aliphatic), 3350(O-H stretching)Figure A5 (appendix A). ^{1}H-NMR (400 MHz, DMSO-d6) δ :3.83(s, 6H, methyl), 6.74(d, 1H, ethylene), 6.80(s, 1H, ethylene), 6.83(d, 1H, ethylene), 7.05(d, 4H, benzene), 7.05(d, 1H, ethylene), 7.14(s, 2H, benzene), 7.15(s, 2H, benzene), 7.55(d, 4H, benzene), 7.57(d, 1H, ethylene), Figure A12 (appendix A).



(1*E*,4*Z*,6*E*)-1,7-bis(2-((*E*)-(4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-methoxyphenyl) -5-hydroxyhepta-1,4,6-trien-3-one

2.2.1.8 Preparation of 3,3'-((((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(propane-1-sulfonic acid). (6)

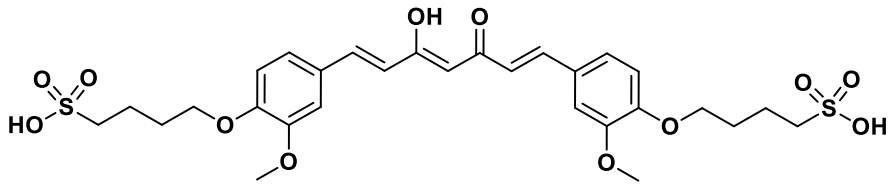
The reaction was carried out in a round-bottom flask fitted with a magnetic stirring bar and a refluxing condenser. A solution of curcumin (**1**, 2.72 mmoles, 1.0 g) and NaOH (powder 2.72 mmol) was prepared in ethanol (30.0 mL). The reaction mixture was stirred for 10 minutes to ensure complete formation of curcumin phenoxide, then 1,3-propanesultone (0.66 g, 5.4 mmol) was added. The reaction flask was kept under reflux for 2.0 hr. The produced solid was collected by suction filtration, rinsed with 5% HOAc solution in methanol (2 x 30 mL) to remove residual sodium hydroxide and the third time with methanol (50 mL). The produced brown solid was recrystallized from a mixture of water-ethanol (1:1 ratio by volume). The product mass 1.23 g (71.92% yield). The melting point 237-240 ^{o}C Figure 2.6. IR: ν_{max} cm^{-1} 1049(C-O stretching) 1135(S-O stretching), 1190(O=S=O stretching), 1512(C=C alkene), 1583(C=C, alkene), 1629(C=C, aromatic), 1732(C=O carbonyl), 3427(O-H stretching), FigureA6 (appendix A). ^{1}H-NMR (400 MHz, DMSO-d6) δ :2.17 (t, 4H), 3.01 (m, 4H), 3.85 (s, 6H, OCH_3), 4.13 (t, 3H, O-CH_2), 6.72 (s, 1H), 6.82 (d, 1H), 6.83 (d, 1H, ethylene),7.05 (m, 5H),7.8 (d, 1H, benzylic vinylic),8.91 (bs, 1H, S-OH), 10.66 (bs, 1H) Figure A13 (appendix A).



3,3'-((((1*E*,3*Z*,6*E*)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(propane-1-sulfonic acid)

2.2.1.9 Preparation of 4,4'-((((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(butane-1-sulfonic acid). (7)

The reaction was carried out in a round-bottom flask fitted with a magnetic stirring bar and a refluxing condenser. A solution of curcumin (2.72 mmoles, 1.0 g) and NaOH (powder 2.72 mmol) was prepared in ethanol (30.0 mL). The reaction mixture was stirred for 10 minutes to ensure complete formation of curcumin phenoxide, then 1,4-butanesultone (5.4 mmol, 0.73 g). The reaction mixture was kept under reflux for 2.0 hr. The produced solid was collected by suction filtration, rinsed with 5% HOAc solution in methanol (2 x 30 mL) to remove residual sodium hydroxide and the third wash was with methanol (50 mL). The produced brown solid was recrystallized from a mixture of water-ethanol (1:1 ratio by volume).Thesolid product was 1.17g with brown color (yield 73.12%).The melting point 275-276^{o}C Figure 2.7. IR: ν_{max} cm^{-1} 1049 (C-O stretching), 1135 (S-O stretching),1190 (O=S=O stretching),1512(C=C alkene), 1583(C=C, alkene),1629(C=C aromatic),1732(C=O carbonyl),3427 (O-H stretching)Figure A7 (appendix A). ^{1}H-NMR (400 MHz, DMSO-d6)*δ:* 1.49 (m, 4H).1.75 (m, 4H), 3.01 (d, 4H),3.85 (s, 6H, OCH_3), 4.06 (t, 3H, O-CH_2), 675 (s, 1H), 6.81 (dd, 1H)), 6.85 (d, 1H, ethylene), 7.03(m, 5H), 7.82 (d, 1H, benzylic vinylic), 8.5 (bs, 1H, S-OH), 10.68 (bs, 1H) Figure A14 (appendix A).



4,4'-((((1*E*,3*Z*,6*E*)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(butane-1-sulfonic acid)

2.3 Antibacterial activities

2.3.1 Materials and methods

2.3.2 Nutrient broth

Nutrient broth (NB) obtained from AcuMedia, USA used in this study was prepared following the instructions given by the manufacturer on the bottle's label. A mass of 4 g of nutrient broth was completely dissolved with 496 mL of distilled water, then each 5-10 mL of dissolved nutrient broth was placed in a test tube. After that, the test tubes

having nutrient broth medium were autoclaved for 15 min at a temperature 121 ºC. The test tubes were then stored in the refrigerator at 4-6 ºC after they cooled.

2.3.3 Nutrient Agar

Nutrient agar (NA) obtained from AcuMedia, USA used in this study was prepared according to information provided on the bottle label. A mass of 11.5 g of NA was mixed thoroughly with a volume of deionized water up to 0.5 L. Then, the agar was dissolved completely by heating. Then agar medium was autoclaved at a temperature 121°C for 15 minutes. The agar temperature was then allowed to reach 50°C-55 °C. After that, 25-30 mL of melted agar was distributed into each sterile Petri dish and left at room temperature to solidify. Then, the dishes were stored at 4°C in the refrigeratoruntil used for bacterial growth culture.

2.3.4 Saline solution (NaCl 0.9%)

A saline solution (0.9% NaCl, MW 58.44) was prepared by dissolving 2.25 grams of sodium chloride were added to a volume of 250 mL of deionized water. The saline solution was then distributed into test tubes each had a volume of 5-10 mL.Then, the test tubes were sealed with a mass of cotton. The test tubes were place in a n autoclave for 15 minutes at a temperature121°C, the test tubes were allowed to cool to room temperature, then stored in refrigerator (4.0 °C-6.0 °C).

2.3.5 Dimethyl sulfoxide (10% DMSO)

The 10% DMSO solution (Aldrich, MW 78.14) was prepared by mixing 10 mL of stock DMSO solution and 90 ml of double distilled water in a 0.25 L bottle. The 10% DMSO solution after being autoclaved at temperature 121 °C for 15 minutes, the solution was cooled to room temperature and stored on a shelf.

2.3.6 McFarland standard solution with 0.5 turbidity

A volume of 0.50 mL of barium chloride dihydrate (BaCl2.2H2O) solution with a concentration of 1.175% (w/v) was added to a volume of 9.95 mL of sulfuric acid with a concentration of 1% (v/v) sulfuric acid in order to reach the McFarland standard of 0.5 turbidity. The 0.5 McFarland standard tube was kept at room temperature and parafilm-sealed to prevent evaporation. Before usage, the 0.5 McFarland standard was well

vortexed. A 0.5 McFarland Standard turbidity is equivalent to a bacterial suspension with 1.5 X 10^8 colony-forming units (CFU)/ml (77).

2.3.7 Microorganisms

In the current study, four different kinds of bacterial strains were used. Two of these strains are Gram-positive bacteria including *Staphylococcus aureus* ATCC6538P (*S. aureus* ATCC6538P) and Methicillin-resistant *S. aureus* (MRSA, clinical isolate), while the other two strains are Gram-negative bacteria in cluding (*K.pneumoniae* ATCC 13883), *Klebsiellapneumoniae* ATCC 13883 and *Escherichia coli* ATCC 25922 (*E. coli* ATCC 25922).

2.3.8 Minimum inhibitory concentration

The Minimum inhibitory concentration**(**MIC) of curcumin-based azo dye compounds was evaluated using a method of two-fold-serial dilution by sterile 96-microtiter plate assay, according to the instruction published previously by Clinical and Laboratory Standard Institute (CLSI) (77). The curcumin-based azo dye compounds (400 µg/ml of 10% DMSO) were diluted two-fold serially in nutrient broth to a final volume of 100 µL in the wells of the microtiter plates. Following that, each well in microtiterplate received a bacterial inoculum of about 1.0 x 10^5 CFU/ml.Different negative control in these assays were also involved such as wells contained 100 µL of NB alone, 100 µL of 10% two-fold-serially diluted DMSO with a bacterial inoculum, or 100 µL of curcumin-based azo and nutrient broth without bacterial inoculum. Each experiment was conducted twice on each azo compound based on curcumin. Then, the microtiterplates were covered and incubated in incubator at 37°C for 18-24 hours.

2.4 Genotoxic Potential test for curcumin-based azo dyes on *E.coli*ATCC 25922strain

2.4.1 Inoculation of *E. Coli*

Under sterile conditions, a few colonies obtained from a 24-hour old *E. coli* ATCC 25922 strain growth culture inoculated on NA medium were sub-cultured into a flask having 15-mL of NB. Then the bacterial growth culture in the flask incubated at 37°C for 1 hour with a constant shaking. Following that, 1 mL of *E. coli* broth culture from the flask was transferred aseptically to different four sterile containers having 24

mL sterile nutrient broth medium. These containers were left in incubator at a temperature of 37°C for 1 hour with regular shaking. The compound 6 which was prepared in 10% DMSO was added to these containers in different concentrations. The final concentration was 100 µg/mL in the first container, 50 µg/mL in the second container, and 25 µg/mL in the third container. However, in the fourth container which is considered as a negative control or untreated one, the final concentration of compound 6 was 0.0 µg/mL.

2.4.2 Genomic DNA Extraction

The entire genome of *E. coli* was prepared for ERIC-PCR analysis by boiling and chillingmethod as described previously (79). Samples from *E. coli* culture broth were obtained after 2 hours, 5 hours and 24 hours and were centrifuged at 11,500 X g for 5 minutes. The bacterial sediment was washed two times in 1 mL of 1X Tris-EDTA buffer (10 mMTris-HCl, 1 mM EDTA [pH 8.0]), and then, the supernatant liquid was removed and the bacterial sediment re-suspended in 0.5 mL of sterile distilled H_2O, boiled for 10-15 minutes. Immediately,the cells were then placed on ice for 10 minutes. The debris was deposited by centrifuging at 11,500 X g for 5 minutes, and the supernatant containing the newly extracted DNA was transferred to a new Eppendorf tube.After determining the concentration of genomic DNA for each sample using a nanodrop spectrophotometer (GenovaNano, Jenway), the genomic DNA samples were kept at -20ºC for ERIC-PCR analysis.

2.4.3 Enterobacterial repetitive intergenic consensus (ERIC) PCR analysis

The primer ERIC1: 5'-ATG TAA GCT CCT GGG GAT TCA C-3' and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3' were used in this study to perform the ERIC-PCR (Ventura et al., 2003). Each 25 µl PCR reaction mix contained the following substances: 10 mM PCR buffer pH 8.3, 3 mM MgCl2, 0.4 mM of each dNTPs, 0.8 mM primer, 1.5 U of Taq DNA polymerase, 5% DMSO, and a specific amount of DNA template (about 30 µg). The thermal cycler (Mastercycler personal, Eppendorf, Germany) was then used for the amplification of DNA at the following thermal conditions: the initial denaturation at 94^{o}C for 180 s, followed by 35 PCR cycles of denaturation at 94^{o}C for 50 seconds, annealing at 50^{o}C for 60 s and extension at 72^{o}C for 180 s, followed by a final extension step at 72ºC for 300 s.The bands of PCR

products were then separated byagarose gel electrophoresis technique using a agarose gel concentration 1.5% (w/v). Changes in the banding pattern profile after amplified DNA obtained from an *E. coli* strain treated with different concentrations of compound 6 were taken into consideration. The alterations in the banding pattern was used to determine the genotoxicitypotential effect. These changes in banding pattern included: the intensity of bands as well as band loss or gain (115).

2.5 Anticancer

2.5.1 Materials and Methods

2.5.2 Different compounds concentrations preparation

To create a stock solution with a final concentration of 2 mg/mL in 2 mL Eppendorf tubes, 40 mg of each curcumin derived compound type (1,2,3,4,5,6 and 7) were dissolved in up to 2 mL freshly generated 1% fresh DMSO in RPMI media and 1% fresh DMSO in RPMI assay media, respectively. The stock solutions were then passed through a 0.25 µm membrane filter for sterilization. By performing two-fold dilutions on those generated working solutions, various concentrations of the final treatment concentrations used in the study—1000, 500, 250, 125, 62.5, and 31.25 µg/mL—were achieved. At the Sterilizer Biosafety cabinet, all of those preparations were completed aseptically.

2.5.3 Cell line and culture medium

The cell lines under investigation were from the ATCC (American Type Culture Collection) and included the B16-F10 skin melanoma, HEPG2 liver carcinoma, MCF-7 breast carcinoma, Colon human cancer, and HeLa cervical carcinoma. The cells were grown in a T25 cell culture flask using liquid Roswell Park Memorial Institute (RPMI 1640) medium. 10% heat-inactivated fetal bovine serum (FBS), 1% v/v penicillin-streptomycin (antibacterial impact), 1% v/v amphotericin (antifungal effect), and 1% v/v L-glutamine (amino acid as energy source) were freshly added to this media. Cells were cultured at 37°C, 95% humidity, and 5% CO_2 in a CO_2incubator (cell culture incubator). The cultivated cells were routinely examined with an inverted microscope to check for contamination and to see if they were attached to the media substrate in the culture flask. Fresh culture medium was changed every three days until 90% cell confluence was obtained.

2.5.4 MTT assay

The MTT reagent (MTT solvent) is reduced by NAD(P)H-dependent oxido-reductase enzymes in the viable cells to an intensely purple-colored insoluble crystalline formazan in this colometric and viability assay. Then, formazan crystals are dissolved in a solubilizing solution (MTT solution), and absorbance is assessed using a plate-reader (ELISA reader) at 500–600 nanometers. The higher the formazan content, which in turn suggests a higher cell viability as shown by more metabolic activity, the higher the observed absorbance.

2.5.5 Method for the MTT assay

As previously noted, cells were separated and counted before being implanted onto 96-well microtiter plates at a density of 20,000 cells per 100 µL of total volume per well (cytotoxic test). The density in the (cytostatic test) was 5,000 cells per 100 µL of total volume per well. In order to achieve final concentrations in each well equal to 31.25-1000 µg/mL by two-fold serial dilution directly in the 96 well microtiter plate, cells were treated with 10 µL of the studied stock chemical compounds solution (1,2,3,4,5,6&7) under research equal to 20 mg/mL. As a standard control, cells exclusively grown in RPMI medium were employed. As a negative control, only the wells with 1% fresh DMSO in RPMI medium were employed. Duplicate treatments of each type were applied in every well. The cultivated plates were then incubated at 5% CO_2, 37°C, for 24 hours for the cytotoxic test, and for 72 hours for the cytostatic test, in a CO_2 incubator. The requisite incubation time (24 or 72 hours) was followed by the removal of the media from each well and a PBS wash. The study's cultivated cells were then re-cultured in 100 µL of serum-free RPMI media with 10 µL of MTT solution (0.5 mg/mL) added to each well, which was then incubated for four hours at a CO_2 incubator. Following the removal of the media and subsequent washing, cells were incubated with 100 µL of acidic isopropanol (0.08N HCL) for 15 minutes in order to dissolve the formazan crystals. Using an ELISA reader, the absorbance of MTT formazan was measured at 570 nm. The percentage of absorbance of treated cells to absorbance of untreated control cells was used to calculate the cell viability.

2.5.6 Statistical analysis

The obtained cytostatic MTT (cell viability data) were analyzed to determine the concentration giving maximal 50% inhibition (IC50) by nonlinear regression calculations with the use of Microsoft Excel. The dose–response curve was obtained by plotting the inhibition percentage (%) versus concentration (µg/mL).

2.5.7 MTT assay procedure

As previously noted, cells were separated and counted before being implanted onto 96-well microtiter plates at a density of 20,000 cells per 100 µL of total volume per well (cytotoxic test). The density in the (cytostatic test) was 5,000 cells per 100 µL of total volume per well. In order to achieve final concentrations in each well equal to 31.25-1000 g/ml by two-fold serial dilution directly in the 96 well microtiter plate, cells were treated with 10 µL of the studied stock chemical compounds solution (1,2,3,4,5,6&7) under research equal to 20 mg/ml. As a standard control, cells were grown in RPMI medium exclusively. As a negative control, wells with 1% fresh DMSO in RPMI media only were employed. Duplicate treatments of each type were applied in every well. After that, culture plates were kept in a 5% CO_2 incubator at 37°C.

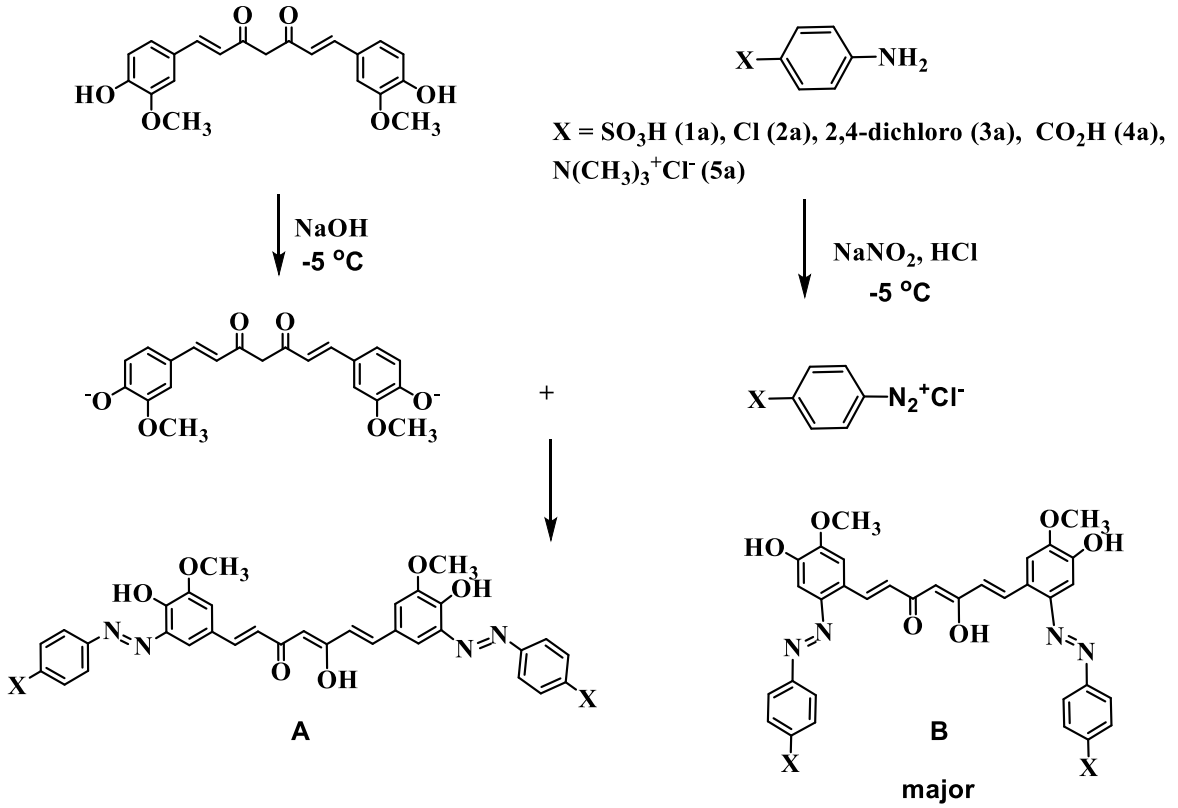
Chapter Three

Results and Discussion

The work presented in this thesis involved the synthesis of two types of curcumin derivatives. The first type contains an azo functionality, and the second type containsalkyl sulfonatemoiety. Both were selected for application in water soluble dye and expected to have various bioactivities including antimicrobial and anticancer. The first step of compounds preparation included reacting curcumin with diazonium salts containing various functional groups as shown in Figure 3.1. The diazonium salts were prepared by reacting various aromatic amino compounds with sodium nitrite and hydrochloric acid at -5^{o}C.The functional groups (X = $CO_2^-Na^+$, $SO_3^-Na^+$, H, Cl, $N(CH_3)_3^+Cl^-$) to impart water solubility and conjugation to the curcumin derivatives. Some of these functional groups also expected to enhance the antimicrobial properties of the target azo compounds. As shown in Figure 3.1 two products are possible A and B. Compound B is expected to be the major. Steric factor and electronic factor propose that compound B could be the major. Since in compound A, the azo group is close to the phenolic hydroxy group. In the second compound (B), the azo groups isp- to the methoxy and o- to the alkyl group. For this reason, the o-position is expected to be more reactive than the m-position. Due to structure similarity and polarity, it was not possible to separate them by column chromatography.

Figure 3.1

A summary of the method used for making the curcumin diazo compounds



3.1 Curcumin-based azo compounds

3.1.1 Synthesis

The prepared azo compoundsare expected to have good bioactivity as antimicrobial and or anticancer agent. Since they all composed of multisite for H-bonding. Most of the azo compoundsare water soluble and forms various colored solution in water (since they are highly conjugated, as shown in Figure 3.1.

Diazo compound 1 was prepared from reacting sulfanilic acid (1a) with curcumin. The reaction was performed by first converting 1a to diazonium salt, followed by coupling with curcumin. The yield was about 81.31%. Analysis by proton NMR and FT-IR showed that compound 1 was the main product.

Diazo compound 2 was prepared from reacting 4-chloroaniline (2a) with curcumin. The reaction was performed by first converting compound (2a) to diazonium salt, followed

by coupling with curcumin. The yield was about 76.31%. Analysis by proton NMR and FT-IR showed that compound 2 was the main product.

Diazo compound 3 was prepared from reacting 2,4-dichloroaniline (3a) with curcumin (2). The reaction was performed by first converting compound (3a) to diazonium salt, followed by coupling with curcumin. The yield was about 72.19%. Analysis by proton NMR and FT-IR showed that compound 3 was the product.

Diazo compound 4 was prepared from reacting 4- aminobenzoic acid (4a) with curcumin. The reaction was performed by first converting compound (4a) to diazonium salt, followed by coupling with curcumin. The yield was about70.48%). Analysis by proton NMR and FT-IR showed that compound 4 was the main product.

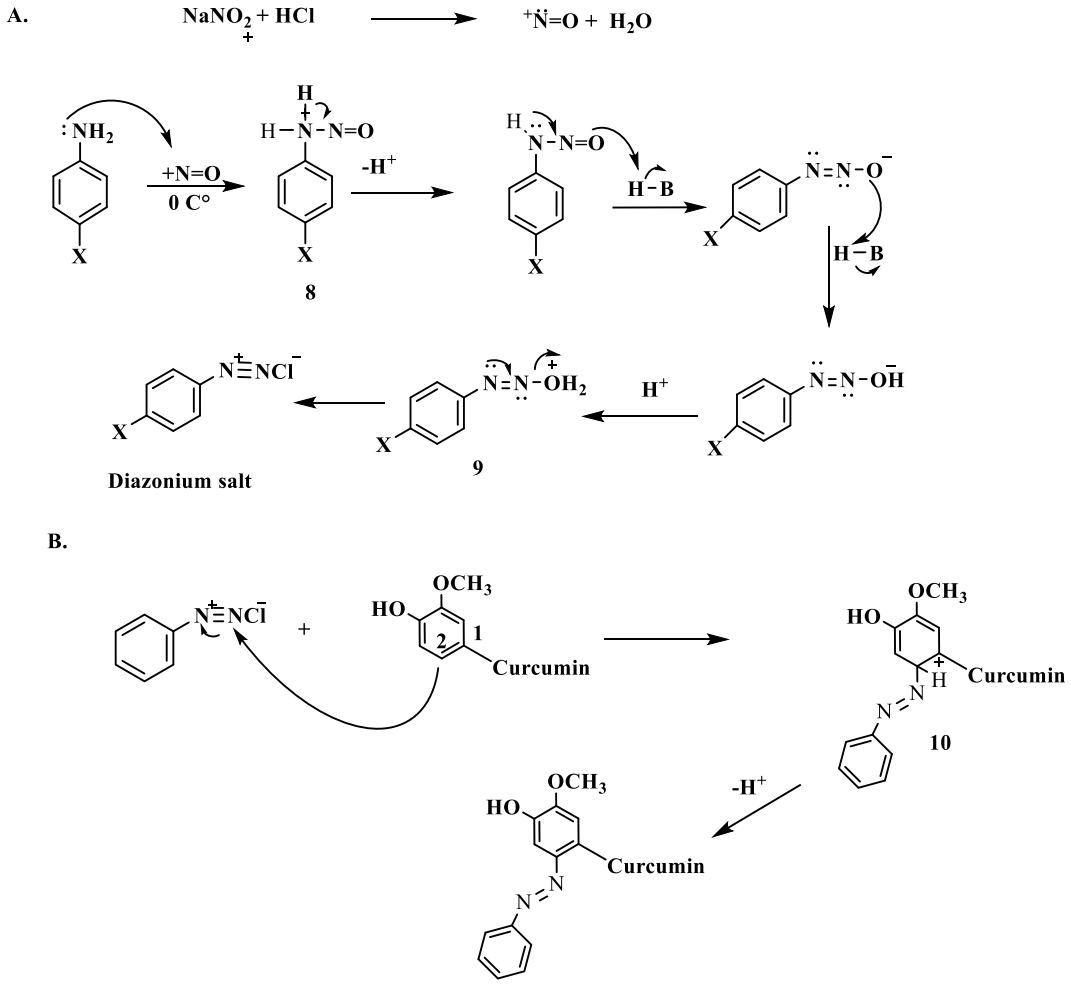
Diazo compound 5was prepared from reacting-Dimethyl-*p*-phenylenediamine (5a) with curcumin. The reaction was performed by first converting compound (5a) to diazonium salt, flowered by coupling with curcumin. The yield was about 81.26%. Analysis by proton NMR and FT-IR showed that compound 5 was the main product.

3.1.2 Reaction mechanism

The mechanism composed of two parts. In part A the diazonium salt is formed by formation of (Nitrosonium ion) which attached by the amine group to form intermediate 8. Intermediate 8 then goes through multiple protonation-deprotonation steps and finally losses of water molecules to the diazonium slat 9 (Figure 3.3).In part B, the diazonium salt 9 undergoes nucleophilic aromatic substitution reaction with curcumin at C-2 to form the intermediate 10 (σ-complex) followed by proton loss to form the target product.

Figure 3.2

The mechanism of the first set with curcumin.

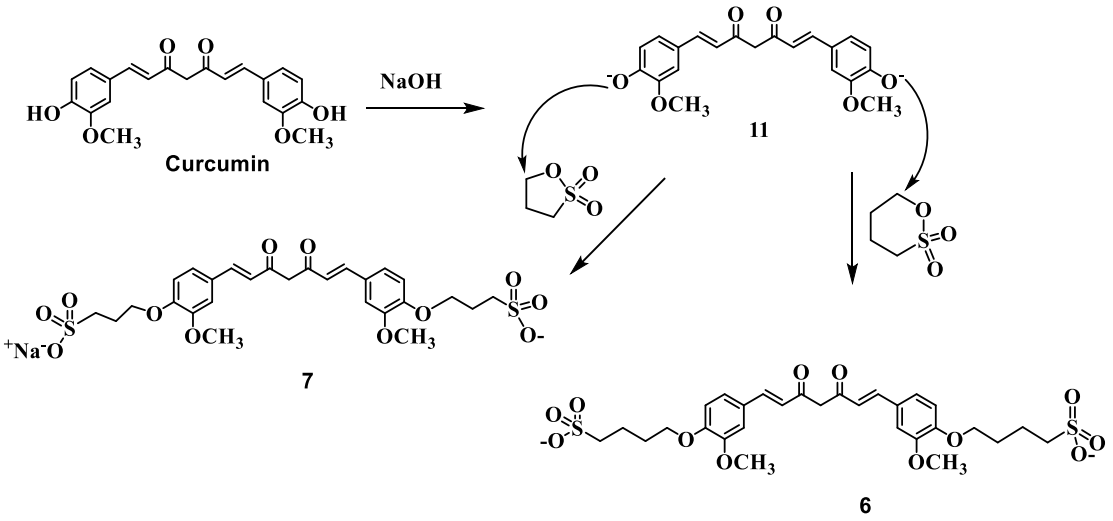


3.2 Curcumin with alkyl sulfonate moiety

The preparation of the curcumin sulfonate compounds involved reacting curcumin with 1,4-butane sultone and 1,3-propane sultone as shown in Figure 3.4. The figure shows the structure of the target products and the reaction mechanism. The first step involves formation of phenoxide11 by loss of the phenolic proton to the NaOH. Intermediate 11 then undergoes S_N2 reaction with sultone to produce the target product in excellent yield. The structures of the products were confirmed by NMR and IR spectroscopy. Both compounds 6 and 7 showed excellent water solubility due to the presence of the sulfonate ionic group.

Figure 3.3

The mechanism of the second set with curcumin

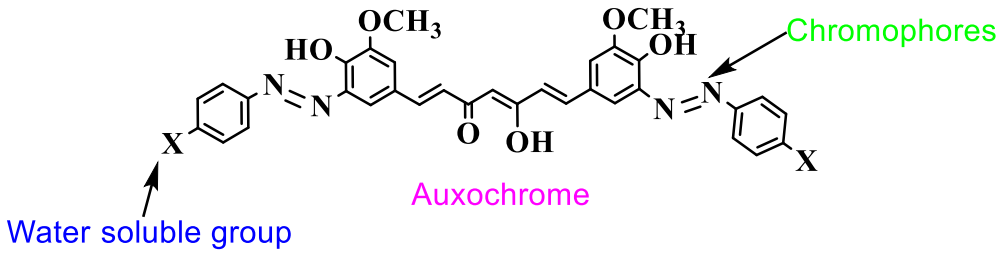


3.3 Performance of the curcumin based azo compounds as dyes.

Prepared diazo compounds have all functionalities required for making them perform as dye. A schematic diagram of the prepared diazo compounds showing functionalities that make them perform as dye is shown in Figure 3.2. The structures show the chromophores (N=N), the water-soluble group (X = carboxyl, sulfonyl and ammonium) and Auxochrome (the conjugated backbone chain)

Figure 3.4

A schematic diagram of the prepared diazo compounds



Solutions of all compounds with a concentration of 0.1% by weight were prepared in in water. The solution colors as a function of pH were studied. Results are summarized in Table 3.1. The change in color could be attributed to the protonation and deprotonation as the pH value changed.

Table 3.1

The color and pH -function of prepared compounds

Compound	1	2	3	4	5	6	7
pH	4.33	-	-	7.17	6.05	7.35	7.32
Color	Orange	Brown	Brown	Yellow	Yellow	Yellow	Red

3.4 Antimicrobial efficacy

As mentioned in the introduction azo compounds showed various bioactivities such as antimicrobial, antivirus and anticancer against certain tumor. The prepared curcumin azo compounds antibacterial activity was evaluated against four different types of bacteria *MRSA*and*Staphylococcus aureus* (Gram-positive), *Escherichia coli and Klebsiella*(Gram-negative).The tested compounds showed various effect ranging from excellent to acceptable.

The MIC value was determined forthe synthesized azo compounds against the selected types of bacteria mentioned above. Results are summarized in Tables 3.2. According to data presented in Table 3.2, all of the evaluated curcumin-based azo compounds showed some degree of inhibition against the selected bacteria strains. In general, the rate of inhibition against *S. aureus*and *E. coli* was higher than that against *Klebsiella* and MRSA. The MIC values of compounds 5, 6, and 7 were in a range of (100 to 800 μg/mL), meanwhile that for compounds 1, 2, 3, and 4was about800 μg/mL against *Klebsiella and E. coli*. The highest obtained antibacterial activity was by compound 6 as it showed MIC of (100 μg/ mL) against S. aureus and E. coli.

