An-Najah National University

Faculty of Graduate Studies

BIOLOGICAL ACTIVITY OF SYNTHESIZED XANTHONE AND THIOXANTHONE ANALOGS

By

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This Thesis Dissertation was Defended Successfully on 5/6/2022 and approved by

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Dr. Nidal Jaradat Internal Examiner	Signature

Dedication

To my father Abd Almajed,

To my mother Olfat,

To my sisters and brother Leen, Dania and Nedal.

Acknowledgments

I would like to thank my supervisors Dr Murad Abualhasan and Dr. Motasem Almasri for their help and support throughout this Master thesis.

I would also thank the lab technicians at An-Najah National University, especially, thanks to Tahrer Shtayeh, Ahmad Mousa, Abd Zarour and Mohammad Nabeel for helping me with the biological tests.

Finally, thanks to my family and friends for their help and support

Declaration

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

BIOLOGICAL ACTIVITY OF SYNTHESIZED XANTHONE AND THIOXANTHONE ANALOGS

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.

Student's Name: Samah Agel

Signature: Sas Z Law

Date: 5/6/2022

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ABSTRACT

Introduction: Xanthone and thioxanthone were used to synthesize a derivative of compounds to test for anticancer, anti-inflammatory, antioxidant and antimicrobial activity. In order to do this, Grignard reagent was added to xanthone and thioxanthone to give a library of tertiary alcohol of phenyl and benzyl xanthone and thioxanthones. After that, some of them were followed by coupling with cysteine to make cysteine analogous. A total of seven compounds were synthesized. The compounds were characterized using the proton and carbon NMR and IR. The synthesized structures were tested for their anticancer, antioxidant, antimicrobial and ant-inflammatory activities.

Results: The test results were promising; compounds III showed a very good activity (IC 50= 2.79 µg/ml) against Colon cancer cells (CaCo). While compound 2 showed a good inhibition activity against hepatic HEPG cells (IC 50= 49.2 µg/ml) and compound I showed a very good inhibition activity against HeLa cells (IC 50= 60 µg/ml). Compound IV has a potent antioxidant inhibition activity (IC_{50}= 4.22 µg/ml). Moreover, compound VII Showed a good anti-inflammatory activity with a COX2 inhibition activity (1.72 µg/ml) and also good selectivity for COX2 (3.81). However, none of the compounds showed antimicrobial activity. The future work requires further an in-vivo testing to understand its pharmacokinetic and pharmacodynamic activity in living system.

Conclusion: Further future work will be carried out to better understand the SAR effects of these compounds.

Keywords: Xanthone, Thioxanthone, Biological, activity.

Chapter One
Introduction

1.1 Cancer

When abnormal cell growth leads to uncontrolled cell proliferation, cancer develops; it can start in practically any organ or tissue of the body and spread beyond its normal boundaries to target other parts of the body and organs. This process is known as metastasizing, and it is a leading cause of cancer-related death (1-3).

Ten million people died in 2020, due to cancer (1). Different types of therapies could be used to treat cancer. However, the most common are chemotherapy, radiation, and surgery. Another less established treatment is immunotherapy (2, 4-7). Cytotoxic drug is a known type of chemotherapy. It works by decreasing the size of tumor, which in the best case could lead to complete remission for some types of cancers such as lymphoma. And in the other cases may prolong the life of the patient in many metastatic cancers, such as osteosarcoma. The type of the tumor and it's histological grade, are some of the factors that are taken into consideration when choosing the type of cytotoxic drug for the treatment. The stage of disease, and the patient’s tolerance for the side effects of the various treatments are other factors that could play a role in choosing the type of the treatment (8). Unfortunately, chemotherapy has two major limitations including : Development of drug resistance(9), and side effects. The toxic side effects include bone marrow suppression, gastrointestinal problems (nausea, vomiting, diarrhea), alopecia and neutropenia (8).

1.2 L-Cysteine

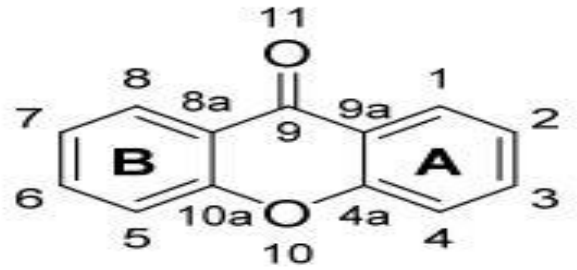
L-Cysteine is a semi-essential amino acid in the diet (10). It plays an important role in many processes such as protein folding stability and trafficking, assembly, biosynthesis of coenzyme A, detoxification of heavy metals and redox balance (11, 12). Due to the strongly reducing environment inside cells, L-cysteine is the predominant type (13). The existence of the thiol group in the structure of the cysteine made it a unique amino acid that may go through a variety of nucleophilic reactions. As a result, cysteines are frequently used as active sites in enzymes, attachment sites for prenylation, palmitoylation tags, and high-affinity metal binding sites, such as zinc or iron (14, 15).

1.3 Xanthone

Xanthone with an IUPAC name of 9H-xanthen-9-one is a heterocyclic compound with a dibenzo-γ-pyrone framework, the basic active compound consists of a tricyclic planar bone with one pyran ring fused with two phenyl rings on both sides. with a basic molecular formula of $C_{13}H_8O_2$ (16, 17). Figure1 shows the structure of xanthone (16).

Figure1

Structure of xanthone



Because this basic tricyclic structure, the molecule demonstrates a wide range of biological actions. It is said to have "privileged structures" (18, 19), because of its capacity to bind to a variety of protein receptors. Xanthone derivatives can conduct a wide range of actions such as antimicrobial, antidiabetic, antioxidant, antiviral, anti-Alzheimer, anti-inflammatory, and anti-tyrosinase actions (20).

Due to its planar structure, xanthones are known to be efficient DNA intercalators. They have also exhibited anticancer activities through non-covalent DNA interaction (21).

MCF-7, HT-29, HL-60, DLD-1, and HeLa cancer cell lines that have shown to have cytotoxic action by xanthones and their derivatives (22).

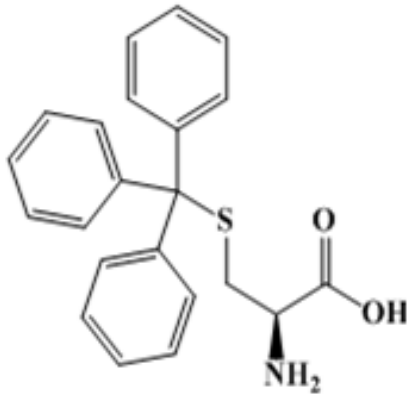
1.4 S-trityl-L-cysteine

S-trityl-L-cysteine is one of a number of cytotoxic compounds that have been used to treat cancer. It is an antimitotic chemotherapeutic medication that is a powerful reversible ATP-noncompetitive inhibitor of human mitotic kinesin Eg5 (23-29). In an NCI 60 tumor cell line screen, STLC was reportedly found to be a potent anticancer drug. It was listed as one of 171 compounds with a "particularly high level of interest at the NCI" in the NCI database of standard agents (30). STLC development was difficult because of the pharmacokinetic problems(24, 31). Because of its amphiphilic nature,

STLC has a low water solubility and low permeability, which affects its bioavailability (31). Alkylation or acylation of the STLC free primary amine to remedy this problem resulted in activity loss (28). Figure 2, shows the structure of S-trityl-L-cysteine (26).

Figure 2

Structure of S-trityl-L-cysteine



2

STLC

1.5 Inflammation

When a noxious stimulus attacks the body and threatens hemostasis, inflammation occurs. This process can range from a localized to a generalized reaction, with fluid and leukocyte buildup resulting to edema and pain (32).

Different physiological and immunological mediators that play a role in acute and chronic inflammation (33) such as prostaglandins, pro-inflammatory cytokines, and chemokines. The most important one is prostaglandin E2 (PGE2) (34). PGE2 is produced when arachidonic acid is catalyzed by the cyclooxygenase enzyme.

COX-1, COX-2, and COX-3 are the three isoforms of the cyclooxygenase enzyme (35).

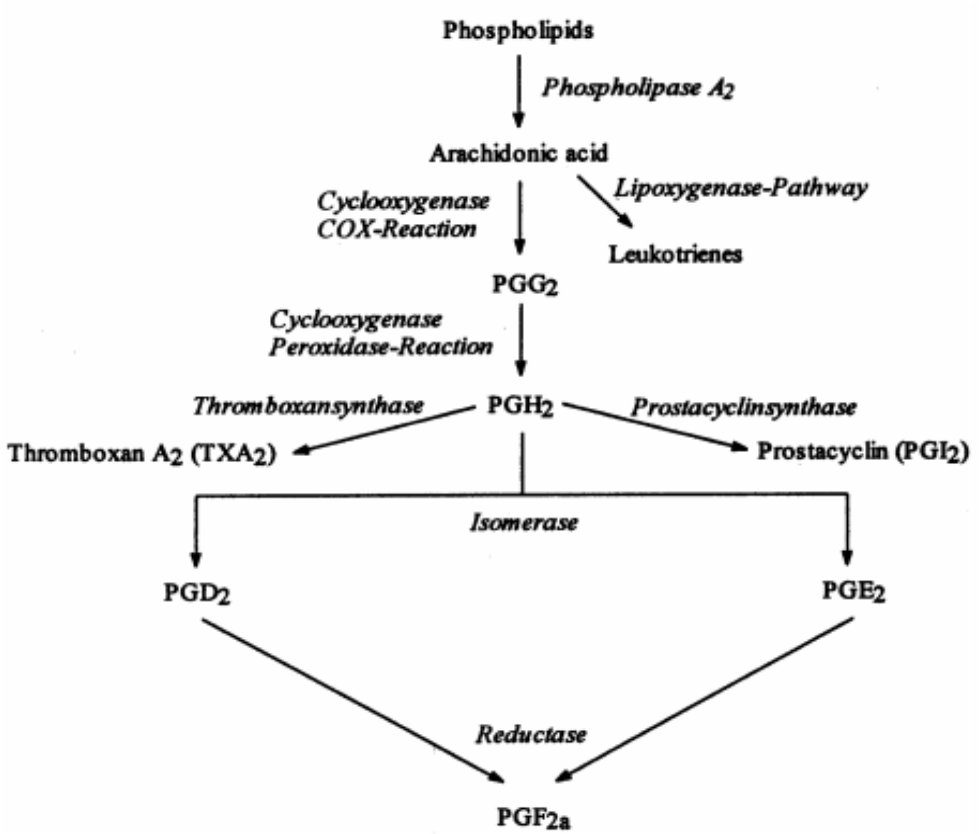
1.5.1 Cyclooxygenase enzyme (COX)

The cyclooxygenase enzyme (COX-1 and COX-2) catalyzes the biosynthesis of prostaglandin H2 and the generation of reactive oxygen species from arachidonic acid which release from membrane phospholipids by the action of phospholipase A_2 (36). Prostaglandin H_2 is the precursor for formation of other prostaglandins that act as

immunomodulaters and inflammatory mediators specially prostaglandin $E_{2,}$ exert their role by vasodilatation, pyretic effects and their proliferation ability (37, 38). Thromboxane $A_{2,}$ Prostaglandin $E_{2,}$ D2, I_2 (Prostacyclin) and $F_{2\alpha}$ are other mediators responsible for many biological responses (39). Figure3, shows the biochemistry of prostanoids and their pathway of formation (40).

Figure 3

Prostanoids biosynthesis pathway.



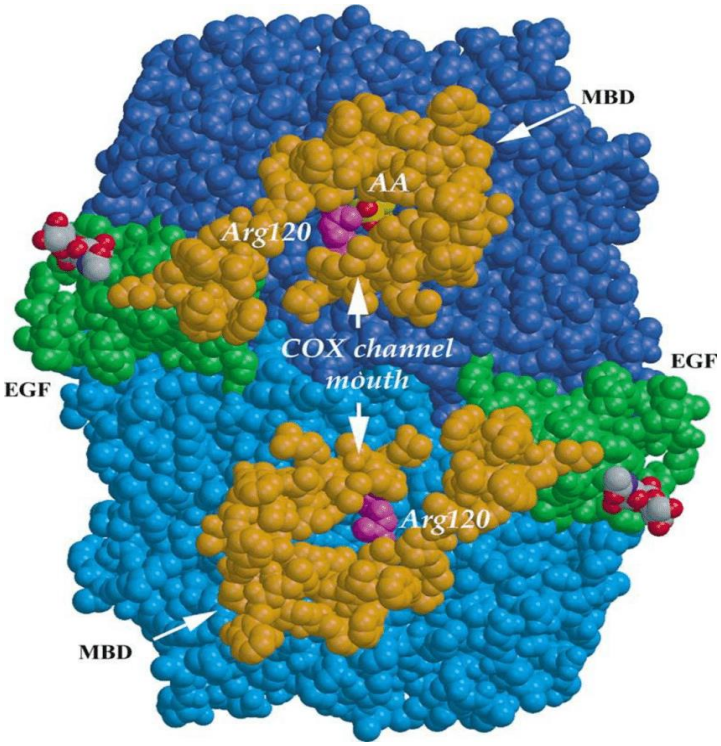
COX-1 and COX-2 isozymes are naturally produced from different genes, with COX-1 being expressed in various cells throughout the body and serving a variety of physiological functions such as gastric mucosa protection, platelet aggregation, and maintaining renal homeostasis, whereas COX-2 is primarily expressed in response to inflammatory mediators such as cytokines (41, 42). This demonstrates its role in both cell growth and inflammation process (43, 44).

COX enzyme is a homodimer with three structural domains that make up its monomer: N-terminal (epidermal growth factor-like), membrane-binding domain that attaches to phospholipid bilayer, and catalytic domain that contains active sites for COX substrate

and inhibitors, as well as heme-containing active sites for peroxidase enzyme. Both of these sites are functionally and structurally linked (45). Figure 4 shows the domains of COX enzyme (46).

