An-Najah National University

Faculty of Graduate Studies

Synthesis of Monocarbonyl Analogues of Curcumin that Required for Biological Screening

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Dedication

To my parents

To my brother

To my sisters

And my friends

I dedicate this work

Acknowledgment

Praise and thanks to my God, the most merciful for assisting and directing me to the right path, without his help my effort would have gone astray.

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الاقرار

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Synthesis of Monocarbonyl Analogues of Curcumin that Required for Biological Screening

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Date: **التاريخ: 9 /5/ 2021**

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List of abbreviations

Symbol	Abbreviations
DNA	Deoxyribonucleic acid
miRNA	microRNA
20^{th}	Twenty Century
WWI	World war one
WBCs	White blood cells
WWII	Second world war
SNi	Substitution Nucleophilic intramolecular
GEFs	Guanine Nucleotide exchange factors
CAMs	Cell adhesion molecules
TOP1	TopoisomeraseI
5-FU	Fluorouracil
MPM	Malignant pleural mesothelioma
CT	Computed Tomography
IV	Intravenous
WHO	World Health Organization
CTR1	Copper Transporter 1
P53	Tumor protein
ROS	Reactive oxygen species
RNS	Reactive nitrogen speacies
OCH_3	Methoxy group
OH	Hydroxy group
Nrf2	Erythroid-2-related factor 2
MCACs	Monocarbonyl curcumin analogues
MCF-7	Breast cancer cell
G2	Second growth phase
M	Mitotic phase
G1	First growth phase
H	Hour
THP	Tetrahydropalmatine
PGE2	Production of prostaglandin E2
$sPLa_2$	Secretory phospholipase A_2
COX-1	Cyclooxygenases

TLC	Thin-layer chrootography
UV	Ultraviolet
IR	Infrared spectroscopy
KOH	Potassium Hydroxide
HCl	Hydrochloric acid
R_f	Retention factor
CC	Column chromatography
CH_2Cl_2	Methylene chloride
m.p	Melting point
$MgSO_4$	Magnesium sulfate
TBDMSCl	Tert-butyldimethylsilyl chloride
Na_2SO_4	Sodium sulfate
NaOH	Sodium hydroxide
NaH	Sodium hydride
DMF	Dimethylformamide
NaCl	Sodium chloride
MeOH	Methanol
mCPBA	Meta-Chloroperoxybenzoic acid
$NaHCO_3$	Sodium bicarbonate
DMSO	Dimethyl sulfoxide
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
UV-Vis	Ultraviolet-Visible
UK	United kindom
CO_2	Carbon Dioxide
w/v	Weight by volume
SN_2	nucleophilic substitution reaction
Ph_3P	Triphenylphosphine
CuI	Copper(I) iodide
MACIR	Microwave-assisted coupling isomerization reaction
Et_3N	Triethyl amine
Min	Minute

H	Hydrogen
Br	Bromo-substituent
Cl	Chloro-substituent
F	Flouro-substituent
NO_2	Nitro-substituent
CH_3	Methyl-substituent
Ml	Milli-meter
β-	Beta-position
MTT	Colorimetric assay
MCF-7	Breast cancer cell line
nm	Nanometer
^oC	Celsius
G	Gram
%	Percent
Hela	cervical cancer cell line
MTS	CellTiter 96 AQueous One Solution Cell Proliferation Assay
µL	Maicro-litter
Mg	Maicro-gram
VF	Volumetric flask
In%	Inhibition percent
A	Absorbance
IC_{50}	The half maximal inhibitory concentration
Eq.	Equation
SD	Standard Deviation
M	Molar concentration
Hep-3B	Human-liver cancer cell line
Hep-G2	Human liver cancer cell line.
TBFA	Tetra-n-butylammonium fluoride
NaOMe	Sodium –methoxide
α-	Alpha-position
TEA	Triethyl amine
$HCCl_3$	Chloroform

µM	Maicro-Molar
BrdU	Bromodeoxyuridin
RNS	Reactive nitrogen species
ORAC	Oxygen radical antioxidant capacity
FRASC	Ferric reducing/antioxidant ascorbic acid chemistry

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Abstract

Curcumin and isoflavone have exhibited wide anti-tumor effect *in vivo* and *in vitro*. It has been developed a new adjuvant chemotherapy protocol of cancer by synthesis of monocarbonyl curcumin analogues and isoflavone derivatives.