Table 3.2

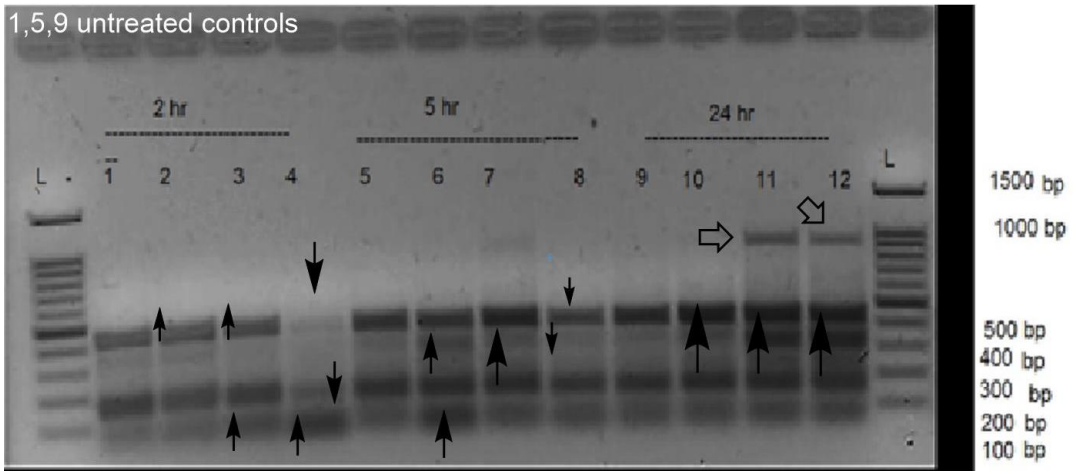
MIC values (μg/ml) of curcuminazo compounds against the four selected bacteria

Curcumin azo	Bacteria Strand			
	S. aureus ATCC 6538P	MRSA (clinical isolate)	*Klebsiella*	*E. coli* ATCC 25922
1	-	-	>400	>400
2	-	-	>400	>400
3	-	-	>400	>400
4	-	-	>400	>400
5	200	>200	>200	>200
6	200	>200	>200	100
7	200	200	>200	>200

The genotoxicity blocking test was conducted on azo compound **6** since it showed the highest antimicrobial efficacy**.** This was carried outusing the profile ERIC-PCR for the DNA extracted from *E. coli*bacterial *strain*. The Genotoxicity results on untreated, and treated *E. Coli* with azo compound 6 at various time intervals are presented in Figure 3.5 The obtained ERIC-PCR profile showed some changes in band intensity in which there is increasing and decreasing in the intensity of bands also appearance of new bands. According to the obtained results, compound 6 interacted with the DNA of the *E. coli.* and it is considered a Genotoxic agent.

Figure 3.5

ERIC-PCR profile of E. coliATCC 25922 strainuntreated and treated with various concentrations of compound 6 at different periods time. Lanes L are 100-bp DNA ladder. Lanes 1,5,9 are negative control treated only with DMSO, lanes (2, 6, 10), (3,7,11) and (4, 8,12) are for ERIC-PCR ofE. coli ATCC 25922 treated with azo 6 at a 100.0µg/ml, 50.0µg/ml and 25.0µg/ml, respectively



3.5 MTT Assay Anticancer Results

MCF-7 breast carcinoma, HEPG2 liver carcinoma, B16-F110 skin melanoma, Colon human cancer and HeLa cervical carcinoma cell lines viability % after 24 hours and 72 hours under curcumin and curcumin modified derived compounds 1: 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (6-hydroxy-5-methoxy-3,1-phenylene)) bis(diazene-2,1-diyl))dibenzenesulfonic acid , 2: (1E,4Z,6E)-1,7-bis (3-((E)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5- hydroxyhepta-1,4,6-trien-3-one, 3: (1E,4Z,6E)- 1,7-bis(3- ((E)- (2,4-dichlorophenyl)-4-hydroxy-5-methoxyphenyl)- 5-hydroxyhepta-1,4,6-trien-3-one, 4:4,4'-((1E,1'E)-(((1E,3Z,6E)-3-

hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (6-hydroxy-5-methoxy-3,1-phenylene)) bis (diazene-2,1-diyl)) dibenzoic acidand 5: (1E,4Z,6E)-1,7-bis(3-((E)- (4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one.have cytotoxic and cytostatic effect is investigated via MTT assay revealing variable results in cell viability percent related to cancer cell line type, compound type and used concentration.

The examined curcumin derived compounds (1, 2, 3, 4 and 5) showed varying cytotoxic and cytostatic effect in comparison with curcumin on MCF-7 breast carcinoma cell line at different concentrations (31.25- 1000 µg/mL) Table 3.3 (appendix B). The cytotoxic and cytostatic viability % of MCF-7 cell line is in the range of 51% at 1000 µg/mL of compound 5 -87% at 31.25 µg/mL of compound 4 Figure A15 (appendix A). and 56% at 1000 µg/mL of compounds 3and 4Figure A16 (appendix A), respectively. The strongest anticancer effect on this cell line among the examined compounds is for compounds 1 and 4, recording pronounced cytostatic effect (83-56%, 86-56% cell viability, respectively) Figure A16 (appendix A). Rather than cytotoxic effect one (85-68%, 87-71% cell viability, respectively Figure A15 (appendix A), in concentration dependent manner. While compounds 2 and 3 showed fluctuating cytotoxic effect rather than cytostatic effect. Also compound 5 has had cytotoxic effect rather than cytostatic effect in comparison to curcumin in a concentration dependent manner. Nevertheless, all examined compounds have had lower cytotoxic effect on MCF-7 cell line Figure A15 (appendix A). As well as better cytostatic effect Figure A16(appendix A), in comparison to the pure curcumin compound. So, in general cell growth of MCF-7 breast carcinoma cell line was decreased as the concentration of the prepared curcuminbasedazocompounds increased as shown in Figures A15 and A16 (appendix A).

Table 3.3

MTT cytotoxic (24 hrs) and cytostatic (72 hrs) MCF-7 cell line viability % effects under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

Cytotoxic and Cytostatic effects MCF-7 Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
1	85	83	83	83	73	69	70	69	68	63	68	56
2	86	90	78	86	73	86	71	83	71	76	70	69
3	86	86	78	83	73	74	71	69	71	63	70	56
4	87	86	80	84	75	71	73	69	73	58	71	56
5	83	88	83	85	69	83	64	82	59	76	51	69
Curcumin	100	100	92	89	88	86	85	80	73	66	67	46

The cell viability percent of HEPG2 liver carcinoma was reduced under the effect of pure curcumin as well as its modified derived examined compounds (1, 2, 3, 4 and 5) showed varying cytotoxic and cytostatic effect at different concentrations (31.25- 1000 µg/mL) Table 3.4 (appendix B). The cytotoxic and cytostatic cell viability % of HEPG2 liver carcinomaisin the range of 98-44% Figure A17 (appendix A). and 103-25% Figure A17 (appendix A), respectively compared to curcumin 98-45% and 99-24%, respectively. Compounds 1, 2 and 4 possess cytotoxic effect rather than cytostatic one in contrast to the cytostatic effect of the curcumin. While, compounds 3 and 5 showed cytostatic effect rather than cytotoxic effect as curcumin showed on HEPG2 cell line. Nevertheless, compound 5 has had more cytostatic effect by recording lower cell viability (65-25%) than curcumin (99-24%) at almost all examined concentrations in a concentration dependent manner Figure A18 (appendix A).

Table 3.4

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) HEPG2 Cell Line Viability % effects under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

Cytotoxic and Cytostatic effects HEPG2 Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
1	98	98	96	103	91	100	89	93	84	93	67	92
2	91	100	89	98	78	98	67	97	67	95	44	67
3	98	90	96	88	89	73	71	67	78	100	71	50
4	91	100	89	101	76	102	73	97	67	83	44	50
5	93	65	91	58	87	48	82	41	76	41	64	25
Curcumin	98	99	95	89	93	79	78	75	75	73	45	24

In addition, under the examined concentrations (31.25-1000 µg/mL) of curcumin derived compounds (1, 2, 3, 4 and 5), the cell viability % of B16-F 10 skin melanoma was reduced within the range 94%-46% for their cytotoxic effect. While, their cytostatic effect showed the reduction of cell viability in the range of 96-28% Table 3.5 (appendix B). Among the examined compounds, compound 5 cytostatic effect on B16-F 10 cell line has had the strongest anticancer effect by reducing cell viability down to 28% which is even lower than achieved by curcumin (42%) at 1000 µg/mL.Compounds1 and 2(62.5-1000 µg/mL) (cell viability 85%-46%) and (70%-44%), respectively is similar to curcumin but to a lower degree Figures A20 (appendix A). In contrast, cytotoxic effect rather than cytostatic effect on the examined cancer cell line was observed by compounds 3 and 4differing in that from curcumin Table 3.5(appendix B). In general, all curcumin derived compounds have higher cytotoxicity except at high concentrations (1000 µg/mL) only compound 5 has the highest Figure A19(appendix A). As well as, their cytostatic effect was less than curcumin, except compound 5 has had better one at 1000 µg/mL Figure A20 (appendix A).

Table 3.5

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) B16-F10 Cell Line Viability % effects under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

Cytotoxic and Cytostatic effects B16-F 10 Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
1	94	96	93	85	92	82	88	74	71	65	65	46
2	93	94	91	70	82	69	71	70	69	57	59	44
3	76	82	79	80	69	78	65	70	64	66	62	66
4	69	83	76	82	71	79	67	76	61	64	53	56
5	92	85	82	83	72	69	60	51	48	49	46	28
Curcumin	100	93	95	67	90	62	85	48	85	45	50	42

On the other hand, the cytotoxic and cytostatic effect assay of compounds 1, 2, 3, 4 and 5 on colon carcinoma cell line revealed a pronounced cytostatic effect (89%-29% cell viability) by compounds 2,3 and 5 rather than cytotoxic one (100%-54% cell viability) under the examined concentrations. However, compound 1 and 4 showed cytotoxic effect (70%-50% and 90%-70% cell viability, respectively) rather than cytostatic effect (74%-57% and 97%-62% cell viability).Except at 1000 µg/mL compound 4 has had cytostatic effect (29% cell viability) rather than cytotoxic one (70% cell viability). The obtained data indicated that compounds 2, 3 and 5 have high anti-proliferative effect against colon cancer cell line at concentration dependent manner. This was similar to curcumin as has cytostatic effect (94-22% cell viability) rather than cytotoxic (94-56% cell viability), respectively Table 3.6(appendix B). As overall, the examined curcumin derived compounds showed more cytotoxicity than curcumin except for compound 1 Figure A21 (appendix A), and better cytostatic one except for compound 4 Figure A22(appendix A).

Table 3.6

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) Colon Cell Line Viability % effects under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

Cytotoxic and Cytostatic effects Colon Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
1	70	74	60	71	50	69	57	66	55	64	50	57
2	99	80	91	78	90	67	70	64	70	51	54	29
3	85	73	80	78	75	66	70	62	60	57	60	53
4	90	97	87	92	80	89	80	81	60	62	70	29
5	100	76	99	71	95	66	90	63	89	59	80	57
Curcumin	97	94	94	72	92	69	89	57	69	47	56	22

Furthermore, HeLa cervical cancer cell line cytotoxic and cytostatic growth behavior has been investigated under the effect of the curcumin derived compounds under study 1, 2, 3, 4 and 5 as well as curcumin. Results revealed that all compounds have cytostatic effect (94%-6% cell viability) rather than cytotoxic one (101%-51% cell viability), except for compound 3. It has showed cytotoxic effect (67%-33% cell viability) rather than cytostatic one (88%-47% cell viability). Among the cytostatic compounds; compound 5 had the strongest effect on HeLa cells line as reduced their viability down to 6% Table 3.7(appendix B),Compounds 2, 3 and 4 had higher cytotoxicity than curcumin among of which compound 3 is the highest. while compounds 1 and 5 have almost similar cytotoxic effect to curcumin except at high concentrations 500-1000 µg/mL Figure A23(appendix A). Nevertheless, they showed better cytostatic effect than curcumin at concentrations under study except at 500-1000 µg/mL concentration. However, compound 5 has had the strongest cytostatic effect on HeLa cell line at all examined concentration in concentration dependent manner Figure A24 (appendix A).

Table 3.7

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) HeLa Cell Line Viability % effects under dnifferent studied concentrations of compounds; 1,2,3,4,5:and pure curcumin

Cytotoxic and Cytostatic effects HeLa Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
1	100	94	99	88	100	82	98	71	97	54	89	47
2	98	83	92	76	89	71	78	56	56	47	51	35
3	67	88	61	85	56	73	56	53	44	49	33	47
4	99	82	98	71	89	68	84	53	72	47	76	27
5	101	44	99	41	99	19	98	8	86	7	78	6
Curcumin	100	99	99	97	97	96	95	59	95	32	93	16

Therefore, the IC50 (Half-maximal inhibitory concentration) of the examined cytostatic compounds on the different cancer cell lines has been calculated via excel to determine their effect under nontoxic concentration in µg/mL in comparison to recorded cytostatic effect IC50 values of curcumin. The obtained results showed pronounced variations among examined curcumin derived compounds depending on the compound type and the cancer cell line under study Table 3.8(appendix B). The strongest anti-proliferative cytostatic effect referred to compound 5 on cervical carcinoma HeLa cell line with the lowest IC50 = 372.3 µg/mL Figure A35 (appendix A). So, this chemical modification to curcumin has reduced the cytostatic effect IC50 of curcumin (516.5 µg/mL Figure A36 (appendix A). Similar reduction of curcumin IC50 on HEPG2 liver carcinoma from 672.9 µg/mL Figure A37 (appendix A). to 514.9 µg/mL Figure A61 (appendix A)by the same compound 5. However, compounds 1, 2, 3, 4 and 5 on the other cytostatic affected cancer cell lines under study have had higher IC50 values than the pure curcumin Figures A39-A49 (appendix A).As a result, the recorded data illustrates that MCF-7 breast carcinoma is not affected in a cytostatic manner by any of the curcumin derived compounds under study. While, for HEPG2 liver carcinoma is only affected by compound 5. Moreover, B16-F 10 skin melanoma is affected by compounds 1 and 2. But colon human cancer is affected by compounds 2, 3 and 5. Furthermore, the cell line which is affected by wide spectrum of the examined compounds is HeLa cervical carcinoma as being inhibited by all compounds except for compound 3 Figure 3.6

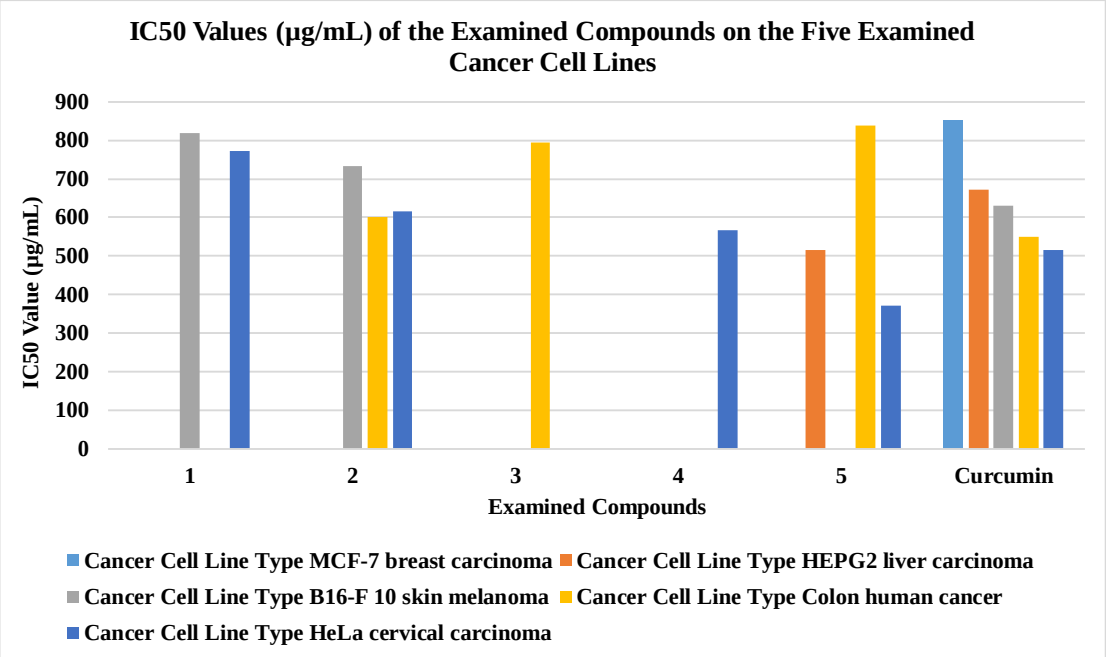
Table 3.8

IC50 values (µg/mL) in the five examined cancer cell lines after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test under the effect of compounds; 1,2,3,4,5andpure curcumin

Compound	Cancer Cell Line Type				
	MCF-7 breast carcinoma	HEPG2 liver carcinoma	B16-F110 skin melanoma	Colon human cancer	HeLa cervical carcinoma
1	-	-	819.6	-	771.6
2	-	-	733.1	602.4	616.5
3	-	-	-	793.6	-
4	-	-	-	-	566.8
5	-	514.9	-	837.5	372.3
Curcumin	850.3	672.9	631.3	550.7	516.5

Figure 3.6

IC50 values (µg/mL) of the five examined cancer cell lines after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test under the effect of compounds 1,2,3,4,5 and pure curcumin



Moreover, MCF-7 breast carcinoma, HEPG2 liver carcinoma, B16-F10 skin melanoma, Colon human cancer and HeLa cervical carcinoma cell lines viability % after 24 hrs and 72 hrs under curcumin and curcumin derived compounds **6:** 3,3'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1phenylene)) bis (propane-1-sulfonic acid), **7:** 4,4'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-

diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(butane-1-sulfonic acid in comparison with the pure curcumin cytotoxic and cytostatic effect is investigated via MTT assay revealing variable results in cell viability % related to cancer cell line type, compound type and used concentration.

Examined compounds 6 and 7 showed varying cytotoxic and cytostatic effect on MCF-7 breast carcinoma cell line at different concentrations (31.25- 1000 µg/mL). They showed cytotoxic effect rather than cytostatic one and vice versa, respectively in comparison to the pure curcumin compounds which has cytostatic effect rather than cytotoxic Table 3.9. In spite of the higher cytotoxic effect of both compounds 6 and 7 than curcumin Figure A25(appendix A), compound 7 has better cytostatic effect with the least cytostatic effect refers for compound 6 Figure A26(appendix A).

While, for the effect of the compounds 6 and 7 on HEPG2 liver carcinoma was cytostatic as reduced cell viability down to 12% and 8%, respectively, as well as achieved by the pure curcumin which decreased cell viability down to 24% in concentration dependent manner Table 3.10. Moreover, the cytotoxicity of compounds 6 and 7 are more than the curcumin on HEPG2 cell line Figure A27 (appendix A). Yetthe cytostatic effect of compounds 6 and 7 are far more than curcumin, out of which compound 7 is the strongest anti-proliferative agent on that cell line Figure A28 (appendix A).

Table 3.9

MTT cytotoxic (24 hrs) and cytostatic (72 hrs) MCF-7 cell line viability % effects under different studied concentrations of compounds; 6,7 and pure curcumin

Cytotoxic and Cytostatic effects MCF-7 Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
6	84	96	81	98	68	98	66	97	59	82	58	56
7	70	76	58	54	53	10	34	8	32	6	17	6
Curcumin	100	100	92	89	88	86	85	80	73	66	67	46

Table 3.10

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) HEPG2 Cell Line Viability % effects under different studied concentrations of compounds; 6,7: and pure curcumin

Cytotoxic and Cytostatic effects HEPG2 Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
6	89	50	87	48	78	47	67	45	44	25	33	12
7	78	45	67	15	56	7.7	44	7.8	33	7.6	22	8
Curcumin	98	99	95	89	93	79	78	75	75	73	45	24

In addition, the B16-F 10 skin melanoma cell line viability was reduced down to 44% by compound 6 in a cytotoxic manner. But was reduced down to 6% and 42% in a cytostatic manner by compound 7 and curcumin, respectively at most concentrations under study Table B1(appendix B). However, in spite of the cytotoxic effect of compound 6, both compounds 6 and 7 have more cytotoxicity effect on B16-F 10 cell line than curcumin Figure A29(appendix A). Furthermore, the highest cytostatic effect against this cell line is by compound 7 in comparison to curcumin Figure A30 (appendix A).

Furthermore, the cytotoxic and cytostatic effect assay of compounds 6 and 7 on colon carcinoma cell line revealed a pronounced cytostatic effect (53%-11% cell viability) rather than cytotoxic one (70%-40% cell viability) under the examined concentrations in comparison to curcumin cytostatic one (94%-22% cell viability)and cytotoxic effect (97%-56% cell viability) Table B2(appendix B). Both compounds 6 and 7 have higher cytotoxicity than curcumin in concentration dependent manner Figure A31(appendix A). This higher cytotoxic effect of both those curcumins derived compounds did not eliminate their strong cytostatic effect compared to curcumin revealing them to be considered some excellent anti-proliferative agents against colon cancer at all examined concentration Figure A32 (appendix A).

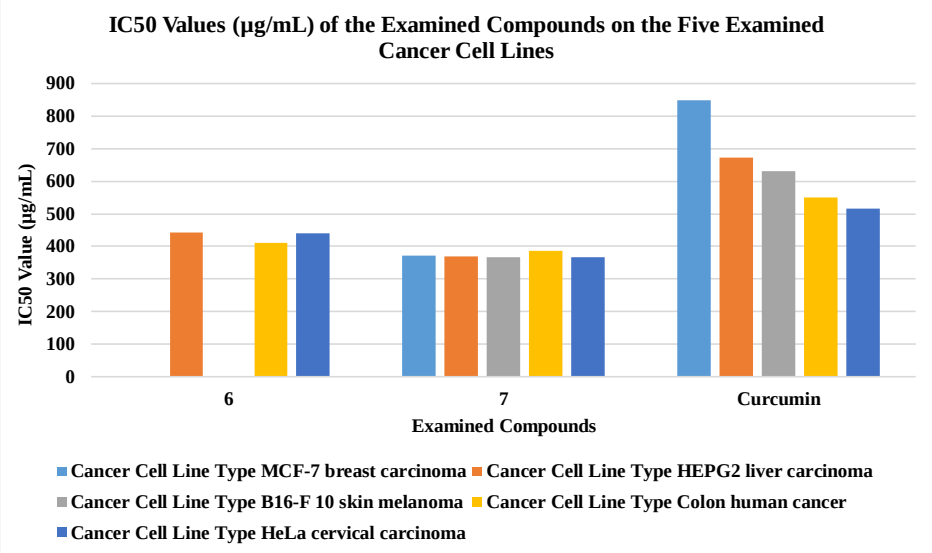
Similar cytostatic effect was recorded on HeLa cervical carcinoma cell line by compounds 6 (61%-9% cell viability, compound 7 (40%-5% cell viability) and curcumin (99%-16% cell viability) rather than cytotoxic one in concentration dependent mannerTableB3(appendix B).After all, the pure curcumin has lower cytotoxic effect/8 than both its derived compounds 6 and 7 on the Hela cell line Figure A33(appendix A). Never the less, compound 7 achieved the most pronounced cytostatic effect on that cancer cell line followed by compound 6 as compared with curcumin Figure A34(appendix A).

As a result, the IC50 (Half-maximal inhibitory concentration) of the examined cytostatic compounds on the different cancer cell lines has been calculated via excel to determine their effect under nontoxic concentration in µg/mL of curcumin and its derived compounds 6 and 7Table B4(appendix B). Compound 7 showed cytostatic effect on MCF-7 breast carcinoma, HEPG2 liver carcinoma, B16-F10 skin melanoma,

Colon human cancer and HeLa cervical carcinoma cell lines viability % after 72 hrswithIC50 non-toxic concentrations 372.5, 370.6, 366.3, 386.6 and 366.3 µg/mL, respectively Figures A50-A54(appendix A). which are lower IC50 values than those recorded by the curcumin itself on the same cancer cell lines under study respectively (850.3, 672.9, 631.3, 550.7 and 516.5 µg/mL) Figures A47, A37, A48, A49 and A36(appendix A). Similarly, the derived curcumin compound 6 also has had lower IC50 values of 442.5, 411.1 and 439.3 µg/mL than curcumin on HEPG2 liver carcinoma, Colon human cancer and HeLa cervical carcinoma cell lines, respectively Figures A55-A57 (appendix A). Among all the examined compounds, compound 7 showed the strongest cytostatic effect with the lowest IC50 concentrations 366.3 µg/mL on both B16-F10 skin melanoma and HeLa cervical carcinoma causing it the most recommended anticancer agent on both cell lines in addition to the other cancer lines. Still, also compound 6 is better anticancer agent than curcumin on the affected cancer lines under study. So the recorded cytostatic effect of the investigated compounds on the studied different cancer cell lines varied depending on the compound and the cancer cell line type as showed different nontoxic IC50 concentrations values Figure 3.7.

Figure 3.7

IC50 values (µg/mL) of the five examined cancer cell lines after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test under the effect of compounds 6,7 and pure curcumin



Therefore, according to (116), anticancer agents possessing anti-proliferative cytostatic properties have been strongly recommended as effective inhibitors of tumor cell lines. These agents induce apoptosis and inhibit growth in cancerous cells while exhibiting non-toxicity towards healthy cells, as reported by (117). The aforementioned compounds analyzed in this study have demonstrated noteworthy importance as potential anticancer agents against various cell lines, including MCF-7 breast carcinoma, HEPG2 liver carcinoma, B16-F110 skin melanoma, Colon human cancer, and HeLa cervical carcinoma, as evidenced by their impact on cell viability percentages. Furthermore, it was observed that all of the analyzed compounds exhibited a consistent pattern, indicating that the effects of various compound types are contingent on their concentration. It was observed that in the MTT assay for cytostatic effect, there was an increase in cell viability over 100% at low concentrations after 72 hours. This finding may be attributed to conflicts within the MTT assay. During the conduction of MTT assays, it is necessary to optimize several parameters, including cell seeding density and number, MTT incubation time, MTT concentration, culture conditions (presence of serum, media type, and phenol red), pH of the solubilization solution, MTT extrusionand/oruptakesuch as cell membrane permeability and integrity, cell number (proliferation), cell metabolism (chemo and radio-induced senescence-like phenotype), cell secretome (e.g., chemo/radio-induced senescence-associated secretory phenotype), background absorbance and scattering, and abiotic reduction of MTT. Therefore, it is possible that these factors could have an impact on the efficacy of the treatments being tested by directly or indirectly influencing the final optical density measurements, as noted by(118).

Hence, it is imperative to explore alternative assay technologies that can provide higher sensitivity compared to MTT assay for screening compounds with cytostatic properties while eliminating those that do not possess such properties. The utilization of colorimetric assays such as MTT, SRB, XTT, among others, is limited to assessing the growth inhibitory effects of compounds of interest rather than indicating cytotoxicity. It is important to note that a 50% reduction in the growth of the cell population under examination does not necessarily imply that 50% of the cells have been killed. It is plausible that half of the cells underwent cytotoxic effects, while the other half experienced growth arrest during the test period, or detachment from the flask's bottom

due to anti-adhesive (anti-metastatic) effects. These three biological processes may have occurred in varying proportions(119).

As a result, it is advisable to utilize additional tools that can offer greater elucidation on the differentiation between cytotoxic and cytostatic effects, in conjunction with colorimetric assays such as phase-contrast microscopy. In addition, to enhance the acquired observations, the utilization of the Trepan blue exclusion assay may prove to be an informative protocol. In conjunction with a more targeted viability assay, such as the LDH assay, it is possible to obtain a more precise understanding of the true antitumor impact of the compounds under investigation (120).

The compounds derived from curcumins that exhibit cytostatic effectiveness are being suggested for use as antitumor agents or as a therapy, either alone or in combination, for the treatment of various cancer lines that are currently under investigation. Furthermore, a crucial concern in this particular area of study is to examine the mechanism behind the cytostatic anticancer effect. The mechanisms that have been identified to combat cancer include the antagonism of estrogen activity, inhibition of cell proliferation, induction of apoptosis, reduction of mitochondrial membrane potential, arrest of cell cycle, increase in the generation of reactive oxygen species (ROS), depletion of intracellular nonprotein thiols, induction of DNA damage, and suppression of inflammation. These mechanisms are known to vary depending on the type of compound and cancer cell line being studied (121). Furthermore, in vivo studies are necessary to enhance comprehension of the therapeutic potential of these substances against various cancer cell lines, including MCF-7 breast carcinoma, HEPG2 liver carcinoma, B16-F110 skin melanoma, Colon human cancer and HeLa cervical carcinoma. In addition, the investigation of the compounds being studied includes the evaluation of their cytotoxic and cytostatic effects on various cancer cell lines. It is important to note that the elements constitute an essential component of the research interventions and concepts pertaining to this expansive domain.

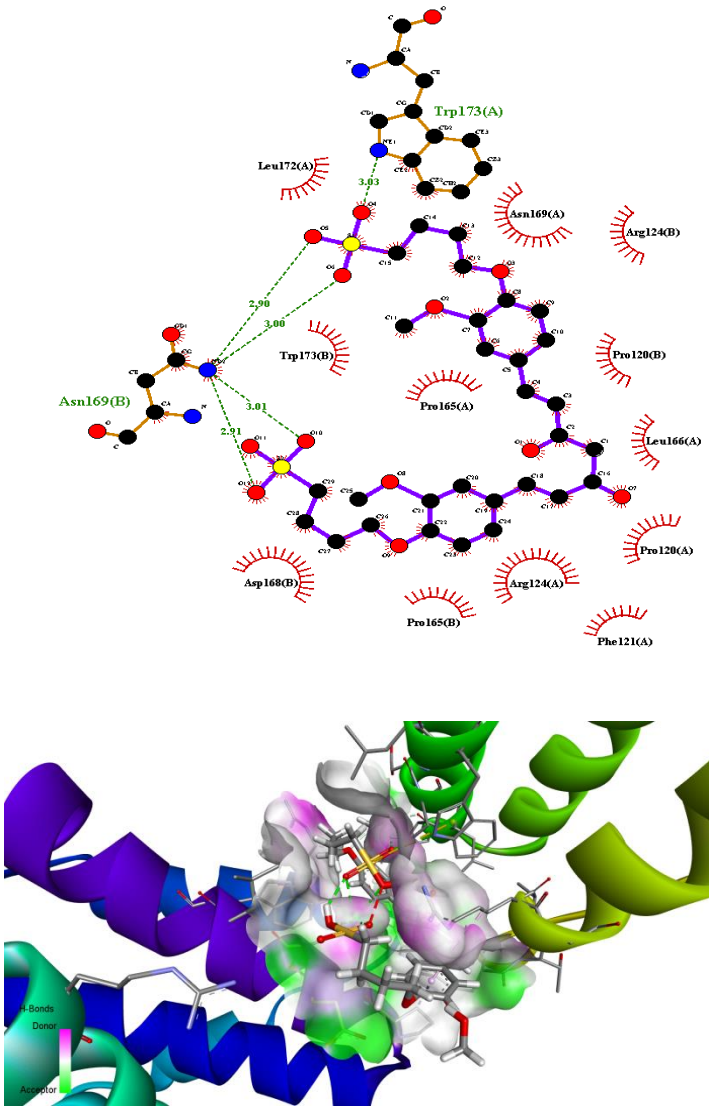
3.6 Molecular Docking

Predicting the interactions that take place between proteins and small molecules is an important step in decoding a broad variety of biological processes, as well as an important step in gaining an advantage in drug development (122). This step plays a significant role in a number of fundamental biological phenomena. Protein–ligand blind docking is an effective method for achieving this goal. This method identifies the areas of a protein that are responsible for binding and simultaneously predicts the binding pose of a molecule (123). Recently, there has been an increasingly urgent necessity of blind docking due to the fact that enormous protein structures have been determined hence opening the doors to the possibility of exploring new target therapies (124). This has resulted in an increase in the number of cases in which blind docking is required. CB-Dock(124-126), which is a server for protein–ligand blind docking was used in this study to evaluate the interaction among the curcumin, and the following proteins: MCF-7 breast carcinoma (4MAN) (127), HEPG2 liver carcinoma (1HNJ)(128), Colon human cancer (2Y9X), and HeLa cervical carcinoma cell lines (2X7F) (129).