Figure 4

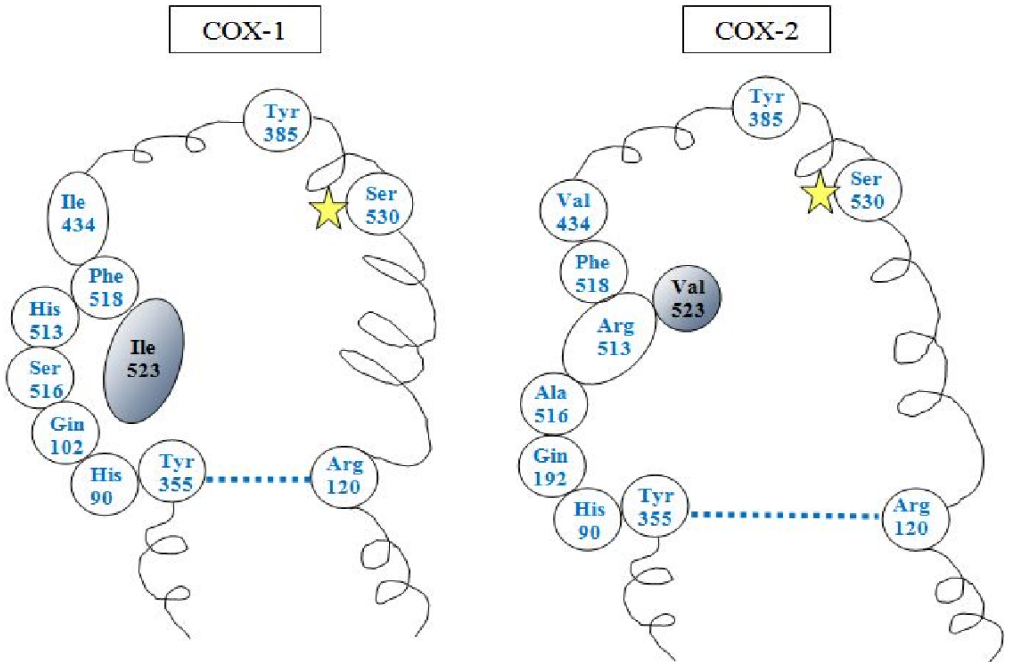
Domains of COX enzyme



COX isoforms are 67% identical in amino acid sequences. At the top of enzyme channel phenylalanine amino acid (Phe503) in COX-1 is replaced by less bulky Leucine (Leu503) in COX-2 to provide the later wider upper space available for binding. However, the most substantial difference is the presence of Valine (Val523) in COX-2 instead of Isolusine (Ilu523) in COX-1 that allows COX-2 to expand to special side pocket with moving back the side chain of Phenylalanine (Phe518). Within COX-2, additional binding interactions may occur. Furthermore, COX-2's side pocket chemistry differs from COX-1's due to the presence of basic Arginine rather than Histidine in COX-1, which allows for the former polar contacts. The overall accessible space in the COX-2 binding region is 25% larger than the space in the COX-1 binding region (47, 48). Figure 5 shows these structural differences (49).

Figure 5

Structure of COX-1 and COX-2 isozymes



1.5.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are chemical groups that are effective at relieving pain, inflammation, and fever (50). NSAIDs are used to treat musculoskeletal, arthritis, osteoarthritis, and other inflammatory disorders (51). The mechanism by which NSAIDs relieve pain and play a role in the inflammatory process is the inhibition of prostaglandin synthesis via the cyclooxygenase enzyme. However, long-term use may result in Gastrointestinal tract ulceration, bleeding, and renal damage (32, 52, 53). It has been reported that osteoarthritis patients who are currently treated with NSAIDs have an increased gastrointestinal risk (54).

1.5.3 Selective cyclooxygenase II inhibitors

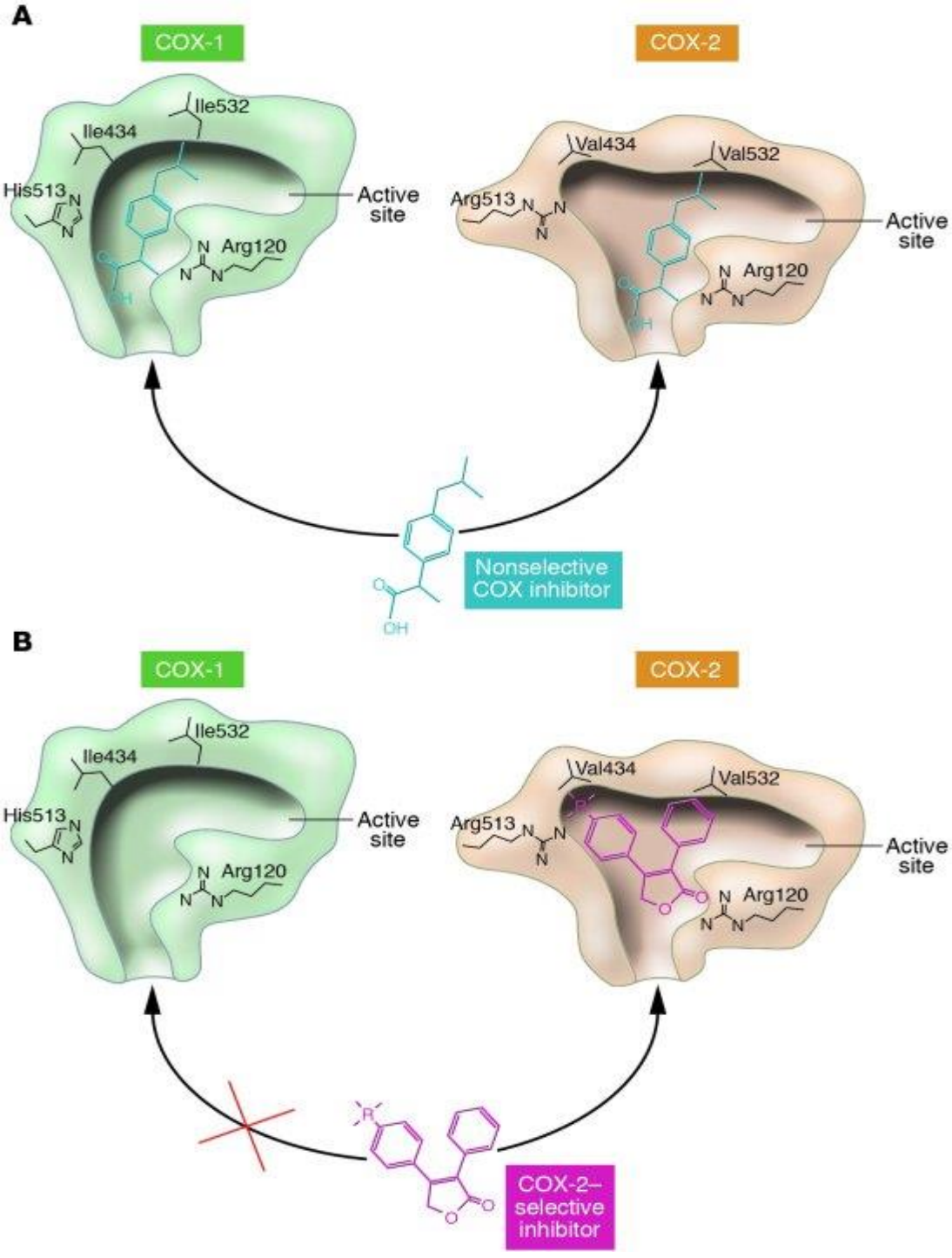
Selective COX-2 inhibitors, a newer class of COX inhibitor, were associated with fewer gastrointestinal side effects than classical NSAIDs, although cardiovascular side effects were a major issue. Rofecoxib was linked to a five-fold increase in myocardial infarctions, according to research (52).

The imbalance between thromboxane A2 and prostacycline synthesis is one of the explanations for cardiotoxicity linked with some selective COX-2 inhibitors. COX-1 is mostly expressed by platelets, whereas COX-2 is primarily expressed by endothelial cells, resulting in this imbalance (55).

Many studies have been conducted to determine COX-2 selectivity over COX-1. Selective COX-2 inhibitors need polar alterations that can form hydrogen bonds with (His90), (Arg513), and (Gln192) in the third region. Polar replacements on diaryl heterocyclics include the phenyl sulfonamide moiety, methyl sulfonamide, and 3-sulfonylvinylbenzophenone moiety. Another method of selectivity was to use electrostatic or hydrophobic bonds to interact with the top lipophilic space of the first region with (Leu503), such as the 3',5'-bis-substitution on flurbiprofen (56, 57). For optimum potency and selectivity, one of the most important studies aiming at synthesizing COX-2 selective inhibitor suggested crucial features must be present in COX-2 inhibitor. The inclusion of a cyclic ring, either hetero or carbon, with two neighboring aryl substituents linked to it, as well as enhancing the flexibility of the compound by lengthening the linker chain, can improve potency and selectivity significantly. There will be more accessible chemicals and greater interactions with amino acid residues in the COX-2 enzyme's sub pocket (58). Figure 6 demonstrates the difference in selectivity between traditional NSAIDs and selective COX-2 inhibitors (39).

Figure 6

Difference in selectivity between traditional NSAIDs and selective COX-2 inhibitors.



1.6 Antioxidant

Reactive oxygen species (ROS) and free radicals may come from internal sources and external sources (59).

ROS activity is concentration dependent; it participates in normal cell processes at low to moderate concentrations, but at high concentrations, it causes detrimental alterations to cell components such as lipids, proteins, and DNA (59-61). When the effect of oxidants exceeds the effect of antioxidants, oxidative stress occurs. Oxidative stress is responsible for cancer, neurological diseases, atherosclerosis, hypertension, ischemia, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma (60, 62, 63). Antioxidant compounds are substances that prevent material oxidation by scavenging free radicals, absorbing UV radiation, binding metal ion catalysts, producing a non-radical species from hydroperoxides, or intercepting single oxygen (64). These substances can repair damage produced by ROS or free radicals, so decreasing the risk of cancer and aging. By transferring a hydrogen from its thiol group, cysteine is supposed to serve as an antioxidant (65-67). The xanthone ring and phenolic hydroxyl group exhibit antioxidant activity (68).

1.7 Antimicrobial activity

Bacteria, fungi, protozoa, viruses, slime molds, and algae are examples of unicellular or multicellular microscopic organisms known as microorganisms or microbes (69).

There are millions of microorganisms in the human body, some of which are necessary for human health. The invasion of body tissues by pathogenic bacteria, their multiplication, and the body's reaction to the infectious agents are all referred to as microbial infection. The host can then be destroyed, and in some cases, died. For a long time, human lives have been plagued by a scarcity of antimicrobial medications and the growth of medication-safe microscopic organisms. Microbes behaving intelligently and recognizing the mechanism of existing medications cause the majority of current drugs to fail. For the aforementioned rationale, to address microbial resistance, there has recently been a rise in interest in creating and enhancing new antimicrobial chemicals from a number of sources (70, 71).

Antimicrobial medications are important for killing or inhibiting the growth of bacteria, especially harmful pathogens (72). They can be classified as antimicrobial chemotherapy for treating infections, bactericidal if the microbe is dead, or biostatic if the medicine limits the microorganism's growth, depending on their purpose.

Xanthone and xanthone derivatives have various biological and pharmacological properties. These compounds proved their activity *against Methicillin-resistant Staphylococcus aureus, Staphylococcus aureus, Escherichia coli, and Enterococcus faecalis* (73, 74).

1.8 Objectives

1-Synthesize a group of xanthone and thioxanthone analogues.

2-Test these drugs for their anticancer activity against variable group of cancer cells including the HeLa cell, hepatocellular carcinoma cell line (HEPG-2) and colon cancer cell line (CaCo).

3-Test their biological activities such as antioxidant, Cyclooxygenase (COX) inhibition activity and antimicrobial activity.

Chapter Two

Methodology

2.1 Reagents and materials

The materials were used to synthesize the tertiary alcohol analogs were: Xanthone (catalog # A14812) and 2-Chlorothioxanthone (catalog # A 18131) were bought from Alfa Aesar (Alfa Aesar Company, England), while benzylmagnesium chloride solution (catalog # 302759) and phenylmagnesium chloride solution (catalog # 224448) were bought from Sigma (Sigma Aldrich, Germany).

The materials were used to synthesize L-cystine analogoues were: boron trifluoride diethyl etherate, 98+% (catalog # A15275) was bought from Alfa Aesar (Alfa Aesar Company, England) while l-cysteine (catalog #168149) was bought from Sigma (Sigma Aldrich, Germany).

The solvents that were used in the synthesis are: ether (catalog #179272) was bought from Sigma (Sigma Aldrich, Germany) while hexane, ethyl acetate, acetone, methanol and dichloromethane, were bought from (C.S. Company, Haifa) and Tetrahydrofuran (THF) (catalog # 487308) was bought from (Carlo Erba Company, MI. Italy).

Chemicals which were used in the extraction process were: sodium bicarbonate, ammonium chloride and sodium sulphate were bought from (C.S. Company, Haifa).

For anti-inflammatory activity the COX (ovine/human) Inhibitor Screening Assay Kit (Item # 560131) was used.

The material was used for anticancer were: For biological test, free Ca++ -phosphate buffered saline (REF # 02-023-1A) and L-glutamine solution (REF # 03-020-1B) were bought from (Biological industries, Jerusalem). RPMI (catalogue # 05669) was bought from (Manassas, VA, USA), Trypsin-EDTA solution 1X (catalog # 59417C) and fetal Bovin Serum (catalog # C8065) were bought from (sigma-aldrich, USA).

The material was used for antioxidant activity was: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (catalog # 224448) was bought from Sigma (Sigma Aldrich, Germany).

The strain of bacteria and fungi that used for determination of antimicrobial activity of the compounds were: *Klebsiella pneumonia* (ATCC13883), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (ATCC8427), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538P), *MRSA* (Clinical sample) *and Candida albicans* (ATCC 90028).

2.2 Instrumentation

Silica gel (Merck, 230-400 mesh) was used for flash chromatography. Positive air pressure was used to elute the columns. For evaporation of solvents, Rota Vapor (Heidolph) was used.

The compounds were characterized by Bruker Avance 500 spectrometer at Jordan University for NMR analysis. The ppm and Hz were used to evaluate the chemical shifts and coupling constants respectively. Pipetting was done with an Accumax Variable micropipette from the United Kingdom. Unilab microplate reader 6000 from the United States was used to read the plate for cell viability test and inhibitory activity against COX. Absorption analysis was conducted on (7315 Spectrophotometer, Jenway, UK) using quartz cuvettes were used to read the absorbance for antioxidant activity. Esco celculture CO2 incubator was used to incubate cell line.