The compounds of monocarbonyl curcumin analogues (MCACs) and isoflavone derivative were synthesized by condensation reaction under basic condition followed by oxidation reaction and identified by IR. They were evaluated for their cytotoxic activity against cervical (Hela), liver (Hep3B, HepG2) and breast (MCF-7) cancer cell lines, and also evaluated as antioxidant agents. Compounds **56**, **58** and **56a** showed no anticancer activity against MCF-7. On the contrary, compounds **57** and **59a** showed anticancer activity against MCF-7 with IC_{50} values of 39.8 μg/ml and 50.11 μg/ml, respectively. Compound **57** displayed inhibitory activity against HepG2 and Hela cancer cell lines with IC_{50} values of 50.11 and 22.3 μg/ml, respectively.

Moreover, compound **56a** showed inhibition of cell growth against HepG2 cancer cell line with IC_{50} value of 7.194 μg/ml better than that of curcumin

(IC_{50} 23.15 μg/ml). These results indicate that compound **56a** has a potent and promising anticancer activity against HepG2 cancer cell line. Compounds **67, 66 and 61** exhibited very weak anticancer activity against all cancer cell lines. On other hand, **56a** and **57a** showed excellent antioxidant activity with IC_{50} values of 11.8 and 15.8 μg/ml, respectively, compared with the strong antioxidant agent Trolox (IC_{50} = 3.4 μg/ml).

Chapter One

Introduction

Cancer is a dangerous and life-threatening disease. It is the second leading cause of death around the world. Cancer is the abnormal growth of cells in the body. In 2018, there were nearly 17 million new cases of cancer worldwide [1]. Factors causing abnormal growth of body cells that lead to cancer, including the exposure to some toxic chemical compounds [2], exposure to harmful radiation, and the presence of genetic genes (a family history) that carry the disease [3].

1.1 Cancer and its formation

Cancer is a disease that affects the growth of cells. The transformation of a normal cell into a neoplastic cell (cancer cell) is caused by changes in genes that control cell growth and differentiation [4]. Genetic and epigenetic changes will happen at several levels, from a take or lack of entire chromosomes, to mutation simulating single DNA nucleotide, or stopping or activating a micro RNA [5]. A tumor suppressor gene are the genes that prevent or stop a cell division, survival, or another characteristic of cancer cells [6].

The tumor suppressor genes are usually obstructed via cancer-promoting genetic changes as shown in the figure 1.1.

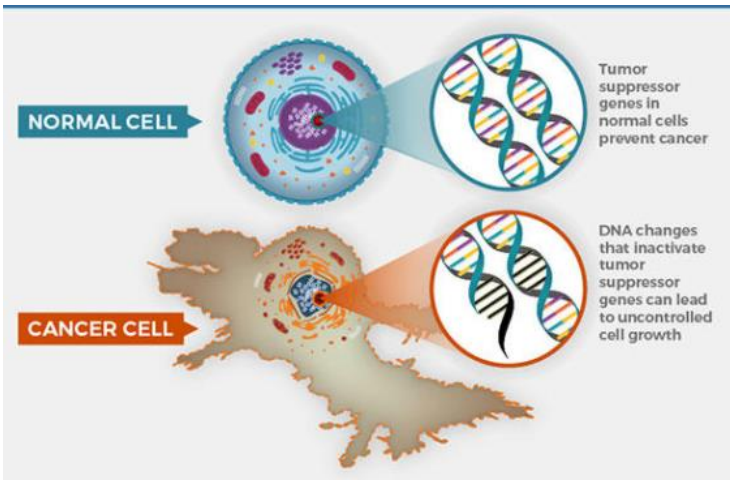

Figure 1.1: Tumer Suppressor Genes in Normal Cell vs Cancer Cell.