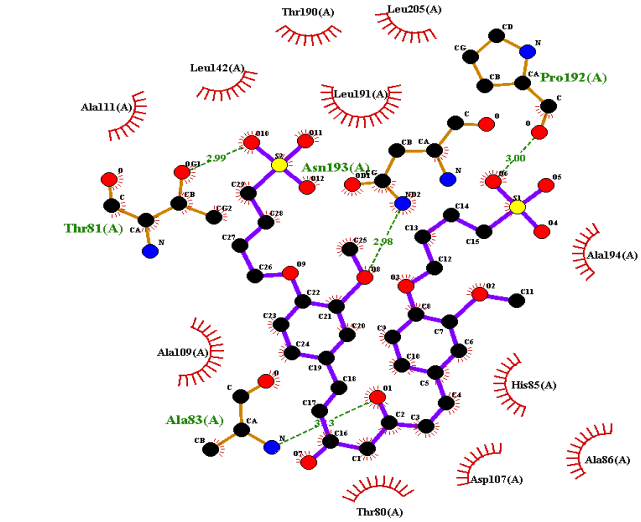
The curcumin molecule is a powerful anticancer candidate drug (as supported from experimental investigation, which exhibited outstanding anticancer activity treatments against many cells), as evidenced from its capacity to from H-bonds during docking with the amino acid side chains and the Vina docking score values as shown in Figure 3.8.

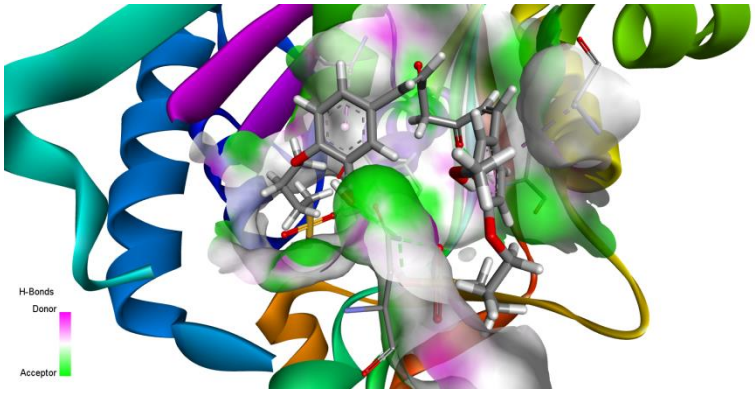
Figure 3.8

H-bonding docking and the Vina docking score values of compound 7 with the amino acid side chains of proteins of various cancer cells

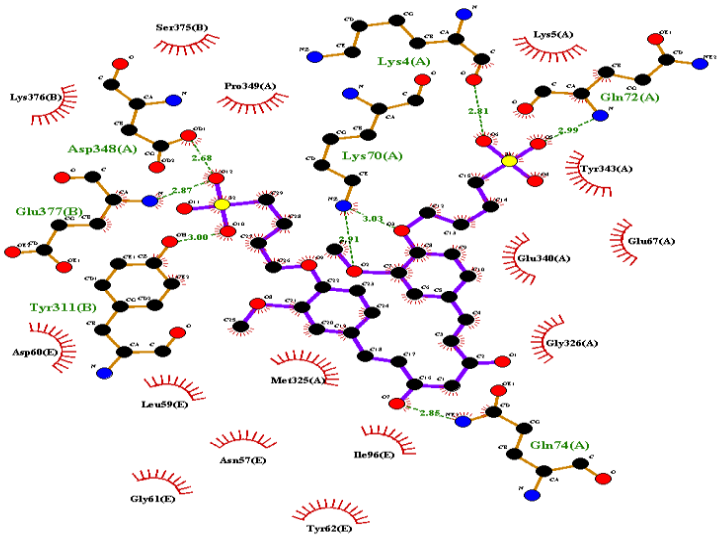


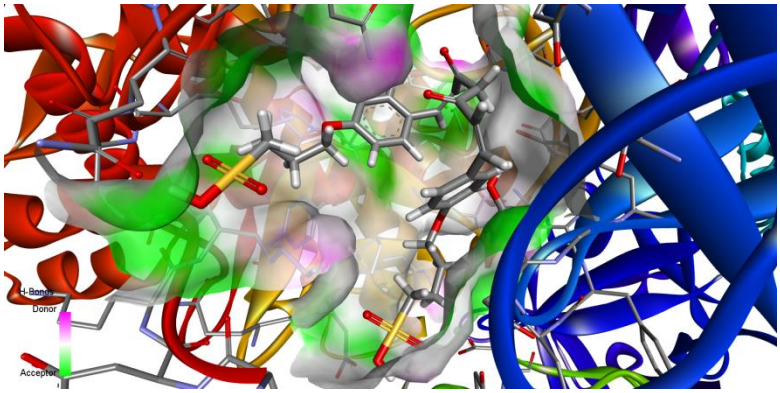
a. 4MAN // Vina Score -8.0 // Contacts: Chain A: PRO120 PHE121 THR122 ARG124 PRO165 LEU166 ASP168 ASN169 LEU172 Chain B: PRO120 PHE121 ARG124 PRO165 LEU166 ASP168 ASN169 LEU172 TRP173.



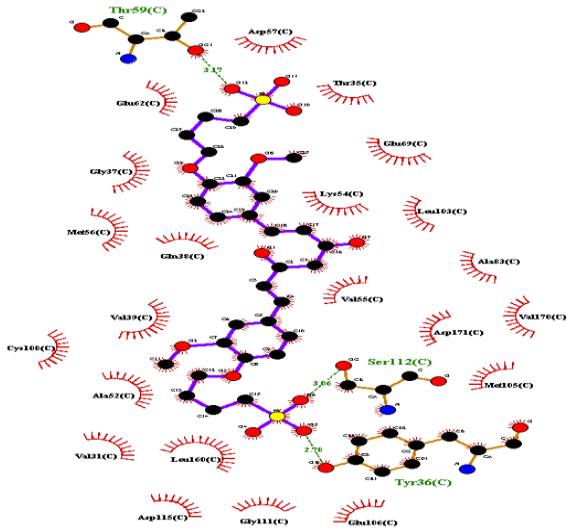


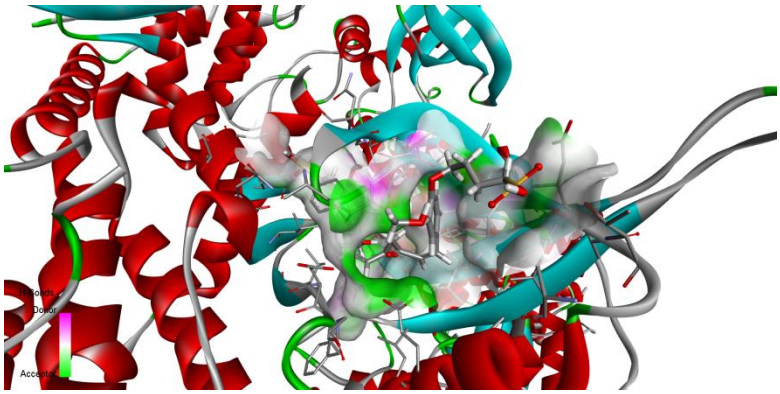
b. 1HNJ // Vina Score -8.6 // Contacts: Chain A: ASP27 THR28 SER29 TRP32 ILE33 ARG36 THR37 CYS112 ARG151 GLY152 THR153 ILE155 ILE156 PHE157 LEU189 MET207 ALA208 GLY209 ASN210 VAL212 PHE213 ALA216 LEU220 PRO243 HIS244 GLN245 ALA246 ASN247 ARG249 ILE250 ILE251 THR254 ASN274 GLU302 ALA303 PHE304 GLY305.





c. 2Y9X // Vina Score -8.3 // Contacts: Chain A: SER2 LYS4 LYS5 GLU67 LYS70 ALA71 GLN72 GLN74 LEU77 TYR82 MET325 GLY326 ILE328 PRO338 GLU340 TYR343 GLN347 ASP348 PRO349 Chain B: TYR311 SER375 LYS376 GLU377 GLU378 Chain E: ASN57 LEU59 ASP60 GLY61 TYR62 SER95 ILE96 GLU97





d. 2X7F // Vina Score -7.7 // Contacts: Chain E: ARG80 ASN81 GLY111 SER112 VAL113 LEU116 ILE117 LYS118 ASN119 LYS126 TRP129 ILE130 TYR132 ILE133 CYS134 ARG135 GLU136 ARG139 VAL159 LEU160 LEU161 ALA165 GLU166 VAL167 THR222 MET226 GLU295 VAL298 ARG299 LEU322.

Chapter Four

Conclusion

4.1 Conclusion

A new novelty Curcumin azo-dyes compound have been prepared, characterized and tested for anticancer and antimicrobial studies; the following conclusion were down from this work:

1. The first set of the prepared compunds including 1: 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (6-hydroxy-5-methoxy-3,1-phenylene)) bis(diazene-2,1-diyl))dibenzenesulfonic acid , 2: (1E,4Z,6E)-1,7-bis(3-((E)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl) -5-hydroxyhepta-1,4,6-trien-3-one, 3: (1E,4Z,6E)-1,7-bis(3-((E)-(2,4-dichlorophenyl)-4-hydroxy-5-methoxyphenyl)- 5-hydroxyhepta-1,4,6-trien-3-one,4:4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (6-hydroxy-5 -methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid, 5: (1E,4Z,6E)-1,7-bis(3-((E)-(4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-methoxyphenyl) -5-hydroxyhepta-1,4,6-trien-3-one. These compunds have been prepared and charecterized for IR, H1-NMR, melting point, and showed different activity aganist different type of bacteria and human cancer cell.

2. The second set fo prepared compounds including compound 3,3'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (6-hydroxy-5-methoxy-3,1-phenylene))bis(propane-1-sulfonic acid), and compound 4,4'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl) bis (6-hydroxy-5-methoxy-3,1-phenylene)) bis (butane-1-sulfonic acid). These compounds have been prepared and charecterized for IR, H1-NMR, melting point, and showed different activity aganist different types of bacteria and showed impresive result againset human cancer cell.

4.2 Future suggestion

It is recommended to investigate the following:

1. Enhance the anticancer activities of the prepared curcumin compounds by converting them to cis-platin analogues. This can be accomplished by reacting the prepared curcumin sulfonate and curcumin based diazo compounds with potassiumtetrachloroplatinate.

2. The color intensity of the curcumin based diazo compounds can be enhanced by complexing them with various transition metals such as cobalt and molybdenum. In this case the complexes can be used in a commercial scale as colorant for fabrics and other materials

List of Abbreviations

Abbreviation	Meaning
FT-IR	Fourier -Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
DMSO	Dimethyl Sulfoxide
Mmol	Millimole
NB	Nutrient Broth
S. aureus	*Staphylococcus aureus*
MRSA	Methicillin-resistant *S. aureus*
K. pneumonia	Klebsiella pneumoniae
E. coli	*Escherichia coli*
CLSI	Clinical and Laboratory Standard Institute
MIC	Minimum Inhibitory concentration
ERIC	Enterobacterial repetitive intergenic consensus
IC50	(Half-maximal inhibitory concentration)
DNA	Deoxyribonucleic acid
ROS	Reaction Oxygen Species
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), yellow tetrazolium salt
LDH	Lactate dehydrogenase
Compound 1	4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid
Compound 2	(1E,4Z,6E)-1,7-bis(3-((E)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one
Compound 3	(1E,4Z,6E)-1,7-bis(3-((E)-(2,4-dichlorophenyl)-4-hydroxy-5-methoxyphenyl)- 5-hydroxyhepta-1,4,6-trien-3-one
Compound 4	4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid
Compound 5	(1E,4Z,6E)-1,7-bis(3-((E)-(4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-

	methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one
Compound 6	3,3'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(propane-1-sulfonic acid)
Compound 7	4,4'-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(butane-1-sulfonic acid)

References

(1) De Graaff JH. The colourful past. Origins, chemistry and identification of natural dyestuffs. 2004:234-5.

(2) Casoli A, Darecchio ME, Sarritzu L. I coloranti nell'arte. I COLORANTI NELL'ARTE: Il Prato Casa Editrice, Collana i Talenti; 2009.

(3) Garfield S. Mauve: how one man invented a color that changed the world: WW Norton & Company; 2002.

(4) Hunger K. Industrial dyes: chemistry, properties, applications: John Wiley & Sons; 2007.

(5) Kuenemann MA, Szymczyk M, Chen Y, Sultana N, Hinks D, Freeman HS, et al. Weaver's historic accessible collection of synthetic dyes: a cheminformatics analysis. Chemical Science. 2017;8(6):4334-9.

(6) Gill M, Mustroph H. From Ferrocyanide Europes largest Film Factory. CHEMIE IN UNSERER ZEIT. 2014;48(6):424-38.

(7) Yum JH, Chen P, Grätzel M, Nazeeruddin MK. Recent developments in solid-state dye-sensitized solar cells. ChemSusChem: Chemistry & Sustainability Energy & Materials. 2008;1(8-9):699-707.

(8) Nakagawa T, Ku S-Y, Wong K-T, Adachi C. Electroluminescence based on thermally activated delayed fluorescence generated by a spirobifluorene donor–acceptor structure. Chemical Communications. 2012;48(77):9580-2.

(9) Cole JM, Pepe G, Al Bahri OK, Cooper CB. Cosensitization in dye-sensitized solar cells. Chemical reviews. 2019;119(12):7279-327.

(10) LZ GMBDH. Stolte M. Würthner F. Adv Mater. 2016;28(19):3615-45.

(11) Decelles C. The story of dyes and dyeing. Journal of chemical education. 1949;26(11):583.

(12) Moeckly S. The History of the Dye Industry. Chemistry. 1998;297(10.15):98.

(13) Matada MN, Jathi K, Rangappa MM, Geoffry K, Kumar SR, Nagarajappa RB, et al. A new sulphur containing heterocycles having azo linkage: Synthesis, structural characterization and biological evaluation. Journal of King Saud University-Science. 2020;32(8):3313-20.

(14) Shankarling GS, Deshmukh PP, Joglekar AR. Process intensification in azo dyes. Journal of environmental chemical engineering. 2017;5(4):3302-8.

(15) Gürses A, Açıkyıldız M, Güneş K, Gürses MS, Gürses A, Açıkyıldız M, et al. Classification of dye and pigments. Dyes and pigments. 2016:31-45.

(16) Shah M. Effective treatment systems for azo dye degradation: a joint venture between physico-chemical & microbiological process. International Journal of Environmental Bioremediation & Biodegradation. 2014;2(5):231-42.

(17) McLaren K. The colour science of dyes and pigments. (No Title). 1986.

(18) El Harfi S, El Harfi A. Classifications, properties and applications of textile dyes: A review. Applied Journal of Environmental Engineering Science. 2017;3(3):00000-3 N° 3 (2017) 311-320.

(19) Collier SW, Storm J, Bronaugh RL. Reduction of azo dyes during in vitro percutaneous absorption. Toxicology and applied pharmacology. 1993;118(1):73-9.

(20) Benkhaya S, Cherkaoui O, Assouag M, Mrabet S, Rafik M, Harfi A. Synthesis of a new asymmetric composite membrane with bi-component collodion: application in the ultra filtration of baths of reagent dyes of fabric rinsing/padding. J Mater Environ Sci. 2016;7(12):4556-69.

(21) Al-Rubaie L, Mhessn RJ. Synthesis and characterization of azo dye para red and new derivatives. E-Journal of Chemistry. 2012;9(1):465-70.

(22) Rauf M, Meetani M, Hisaindee S. An overview on the photocatalytic degradation of azo dyes in the presence of TiO2 doped with selective transition metals. Desalination. 2011;276(1-3):13-27.

(23) Roberts RM. Serendipity: Accidental discoveries in science1989.

(24) Skelton H. A colour chemist's history of Western art. Review of Progress in Coloration and Related Topics. 1999;29(1):43-64.

(25) Marchevsky E, Olsina R, Marone C. 2-[2-(5-Chloropyridyl) azo]-5-dimethylaminophenol as indicator for the complexometric determination of zinc. Talanta. 1985;32(1):54-6.

(26) Marcus B. Tropical Forests: Jones & Bartlett Learning; 2009.

(27) Ott AM. Backpacking to reading success: parents and teachers working together to promote literacy development. 1997.

(28) Crozier W. The psychology of colour preferences. Review of Progress in Coloration and Related Topics. 1996;26(1):63-72.

(29) Ricks D. The shade of Homer: a study in modern Greek poetry: Cambridge University Press; 1989.

(30) Hamidian H. Synthesis of novel compounds as new potent tyrosinase inhibitors. BioMed Research International. 2013;2013.

(31) Dembitsky VM, Gloriozova TA, Poroikov VV. Pharmacological and predicted activities of natural azo compounds. Natural products and bioprospecting. 2017;7:151-69.

(32) Matsuoka M. Infrared absorbing dyes: Springer Science & Business Media; 2013.

(33) Rosales AG. Managing stress in broiler breeders: a review. Journal of Applied Poultry Research. 1994;3(2):199-207.

(34) El Mekkawi D, Abdel-Mottaleb M. The interaction and photostability of some xanthenes and selected azo sensitizing dyes with TiO 2 nanoparticles. International Journal of Photoenergy. 2005;7:95-101.

(35) Etesami H, Mansouri M, Habibi A, Jahantigh F. S7ynthesis and investigation of double alternating azo group in novel para-azo dyes containing nitro anchoring group for solar cell application. Journal of Molecular Structure. 2020;1203:127432.

(36) Farghaly TA, Abdallah ZA. Synthesis, azo-hydrazone tautomerism and antitumor screening of N-(3-ethoxycarbonyl-4, 5, 6, 7-tetrahydro-benzo [b] thien-2-yl)-2-arylhydrazono-3-oxobutanamide derivatives. Arkivoc. 2008;17:295-305.

(37) Zhi-Gang Y, Chun-Xia Z, De-Feng Z, Freeman HS, Pei-Tong C, Jie H. Monoazo dyes based on 5, 10-dihydrophenophosphazine, Part 2: Azo acid dyes. Dyes and Pigments. 2009;81(2):137-43.

(38) Park C, Lim J-S, Lee Y, Lee B, Kim S-W, Lee J, et al. Optimization and morphology for decolorization of reactive black 5 by Funalia trogii. Enzyme and Microbial Technology. 2007;40(7):1758-64.

(39) PaulEhrlich. en.wikipedia.org; 1935 [Available from: http://en.wikipedia.org/wiki/PaulEhrlich.1909-1935.

(40) GerhardDomagk. en.wikipedia.org; 1935 [Available from: http://en.wikipedia.org/wiki/GerhardDomagk.1935.

(41) Ottenberg R. The Treatment of Hemolytic Streptococcus Infections and the Newer Applications of Sulphanilamide. Bulletin of the New York Academy of Medicine. 1938;14(8):453.

(42) Ugras HI, Basaran I, Kilic T, Cakir U. Synthesis, complexation and antifungal, antibacterial activity studies of a new macrocyclic schiff base. Journal of heterocyclic chemistry. 2006;43(6):1679-84.

(43) Morad F, Elajaily M, Ben Gweirif S. Preparation, physical characterization and antibacterial activity of Ni (II) Schiff base complex. Journal of Science and Its Applications. 2007;1(1):72-8.

(44) Aanandhi V, George S, Vaidhyalingam V. Synthesis and antimicrobial activities of 1-(5-substituted-2-oxo indolin-3-ylidene)-4-(substituted pyridin-2-yl) thiosemicarbazide. Arkivoc. 2008;11:187-94.

(45) Khalil R, Jalil A, Abd-Alrazzak A. Application of a Schiff base derived from sulfanilamide as an acid-base indicator. Journal of the Iranian Chemical Society. 2009;6:345-52.

(46) Kavitha T, Velraj G. Molecular structure, spectroscopic and docking analysis of 1, 3-diphenylpyrazole-4-propionic acid: A good prostaglandin reductase inhibitor. Journal of Molecular Structure. 2018;1155:819-30.

(47) Raman N, Dhaveethu Raja J, Sakthivel A. Synthesis, spectral characterization of Schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies. Journal of Chemical sciences. 2007;119:303-10.

(48) Chittilappilly PS, Yusuff K. Synthesis, characterization and biological properties of ruthenium (III) Schiff base complexes derived from 3-hydroxyquinoxaline-2-carboxaldehyde. 2008.

(49) Vančo J, Švajlenová O, Račanská E, Muselík J, Valentová J. Antiradical activity of different copper (II) Schiff base complexes and their effect on alloxan-induced diabetes. Journal of Trace Elements in Medicine and Biology. 2004;18(2):155-61.

(50) Hearn MJ, Cynamon MH. Design and synthesis of antituberculars: preparation and evaluation against Mycobacterium tuberculosis of an isoniazid Schiff base. Journal of Antimicrobial Chemotherapy. 2004;53(2):185-91.

(51) Cukurovali A, Yilmaz I, Ozmen H, Ahmedzade M. Schiff base ligands containing cyclobutane and their metal complexes with Co (II), Cu (II), Ni (II) and Zn (II) complexes of two novel Schiff base ligands and their antimicrobial activity. Trans Met Chem. 2002;27:171-6.

(52) Dharmaraj N, Viswanathamurthi P, Natarajan K. Ruthenium (II) complexes containing bidentate Schiff bases and their antifungal activity. Transition Metal Chemistry. 2001;26:105-9.

(53) Nair R, Shah A, Baluja S, Chanda S. Synthesis and antibacterial activity of some Schiff base complexes. Journal of the Serbian Chemical Society. 2006;71(7):733-44.

(54) Shabani F, Saghatforoush LA, Ghammamy S. Synthesis, characterization and anti-tumour activity of Iron (III) Schiff base complexes with unsymmetric tetradentate ligands. Bulletin of the Chemical society of Ethiopia. 2010;24(2).

(55) Eşme A, Sağdınç S. Spectroscopic (FT–IR, FT–Raman, UV–Vis) analysis, conformational, HOMO-LUMO, NBO and NLO calculations on monomeric and dimeric structures of 4–pyridazinecarboxylic acid by HF and DFT methods. Journal of Molecular Structure. 2017;1147:322-34.

(56) Sudhakar A. History of cancer, ancient and modern treatment methods. Journal of cancer science & therapy. 2009;1(2):1.

(57) Saini A, Kumar M, Bhatt S, Saini V, Malik A. Cancer causes and treatments. International Journal of Pharmaceutical Sciences and Research. 2020;11(7):3121-34.

(58) Abbas Z, Rehman S. An overview of cancer treatment modalities. Neoplasm. 2018;1:139-57.

(59) what is cancer. national cancer institute. 2015.

(60) intriduction to cancer biology.

(61) Hegde MV, Mali AV, Chandorkar SS. What is a cancer cell? Why does it metastasize? Asian Pacific Journal of Cancer Prevention. 2013;14(6):3987-9.

(62) Eldridge L. Cancer cells vs. normal cells: how are they different. Verywell Health. 2022.

(63) progmedphys. 2020.

(64) Angiogenesis Inhibitors Therapy: National Cancer Institute. A fact sheet that describes the process of eliminating the blood supply to tumors. National Cancer Institute.

(65) Huang J, Li Y, Orza A, Lu Q, Guo P, Wang L, et al. Magnetic nanoparticle facilitated drug delivery for cancer therapy with targeted and image-guided approaches. Advanced functional materials. 2016;26(22):3818-36.

(66) Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come? Pharmaceutical research. 2010;27:950-61.

(67) Mahasneh AM, El-Oqlah AA. Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. Journal of Ethnopharmacology. 1999;64(3):271-6.

(68) Kintzios SE. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Critical reviews in plant sciences. 2006;25(2):79-113.

(69) Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981− 2002. Journal of natural products. 2003;66(7):1022-37.

(70) Sivalokanathan S, Ilayaraja M, Balasubramanian M. Efficacy of Terminalia arjuna (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. 2005.

(71) Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. Journal of ethnopharmacology. 2005;100(1-2):72-9.

(72) Ramli RM, Sinrang AW, Aminuddin A. Levels of Alpha-1 Acid Glycoprotein (AGP) in Stunting and Non Stunting Tolls Age 36-60 Months. International Journal of Health and Medical Sciences.4(1):145-9.

(73) Lodge M. The role of the Commonwealth in the wider cancer control agenda. The Lancet Oncology. 2020;21(7):879-81.

(74) Health annual report Palestine. 2017 [Available from: Health annual report Palestine. 2017. Retrieved fromhttp://site.moh.ps/.

(75) Erejuwa OO, Sulaiman SA, Wahab MSA. Effects of honey and its mechanisms of action on the development and progression of cancer. Molecules. 2014;19(2):2497-522.

(76) Gupta A, Khan S, Manzoor M, Yadav A, Sharma G, Anand R, et al. Anticancer curcumin: natural analogues and structure-activity relationship. Studies in natural products chemistry. 2017;54:355-401.

(77) Alibeiki F, Jafari N, Karimi M, Peeri Dogaheh H. Potent anti-cancer effects of less polar Curcumin analogues on gastric adenocarcinoma and esophageal squamous cell carcinoma cells. Scientific reports. 2017;7(1):2559.

(78) Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as “Curecumin”: from kitchen to clinic. Biochemical pharmacology. 2008;75(4):787-809.

(79) Nagahama K, Utsumi T, Kumano T, Maekawa S, Oyama N, Kawakami J. Discovery of a new function of curcumin which enhances its anticancer therapeutic potency. Scientific reports. 2016;6(1):30962.

(80) Aggarwal BB, Deb L, Prasad S. Curcumin differs from tetrahydrocurcumin for molecular targets, signaling pathways and cellular responses. Molecules. 2014;20(1):185-205.

(81) Ohtsu H, Xiao Z, Ishida J, Nagai M, Wang H-K, Itokawa H, et al. Antitumor agents. 217. Curcumin analogues as novel androgen receptor antagonists with potential as anti-prostate cancer agents. Journal of medicinal chemistry. 2002;45(23):5037-42.

(82) Lin L, Shi Q, Su C-Y, Shih CC-Y, Lee K-H. Antitumor agents 247. New 4-ethoxycarbonylethyl curcumin analogs as potential antiandrogenic agents. Bioorganic & Medicinal Chemistry. 2006;14(8):2527-34.

(83) Nieto CI, Andrade A, Sanz D, Claramunt RM, Torralba MC, Torres MR, et al. Curcumin Related 1, 4-Diazepines: Regioselective Synthesis, Structure Analysis, Tautomerism, NMR Spectroscopy, X-ray Crystallography, Density Functional Theory and GIAO Calculations. ChemistrySelect. 2017;2(13): 3732-8.

(84) Choudhary A, Naughton LM, Montánchez I, Dobson AD, Rai DK. Current status and future prospects of marine natural products (MNPs) as antimicrobials. Marine drugs. 2017;15(9):272.

(85) Hamed O, Fares O, Taleeb S, Adwan G, Saadeh H, Jodeh S, et al. New Insights Towards 1, 4-Benzodiazepines from Curcumin. Design, Synthesis and Antimicrobial Activities. Medicinal Chemistry. 2020;16(8):1112-23.

(86) Khajeh Dangolani S, Panahi F, Khalafi-Nezhad A. Synthesis of new curcumin-based aminocarbonitrile derivatives incorporating 4 H-pyran and 1, 4-dihydropyridine heterocycles. Molecular Diversity. 2021;25:2123-35.

(87) Padhye S, Banerjee S, Chavan D, Pandye S, Swamy KV, Ali S, et al. Fluorocurcumins as cyclooxygenase-2 inhibitor: molecular docking, pharmacokinetics and tissue distribution in mice. Pharmaceutical research. 2009;26:2438-45.

(88) Padhye S, Yang H, Jamadar A, Cui QC, Chavan D, Dominiak K, et al. New difluoro Knoevenagel condensates of curcumin, their Schiff bases and copper complexes as proteasome inhibitors and apoptosis inducers in cancer cells. Pharmaceutical research. 2009;26:1874-80.

(89) Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Molecular pharmaceutics. 2007;4(6):807-18.

(90) Ravindranath V, Chandrasekhara N. Metabolism of curcumn-studies with [3H] curcumin. Toxicology. 1981;22(4):337-44.

(91) Khan S, Imran M, Butt TT, Shah SWA, Sohail M, Malik A, et al. Curcumin based nanomedicines as efficient nanoplatform for treatment of cancer: new developments in reversing cancer drug resistance, rapid internalization, and improved anticancer efficacy. Trends in Food Science & Technology. 2018;80:8-22.

(92) Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas P. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta medica. 1998;64(04):353-6.

(93) Ireson CR, Jones DJ, Orr S, Coughtrie MW, Boocock DJ, Williams ML, et al. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. Cancer Epidemiology Biomarkers & Prevention. 2002;11(1):105-11.

(94) Metzler M, Pfeiffer E, Schulz SI, Dempe JS. Curcumin uptake and metabolism. Biofactors. 2013;39(1):14-20.

(95) Gurung RB, Gong SY, Dhakal D, Le TT, Jung NR, Jung HJ, et al. Synthesis of curcumin glycosides with enhanced anticancer properties using one-pot multienzyme glycosylation technique. 2017.

(96) Rodrigues FC, Kumar NA, Thakur G. Developments in the anticancer activity of structurally modified curcumin: An up-to-date review. European journal of medicinal chemistry. 2019;177:76-104.

(97) Vyas A, Dandawate P, Padhye S, Ahmad A, Sarkar F. Perspectives on new synthetic curcumin analogs and their potential anticancer properties. Current pharmaceutical design. 2013;19(11):2047-69.

(98) Rajasekhar Reddy A, Dinesh P, S Prabhakar A, Umasankar K, Shireesha B, Bhagavan Raju M. A comprehensive review on SAR of curcumin. Mini reviews in medicinal chemistry. 2013;13(12):1769-77.

(99) Jankun J, Wyganowska-Świątkowska M, Dettlaff K, Jelińska A, Surdacka A, Wątróbska-Świetlikowska D, et al. Determining whether curcumin degradation/condensation is actually bioactivation. International journal of molecular medicine. 2016;37(5):1151-8.

(100) Zhang H-Z, Zhao Z-L, Zhou C-H. Recent advance in oxazole-based medicinal chemistry. European journal of medicinal chemistry. 2018;144:444-92.

(101) Rodrigues FC, Kumar NA, Thakur G. The potency of heterocyclic curcumin analogues: An evidence-based review. Pharmacological Research. 2021;166:105489.

(102) Hutzen B, Friedman L, Sobo M, Lin L, Cen L, De Angelis S, et al. Curcumin analogue GO-Y030 inhibits STAT3 activity and cell growth in breast and pancreatic carcinomas. International journal of oncology. 2009;35(4):867-72.

(103) Olivera A, Moore TW, Hu F, Brown AP, Sun A, Liotta DC, et al. Inhibition of the NF-κB signaling pathway by the curcumin analog, 3, 5-Bis (2-pyridinylmethylidene)-4-piperidone (EF31): Anti-inflammatory and anti-cancer properties. International immunopharmacology. 2012;12(2):368-77.

(104) Selvendiran K, Ahmed S, Dayton A, Kuppusamy ML, Tazi M, Bratasz A, et al. Safe and targeted anticancer efficacy of a novel class of antioxidant-conjugated difluorodiarylidenyl piperidones: differential cytotoxicity in healthy and cancer cells. Free Radical Biology and Medicine. 2010;48(9):1228-35.

(105) Selvendiran K, Tong L, Bratasz A, Kuppusamy ML, Ahmed S, Ravi Y, et al. Anticancer efficacy of a difluorodiarylidenyl piperidone (HO-3867) in human ovarian cancer cells and tumor xenografts. Molecular cancer therapeutics. 2010;9(5):1169-79.

(106) Tan X, Sidell N, Mancini A, Huang R-P, Wang S, Horowitz IR, et al. Multiple anticancer activities of EF24, a novel curcumin analog, on human ovarian carcinoma cells. Reproductive Sciences. 2010;17(10):931-40.

(107) Fuchs JR, Pandit B, Bhasin D, Etter JP, Regan N, Abdelhamid D, et al. Structure–activity relationship studies of curcumin analogues. Bioorganic & medicinal chemistry letters. 2009;19(7):2065-9.

(108) Paulraj F, Abas F, H. Lajis N, Othman I, Naidu R. Molecular pathways modulated by curcumin analogue, diarylpentanoids in cancer. Biomolecules. 2019;9(7):270.