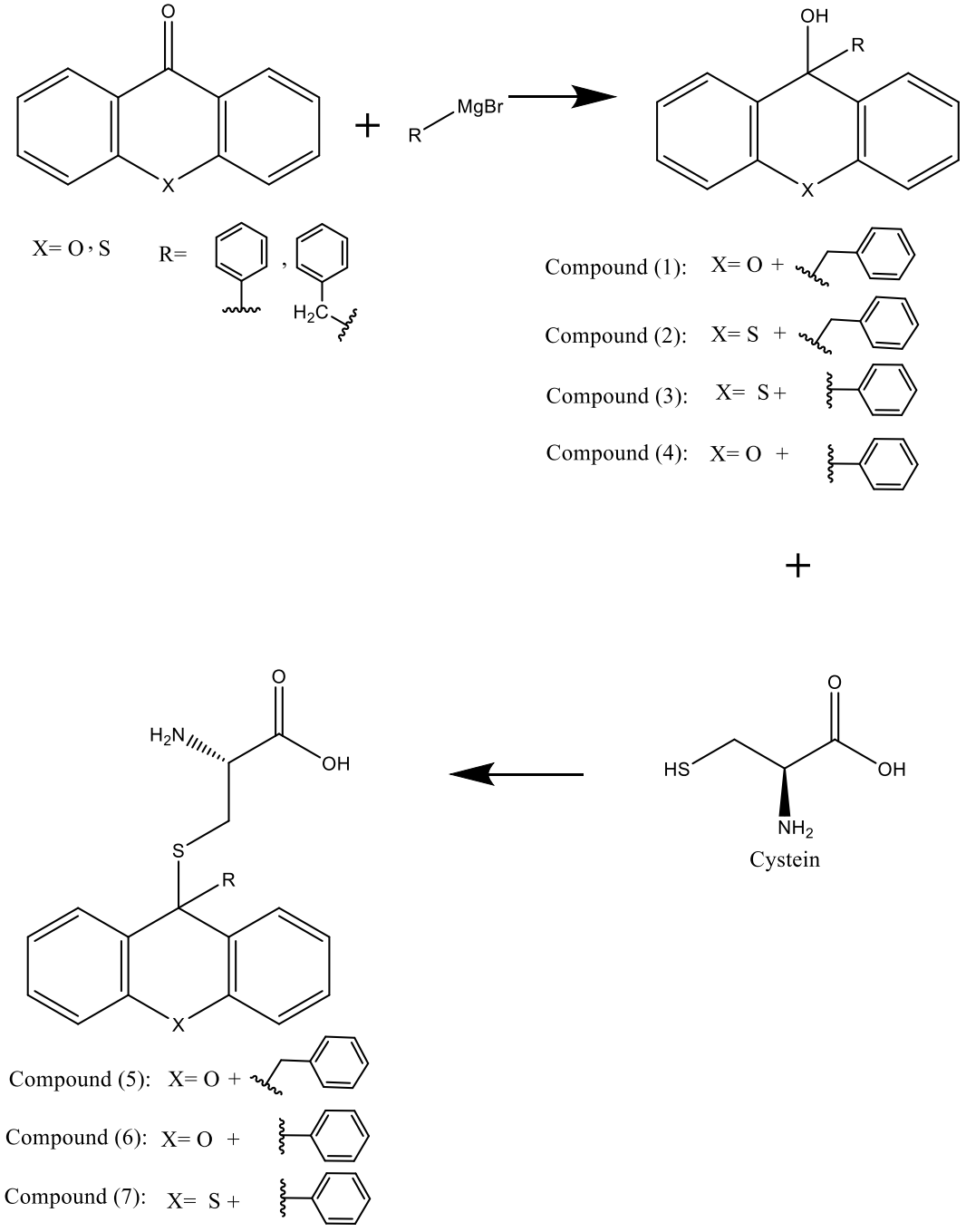
2.3 Chemical synthesis and characterization of the products

At An-Najah National University, all synthetic methods, enzyme screening kit testing, anticancer activity testing, antimicrobial activity testing and antioxidant activity testing were completed. While at Jordan University NMR measurements were accomplished.

The compounds were synthesized in two stages. At first tertiary alcohols were synthesized then were coupled with cysteine. The synthetic scheme is shown in Figure 7.

Figure 7

synthetic scheme

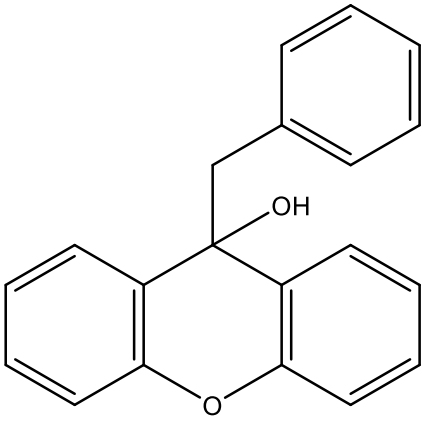


2.3.1 Synthesis of tertiary alcohol compounds

The ketone was transferred to a round bottom flask after that Grignard reagent was added to the RBF in a nitrogen environment at 0 °C and placed on the stirrer for the 24 hours.

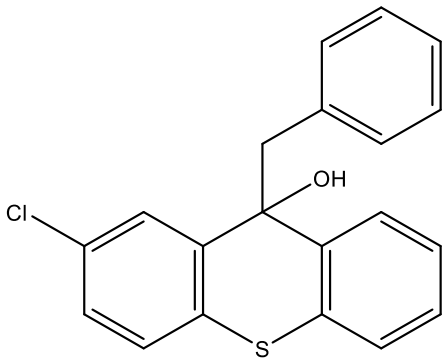
Then, Saturated ammonium chloride (10mL) was used to quench the mixture. Then the mixture was extracted with ethyl acetate (3 x 20 mL), dried over sodium sulphate, and evaporated under reduced pressure. Silica gel chromatography was used to purify the residue [ethyl acetate: Hexane (1:2)].

Synthesis of Compound I



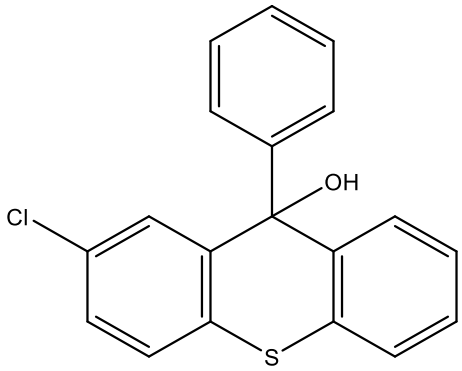
To obtain compound I: Xanthone (196.21mg,1mmole) was added to benzylmagnesium chloride(292.7µl,2mmole), DCM(5ml). A pure yellow powder was obtained (180 mg, 60 % yield). R_f: 0.5 (ethyl acetate/hexane (1:2)) M.P. 200°C -202°C. IR: ATR, υ_{max} (cm^{-1}) : 3367.2 (OH stretch for alcohol). . ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 4.5 (1H, s, OH), 2.34 (1H, s, -CH_2-), 6.97-7.50 (9H, m, Ar-H), 7.5 (1H, t, *J* = 8.2. 7 Hz, Ar-H), 7.68 (1H, d, *J* = 8 Hz, Ar-H), 7.89 (1H, t, *J* = 8.1, 7 Hz, Ar-H), 8.21 (1H, d, *J* = 8.7 Hz, Ar-H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 156.50, 136.05, 126.85, 126.46, 124.86, 121.60, 118.67, 63.34, 55.67.

Synthesis of compound II



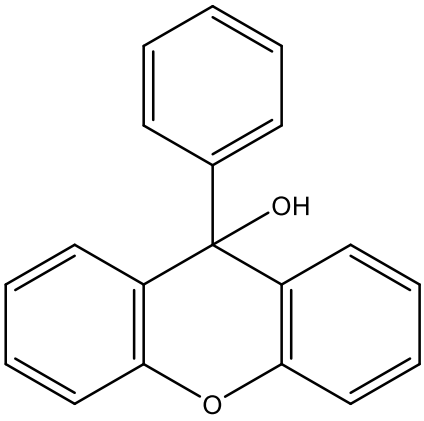
To obtain compound II: 2-chlorothioxanthone (246.71mg, 1mmole) was added to benzylmagnesium chloride (292.7 µl,2mmole), DCM(5ml). A pure yellow powder was obtained (155mg, 55 % yield). R_f: 0.55 (ethyl acetate/hexane (1:2)), M.P:185°C -188°C. IR: ATR, υ_{max} (cm^{-1}): 3321.2 (OH stretch for alcohol). ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 4.5 (1H, s, -CH2-,), 7.08-7.13 (4H, m, Ar-H), 7.23 (1H, D, *J* = 4.1 Hz, Ar-H), 7-34-7.69 (6H, m, Ar-H), 7.83 (1H, d, *J* = 8Hz, Ar-H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 158.40, 145.15, 131.85, 123.46, 1 21.86, 120.50, 118.67, 63.34, 55.67.

Synthesis of compound III



To obtain compound III: 2-chlorothioxanthone (246.71mg, 1mmole) was added to phenylmagnesium chloride (268.35µl, 2mmole), DCM(5ml). A pale-yellow powder was obtained (150mg, 50 % yield). R_f : 0.4 (ethyl acetate/hexane (1:2)) M.P190°C -192°C. IR: ATR, υ_{max} (cm^{-1}): 3226.9 (OH stretch for alcohol).. ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 5.5 (1H, s, -OH), 7.39 (1H, s, Ar-H), 7.76-7.82 (10H, m, Ar-H), 8.12-8.15 (1H, *J* = 15.1 Hz Ar-H. ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 143.01, 142.23, 141.03, 140.47, 121.86, 120.50, 118.67, 139.21, 135.95 130.75, 128.76, 128.76, 127.70, 126.22, 125.62, 123.50

Synthesis of compound IV



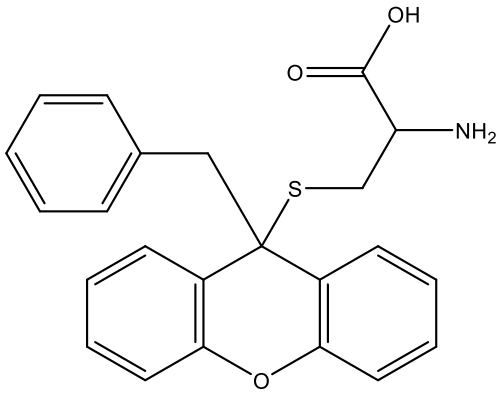
To obtain compound IV: Xanthone (196.21mg,1mmole) was added to phenylmagnesium chloride(268.35 µl,2mmole),THF(5ml).A pure yellow powder was obtained (90mg, 42% yield). R_f: 0.5 (ethyl cetate/hexane (1:2)) M.P:205°C -208°C. IR: ATR, υ_{max} (cm^{-1}): 3549.15 (OH stretch for alcohol).^{1}H NMR (DMSO-d^{6}, 500 MHz) δ ppm: 6.5 (1H, s, OH), 7.1-7.51 (9H, m, Ar-H), 7.6-8.1 (4H,m, Ar-H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 152.40, 135.04, 127.75, 125.36, 123.76, 120.50, 117.57, and 73.34.

2.3.2 Synthesis of L-cysteine analogues

L-Cysteine and the tertiary alcohol were dissolved in 0.5 mL acetic acid. Boron trifluoride di ethyl etherate was added dropwise under nitrogen atmosphere at 0 °C. After that, the reaction mixture was left on the stirrer at 0 °C for 2h.

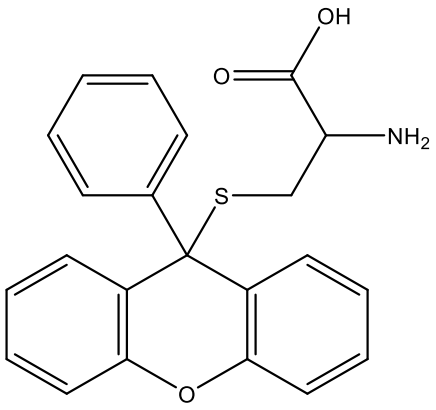
TLC was used to observe the reaction progress. The reaction was then quenched by adding 1.5 mL of 10 % sodium acetate and 1.5 mL of water. The resulting precipitate was filtered, washed with water and then ether and dried in a vacuum oven (40°C for 24 h) to give the corresponding L-cysteine derivatives.

Synthesis of compound V



To obtain compound V: L-Cysteine (58mg, 0.48mmole) was reacted with Compound I (138.4mg,0.48mmole), boron trifluoride diethyl etherate (207 µl,1.68mmole). A pure white powder was obtained (78mg, 40% yield). R_f: 0.3(DCM/ MeOH (9:1)), M.P:230°C -233. °C IR: ATR, υ_{max} (cm^{-1}): 3388.15 (OH stretch for alcohol). ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 1.9 (2H, d, J= 12.1 Hz -C<u>H$_2$</u>-CH-), 3.2 (2H, s, -C<u>H$_2$</u>-), 3.9 (2H, t, J= 10, 11.3Hz -CH$_2$-C<u>H</u>-), 7.1 (1H, s, Ar-H), 7.13-7.42 (10H, m, Ar-H), 7.93 (1H, D, *J* = 15.1 Hz Ar-H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 152.71, 151.2, 137.90, 130.17, 129.61, 129.01, 128.77, 127.62, 126.06 124.72, 124.72, 124.08, 123.44, 123.11, 121.10, 117.5, 55.12, 49.12, 48.50, 31.29.

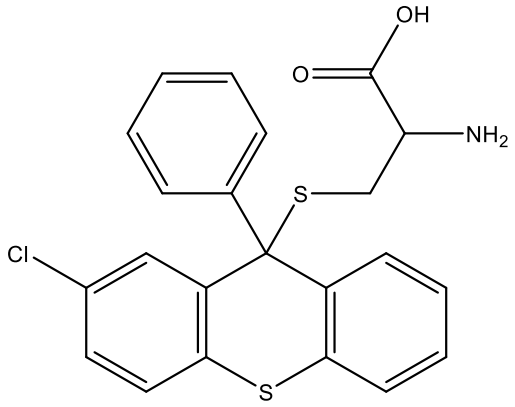
Synthesis of compound VI



To obtain compound VI: L-Cysteine (58mg, 0.48mmole) was reacted with Compound III (132mg, 0.48mmole), boron trifluoride diethyl etherate (207 µl,1.68mmole). A pure yellow powder was obtained (90mg, 47% yield). R_f: 0.1(DCM/ MeOH (9:1)), M.P:235°C -238°C. IR: ATR, υ_{max} (cm^{-1}): 1620.06 (C=O stretch). ^{1}H NMR (DMSO-d^6,

500 MHz) δ ppm: 1.82 (2H, d, J= 12.1 Hz -C$\underline{H_2}$-CH-), 3.71 (2H, t, J= 11, 12.3Hz -CH_2-C$\underline{H}$-), 72.13-7.52 (13H, m, Ar-H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 151.73, 150.2, 138.90, 131.18, 128.51, 127.91, 127.6, 127.42, 125.76 124.72, 124.08, 123.24, 123.11, 121.30, 118.5, 55.13, 48.12, 30.28.

Synthesis of compound VII



To obtain Compound VII: L-Cysteine (58mg, 0.48mmole) was reacted with compound IV(155mg,0.48mmole), boron trifluoride diethyl etherate (207 µl,1.68mmole). A pure yellow powder was obtained (120mg, 55% yield). R_f: 0.3 (DCM/ MeOH (9:1)). M.P:250°C-252°C. IR: ATR, υ_{max} (cm^{-1}): 1620.95 (C=O stretch).^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 1.81 (2H, d, J= 12.1 Hz -C$\underline{H_2}$-CH-), 3.69 (2H, t, J= 12.1, 11.3Hz -CH_2-C$\underline{H}$-), 7.01 (1H, ms, Ar-H), 7.17-7.2 (3H, m, Ar-H), 7.35-7.5 (6H, m, Ar-H), 8.1 (2H, m, Ar-H). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 153.63, 151.1, 139.80, 132.17, 129.61, 128.81, 128.6, 127.52, 126.66 125.62, 124.88, 123.34, 123.22, 122.10, 119.5, 65.13, 55.12, 40.28.

2.4 Anticancer test

2.4.1 Cell line

HeLa, HepG-2and Caco-2 cells were the cell lines that used to study the anticancer activity of the compounds.

2.4.2 Cell culture

10% fetal bovine serum (FBS), L-glutamine, PRMI and penicillin/streptomycin which were used to prepare the culture growth medium (CGM) was added to a 15-cm^2 plastic culture plate in order to implant the HeLa, hepG-2 and Caco2 cells. Then, at 37°C in a humidified atmosphere containing 5% CO_2 all cells were incubated.

For sub-culturing, in a 15-cm^2 culture plate the CGM was removed from the all plates by suction, then 15 ml of Ca^{2+}-free phosphate buffer saline was used to wash the cells for two times.

Then, at 37 $^{\circ}$C in a humidified atmosphere containing 5% CO_2 the cells were incubated for 5 minutes but before that 5ml of trypsin was added to the cells this have been done in order to allow a sufficient cell could detached the plate surface.