(109) Suarez JAQ, Rando DG, Santos RP, Gonçalves CP, Ferreira E, de Carvalho JE, et al. New antitumoral agents I: In vitro anticancer activity and in vivo acute toxicity of synthetic 1, 5-bis (4-hydroxy-3-methoxyphenyl)-1, 4-pentadien-3-one and derivatives. Bioorganic & medicinal chemistry. 2010;18(17):6275-81.

(110) Adams BK, Ferstl EM, Davis MC, Herold M, Kurtkaya S, Camalier RF, et al. Synthesis and biological evaluation of novel curcumin analogs as anti-cancer and anti-angiogenesis agents. Bioorganic & medicinal chemistry. 2004;12(14):3871-83.

(111) Citalingam K, Abas F, Lajis NH, Othman I, Naidu R. Anti-proliferative effect and induction of apoptosis in androgen-independent human prostate cancer cells by 1, 5-bis (2-hydroxyphenyl)-1, 4-pentadiene-3-one. Molecules. 2015;20(2):3406-30.

(112) Liang G, Shao L, Wang Y, Zhao C, Chu Y, Xiao J, et al. Exploration and synthesis of curcumin analogues with improved structural stability both in vitro and in vivo as cytotoxic agents. Bioorganic & medicinal chemistry. 2009;17(6):2623-31.

(113) Lin L, Shi Q, Nyarko AK, Bastow KF, Wu C-C, Su C-Y, et al. Antitumor agents. 250. Design and synthesis of new curcumin analogues as potential anti-prostate cancer agents. Journal of medicinal chemistry. 2006;49(13):3963-72.

(114) Cen L, Hutzen B, Ball S, DeAngelis S, Chen C-L, Fuchs JR, et al. New structural analogues of curcumin exhibit potent growth suppressive activity in human colorectal carcinoma cells. BMC cancer. 2009;9:1-8.

(115) Abdulridha RN, Saliem AH. Evaluation the Activity of Capparis Spinosa Fruits Extract Against Resistant E. Coli O157: H7. HIV Nursing. 2023;23(1):407-14.

(116) Sivaraj R, Rahman PK, Rajiv P, Narendhran S, Venckatesh R. Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;129:255-8.

(117) Shahneh F, Baradaran B, Majidi J, Babaloo Z. Echinophora platyloba DC (Apiaceae) crude extract induces apoptosis in human prostate adenocarcinoma cells (PC 3). Biomedical journal. 2014;37(5).

(118) Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. International journal of molecular sciences. 2021;22(23):12827.

(119) Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, et al. Cell viability assays. Assay Guidance Manual [Internet]. 2016.

(120) Anttila JV, Shubin M, Cairns J, Borse F, Guo Q, Mononen T, et al. Contrasting the impact of cytotoxic and cytostatic drug therapies on tumour progression. PLoS computational biology. 2019;15(11):e1007493.

(121) Wang X, Simpkins JW, Dykens JA, Cammarata PR. Oxidative damage to human lens epithelial cells in culture: estrogen protection of mitochondrial potential, ATP, and cell viability. Investigative ophthalmology & visual science. 2003;44(5):2067-75.

(122) Jacob L, Vert J-P. Protein-ligand interaction prediction: an improved chemogenomics approach. bioinformatics. 2008;24(19):2149-56.

(123) Hetényi C, van der Spoel D. Efficient docking of peptides to proteins without prior knowledge of the binding site. Protein science. 2002;11(7):1729-37.

(124) 124. Liu Y, Yang X, Gan J, Chen S, Xiao Z-X, Cao Y. CB-Dock2: Improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic acids research. 2022;50(W1):W159-W64.

(125) Liu Y, Grimm M, Dai W-t, Hou M-c, Xiao Z-X, Cao Y. CB-Dock: A web server for cavity detection-guided protein–ligand blind docking. Acta Pharmacologica Sinica. 2020;41(1):138-44.

(126) Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry. 2009;30(16):2785-91.

(127) Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nature medicine. 2013;19(2):202-8.

(128) Qiu X, Janson CA, Smith WW, Head M, Lonsdale J, Konstantinidis AK. Refined structures of β-ketoacyl-acyl carrier protein synthase III. Journal of molecular biology. 2001;307(1):341-56.

(129) Crystal structure of the kinase domain of human Traf2- and Nck- interacting Kinase with Wee1Chk1 inhibitor, https://www.rcsb.org/structure/2x7f 2010 [Available from: https://www.rcsb.org/structure/2x7f.

Appendices

Appendix A

Figures of Study

Figure A1

FT-IR of 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid. (1)

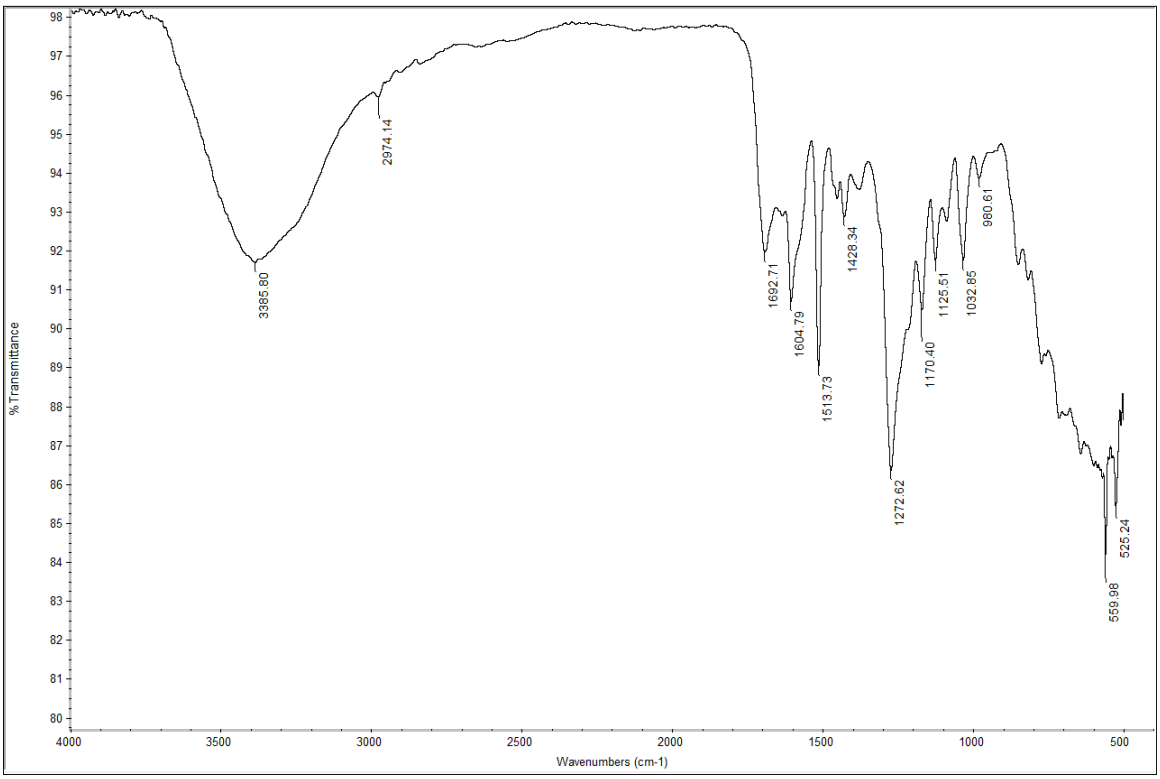


Figure A2

FT-IR Spectra of (1E,4Z,6E)-1,7-bis(3-((E)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (2)

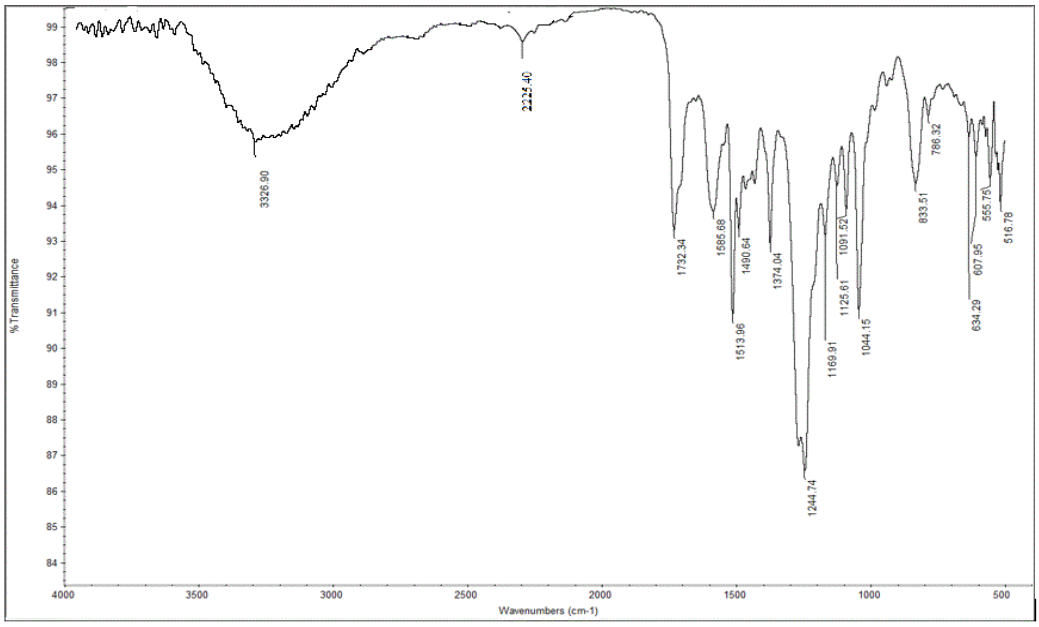


Figure A3

FT-IR of (1E,4Z,6E)-1,7-bis(3-((E)-(2,4-dichlorophenyl)-4-hydroxy-5-methoxyphenyl)- 5-hydroxyhepta-1,4,6-trien-3-one. (3)

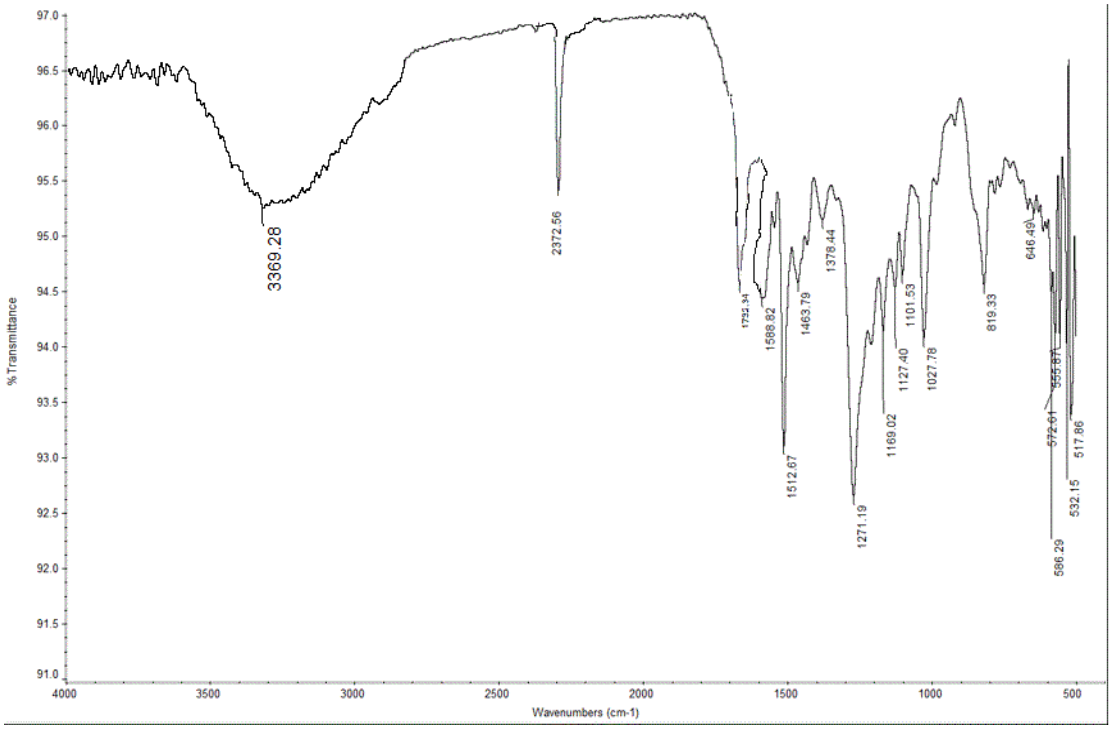


Figure A4

FT-IR Spectra of 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid. (4)

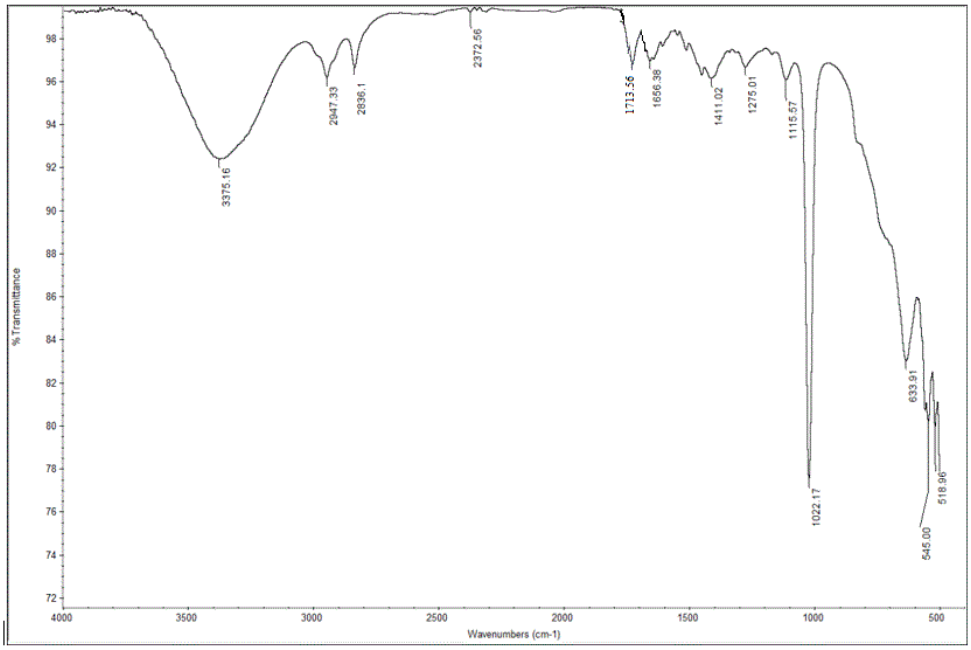


Figure A5

FT-IR Spectra of (1E,4Z,6E)-1,7-bis(3-((E)-(4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (5)

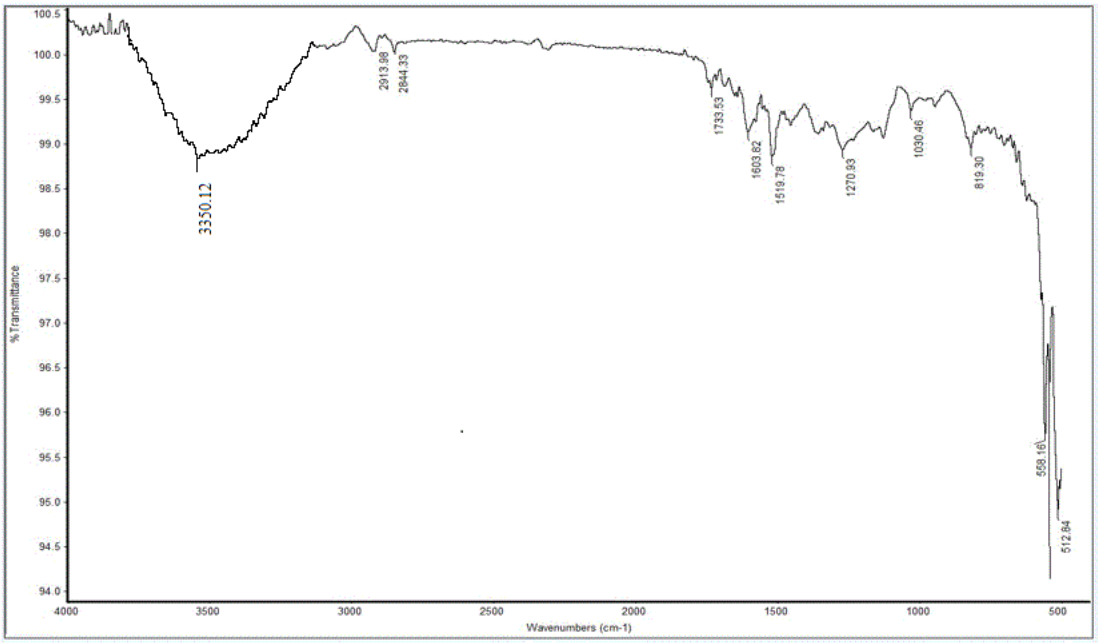


Figure A6

FT-IR Spectra of FT-IR Spectra of 3,3'-((((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(propane-1-sulfonic acid).(6)

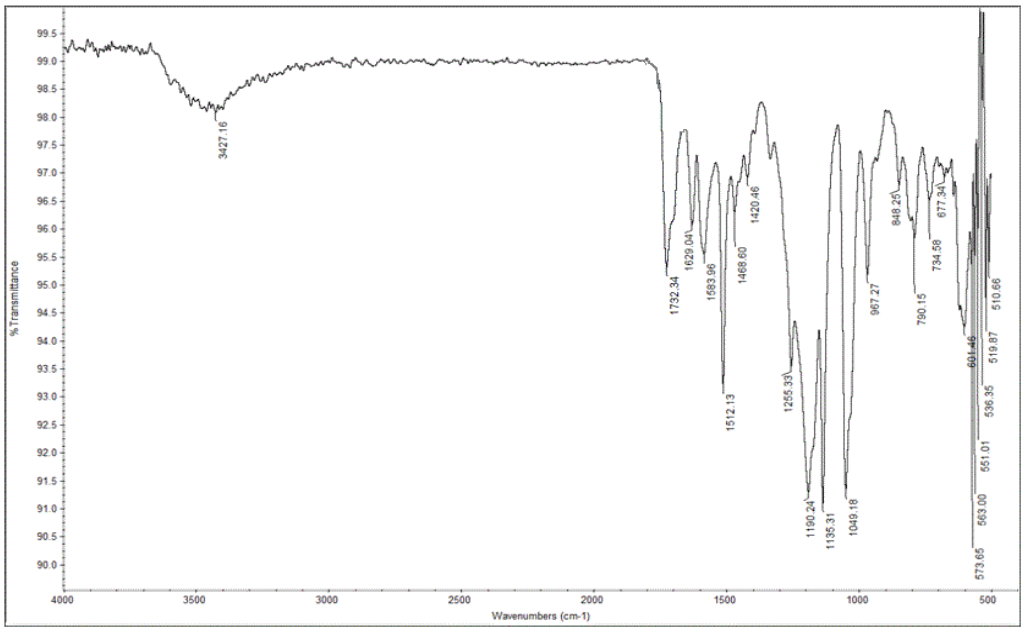


Figure A7

4,4'-((((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene)) bis(oxy))bis(butane-1-sulfonic acid).(7)

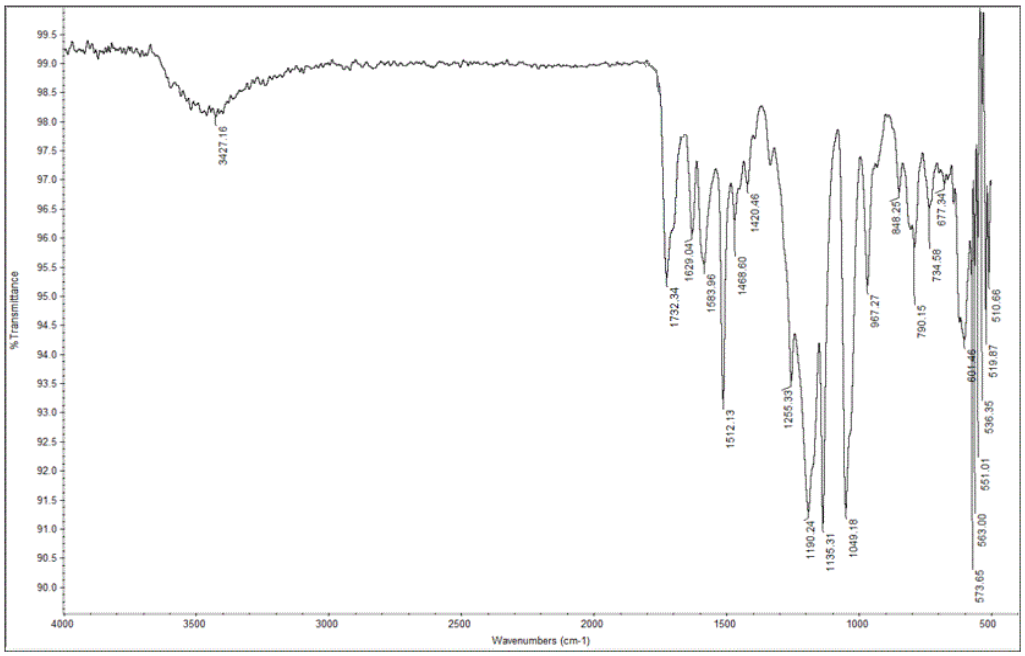


Figure A8

[1]H-NMR Spectra 4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzenesulfonic acid. (1)

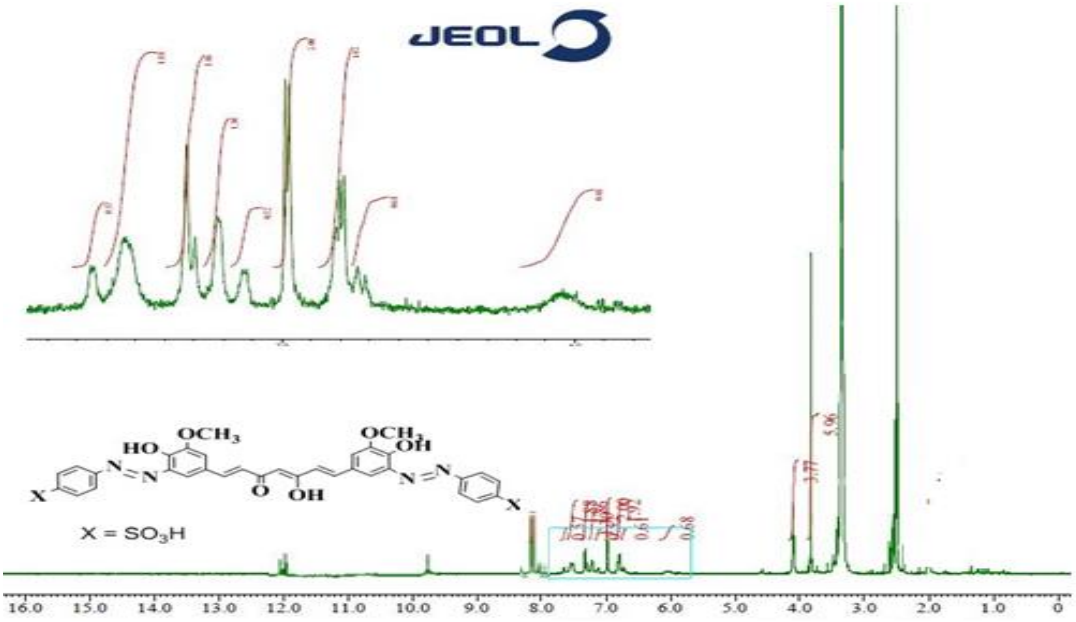


Figure A9

[1]H-NMR Spectra of (1E,4Z,6E)-1,7-bis(3-((E)-(4-chlorophenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (2)

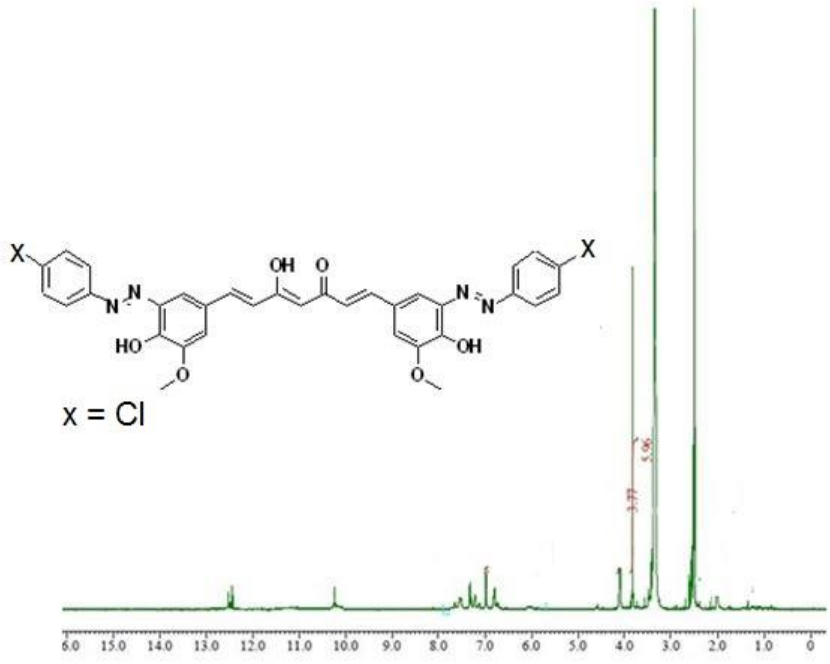


Figure A10

[1]H-NMR Spectra of (1E,4Z,6E)-1,7-bis(3-((E)-(2,4-dichlorophenyl)-4-hydroxy-5-methoxyphenyl)- 5-hydroxyhepta-1,4,6-trien-3-one. (3)

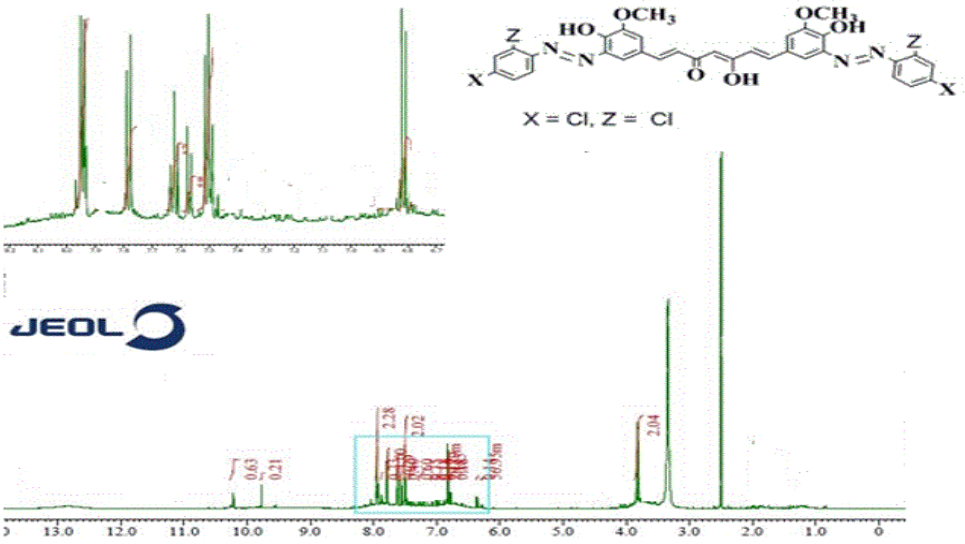


Figure A11

[1]H-NMR Spectra of4,4'-((1E,1'E)-(((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(6-hydroxy-5-methoxy-3,1-phenylene))bis(diazene-2,1-diyl))dibenzoic acid. (4)

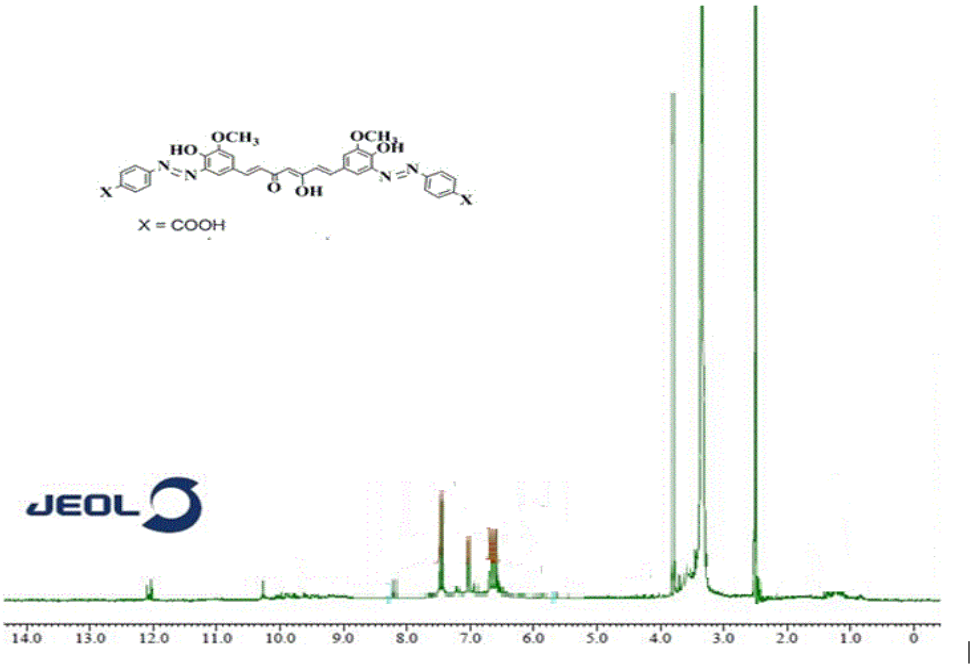


Figure A12

[1]H-NMR Spectra of (1E,4Z,6E)-1,7-bis(3-((E)-(4-(dimethylamino)phenyl)diazenyl)-4-hydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one. (5)

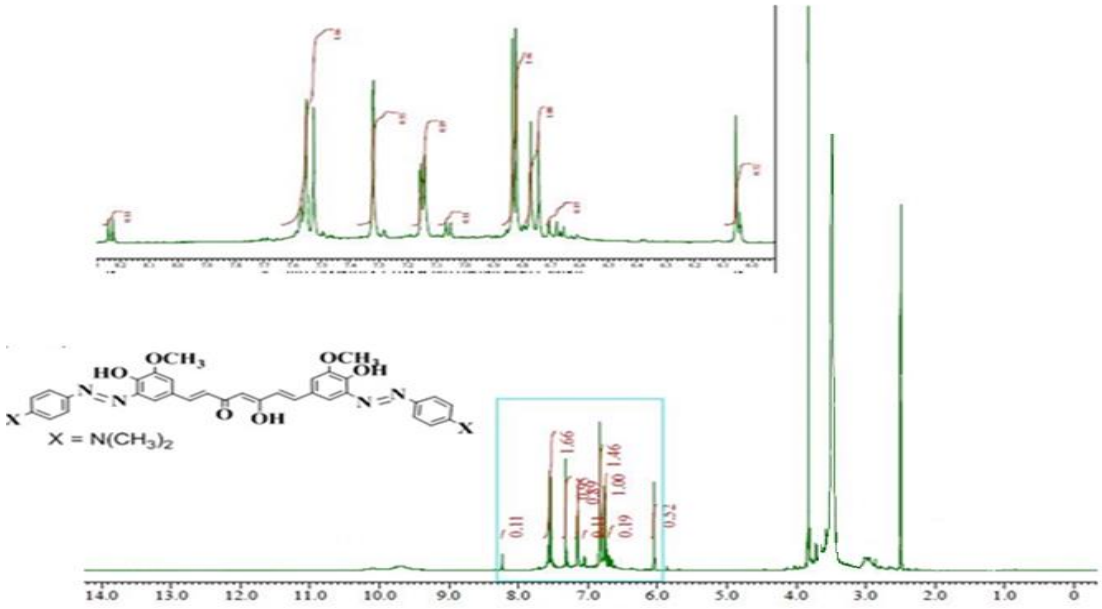


Figure A13

[1]H-NMR Spectra of 3,3'-((((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(propane-1-sulfonic acid). (6)