After that, 10 ml of CGM was added to the cells in order to inactivate the trypsin. Then, in a 96-well plate the cells were distributed and incubated for 24h after the cells suspension were assembled and diluted

2.4.3 Cell viability test

After 24 h, in a 96 well plate which contained the cells a 100 µl of various compounds concentrations were added to it and incubated for 48 hours. After 48 hours, in order to know the cell viability a 20 µl of MTS solution was supplied to each well plate and left in the incubation for 2 h at 37 $^{\circ}$C. Then the absorbance were measured of all plates using the plate reader (75).

2.5 In vitro test on COX-1 and COX-2 enzymes screening kits

The COX (human) Inhibitor Screening Assay Kit (provided by Cayman chemicals (Item # 701230), Ann Arbor, MI, USA) was used to assess the inhibitory activity of our produced compounds against COX-1 and COX-2.The reagent preparation and testing method were carried out according to the manufacturer's instructions.

The inhibitors were dissolved in dimethylsulfoxide (DMSO) to prepare two concentration (10 µM and 50 µM) after that 160 µl of reaction buffer,10 µl of Heme and 10 µl of COX-1 or COX-2 diluted enzyme where added to it and incubated for 10

minutes at37ºC. In order to initiate the reaction 10 µl of Arachidonic acid was added to all reaction tubes then quickly mixed and followed by incubation at 37 ºC for two minutes.

In order to stop the reaction 30 µl of stannous chloride solution was added to each reaction tubes followed by incubation of all reaction tubes for five minutes at room temperature. The mixture became cloudy. Prostaglandins have been made by cox reactions in the samples. Then, it was quantified via enzyme-linked immune-sorbent assay (ELISA). Plastic film was used to cover the 96-well plate, and the samples were stored in an orbital shaker for 18 hours at room temperature. After that, the plate wells were emptied from the cox reaction, and then washed buffer was used to rinse the plate wells. The rinsing procedure was repeated five times then; Ellman's reagent with a volume of 200 µl was added to each well. The plate was covered with the plastic film and flat cover to allow the plate to develop in the dark.

The Bo value should be between 0.3 and 0.8 in the 405-420nm in order to achieve this the plates were incubated for 60-90 minutes at room temperature on the orbital shaker. After that, ELISA plate reader Unilab microplate readers 6000 were used to read the plates. The control inhibitory percentage was compared with the measured inhibitory percentage for the different tested concentrations. After that, the concentration inhibition response curve was produced and used to calculate the IC_{50}.

In order to know the selectivity of each compound on COX-1 and COX-2 the IC_{50} COX-1 was divided by IC_{50} of COX-2 to produce the selectivity index (SI) (76).

2.6 Antioxidant test

Antioxidant activity was examined through DPPH assay. Seven synthetic compounds were tested for antioxidant activities. In order to get a concentration 100 µg/ml, a 10 mg from each compound was weighted and dissolved in 100 ml of methanol then the solution was used to prepare different concentration 1, 2, 3, 5, 7, 10, 20, 30, 50 and 80 µg/ml. The DPPH solutions were prepared by weighting 2 mg of DPPH and dissolve it in 100 ml methanol to have a concentration (0.002% w/v). After that the products solutions were mixed with DPPH solution and methanol in a ratio of 1:1:1(compound: DPPH: methanol). The methanol solution was used as a blank. All the solution were stored for 30 minutes in the dark at room temperature. UV-vis spectrophotometer was

used to measure the absorbance values, at wave length 517nm. The antioxidant potential percentage of each compound was calculated according to this formula:

$$I (\%) = ((Ab - As)/Ab) \times 100\% \quad (1)$$

Where:

Ab: the blank absorbance value which contained 2 ml of methanol and 1 ml of DPPH.

As: the absorbance value of the sample.

At the end, a curve was drawn between inhibition percentage and the compound concentration to calculate the IC_{50} of each compound (77).

2.7 Antimaicrobial activity

The strain of bacteria and fungi that used for determination of antimicrobial activity of the compounds were: *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, MRSA and Candida albicans.* The method that was used is broth microdilution.

From each type of bacteria and fungi, a swap was taken and put in saline solution. Then, a UV spectroscopy at wave length 620nm was used to measure the turbidity for each type of bacteria and fungi. The absorbance of microbial suspension should be more than 0.08. So, if the value is less than that, the mass of bacteria or fungi should be increased. However, the maximum value for the turbidity is 0.12 and the excessed of this value means that more saline should be added to the sample. The bacterial suspension was diluted 1:3 in Mueller–Hinton broth and *Candida albicans* was diluted 1:20 then 1:50. Each compound was dissolved in a solution containing 10% Dimethylsulfoxide. Serial dilution was done for all of the compounds, where into microtiter 100 µl was placed into each well. Into the first well 100 µl of the tested compound was pipetted, then mixed and from it 100 µl was transferred to the next well. This was repeated up to well 11 and well number 12 was free from the compound. To all wells expect well 11, the bacteria suspension (1 µl) and yeast (100 µl) were added and incubated to 37 ºC for 24 hours (for bacteria) and 48 hours (for yeast).

There were 2 controls for all bacteria and fungi tested: first one (negative control of microbial growth), which contained only media and compound (well 11), second one (well 12), which represents positive control of microbial growth, which contained media and bacteria (78).

Chapter Three

Results and discussion

3.1 Synthesis of xanthone and thioxanthone analogs

In order to synthesize xanthone and thioxanthone analogs, first step was the synthesis of tertiary alcohol compounds by reacting xanthone and thioxanthone with phenyl or benzyl magnesium chloride in THF or DCM. Then, tertiary alcohol compounds were reacted with L-cysteine in acetic acid using boron trifluoride diethyl etherate as acid catalyst. All synthesized compounds were purified by flash chromatography. The structures of these compounds were confirmed by IR analysis, ^{13}C NMR and ^{1}H NMR spectral data. (See appendix)

3.2 Antioxidant activity

DPPH is a stable molecule characterized by its deep purple color and its solubility in methanol. It is used to determine the compounds' antioxidant activity through the compound's ability to work as a radical scavenger.

Seven compounds were tested for their antioxidant activity at different concentrations from 1-80 µg/ml. The test was performed against the DPPH. The calculated percentage of inhibition is shown in Table 1. The results showed variable antioxidant activity. In general, moderate antioxidant activity was observed for the synthesized compounds. The maximum inhibition was obtained from compound IV (IC_{50}= 4.22 µg/ml). This compound has tertiary alcohol which could help in the antioxidant activity. The results showed that free tertiary alcohols had better antioxidant activity than cysteine-coupled compounds. The IC_{50} ranges from 4.22- 79.98 µg/ml. The detailed results are illustrated in Figure 8.

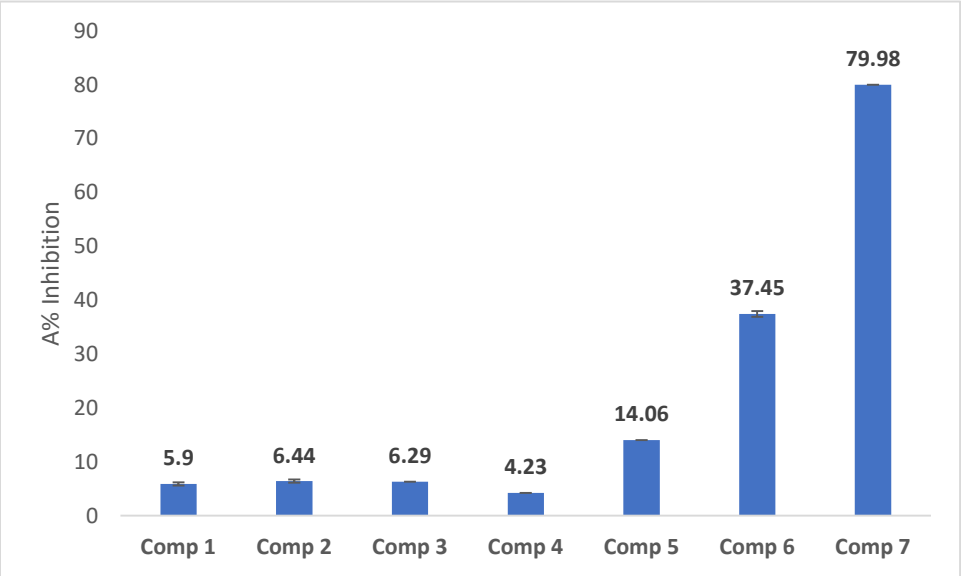
Table1

Antioxidant activity for the synthesized compounds

Conc µg/ml	Comp (1)	Comp (2)	Comp (3)	Comp (4)	Comp (5)	Comp (6)	Comp (7)
1	0.31 ±0.02	0.26 ± 0.01	0.14 ± 0.02	No Inh	5.0 ± 0.01	18.0 ± 1.02	22.0 ± 3.31
2	0.36 ± 0.02	0.26 ± 0.03	0.15± 0.01	No Inh	6.0 ± 0.02	18.0 ± 2.03	22.0 ± 3.11
3	0.36 ± 0.01	0.33 ± 0.04	0.15 ± 0.03	No Inh	7.0 ± 0.01	22.0 ± 1.01	22.0 ± 2.22
5	0.31 ± 0.01	0.33 ± 0.01	0.29 ± 0.01	33.0 ± 1.21	7.0 ± 0.51	28.0 ± 0.01	29.0 ± 4.22
7	36.40 ± 4.11	26.00 ± 7.20	18.20 ± 1.40	33.0 ± 2.13	7.0 ± 0.67	28.0 ± 0.01	22.0 ± 1.22
10	36.40 ± 3.31	27.00 ± 1.03	18.00 ± 2.37	35.0 ± 2.31	7.0 ±0.35	30.0 ± 0.01	26.0 ± 3.44
20	40.20 ± 5.21	28.00 ± 2.14	15.60 ± 2.01	35.0 ± 3.31	11.4 ± 1.01	30.0 ± 0.01	23.0 ± 1.11
30	35.50 ± 1.01	29.00 ± 1.15	19.90 ± 1.0	39.0 ± 5.51	11.4 ± 2.10	30.0 ± 0.01	23.0 ± 1.21
50	35.10 ± 3.31	32.00 ± 3.75	20.00 ± 3.12	39.4 ± 2.44	12.0 ± 1.33	30.0 ± 0.01	23.0 ± 3.10

Figure 8

IC50s of the antioxidant activities of the synthesized compounds



3.3 Anticancer activity

The anti-cancer activities were tested against different cancer cell lines, including Hella cells, hepatic (HEPG), and colon cancer (CaCo). Some of the synthesized compounds have shown promising results.

3.3.1 Anti-cancer activity against Hella cell

The drug activity against Hella cells was with variable results. Compound I showed very good inhibition activity (IC50=60 µg/ml). Compound IV and Compound I had (98% and 96%) inhibition for the Hella cells, respectively which was achieved for these compounds at 500µg/ml. However, Compound VII showed no activity at all. In Table 2, the results demonstrate that tertiary alcohols have much better activity than compounds that have been coupled with cysteine (Compounds V, and VI). The IC50 was calculated and the detailed results are illustrated in figure 9.

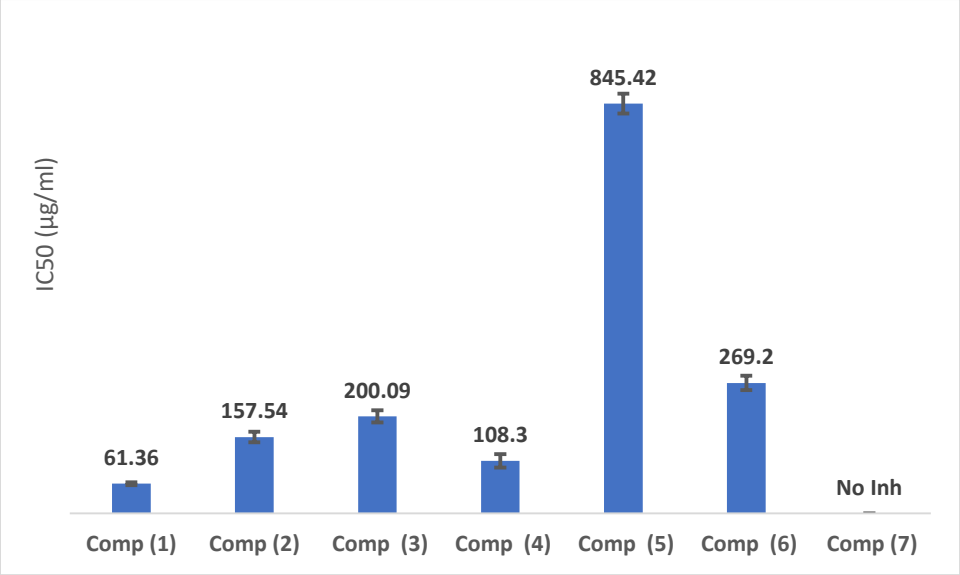
Table2

The % inhibition of the synthesized compounds against Hella cells at different concentration

Conc. (µg)	Comp (1)	Comp (2)	Comp (3)	Comp (4)	Comp (5)	Comp (6)	Comp (7)
500	96.03 ± 3.4	89.95 ±4.8	89.91 ± 5.7	98.31 ± 6.7	84.0 ± 8.7	21.71 ± 2.1	No inh
250	92.96 ± 4.6	80.11 ± 7.3	60.01 ± 5.5	91.74 ± 5.4	50.73 ± 7.4	6.40 ± 1.2	No inh
125	88.96 ± 4.3	79.6 ± 5.4	26.40 ± 2.1	74.88 ± 2.9	30.00 ± 4.7	No inh	No inh
62.5	47.29 ± 3.7	69.42 ± 3.8	No Inh	54.56 ± 6.1	19.0 ± 1.1	No inh	No inh

Figure 9

The IC50 of inhibition activity of the synthesized compounds against Hela cells



3.3.2 Anti-cancer activity against HEPG cell

The compounds activity for the HEPG cells was also variable. Compound II showed very good inhibition activity (IC50= 49.05µg/ml)). Compound IV and Compound II had (99%) inhibition for the HEPG-2 cells which was achieved for these compounds at 500µg/ml. However, some compounds such as Compound VI and compound VII showed no inhibition at all Table 3. The detailed IC50 are shown in figure 10.

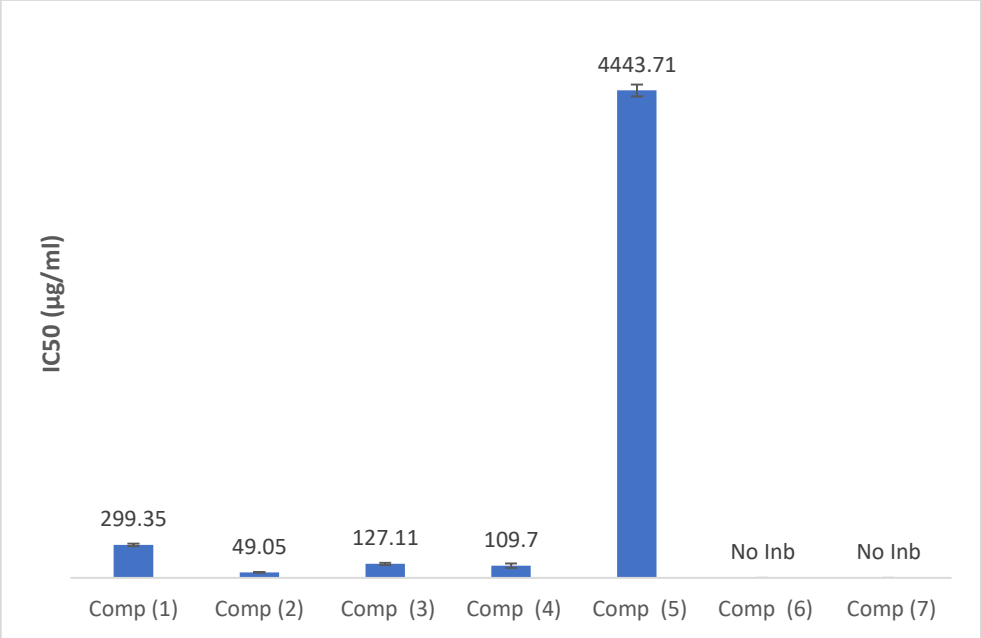
Table 3

The %inhibition of the synthesized compounds against HEPG cells at different concentration

Conc (µM)	Comp (1)	Comp (2)	Comp (3)	Comp (4)	Comp (5)	Comp (6)	Comp (7)
500	72 ± 3.1	99 ± 3.1	70 ± 5.7	99 ± 5.2	44 ±1.8	No inh	No inh
250	13 ± 1.2	88 ± 4.5	58 ± 6.1	80 ± 5.3	28 ± 1.2	No inh	No inh
125	No inh	76 ± 5.1	34 ± 2.2	55 ± 3.2	16 ± 0.8	No inh	No inh
62.5	No inh	43 ± 2.2	No inh	31 ± 1.3	12 ± 0.7	No inh	No inh
31.25	No inh	8 ±0 .9	No inh	9 ± 0.7	0	No inh	No inh

Figure 10

The IC50 of inhibition activity of the synthesized compounds against HEPG cells



3.3.3 Anti-cancer activity against CaCo cell

Moreover, the compounds were tested against the colon cancer (CaCo). Again compound IV showed potent anticancer activity with (IC50 6.74 µg/ml). Compound III was the most potent with IC50 = 2.79 µg/ml. Compound I, Compound III had Compound IV had (96%, 92%, 98%) inhibition for the CaCo cells respectively which was achieved for these compounds at 500µg/ml.While compound VII showed no inhibition activity. The detailed inhibition and the IC50 are shown in Table 4 and Figure 11in Appendix A.

Table 4

The %inhibition of the synthesized compounds against CaCo cells at different concentration

Conc. µg/ml	**Comp (1)**	**Comp (2)**	**comp (3)**	**comp (4)**	**comp (5)**	**Comp (6)**	**comp (7)**
500	96 ± 3	69 ±4.3	92 ±7.8	98 ±4.6	76 ±8.9	64 ±3.1	No inh
250	26 ±2	18 ±3.3	68 ±4.8	90 ±5.9	53 ±5.3	30 ±2.5	No inh
125	No inh	7 ±2.4	57 ±5.6	87 ±7.1	18 ±4.1	3 ±0.8	No inh
62.5	No inh	4 ±1.7	27 ±1.5	82 ±8.1	14 ±1.5	No inh	No inh
31.25	No inh	3 ±1.1	1 ±0.2	50 ±4.1	3 ±0.9	No inh	No inh

3.4 Cyclooxygenase inhibition activity

The synthesized drugs were tested against COX isoenzymes. The results were good for most of the synthesized compounds. The IC50 was in the range of 1.4-6.4 µg/ml. However, the best IC50 for COX1 inhibition was for compound 6 (1.59 µg/ml). While compound 1 was the most effect COX 2 inhibitor (IC50 = 141 µg/ml). Moreover the compound 7 has a very good inhibition activity for COX2 (1.72 µg/ml) and also good selectivity for COX2 (3.81), The results illustrate that adding a cysteine to the tri aromatic structure will hugely increase the COX2 inhibition activity. The detailed IC50 are illustrating in results illustrated in Table 5. The selectivity for COX2 are illustrated in Figure 12 in Appendix A.

Table 5

COX inhibition activity for the synthesized compounds

Name of compound	IC50 (COX1) µg/ml	IC50(COX2) µg/ml	Selectivity of COX2
Comp (1)	1.61 ±0.11	1.41 ±0.07	1.14
Comp (2)	1.71 ±0.23	2.19 ±0.07	0.78
Comp (3)	3.71 ±0.12	2.23 ±0.04	1.66
Comp (5)	1.65 ± 0.09	2.35 ±0.05	0.70
Comp (4)	2.55 ±0.34	5.46 ±1.2	0.47
Comp (6)	1.59 ±.07	4.87 ±1.2	0.33
Comp (7)	6.58 ± 1.33	1.72 ±0.08	3.81

3.5 Antimicrobial activity

All compounds were tested against six types of bacteria and one type of fungus that cause human infections. All compounds showed no activity against bacteria and fungus.

Chapter Four

Conclusion

In this study we successfully synthesized a group of tertiary alcohols of benzyl and phenyl xanthones and thioxanthones. The synthesized structures were tested against varieties of cancer cell line. Some of the compounds were very active such as compound 2. Moreover, these compounds were tested for their COX inhibition activities and compound 7 showed a good COX2 inhibition activity with COX2 inhibition selectivity. A future work require further synthesis and testing of this chemical group and the synthetized compounds require in-vivo testing to understand its pharmacokinetic and pharmacodynamic activity in living system. Moreover, more synthesis of these analogues to better understand the SAR of this important group of compounds.

List of Abbreviations

Symbol	Abbreviation
C	Concentration
CaCo	Colon cancer cells
COX	Cyclooxygenase
COX-1	Cyclooxygenase 1
COX-2	Cyclooxygenase 2
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GIT	Gastrointestinal tract
HEPG2	Hepatic cancer cells
Hz	Hertz
IC_{50}	The half maximal Inhibitory concentration
λ_{max}	Lambda max
µg	Microgram
Mg	Milligram
MHz	Mega Hertz
ml	Milliliter
µM	Micro molar
NIR	Near infrared
NMR	Nuclear Magnetic Resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
R_f	Retention factor
RBF	Round bottom flask
ROS	Reactive oxygen species
STLC	S-trityl-L-cysteine
TLC	Thin Layer Chromatography
UV-Vis	Ultraviolet-Visible

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Appendices

Appendix A

Figure 11

The IC50 of inhibition activity of the synthesized compounds against CaCo cells

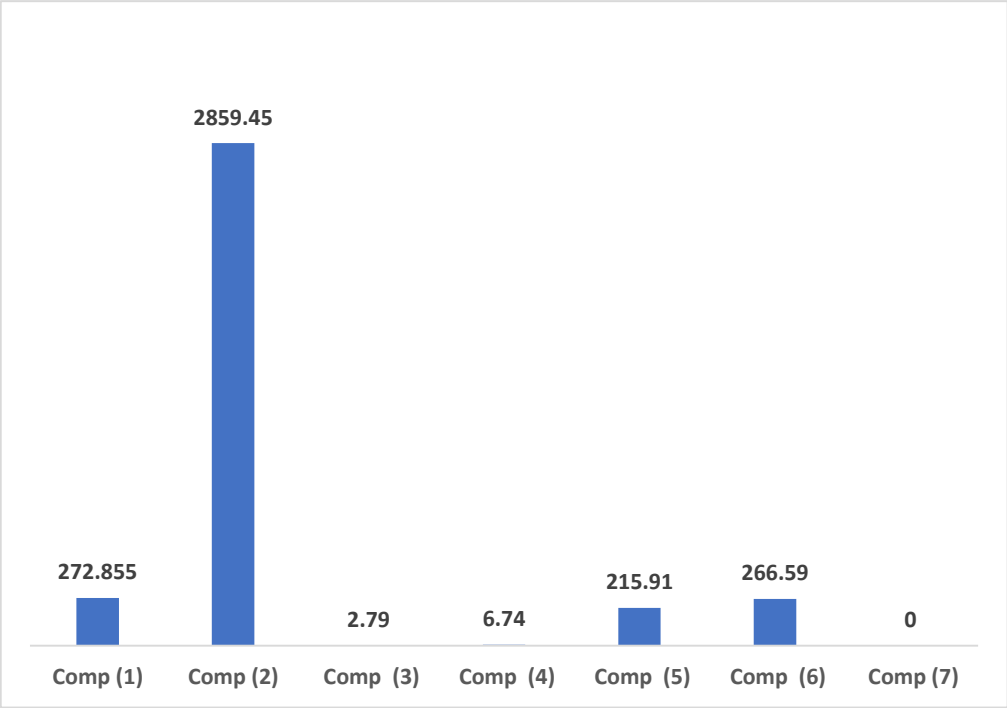
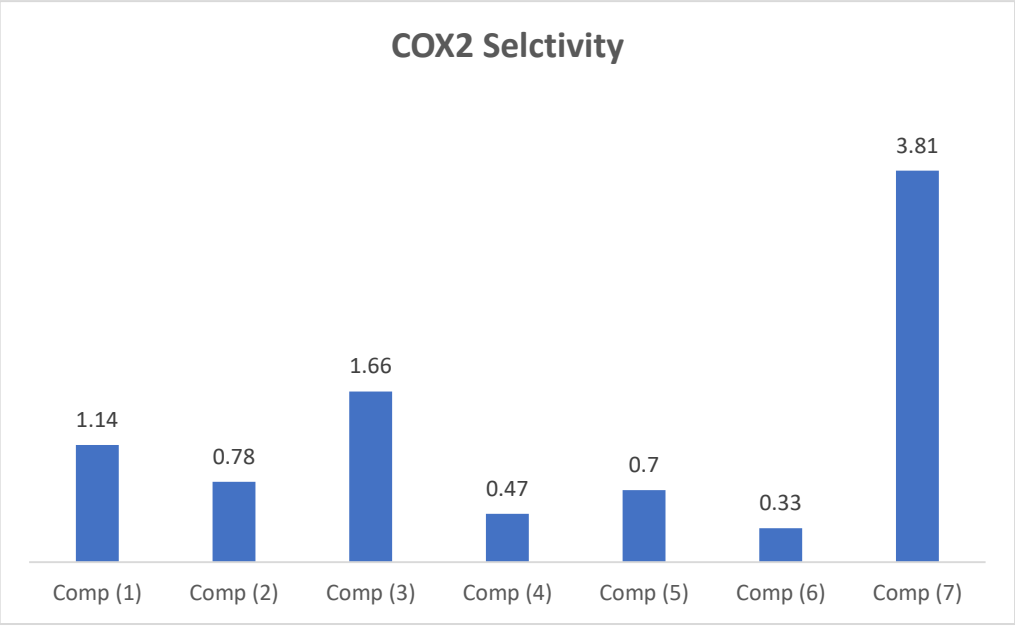


Figure 12

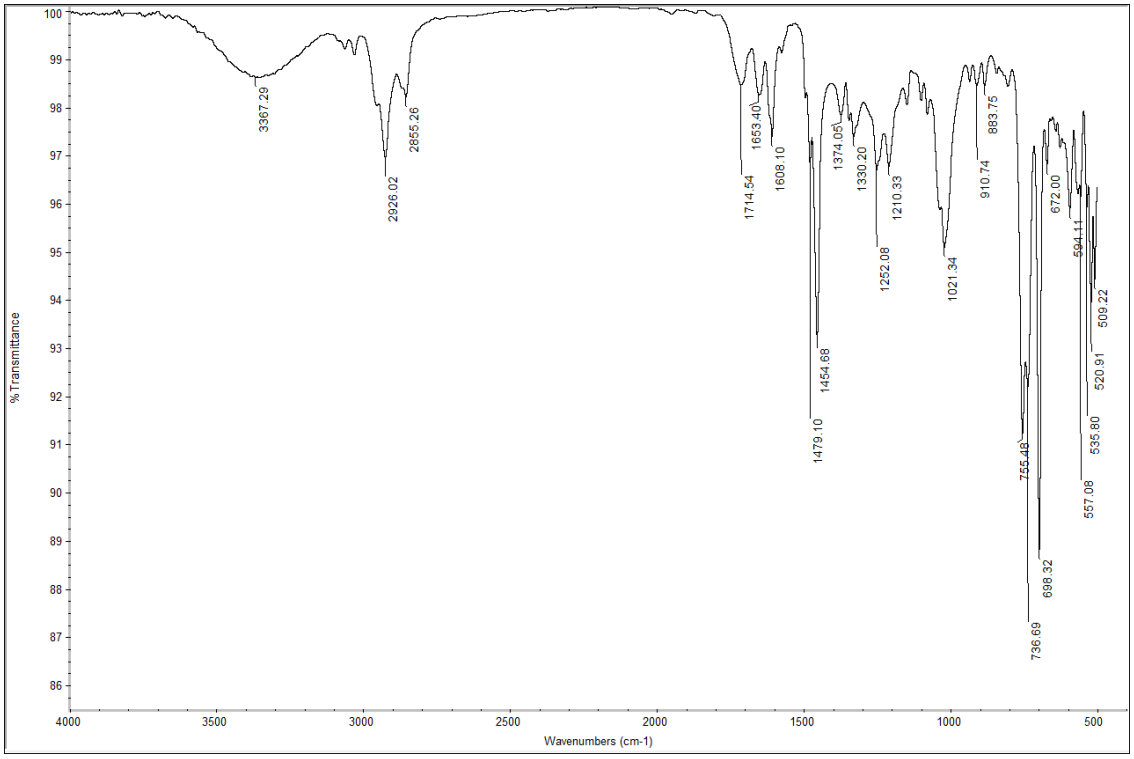
The COX2 ihibtion selctivity of the synthesized compounds