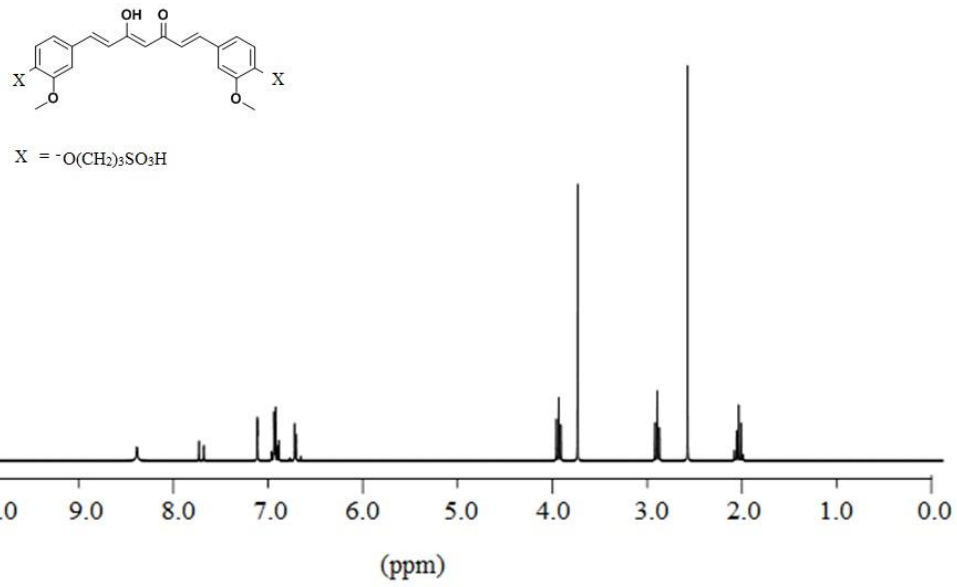


Figure A14

^{1}H-NMR Spectra 4,4'-((((1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(butane-1-sulfonic acid). (7)

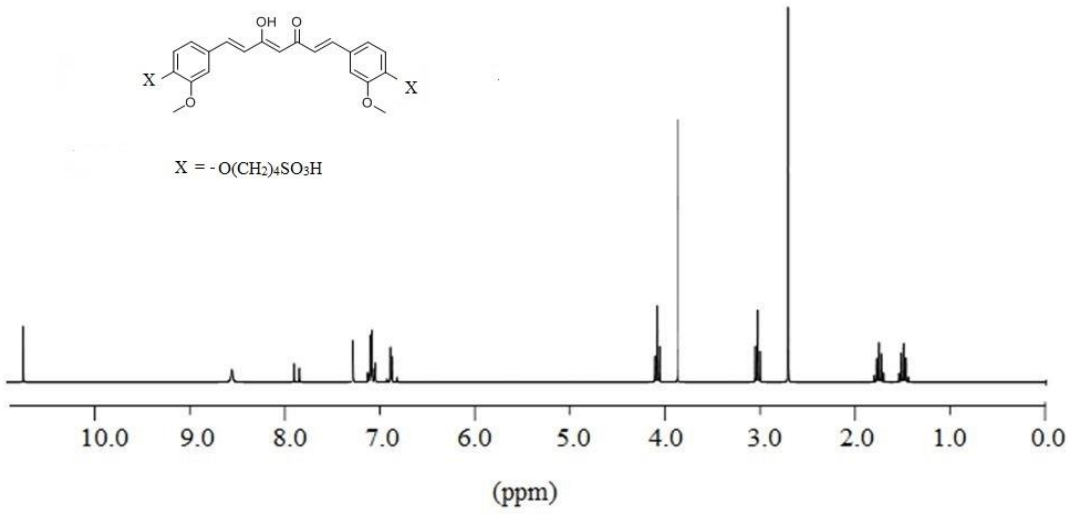


Figure A15

MTT Assay of MCF-7 Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

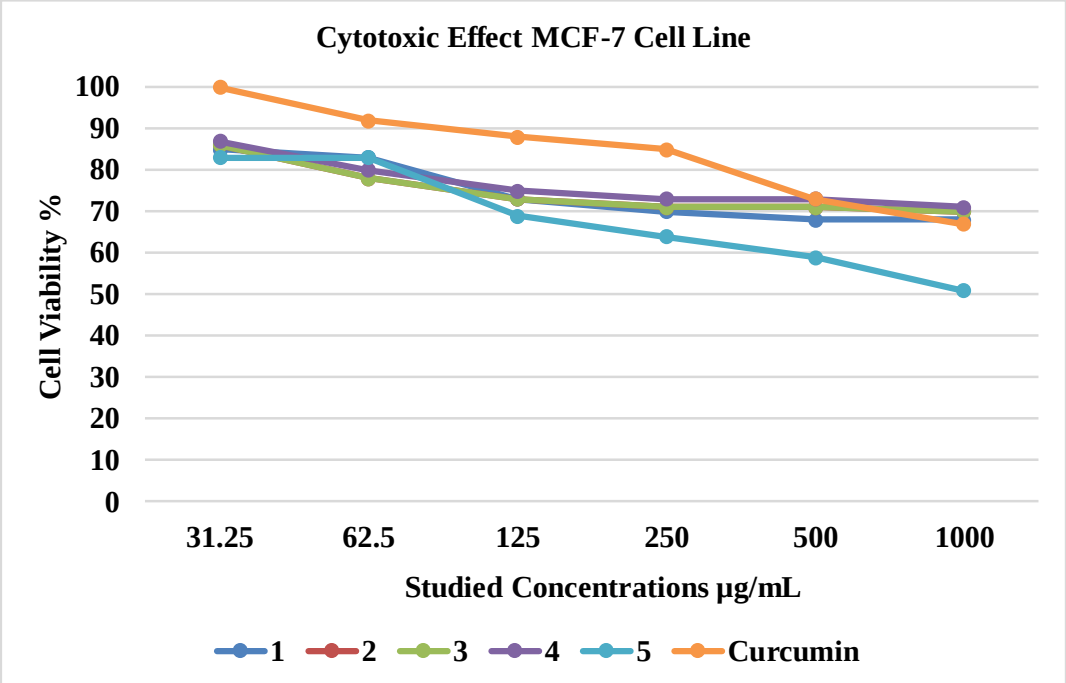


Figure A16

MTT Assay of MCF-7 Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

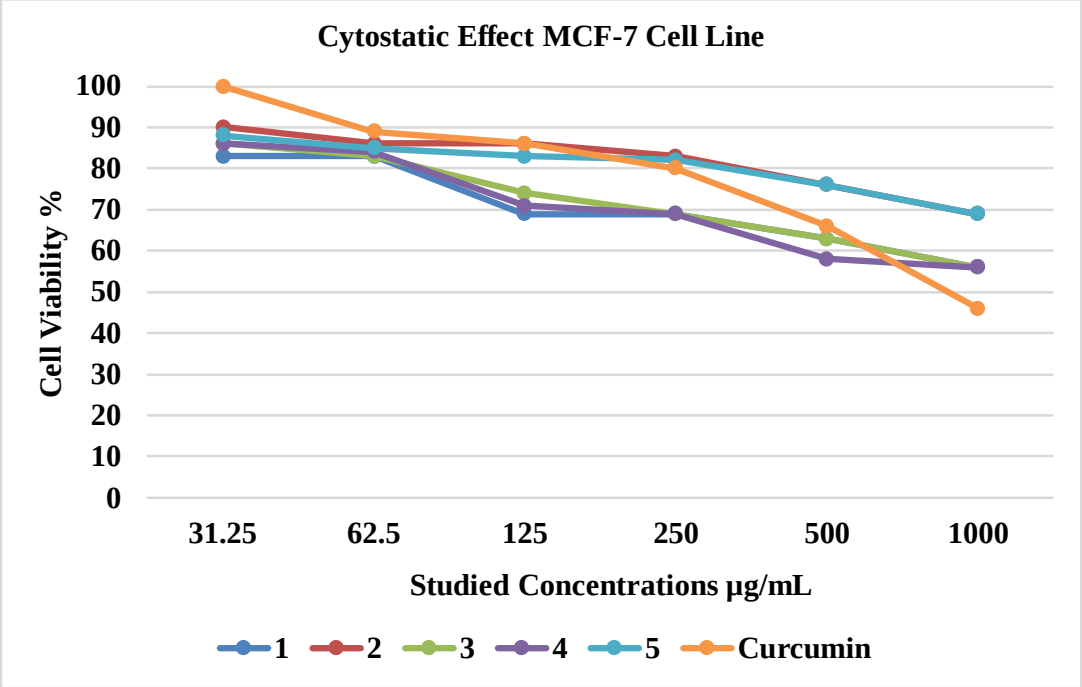


Figure A17

MTT Assay of HEPG2 Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

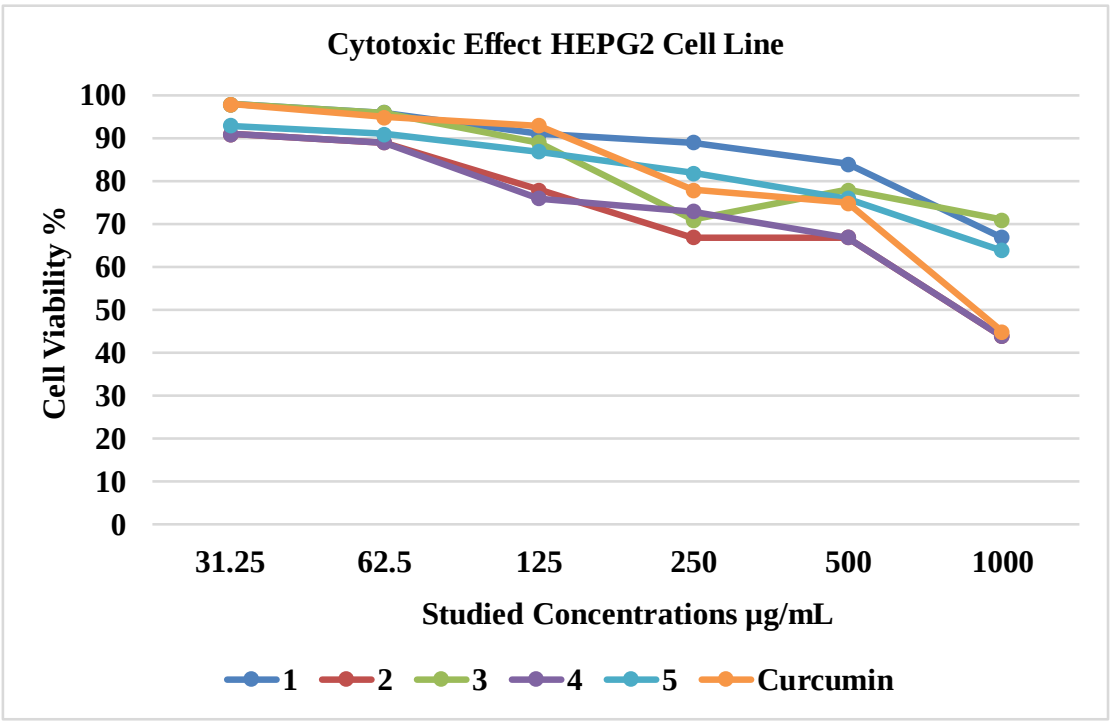


Figure A18

MTT Assay of HEPG2 Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

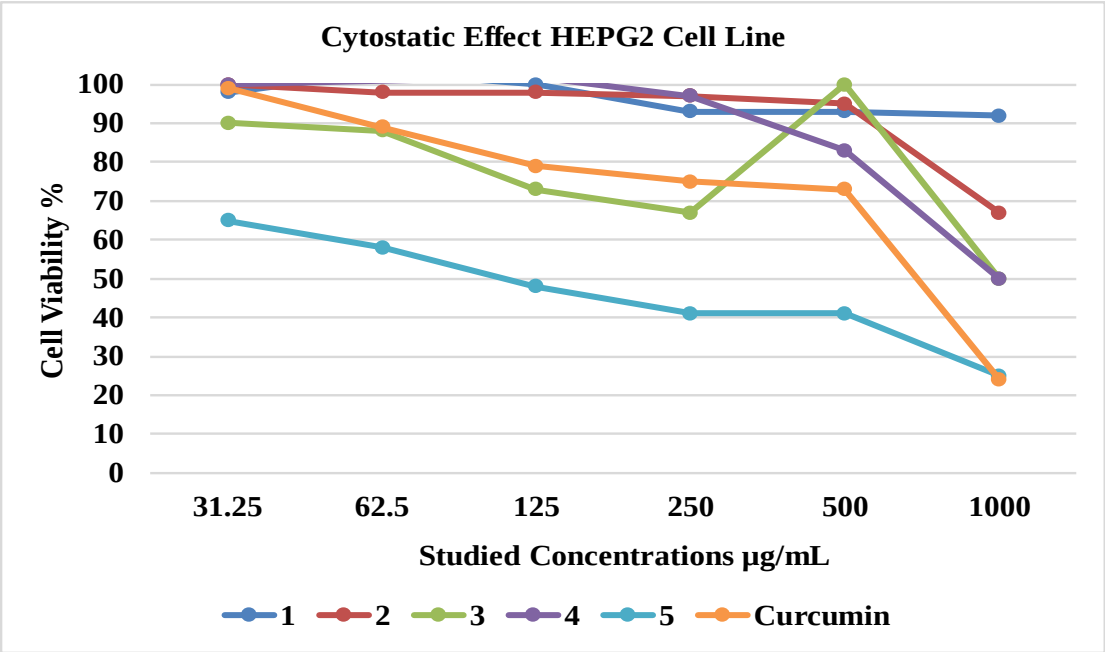


Figure A19

MTT Assay of B16-F10 Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

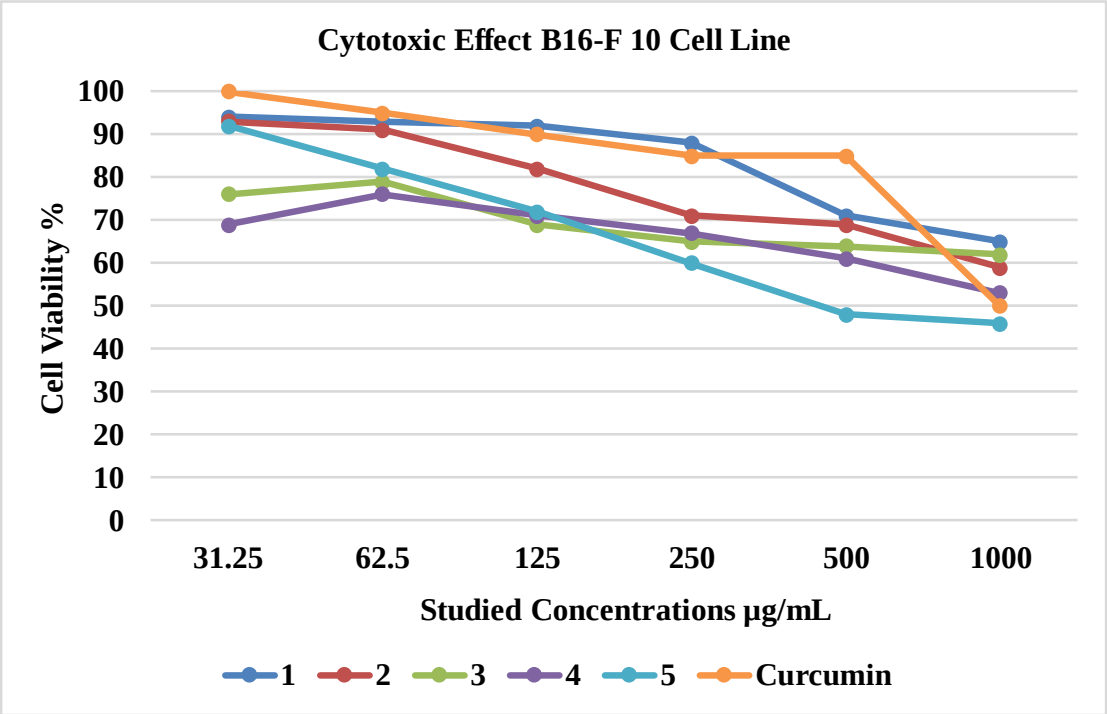


Figure A20

MTT Assay of B16-F10 Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin

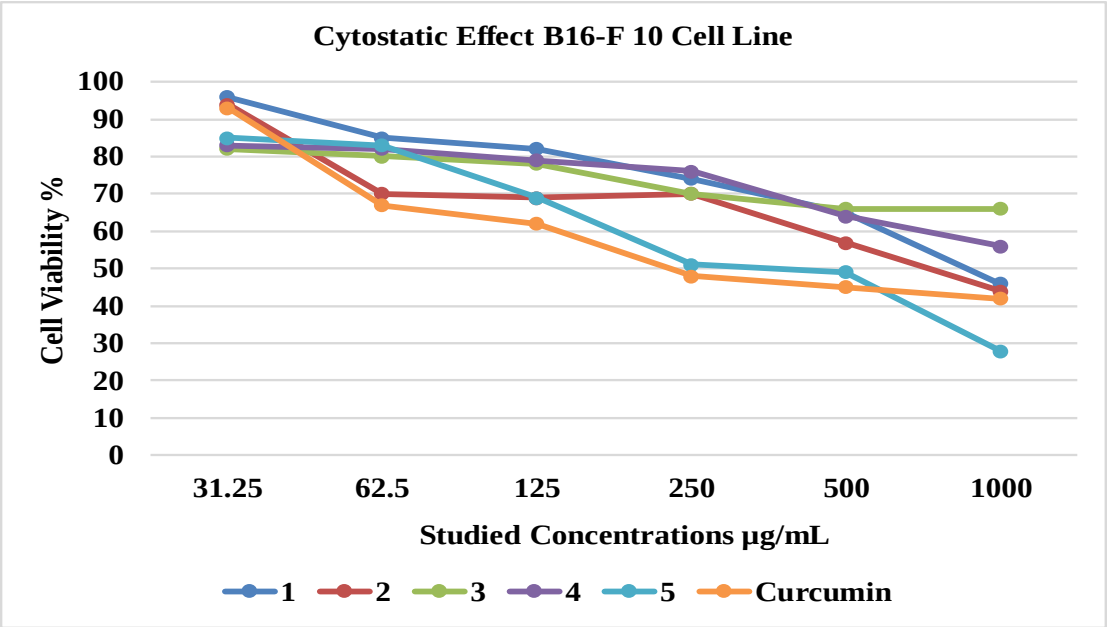


Figure A21

MTT Assay of Colon Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin.

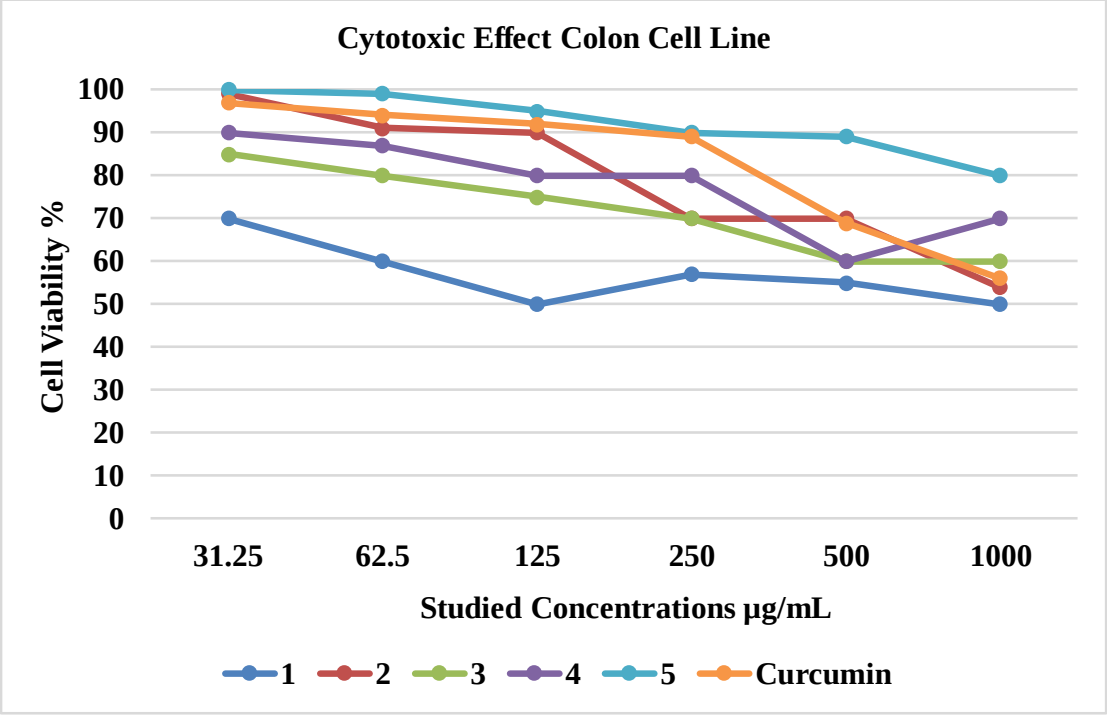


Figure A22

MTT Assay of Colon Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin.

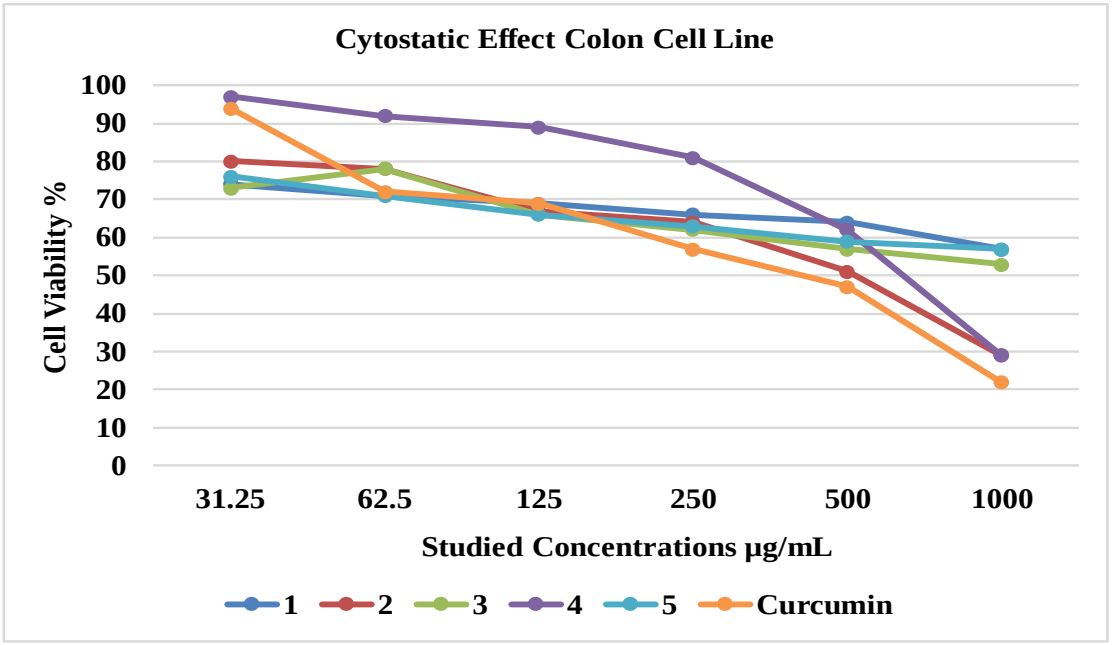


Figure A23

MTT Assay of HeLa Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin.

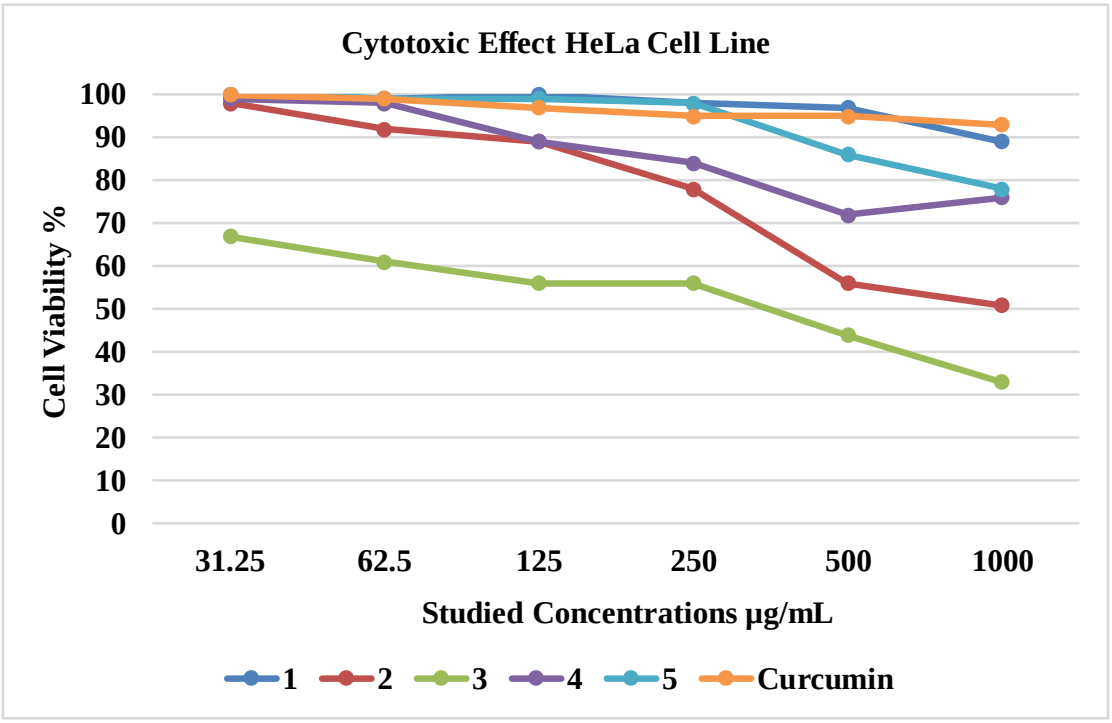


Figure A24

MTT Assay of HeLa Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 1,2,3,4,5 and pure curcumin.

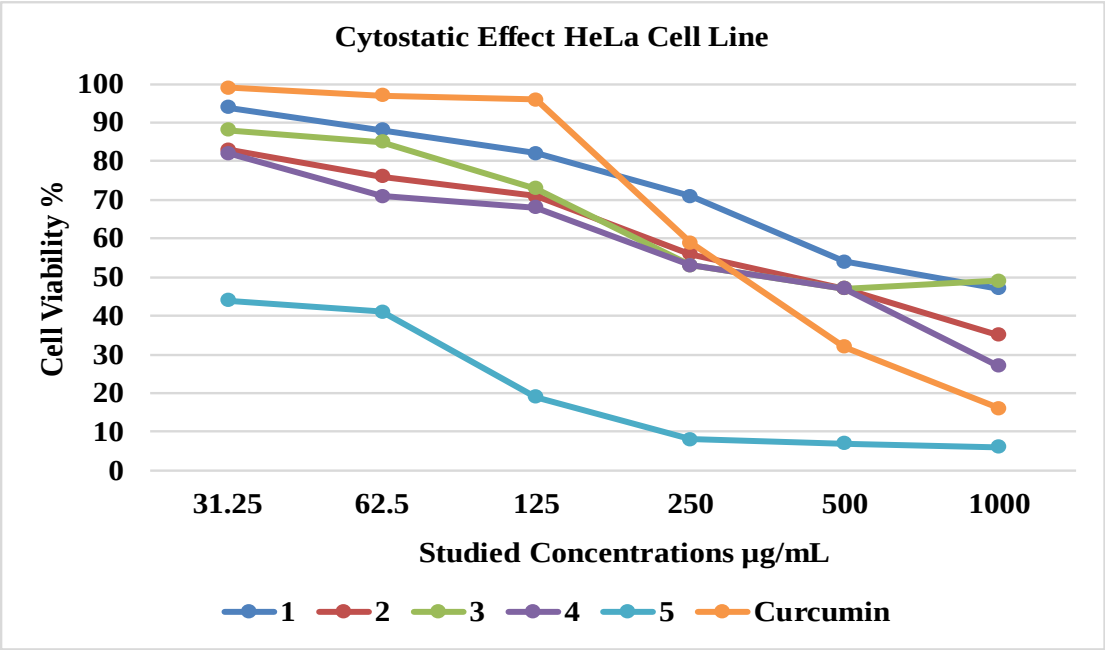


Figure A25

MTT Assay of MCF-7 Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin.

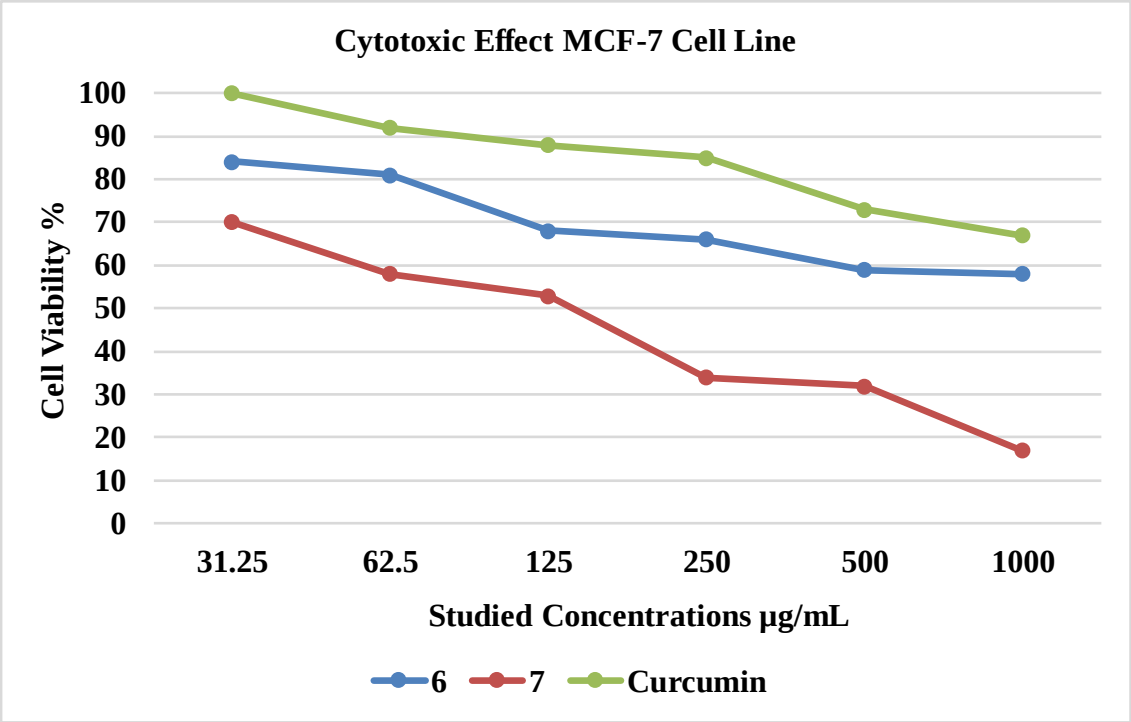


Figure A26

MTT Assay of MCF-7 Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin

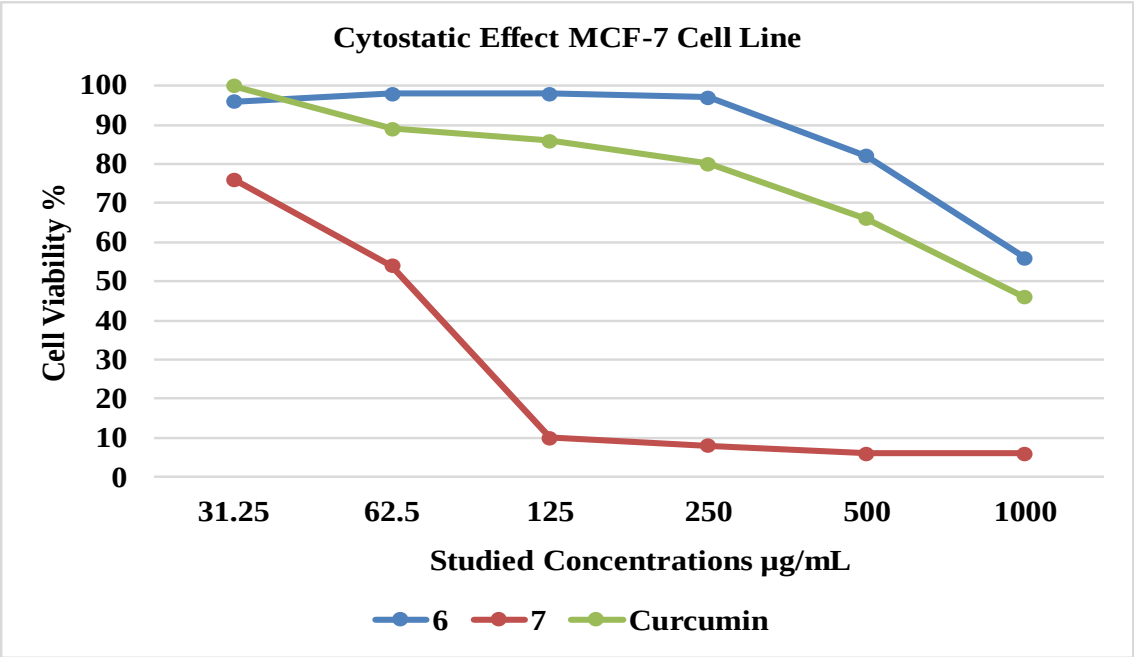


Figure A27

MTT Assay of HEPG2 Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin.