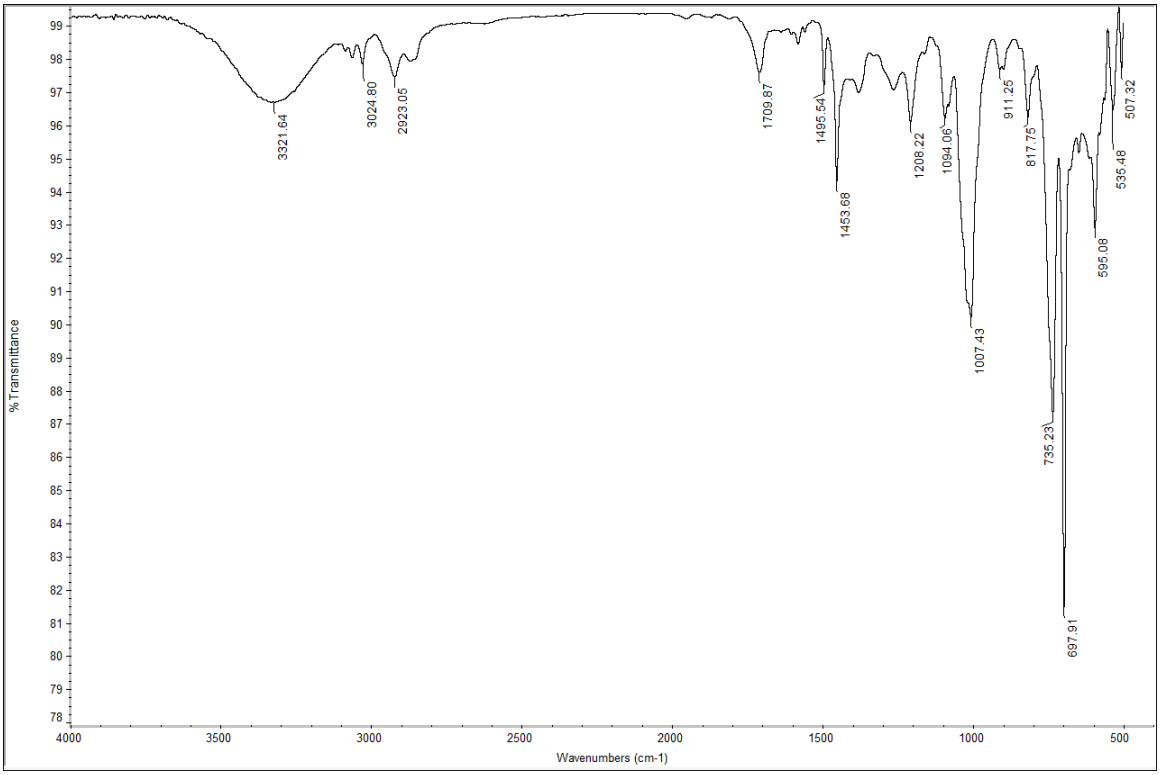
Appendix B

IR spectra

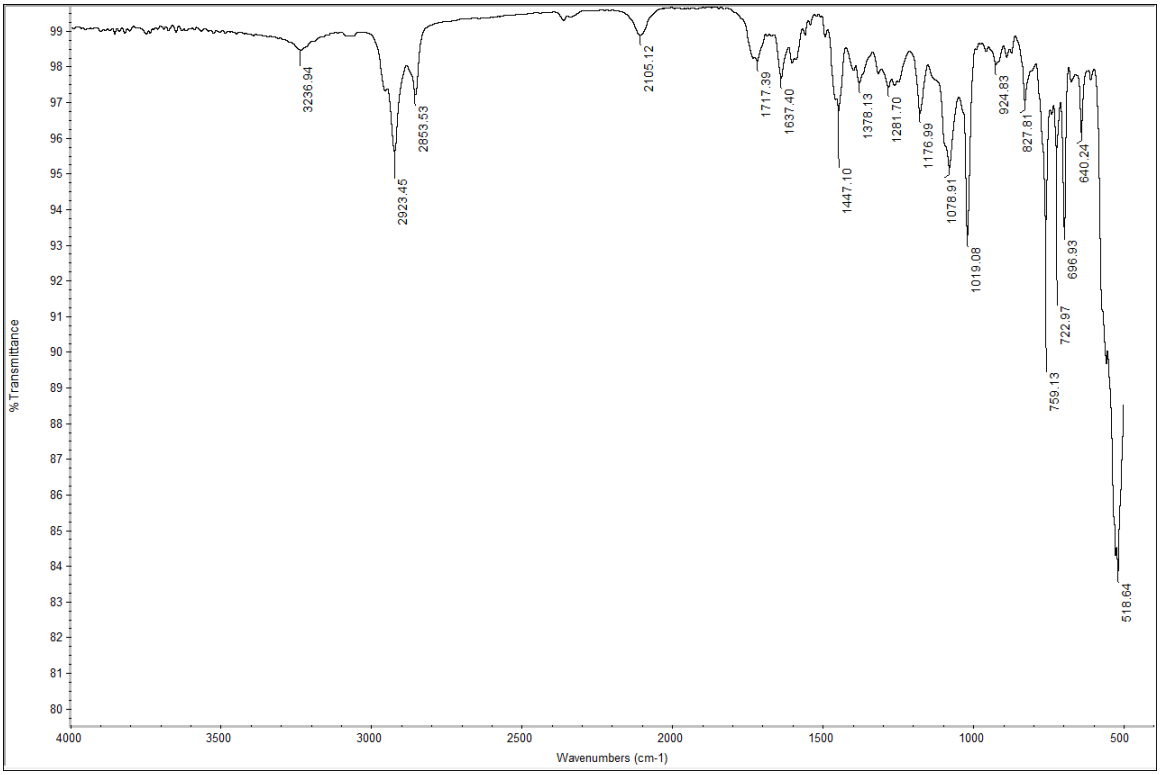
IR spectra for compound I



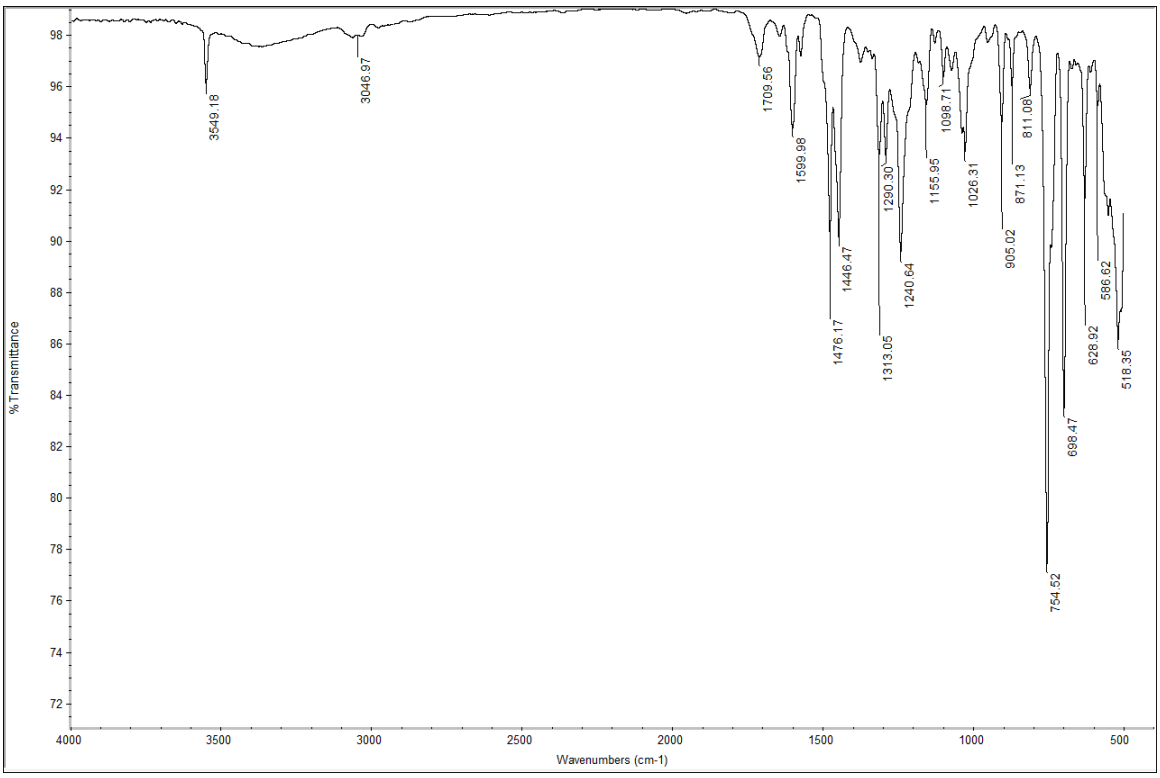
IR spectra for compound II



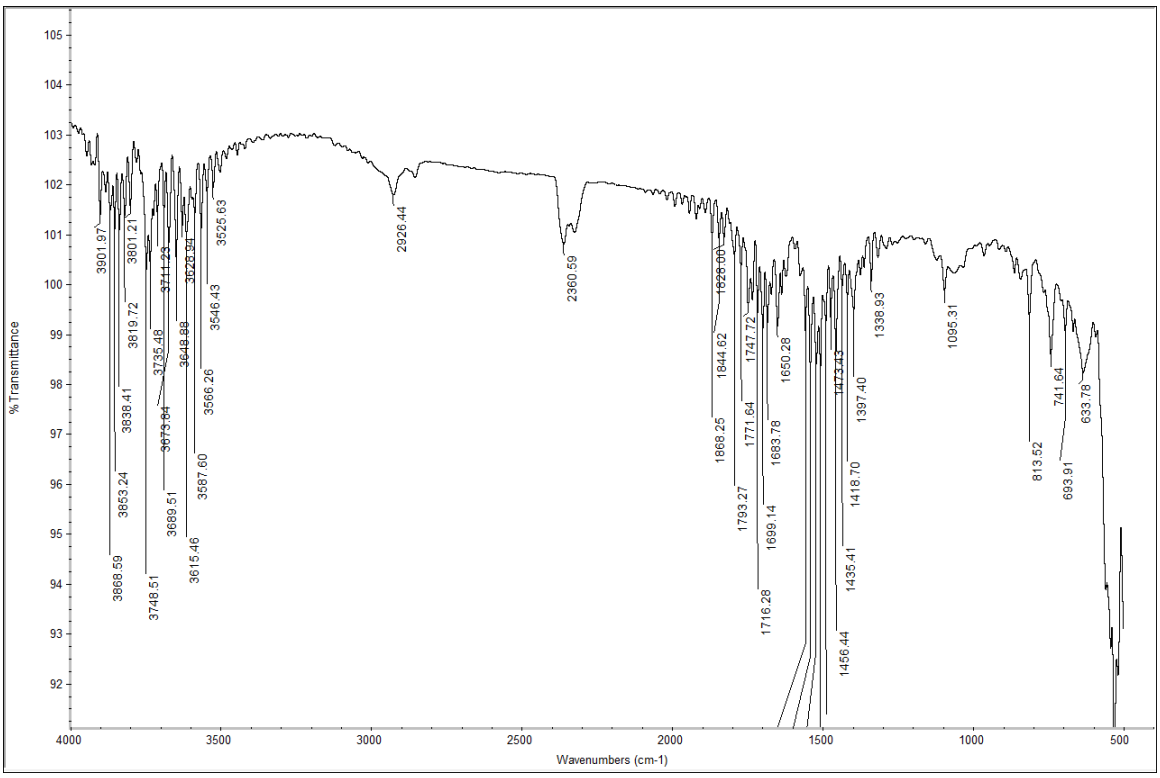
IR spectra for compound III



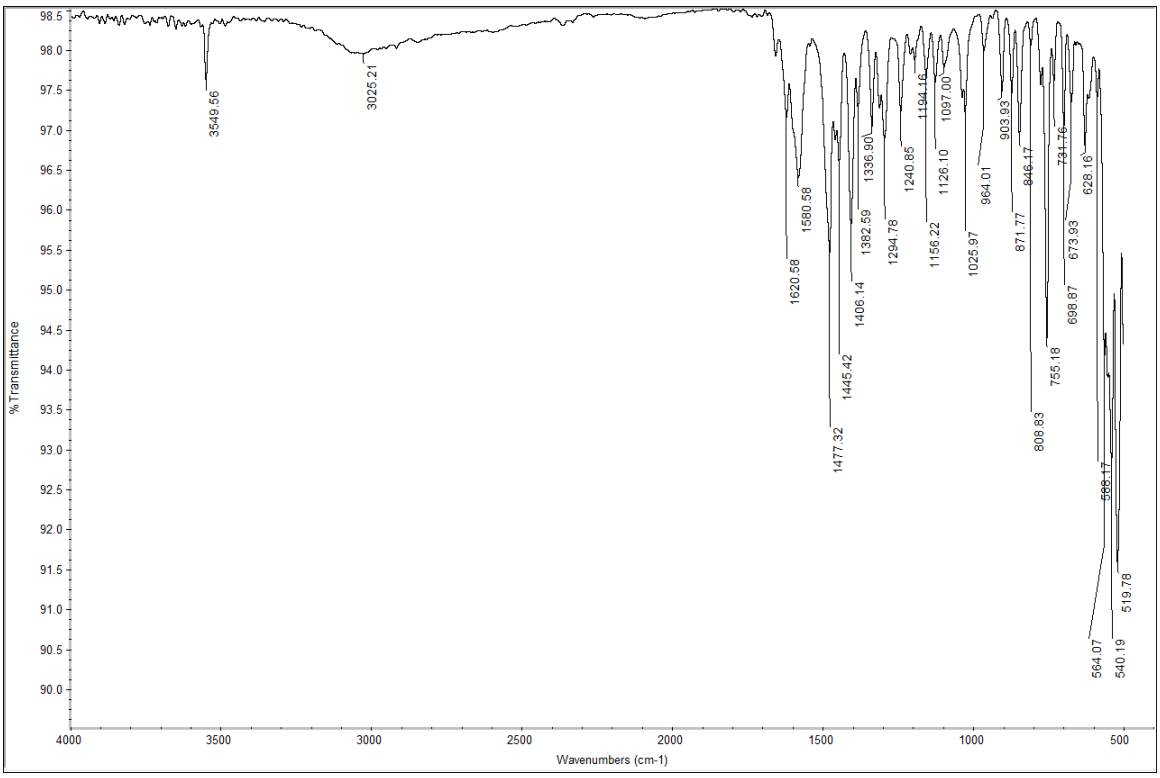
IR spectra for compound IV



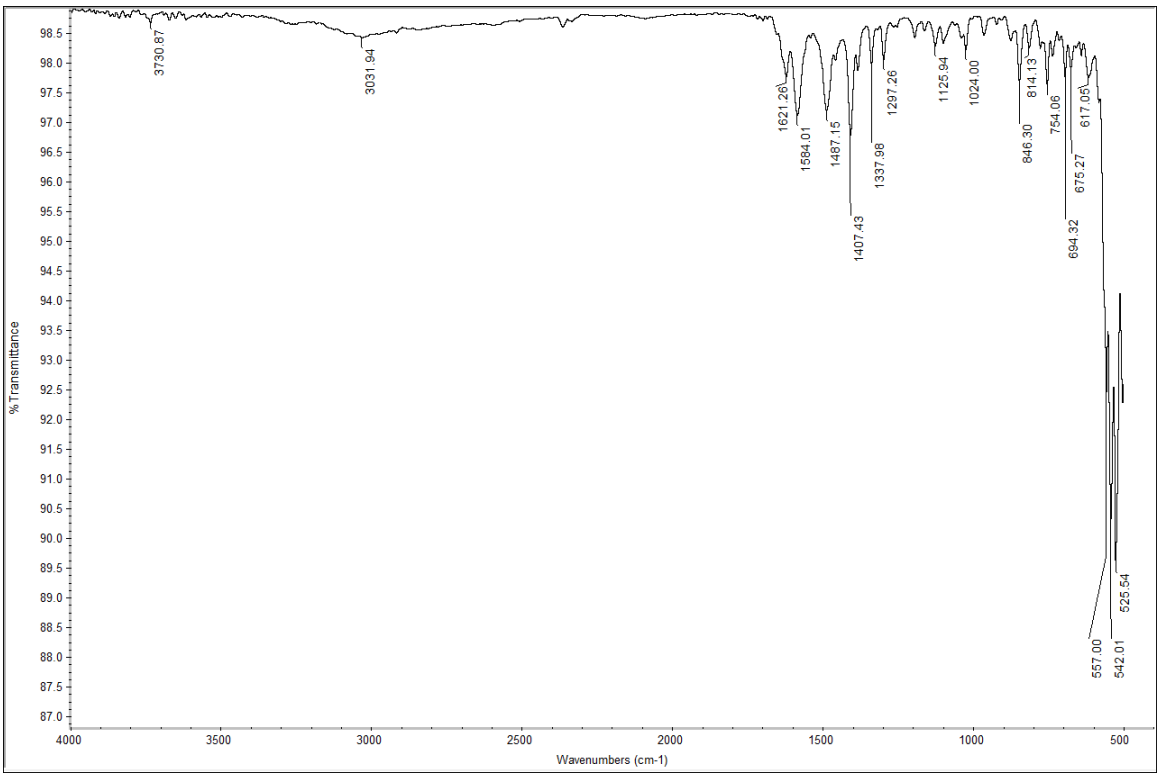
IR spectra for compound V



IR spectra for compound VI



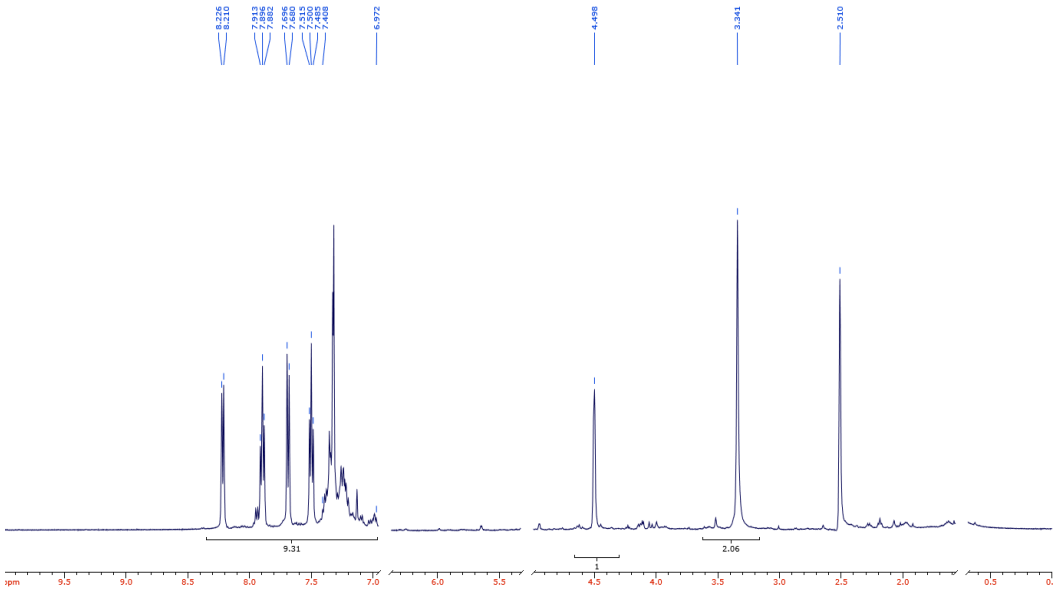
IR spectra for compound VII

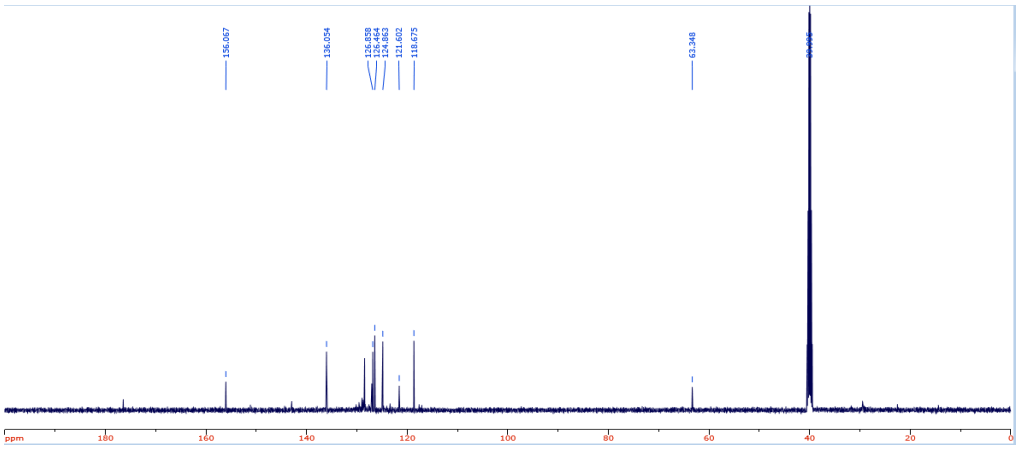


Appendix C

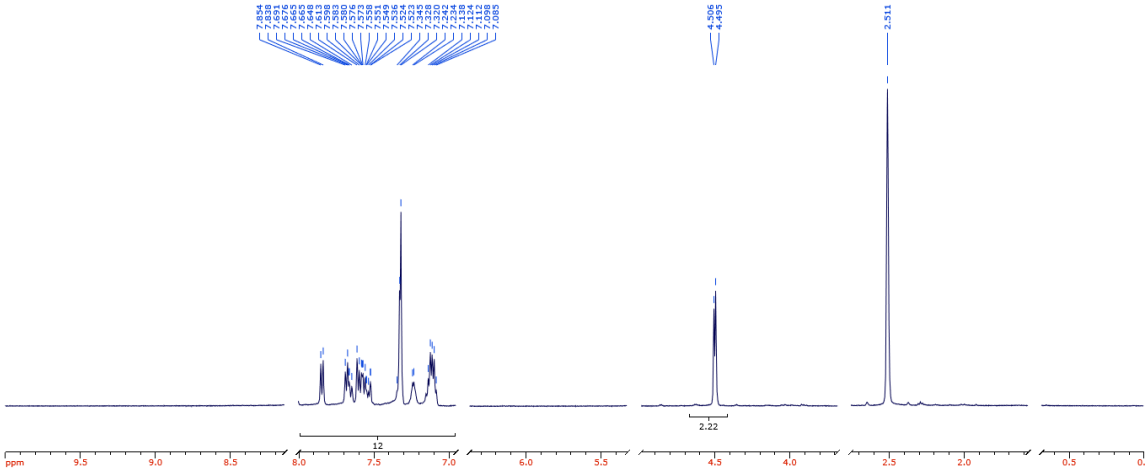
NMR spectra

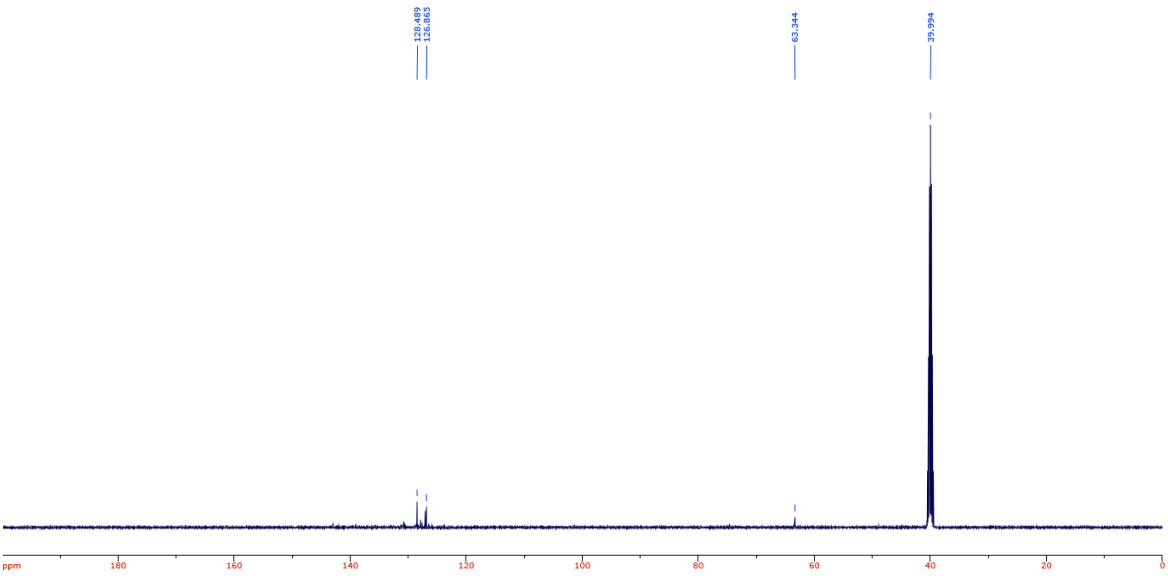
NMR spectra for compound I



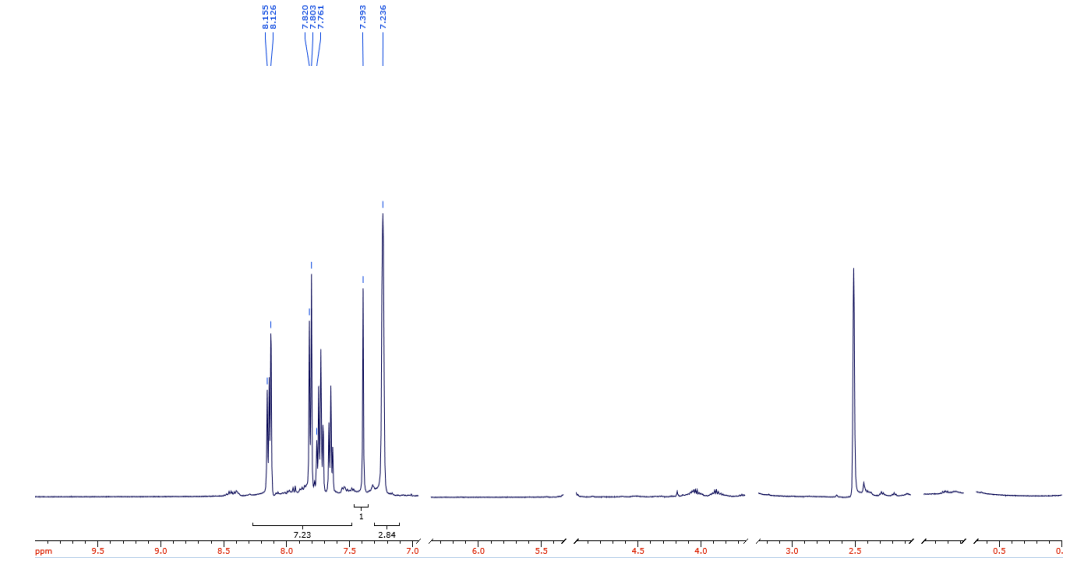


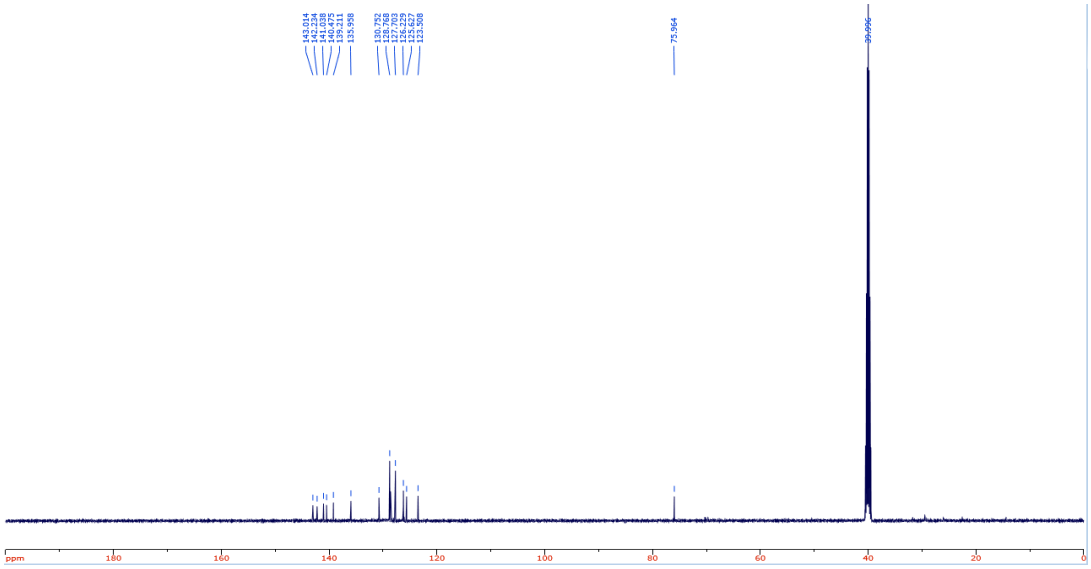
NMR spectra for compound II



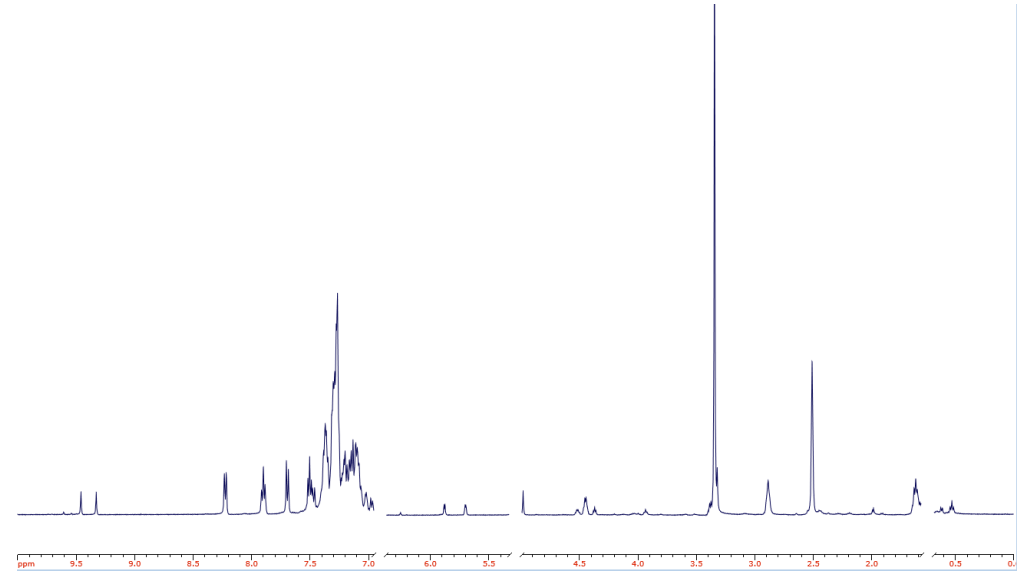


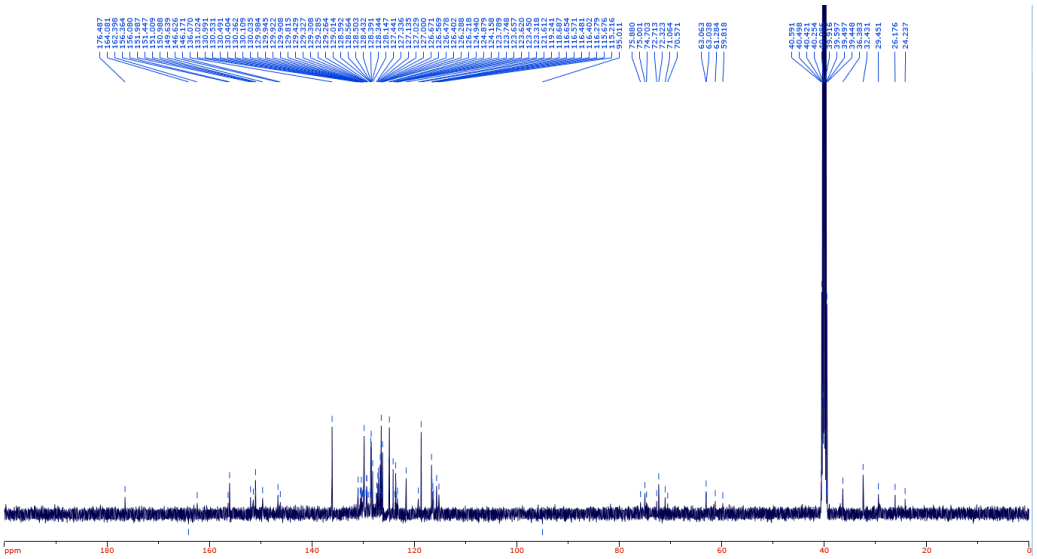
NMR spectra for compound III



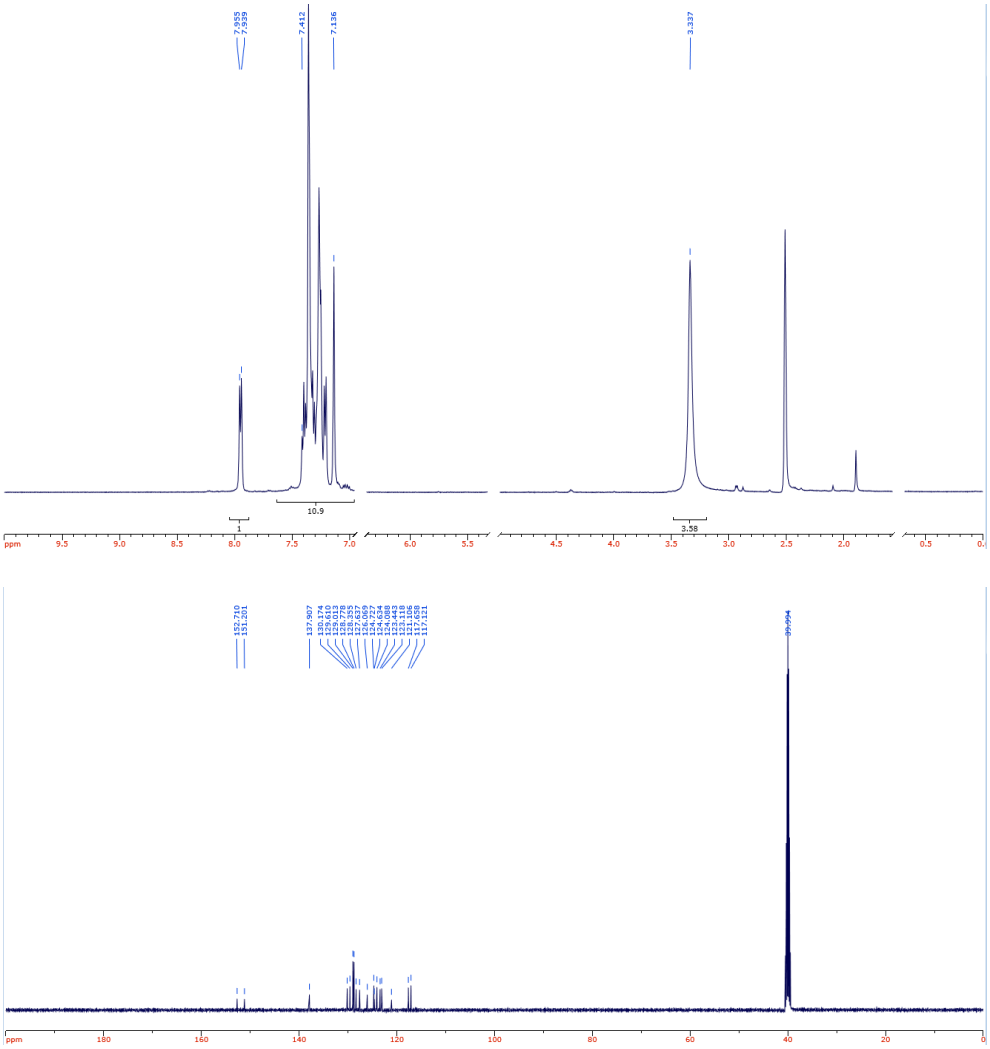


NMR spectra for compound IV

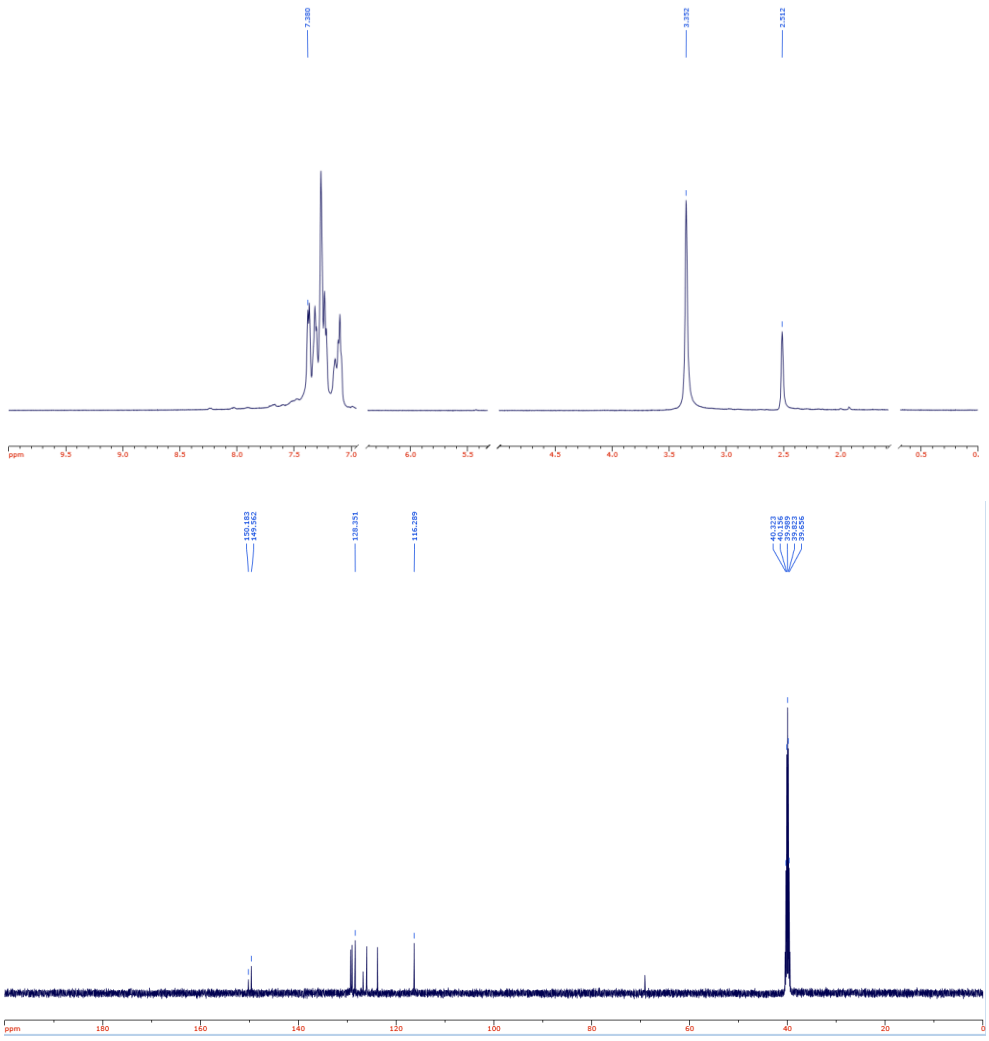