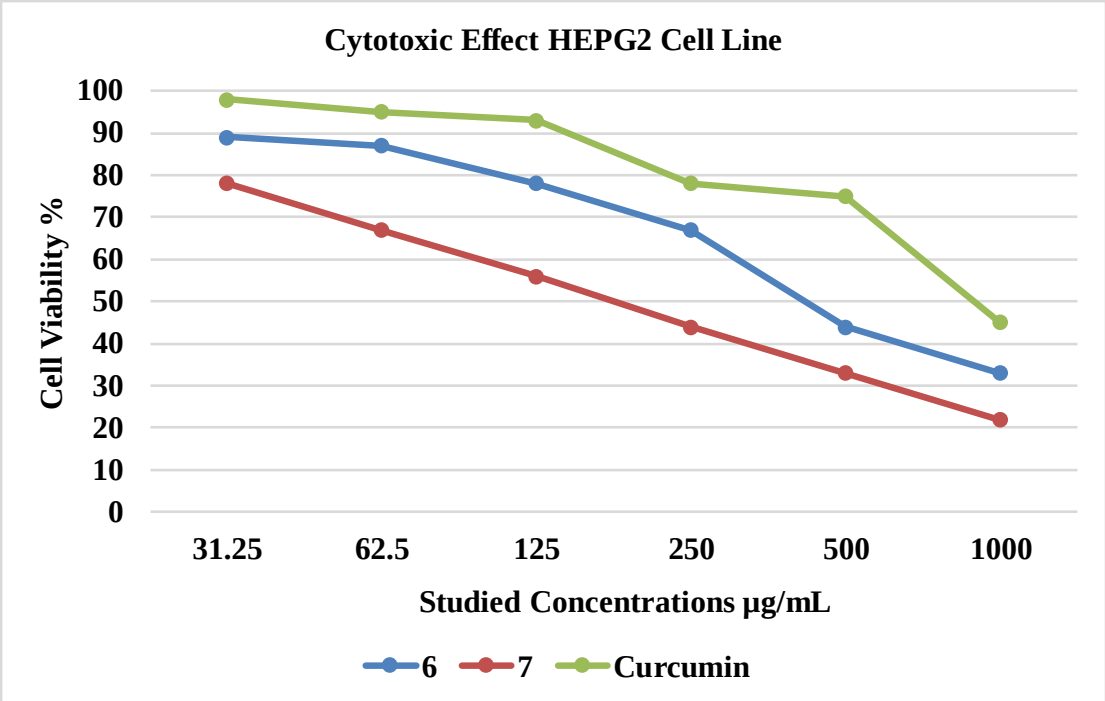


Figure A28

MTT Assay of HEPG2 Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin.

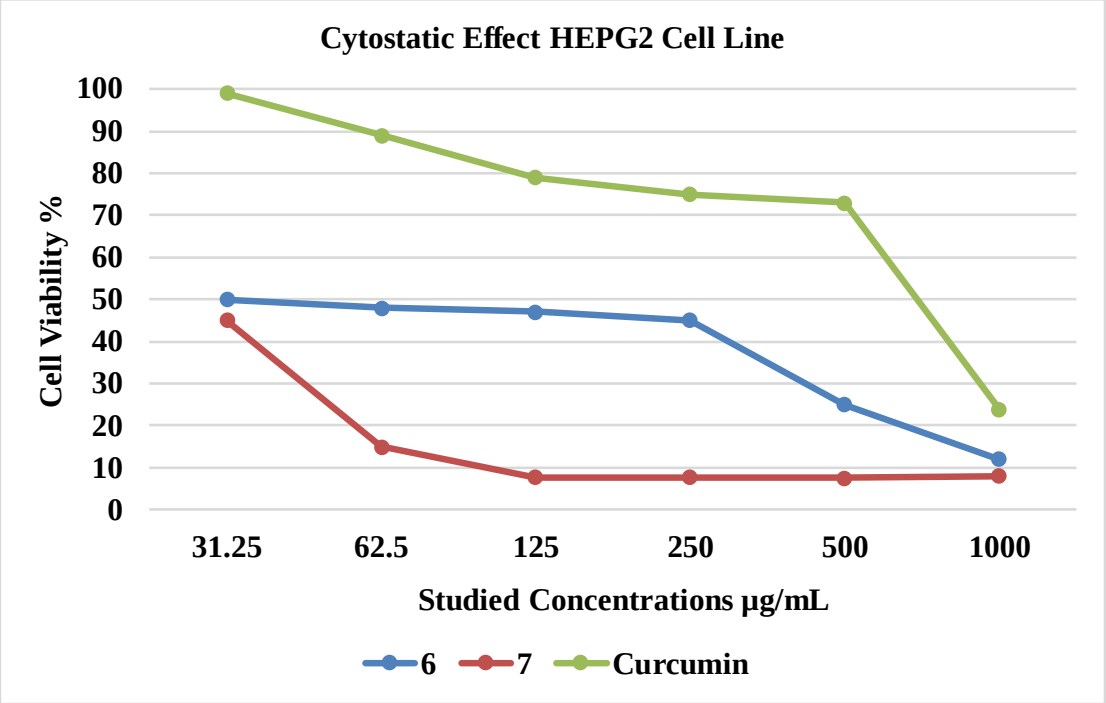


Figure A29

MTT Assay of B16-F10 Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin.

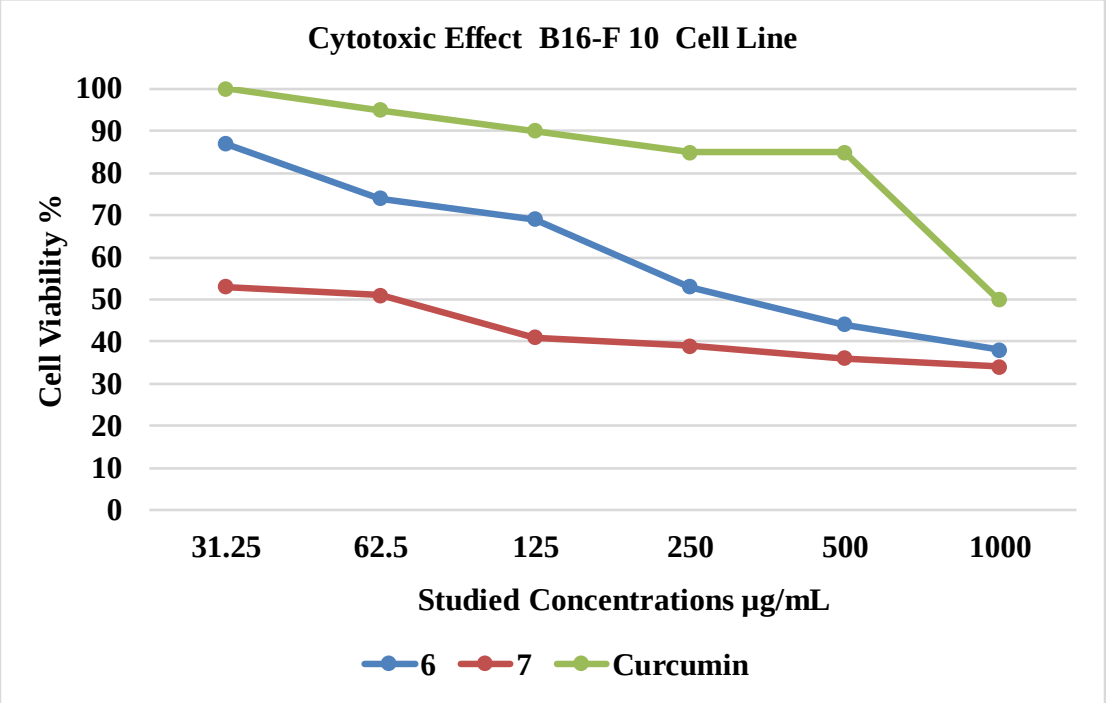


Figure A30

MTT Assay of B16-F10 Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 6,7and pure curcumin.

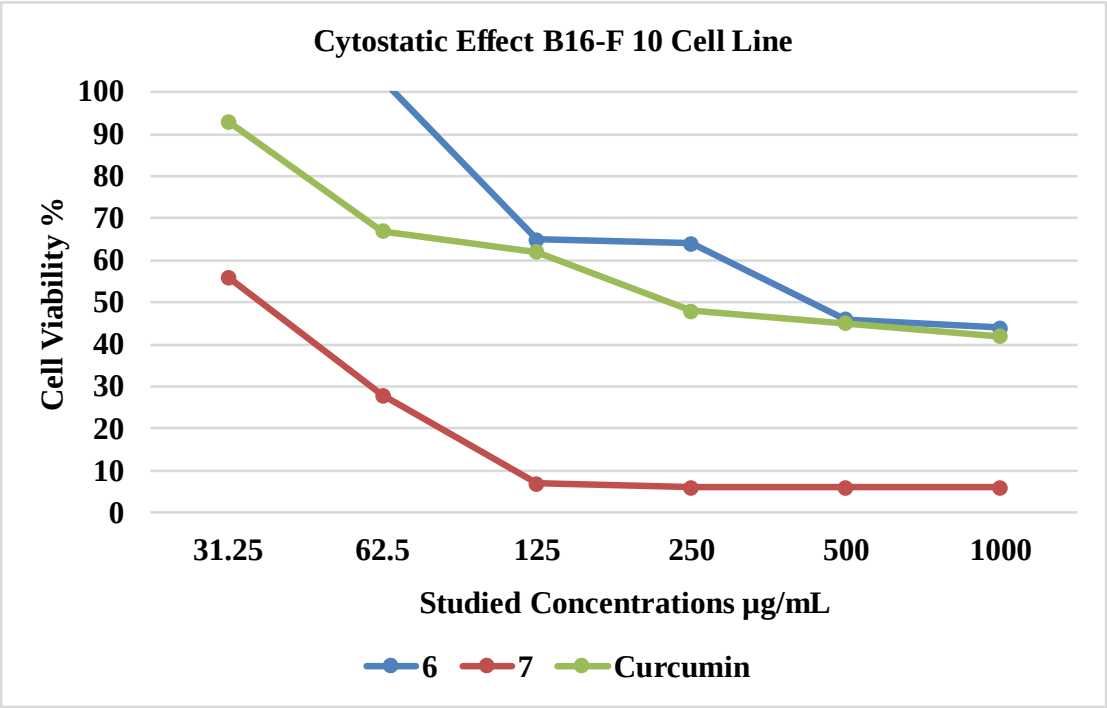


Figure A31

MTT Assay of Colon Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 6,7and pure curcumin.

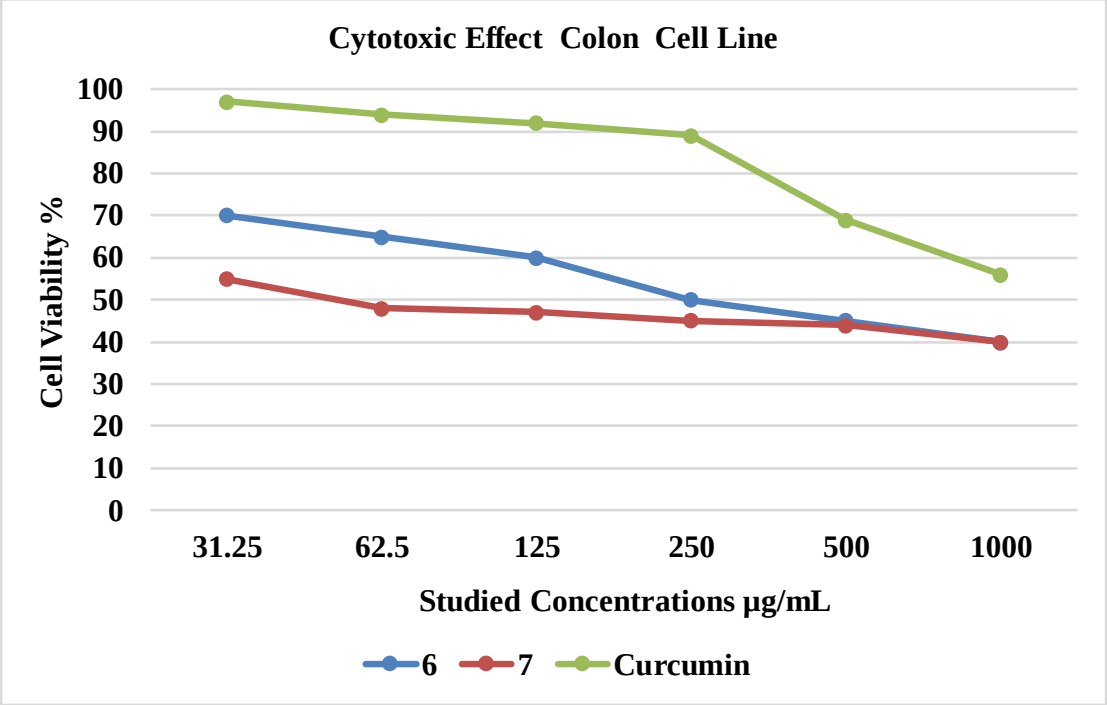


Figure A32

MTT Assay of Colon Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin.

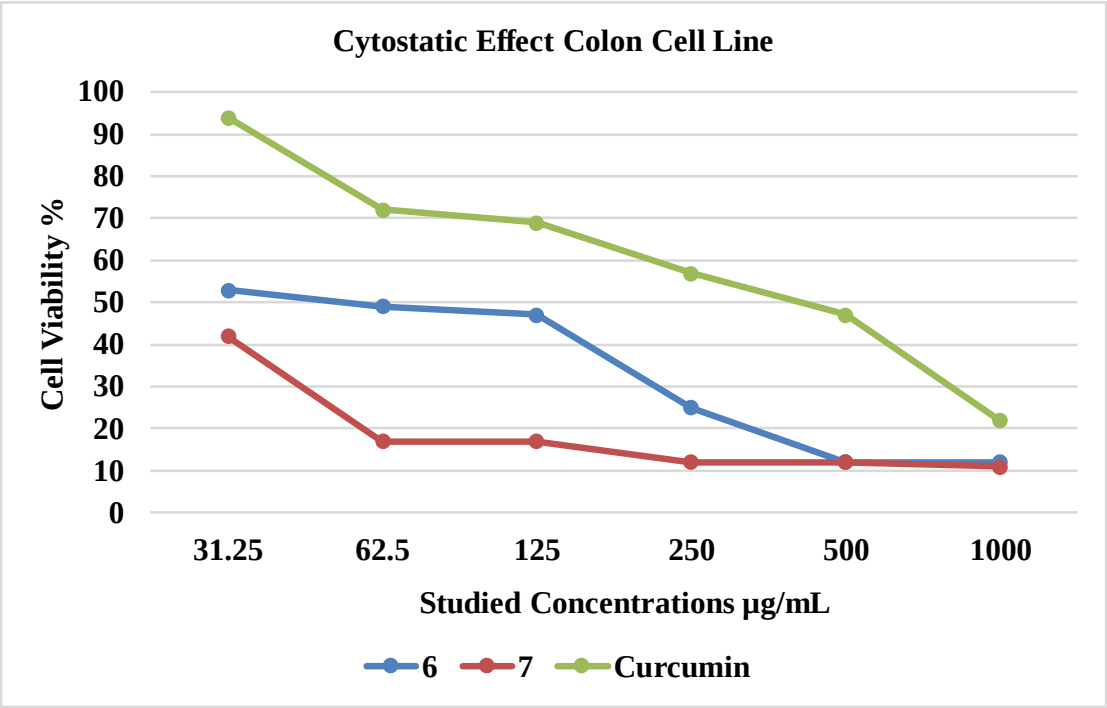


Figure A33

MTT Assay of HeLa Cell Line Viability % Cytotoxic (24 hrs) effect under different studied concentrations of compounds; 6,7and pure curcumin.

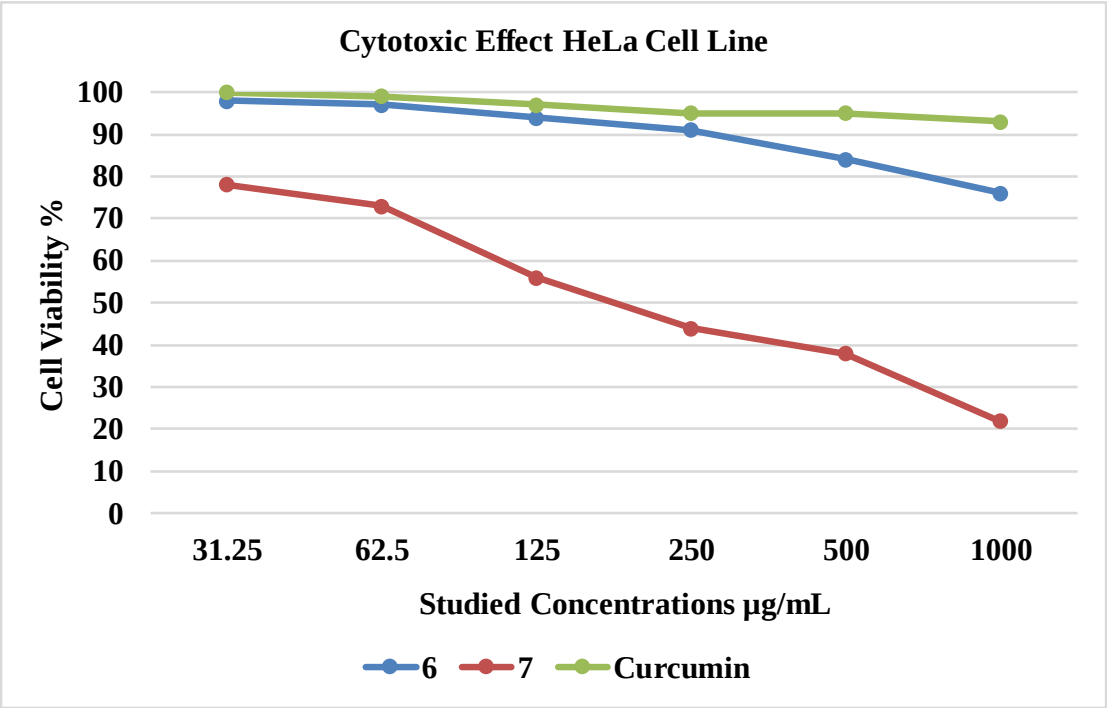


Figure A34

MTT Assay of HeLa Cell Line Viability % Cytostatic (72 hrs) effect under different studied concentrations of compounds; 6,7 and pure curcumin.

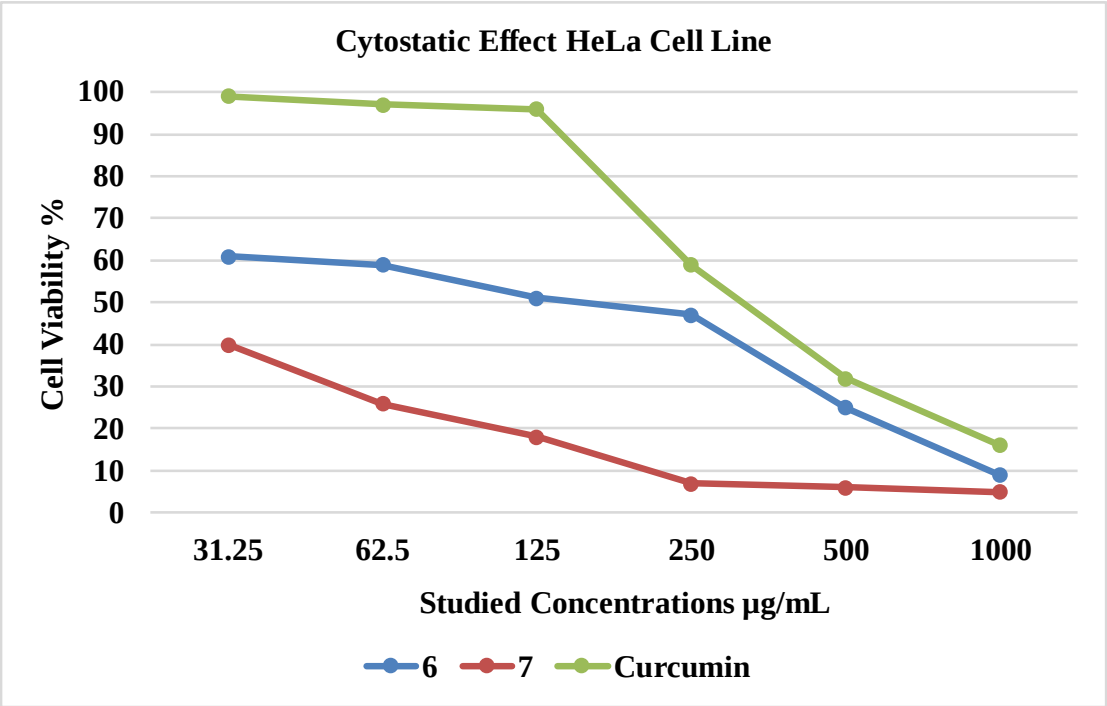


Figure A35

IC50 value (372.3 µg/mL) of compound 5 on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

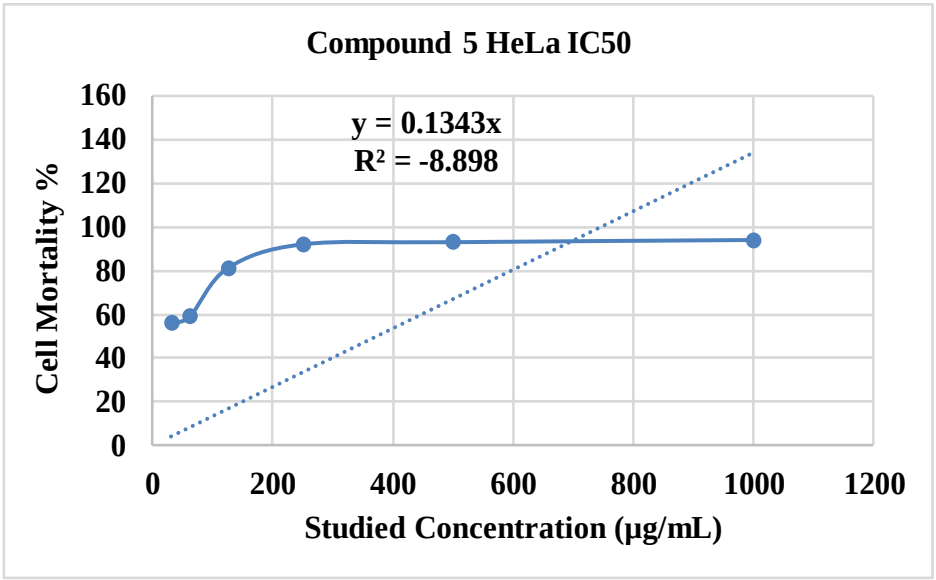


Figure A36

IC50 value (516.5 µg/mL) of curcumin on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

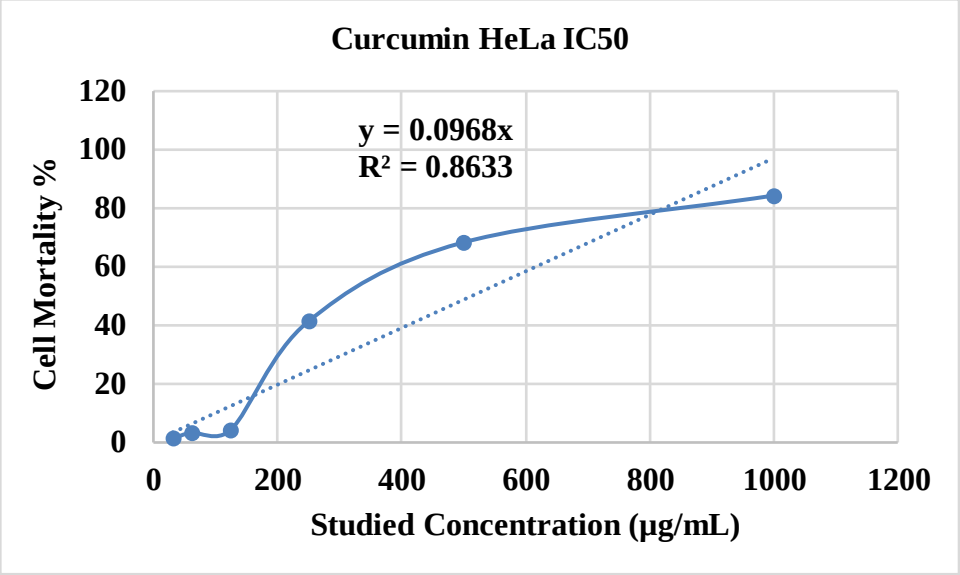


Figure A37

IC50 value (672.9 µg/mL) of curcumin on HEPG2 liver carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

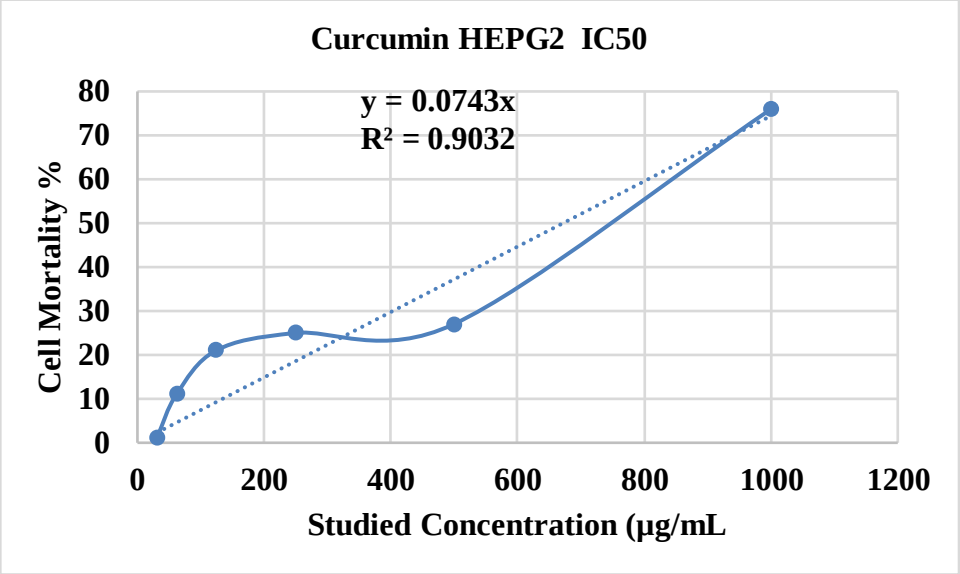


Figure A38

IC50 value (514.9 µg/mL) of compound 5 on HEPG2 liver carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

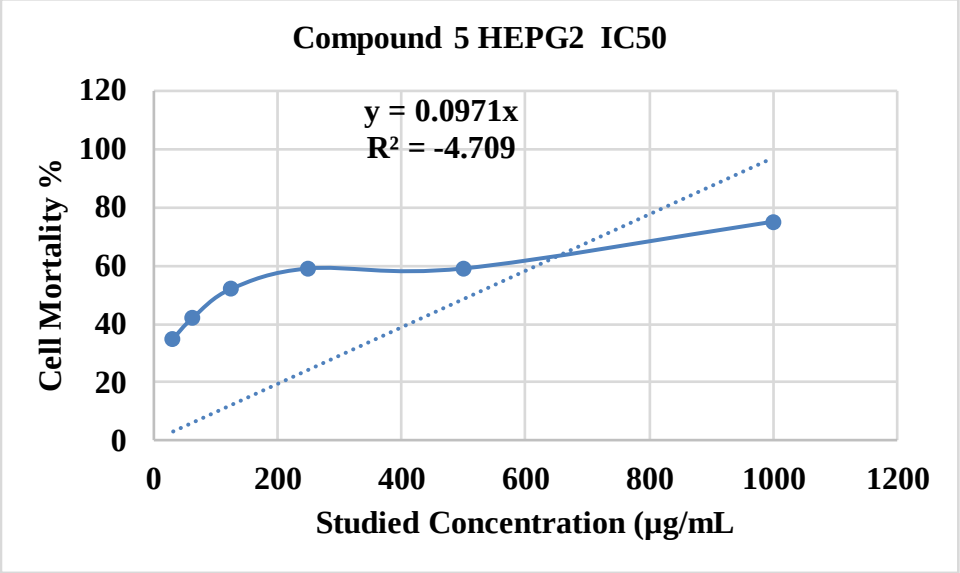


Figure A39

IC50 value (819.6 µg/mL) of compound 1on B16-F10 skin melanoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

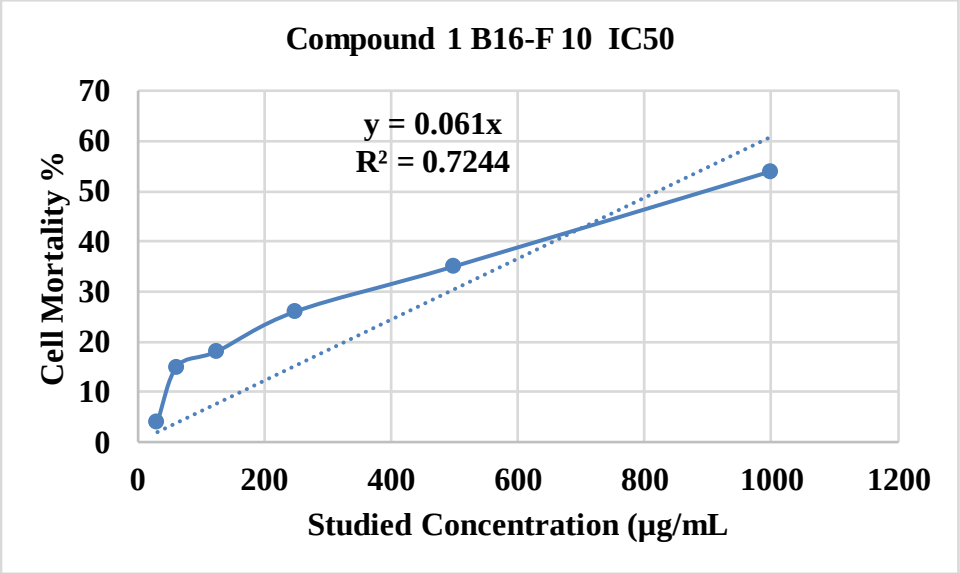


Figure A40

IC50 value (771.6 µg/mL) of compound 1 on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

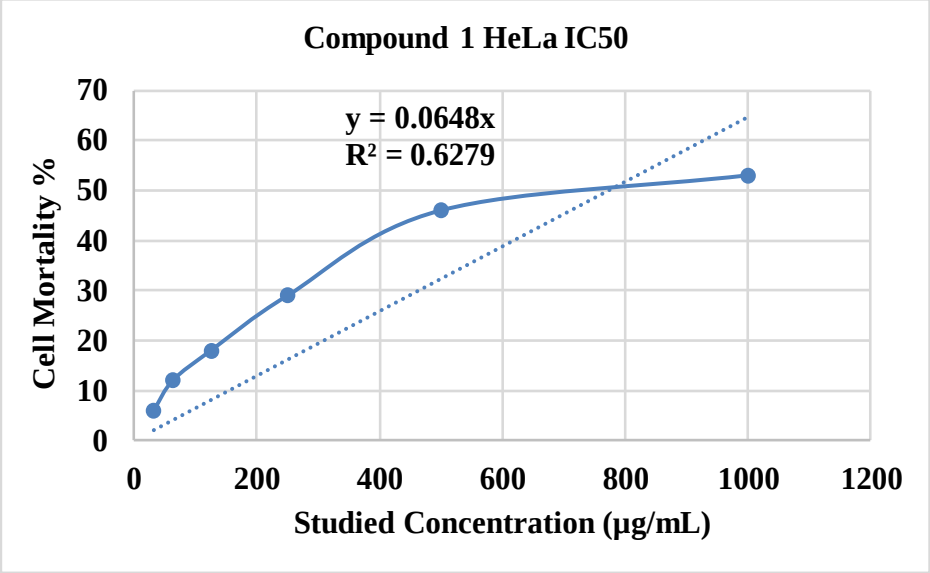


Figure A41

IC50 value (733.1µg/mL) of compound 2on B16-F10 skin melanoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

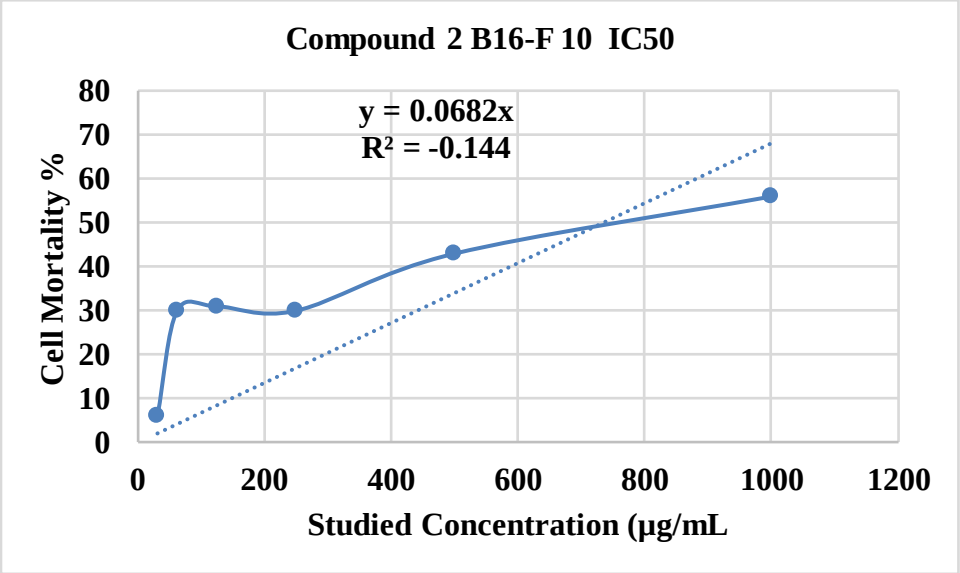


Figure A42

IC50 value (602.4 µg/mL) of compound 2on Colon human cancer cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

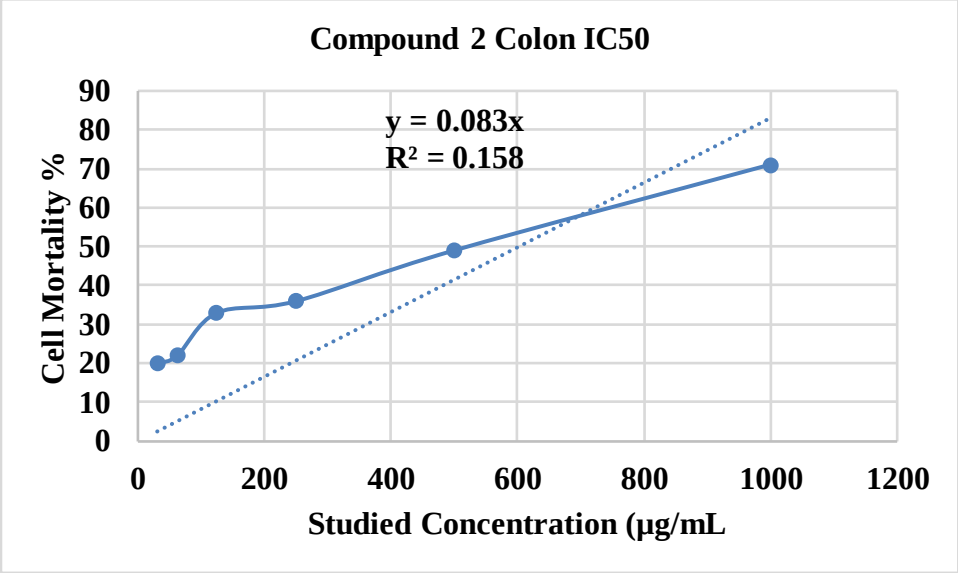


Figure A43

IC50 value (616.5 µg/mL) of compound 2 on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

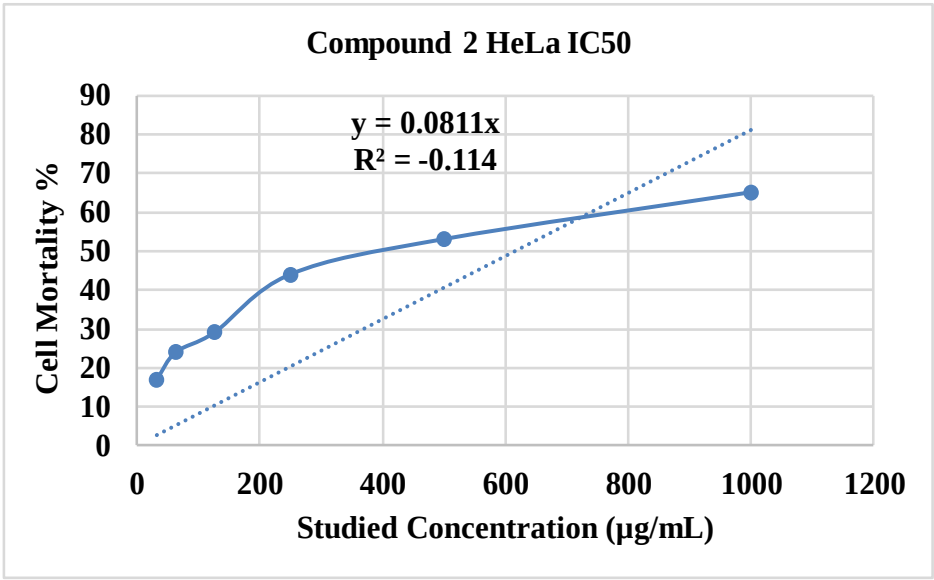


Figure A44

IC50 value (793.6 µg/mL) of compound 3on Colon human cancer cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

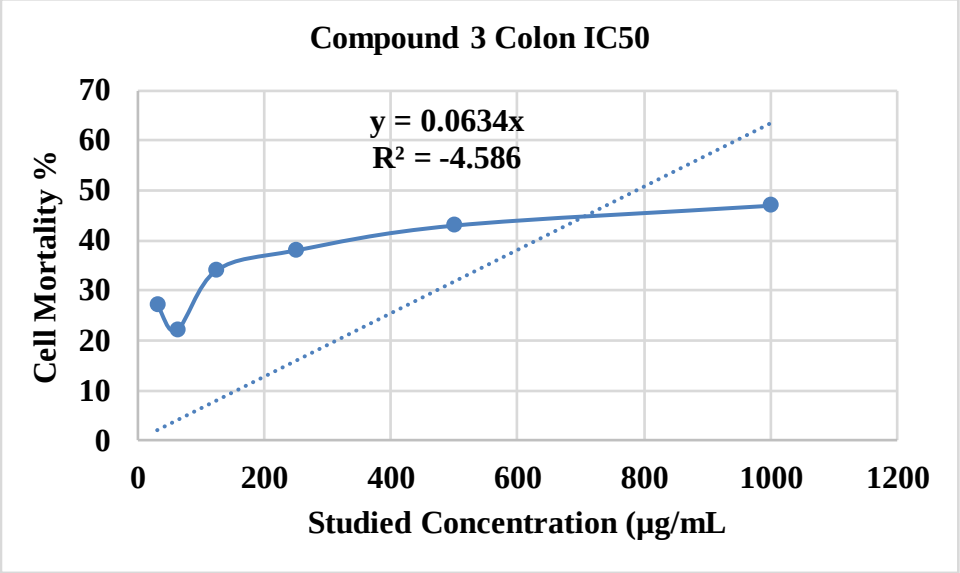


Figure A45

IC50 value (566.8 µg/mL) of compound 4 on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

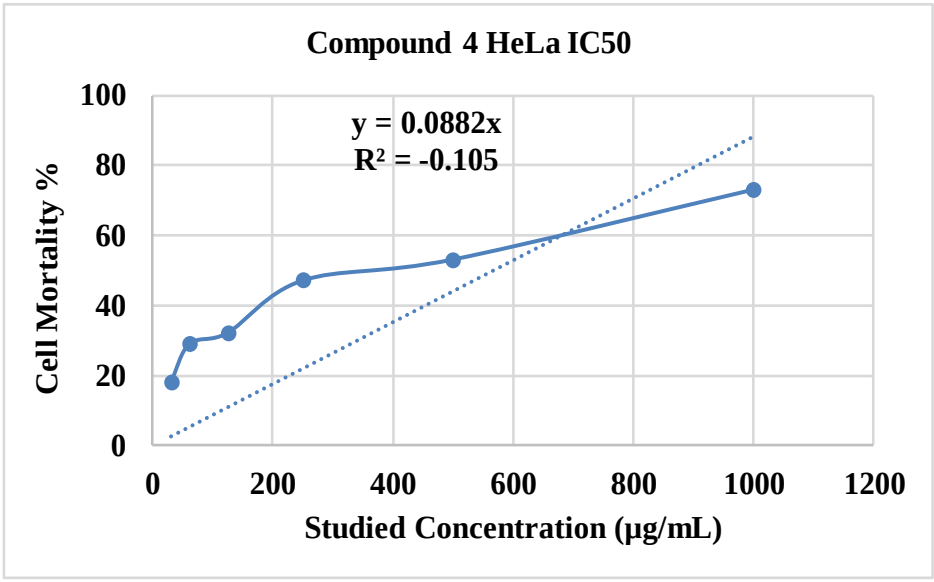


Figure A46

IC50 value (837.5 µg/mL) of compound 5 on Colon human cancer cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

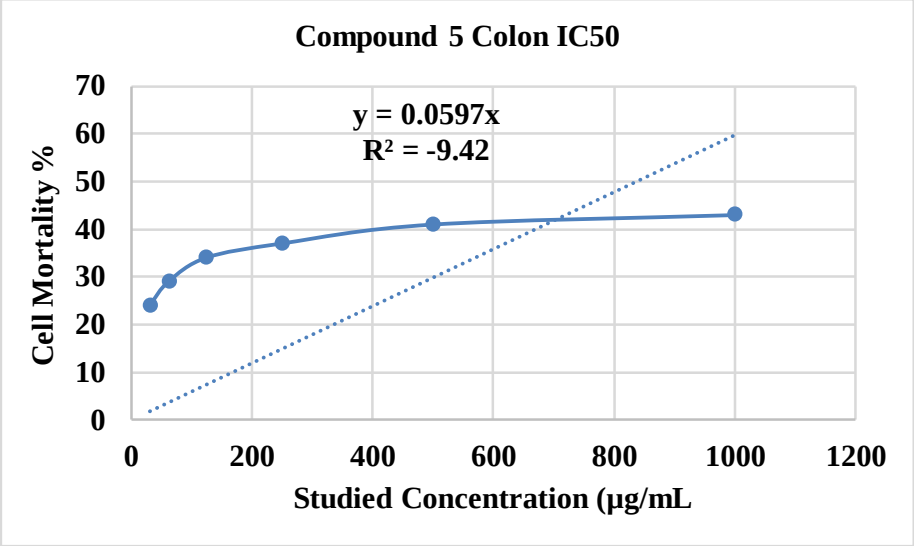


Figure A47

IC50 value (850.3 µg/mL) of curcumin on MCF-7 breast carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

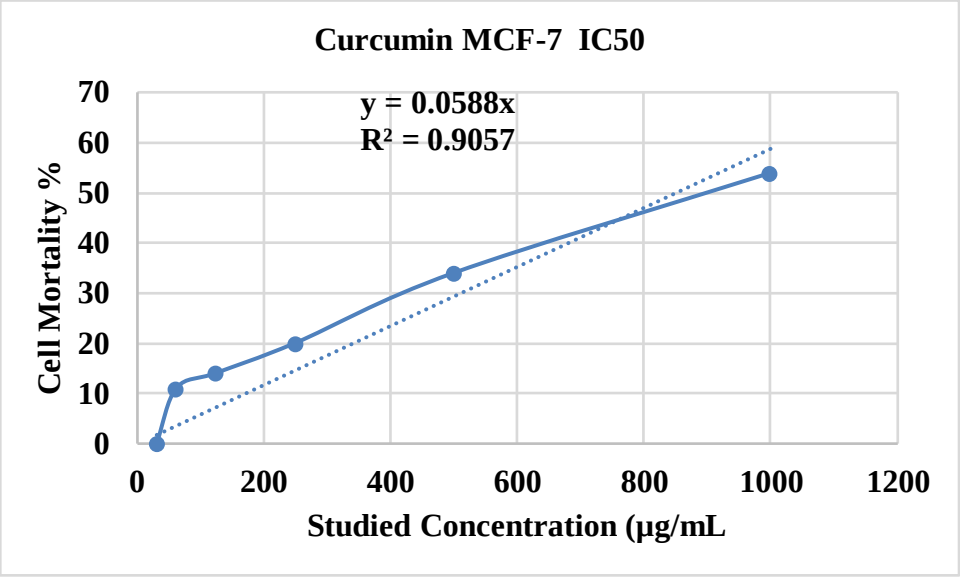


Figure A48

IC50 value (631.3 µg/mL) of curcumin on B16-F 10 skin melanoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

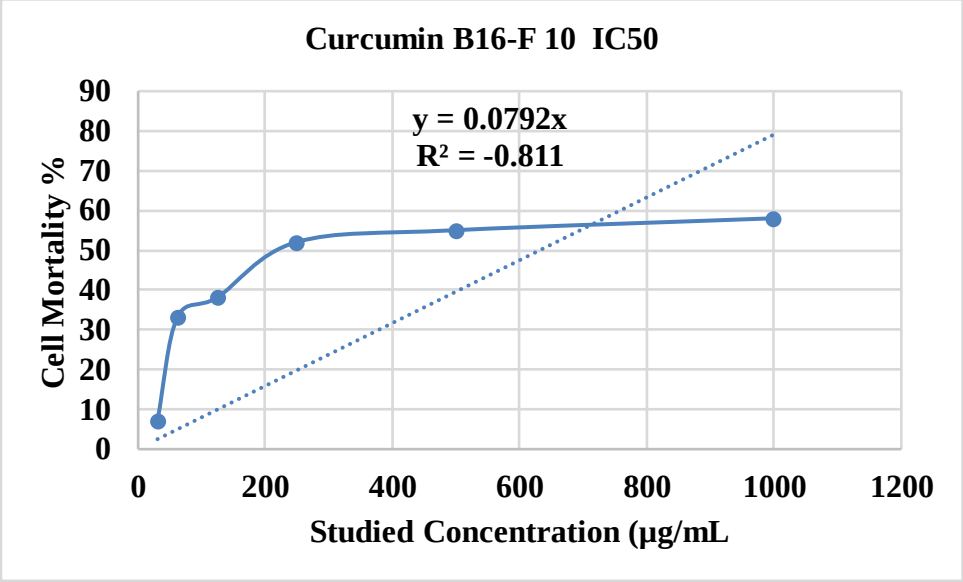


Figure A49

IC50 value (550.7 µg/mL) of curcumin on Colon human carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

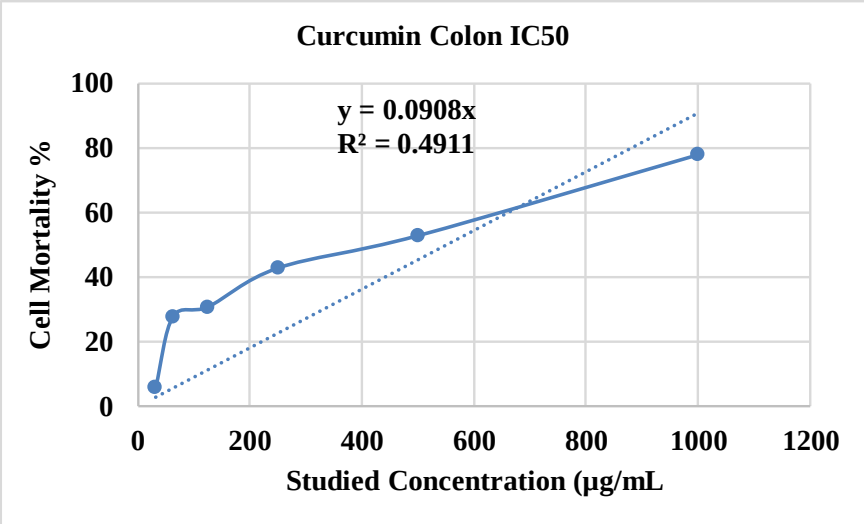


Figure A50

IC50 value (372.5 µg/mL) of compound 7on MCF-7 breast carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

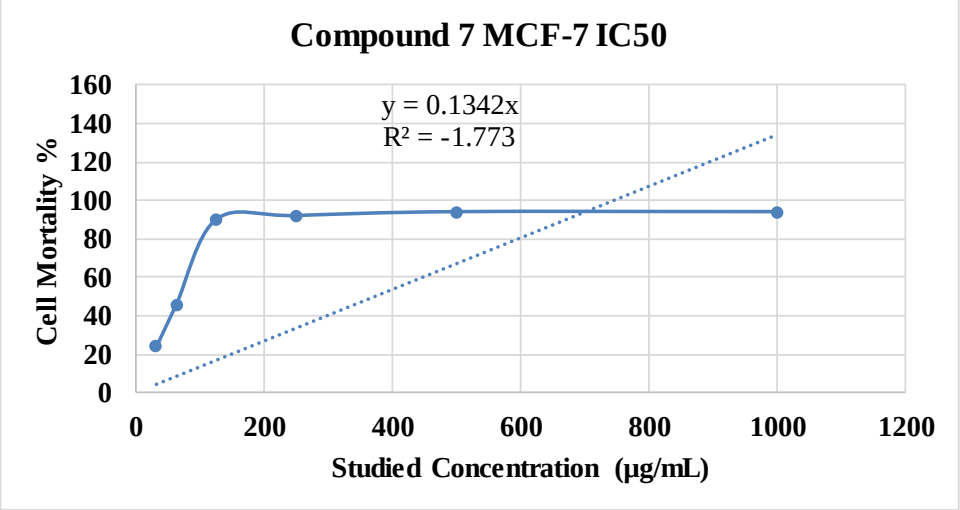


Figure A51

IC50 value (370.6 µg/mL) of compound 7on HEPG2 liver carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

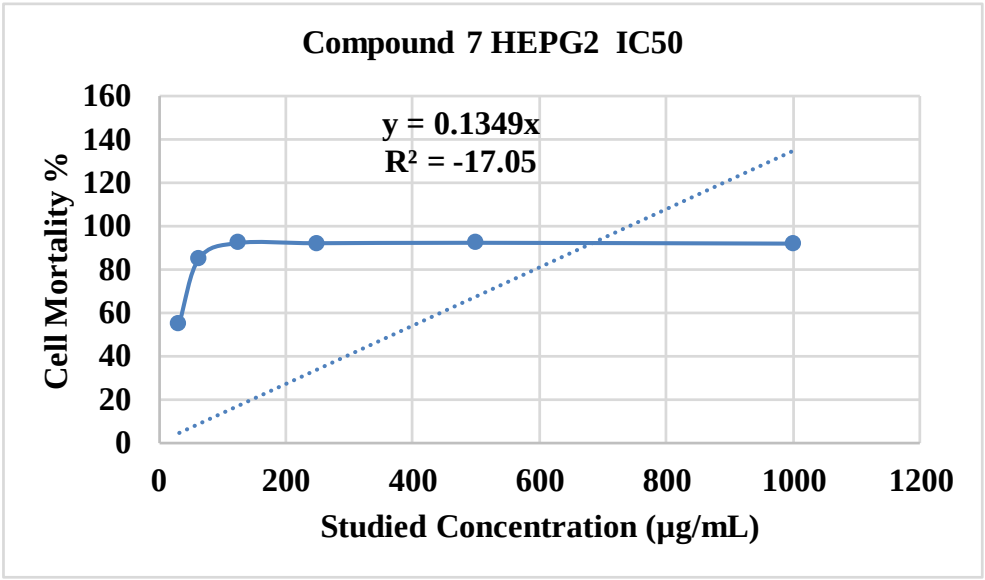


Figure A52

IC50 value (366.3 µg/mL) of compound 7on B16-F110 skin melanoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

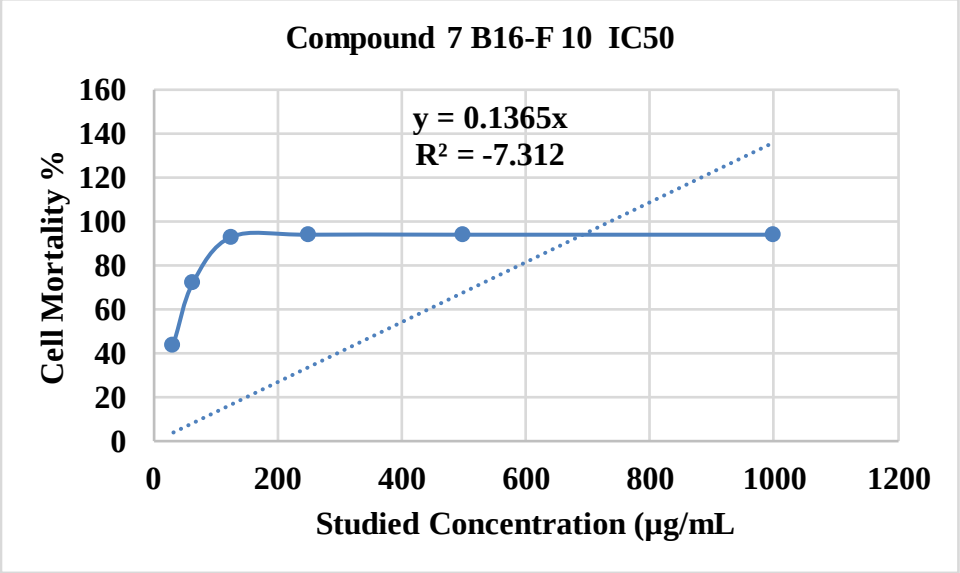


Figure A53

IC50 value (386.6 µg/mL) of compound 7on Colon human cancer cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

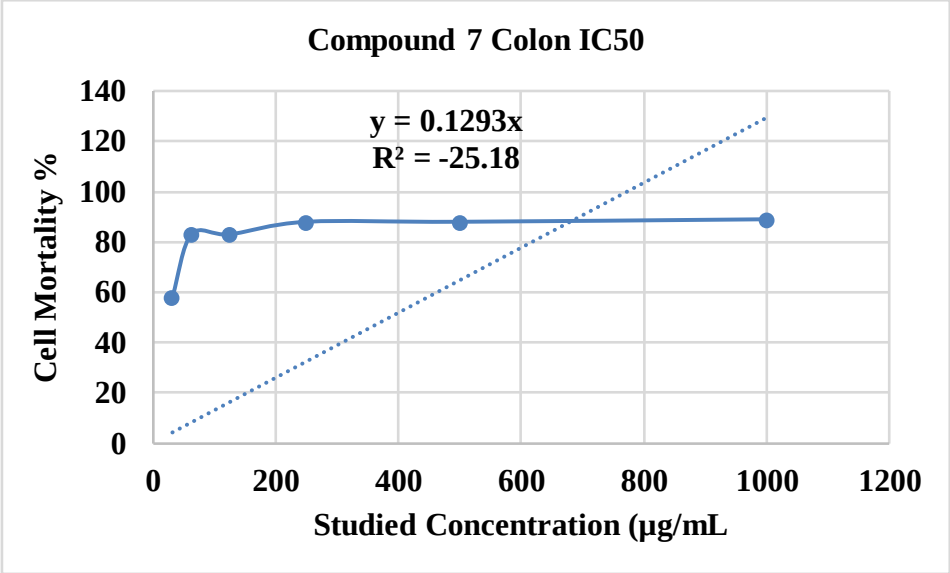


Figure A54

IC50 value (366.3 µg/mL) of compound 7on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

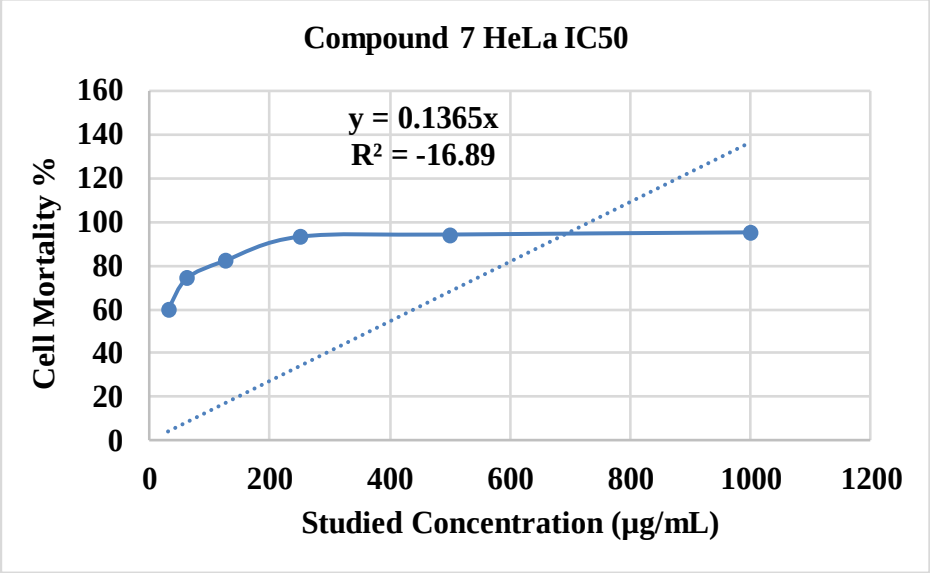


Figure A55

IC50 value (442.5 µg/mL) of compound 6on HEPG2 liver carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

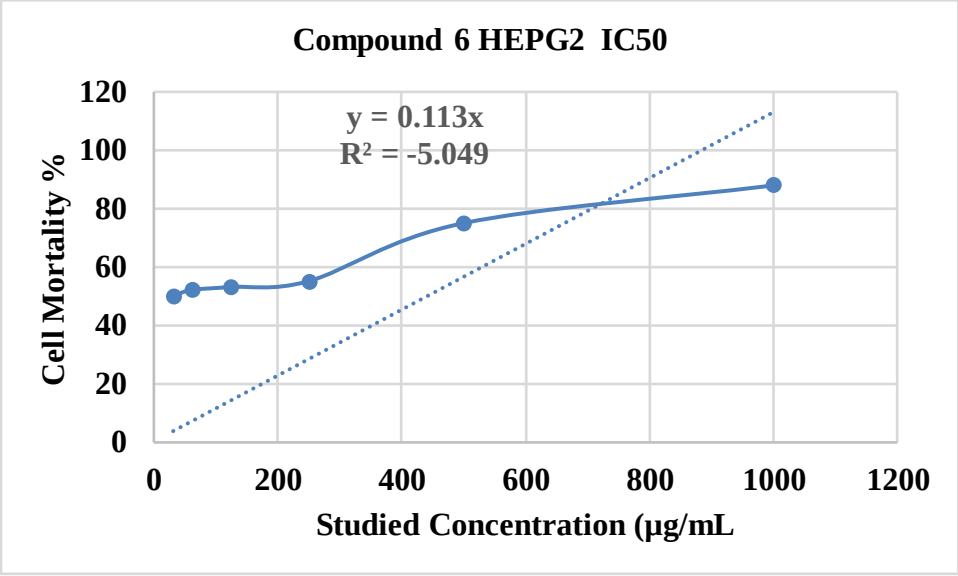


Figure A56

IC50 value (411.1 µg/mL) of compound 6 on Colon human cancer cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

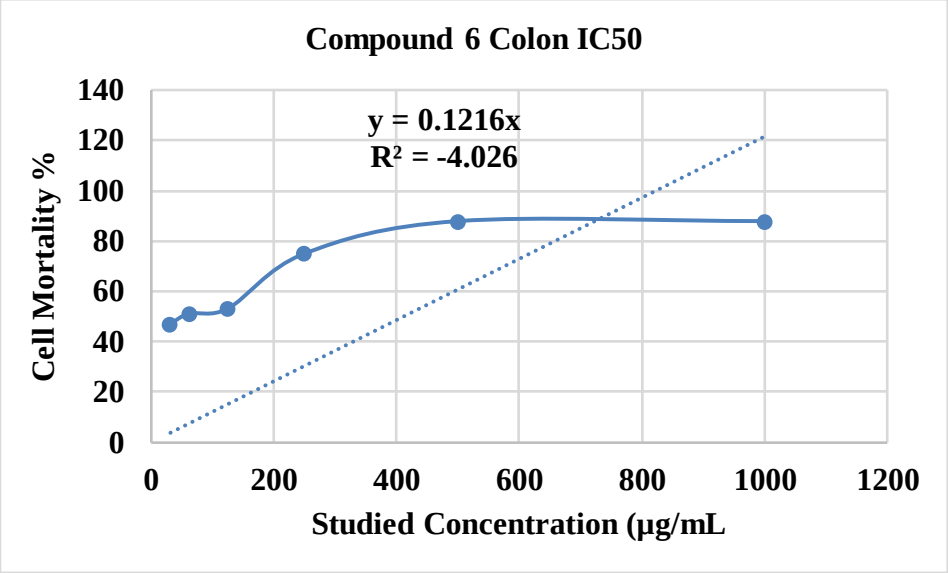


Figure A57

IC50 value (439.3 µg/mL) of compound 6on HeLa cervical carcinoma cell line after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test.