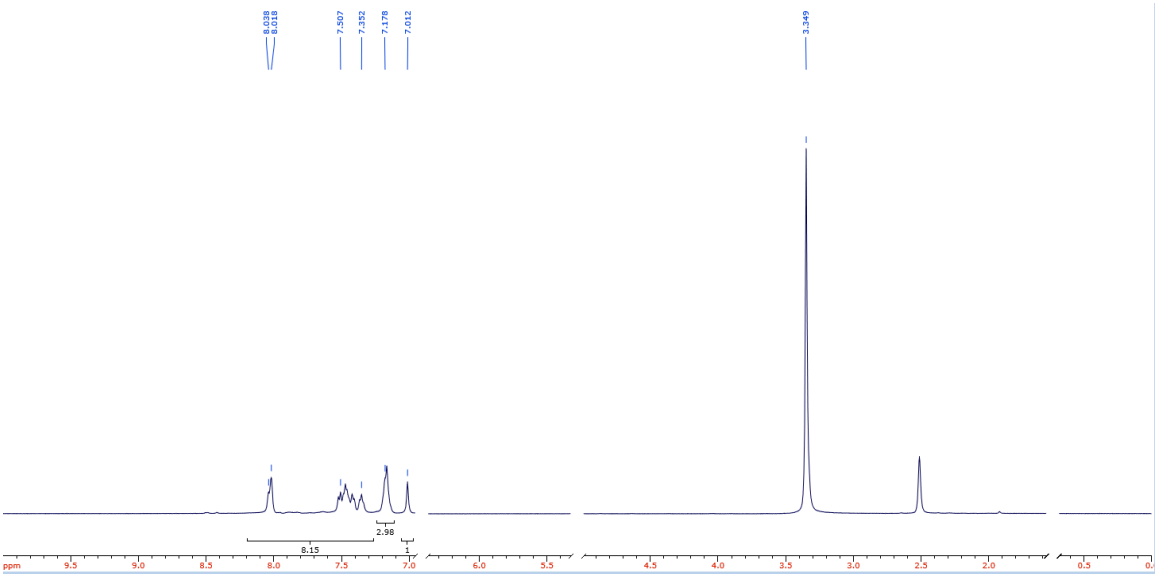
NMR spectra for compound V

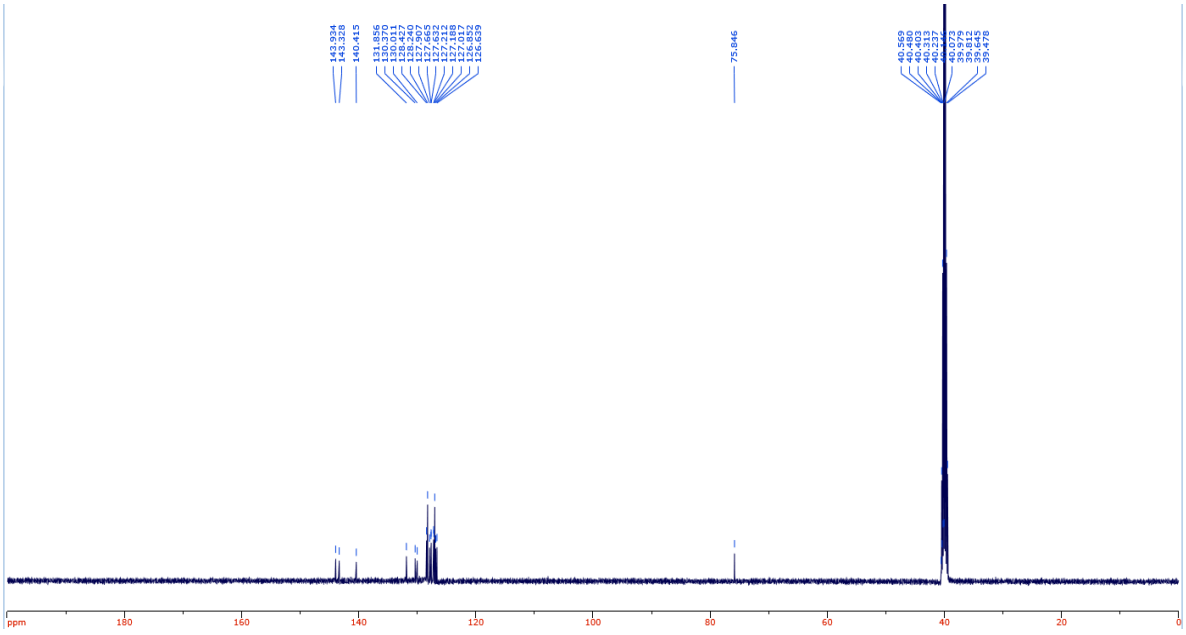


NMR spectra for compound VI



NMR spectra for compound VII





جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

تقييم الفعالية البيولوجية لمركبات مصنعة لمشتقات زانثون وثيوزانثون

إعداد

سماح عبد المجيد توفيق عقل

إشراف

د. مراد ابو الحسن

د. معتصم المصري

قدمت هذه الرسالة استكمالا لمتطلبات الحصول علي درجه الماجستير في العلوم الصيدلانية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2022

تقييم الفعالية البيولوجية لمركبات مصنعة لمشتقات زانثون و ثيوزانثون

إعداد

سماح عبد المجيد توفيق عقل

إشراف

د. مراد ابو الحسن

د. معتصم المصري

الملخص

المقدمة: تم استخدام زانثون وثيوزانثون لتصنيع مكتبة من المركبات واختبار هذه المركبات كمضادات سرطان ومضادات للالتهاب ومضادات للأكسدة ومضادات للميكروبات.

من اجل القيام بذلك، تم اضافة كاشف جرينيارد الى زانثون وثيوزانثون وذلك لانتاج مكتبة من الكحولات الثلاثية للفينيل والبينزل للكزانثون والثيوكزانثون وبعض هذه المركبات تم ربطها مع السيستين لانتاج مشتقات السيستين. تم تصنيع سبع مركبات بنجاح وتم اختبار هذه المركبات كمضادات سرطان ومضادات للالتهاب ومضادات للأكسدة ومضادات للميكروبات.