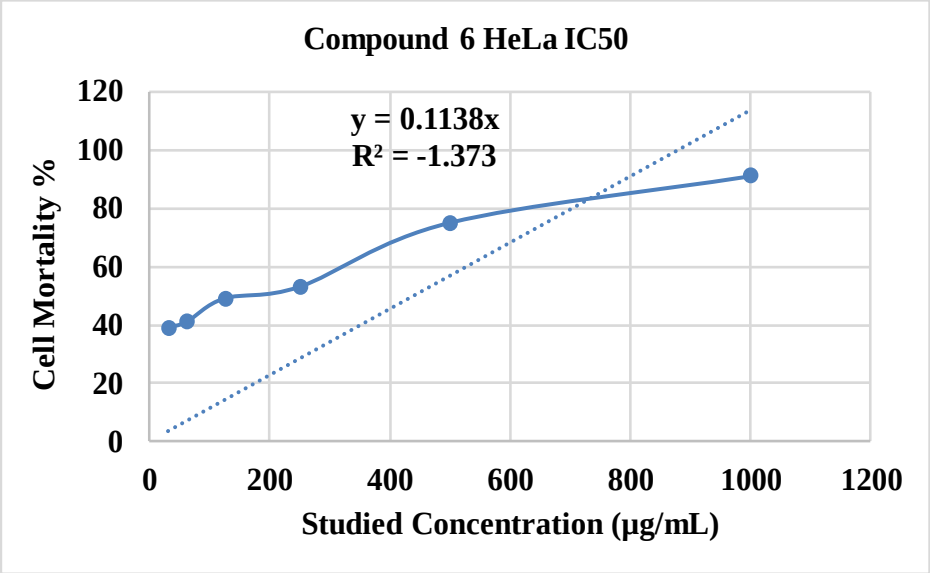


Figure A58

Chemical structure of Mauveine (3)

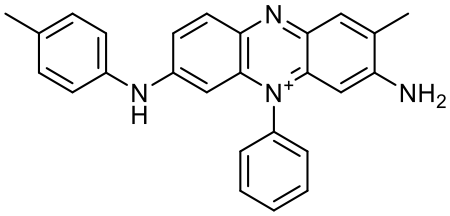


Figure A59

First examples of Azo dyes prepared by Bismark Brown 1958

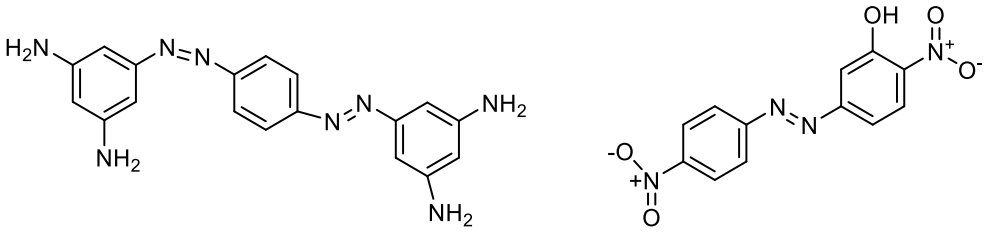


Figure A60

The chemical structure of disperse orange(18)

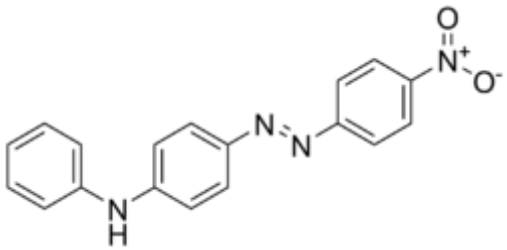


Figure A61

Classification of azo dye functional group

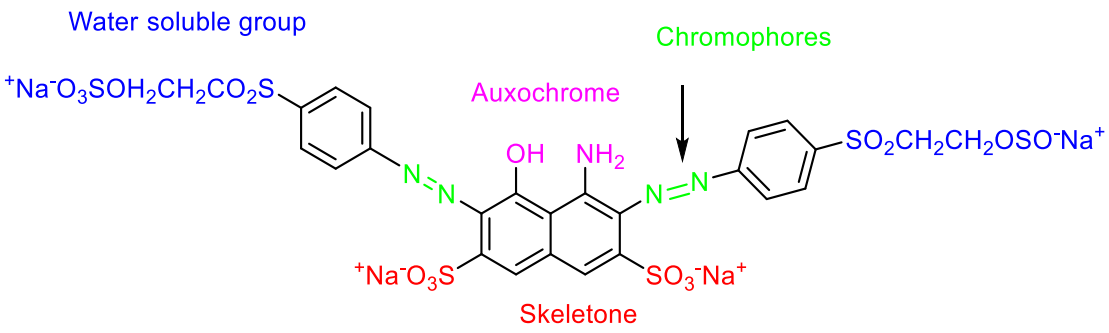


Figure A62

A coupling of diazonium slat with a nucleophile

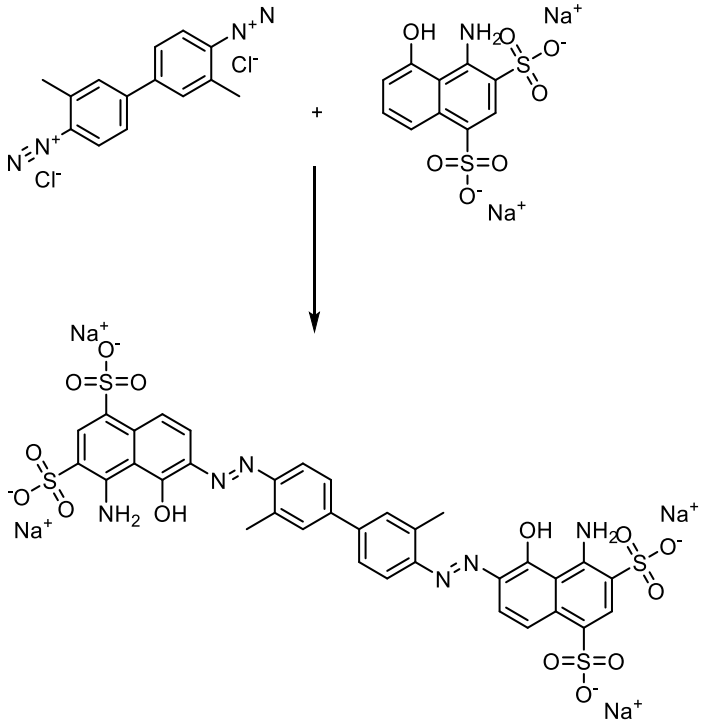


Figure A63

a: Metanil yellow, b: Orange II

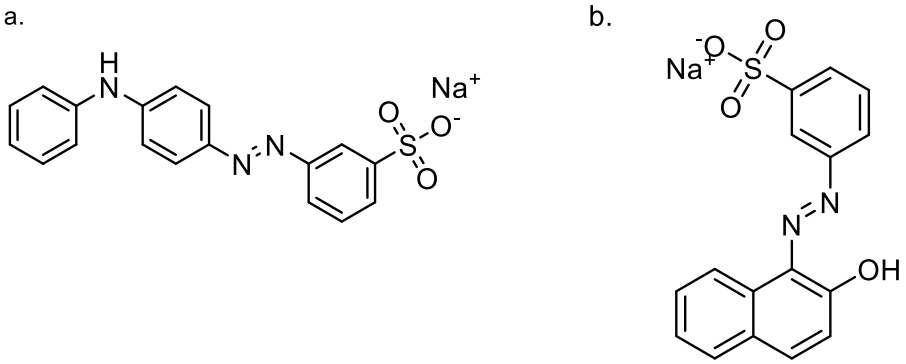


Figure A64

Acid black

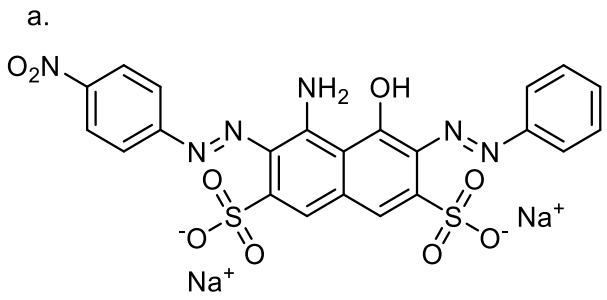


Figure A65

a: Methyl Orange, b: Orange G

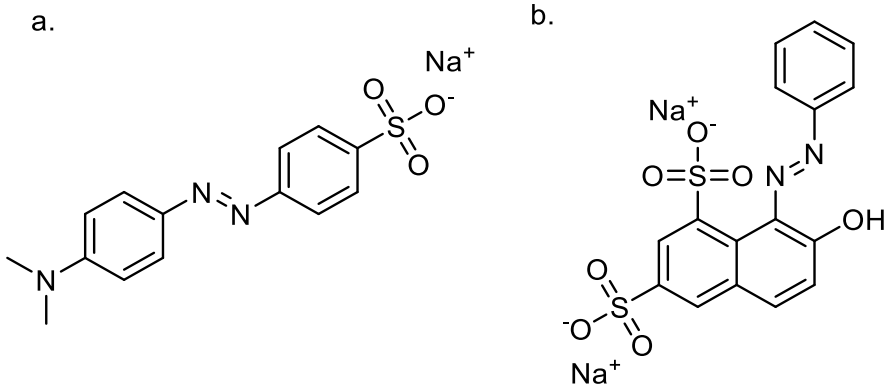


Figure A66

a: Aniline yellow, b: Butter yellow

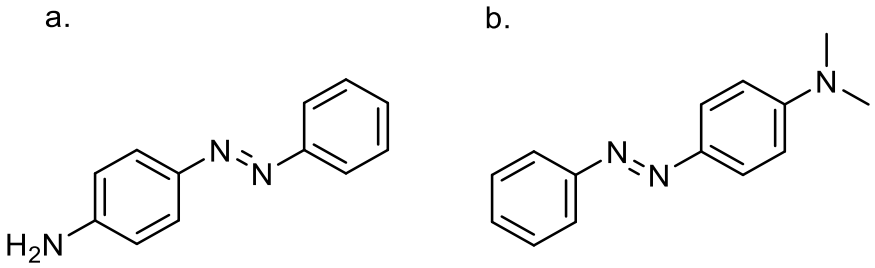


Figure A67

Congo red

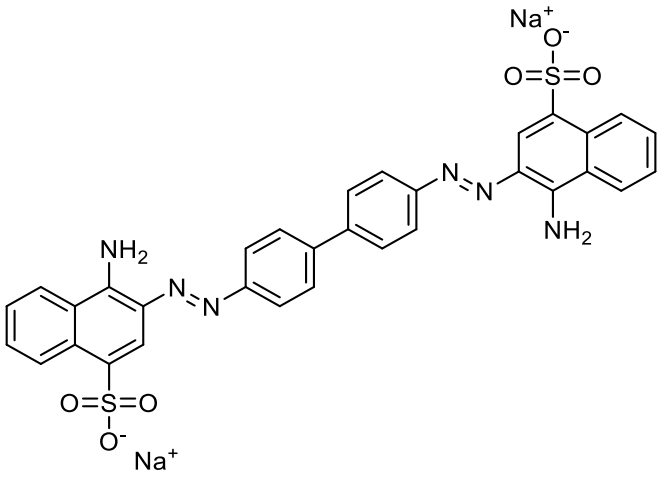


Figure A68

4-((2,4-diaminophenyl)diazenyl)4-amino benzenesulfonamide

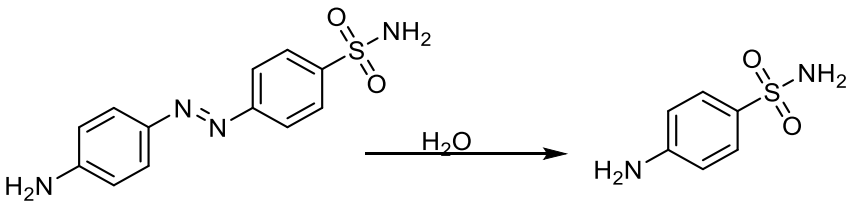


Figure A69

4-((2,4-diaminophenyl)diazenyl)benzenesulfonamide

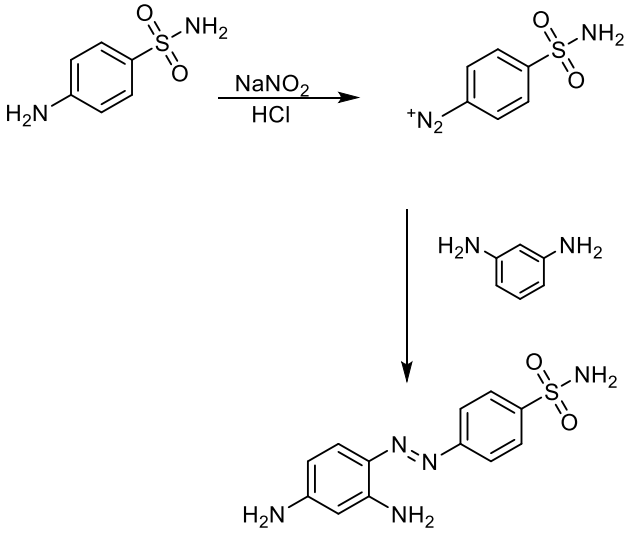


Figure A70

Prontosil soluble

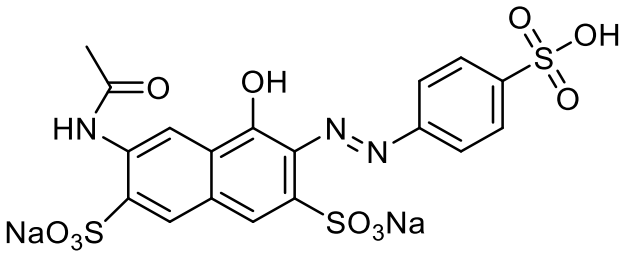


Figure A71

Cancer and normal cells with Uncontrollable growth (57).

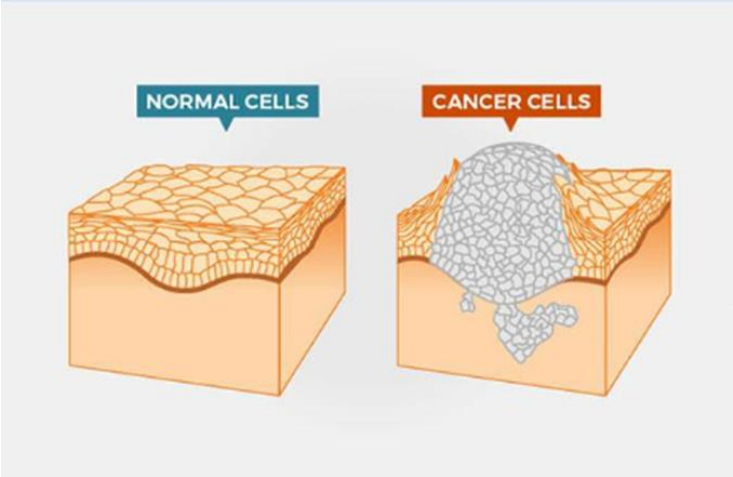


Figure A72

Important sites in curcumin structure.

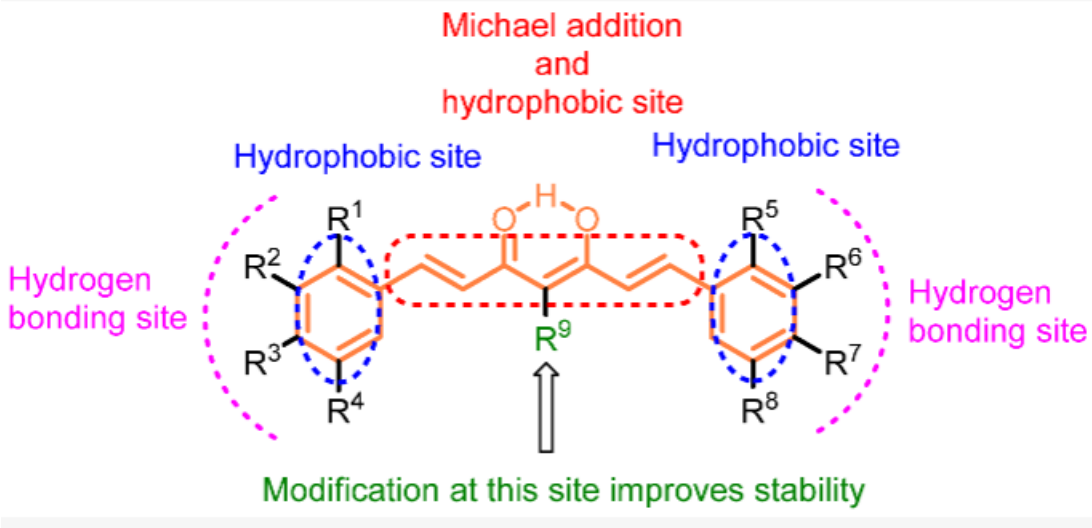


Figure A73

Chemical Structures of various DAP compounds

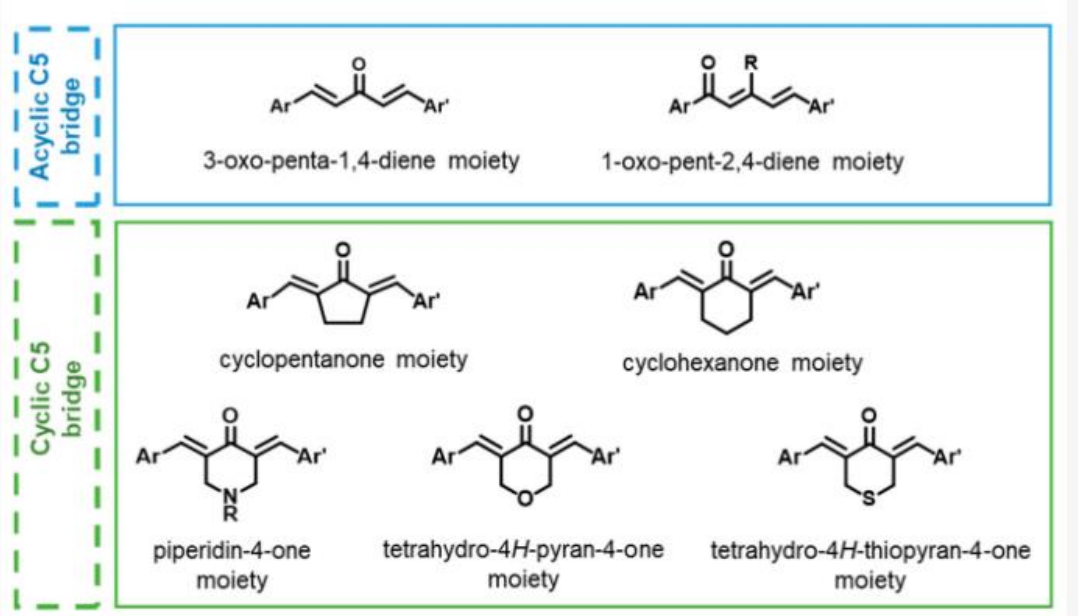


Figure A74

Chemical Structure of MDA

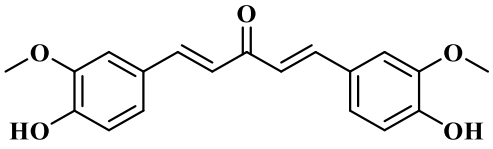


Figure A75

The Gram-positive bacteria retain a purple-colored stain but the Gram-negative bacteria lose the crystal violet and stain pinkish or red [8].

Figure A76

The composition of the Gram-positive bacteria cell wall [5].

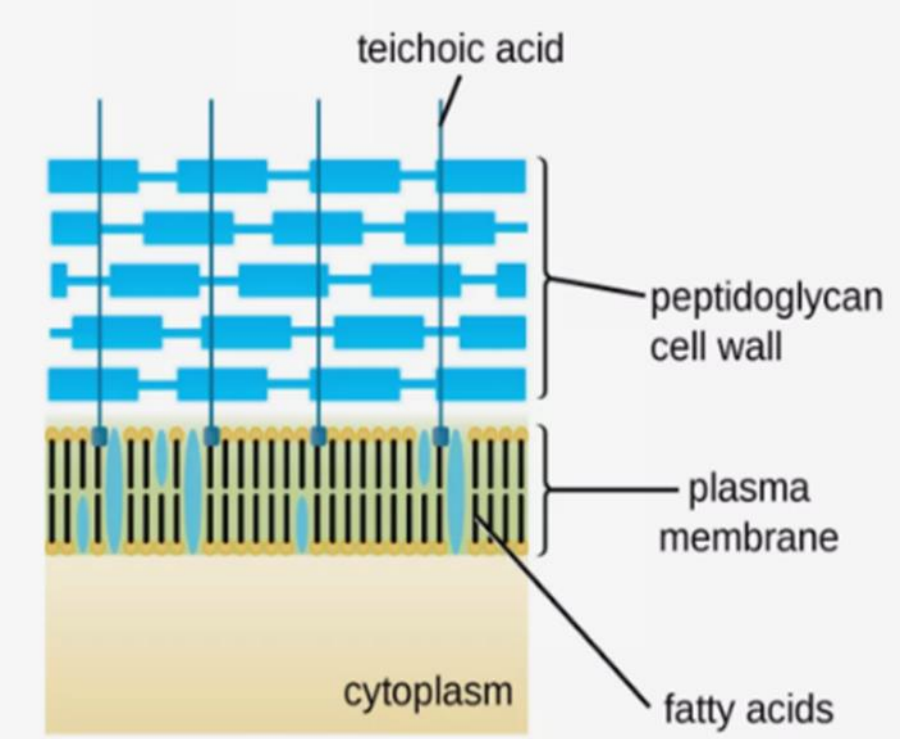


Figure A77

The composition of the Gram-negative bacteria cell wall [5].

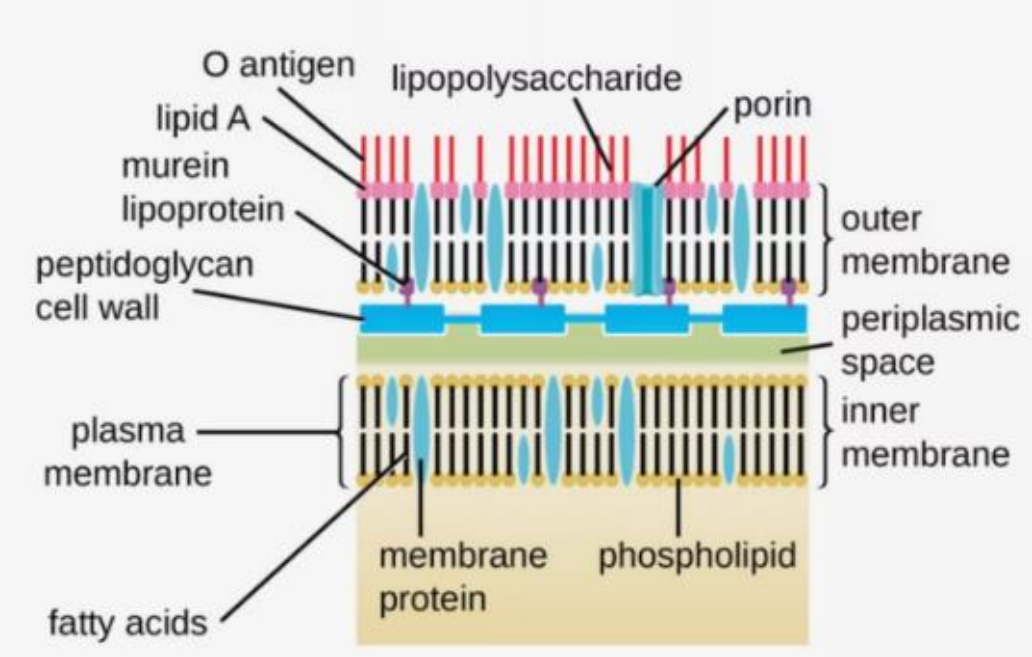


Figure A78

Staphylococcus aureus Gram-positive bacteria [9].

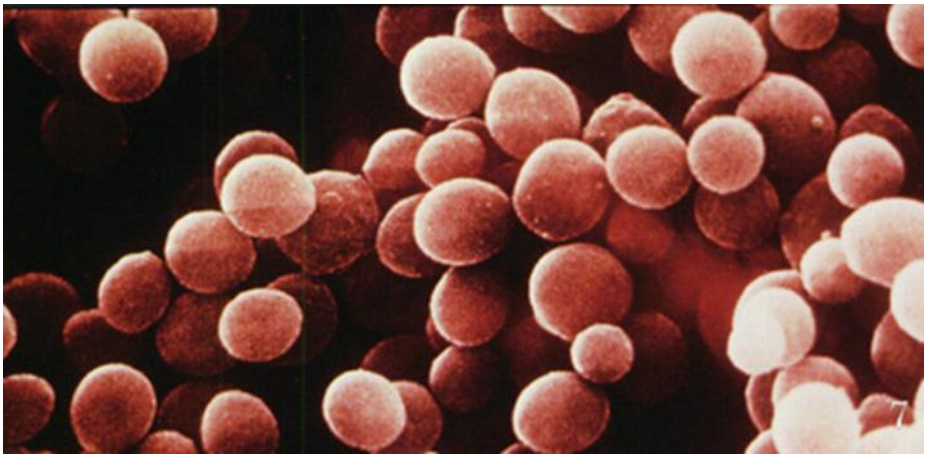


Figure A79

Methicillin-resistant Staphylococcus aureus (MRSA) [22].

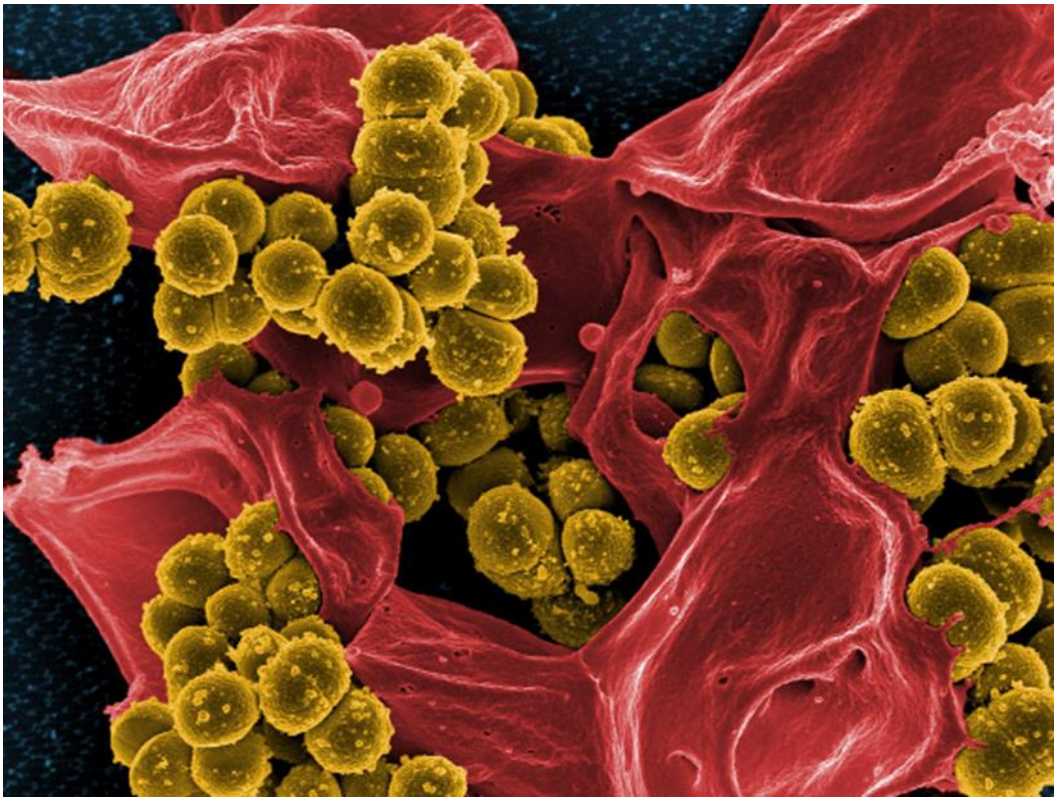
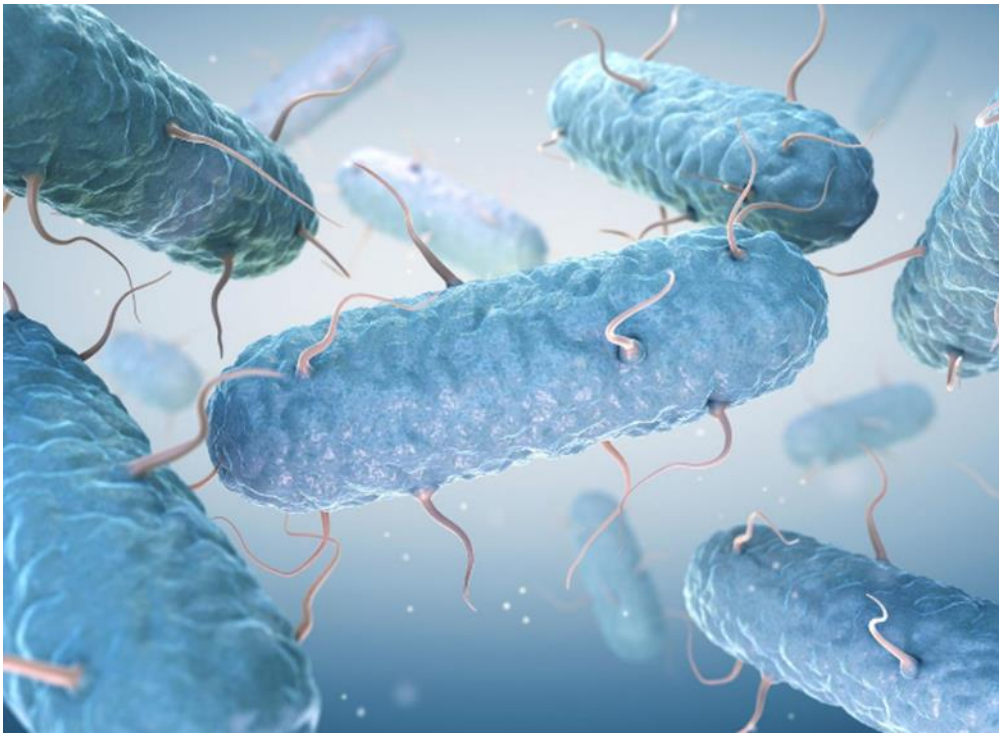


Figure A80

Klebsiella is Gram-negative bacteria [28].

Figure A81

Escherichia coli Gram-negative bacteria [33].



Appendix B

Tables of Study

Table B1

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) B16-F10 Cell Line Viability % effects under different studied concentrations of compounds; 6,7: and purecurcumi

Cytotoxic and Cytostatic effects B16-F 10 Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
6	87	104	74	103	69	65	53	64	44	46	38	44
7	53	56	51	28	41	7	39	6	36	6	34	6
Curcumin	100	93	95	67	90	62	85	48	85	45	50	42

Table B2

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) Colon Cell Line Viability % effects under different studied concentrations of compounds; 6,7 and pure curcumin

Cytotoxic and Cytostatic effects Colon Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
6	70	53	65	49	60	47	50	25	45	12	40	12
7	55	42	48	17	47	17	45	12	44	12	40	11
Curcumin	97	94	94	72	92	69	89	57	69	47	56	22

Table B3

MTT Cytotoxic (24 hrs) and Cytostatic (72 hrs) HeLa Cell Line Viability % effects under different studied concentrations of compounds; 6,7 and pure curcumin

Cytotoxic and Cytostatic effects HeLa Cell Line												
Cell Viability %												
Compound	Studied Concentration (µg/mL)											
	31.25		62.5		125		250		500		1000	
	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic	Cytotoxic	Cytostatic
6	98	61	97	59	94	51	91	47	84	25	76	9
7	78	40	73	26	56	18	44	7	38	6	22	5
Curcumin	100	99	99	97	97	96	95	59	95	32	93	16

Table B4

IC50 values (µg/mL) in the five examined cancer cell lines after 72 hrs at cell density of 5000 cell/well (cytostatic) using the MTT test under the effect of compounds; 6,7 and pure curcumin

Compound	Cancer Cell Line Type				
	MCF-7 breast carcinoma	HEPG2 liver carcinoma	B16-F10 skin melanoma	Colon human cancer	HeLa cervical carcinoma
6	-	442.5	-	411.1	439.3
7	372.5	370.6	366.3	386.6	366.3
Curcumin	850.3	672.9	631.3	550.7	516.5

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

بناء وتشخيص طيفي وفحص بيولوجي للكركم المرتبط بمعقدات صبغات الآزو وصبغات الآوز المعدنية

إعداد

الاء عبد الرحيم جانم

إشراف

أ. د. عثمان حامد

قُدّمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الدكتوراه في الكيمياء، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2023

بناء وتشخيص طيفي وفحص بيولوجي للكركم المرتبط بمعقدات صبغات الآزو وصبغات الآوز المعدنية

إعداد

الاء عبد الرحيم جانم

إشراف

أ. د. عثمان حامد

الملخص

الخلفية: يعتبر الكركمين ومشتقاتة مكون طبيعي وحيوي وهام بسبب أصلها الطبيعي، وعدم سميته أيضًا لخصائصها الوظيفية الحيوية بما في ذلك مضادات الأكسدة ومضادات الالتهابات ومضادات للأورام السرطانية. وله تأثير على مراحل تطور السرطان مثل تنشيط الجينات الورمية، وتكاثر الخلايا، على عكس أدوية العلاج الكيميائي التي تعمل على مراحل محددة من تطور السرطان، مثل موت الخلايا المبرمج ونمو الخلايا.

الهدف: في هذه الدراسة، تم تحضير مجموعة جديدة من مركبات الآزو القائمة على الكركمين.

المنهجية: تتضمن الطريقة تحويل الأمينات الأروماتية بمجموعات السلفونيل والكربوكسيل والأمونيوم إلى ملح الديازونيوم ثم ربط ملح الديازونيوم بالكركمين.

النتائج: في هذه الدراسة تم توليف مركبات جديدة استناداً للكركمين وتشخيصها بتقنية ^{1}H NMR و$C^{13}NMR$ وFT-IR ودرجة الانصهار. ثم تم تقييم أداء مركبات الآزو كأصباغ. حيث درس تغير لون الآزو مع تغير عامل الرقم الهيدروجيني. وتم تقييم بعض الأنشطة الحيوية لمركبات ومجمعات الآزو مثل مضادات الميكروبات والسرطان ضد سلالات البكتيريا:

Escherichiacoli), Staphylococcus aureus, klebsiella pneumonia ,Staphylococcus aureus, MCF-7, HEPG2, B16-F110, Colon and HeLa cell lines viability MTT assay in Palestine)

لذلك فإن الدراسة تثبت أن تأثير النشاط التثبيطي للخلايا المسجل للمركبات الجديدة المشتقة من الكركمين والتي تم فحصها على خطوط الخلايا السرطانية المختلفة المدروسة قد اختلف باختلاف المركب ونوع خط الخلية السرطانية كما أظهر تراكيز مختلفة غير سامة

الخلاصة: تم تحضير مجموعة جديدة من مركبات الكركمين azo dyes، وأظهرت المركبات المحضرة نشاط مضاد حيوي مختلف ضد أنواع مختلفة من البكتيريا وكذلك أنواع مختلفة من خط الخلايا السرطانية.

الكلمات المفتاحية: الكركمين، الآزو، مضاد للسرطان، الكركمين سلفونات، سلطون، سام للجينات.