النتائج: نتائج الفحوصات كانت ناجحة بحيث ان المركب رقم 3 اعطى فعالية جيدة جدا ضد خلايا الكولون السرطانية بتكيز تثبيط نصفي =2.79 مايكرو غرام لكل مل والمركب رقم 2 اعطى فعالية جيدة ضد خلايا الكبد السرطانية بتركيز تثبيط نصفي=49.2 مايكرو غرام لكل مل رو المركب رقم 1 اعطى فعالية جيدة ضد خلايا الهيلا بتركيز تثبيط نصفي=60 مايكرو غرام لكل مل بينما المركب رقم 4 كان فعال كمضاد اكسدة بتركيز نصفي =4.22 مايكرو غرام لكل مل بالإضافة الى ذلك المركب رقم 7 اعطى فعالية جيدة مضادة للالتهاب مع نشاط تثبيط لانزيم السايكلو اكسيجيناز 2بتركيز تثبيط نصفي 1.72 مايكرو غرام لكل مل وكذلك انتقائية جيدة لانزيم السايكلو اكسيجيناز2. بينما لا يوجد احد من المركبات قد اعطى نتيجة كمضاد ميكروبات.

الخاتمة: يتطلب العمل المستقبلي مزيداً من الاختبارات في الجسم الحي لفهم الحركة الدوائية في الاجسام الحية. بالإضافة الى الحاجة الى تصنيع مركبات اخرى من هذه النظائر للتمكن من فهم افضل لشكل هذه المركبات وعلاقتها بالنشاط الذي تعطيه.

الكلمات المفتاحية: زانثون، ثيوزانثون، البيولوجية، الفعالية.