There are several compilations for the distinct genetic changes that may be involved in the generation of cancer cells. Considerable of these changes are mutations [7], or changes in the nucleotide series of the genomic DNA [8], and also neuploidy, that is the existence of abnormal number of chromosomes, is a genomic modification that may include any gain or lack of one or more chromosomes out of errors in the significant motivating to cause the cancer [9].

DNA damage is the major cause of cancer formation [10]. The sources of this damage may include substances released by the body such as reactive oxygen species produced by certain cells, which cause DNA damage that stimulate the production of mutagenesis, which then leads to the formation of tumoriggenesis [11]. Some external factors such as the exposure to ultraviolet rays could cause the DNA damage, therefore the risk for cancer is increasing [12]. Other sources of DNA damage are the replication stress, the cell metabolism, the viral infection and chemotherapy [13] as shown in

the figure 1.2. In addition, contribution of field defects [14] and a genome instability could trigger cancer [15].

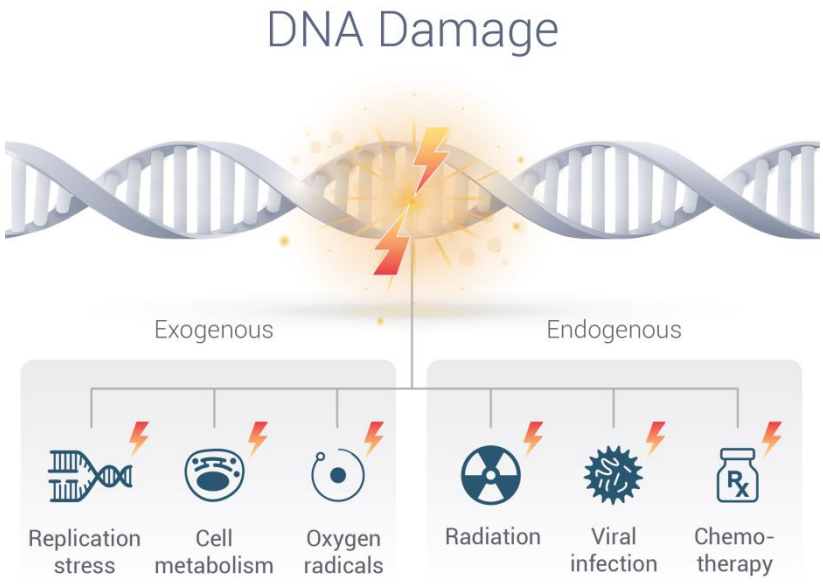

Figure 1.2: The Sources of DNA Damage.

There are many kinds of cancer such as the lung, lymphoma, breast, skin, colon and prostate cancer. Symptoms differ from one type to another, as each type has its own symptoms. There are several symptoms that are general for some types, such as weight loss, pain, the change in the bowel, the bladder function, persistent coughing and the appearance of some lumps [16]. There are many treatments methods that can be used to treat cancer. The choice of treatment type depends on the awareness of the location of the tumor, its size, what a stage it is, and the health status of the patient [17]. Surgery, chemotherapy, radiotherapy, immunotherapy and hormonal therapy or the treatment with antibodies, are main treatments methods for cancer [18]. The goal of the treatment is to allow the patient to lead normal life that may or may not be possible. If it does not succeed in removing a cancer cell

completely, it may reduce it or slow its growth and a spread as much as possible [18].

1.2 Chemotherapy treatment

The Chemotherapy is the treatment of diseases using chemicals to kill cancer cell. Chemotherapy refers to the use of anti-tumor drugs to treat cancer or to be antibiotics for non-cancerous diseases [19]. Drug treatment was started in the early 20th century, without intent. The roots of the first efficient chemotherapy for cancer depend on rigorous analysis and also on accidental findings during World War I (WWI) when mustard gas was used as a weapon [20]. Despite the horrible use of mustard gas **1** during WWI, there was a good side by the discovery of the first new chemotherapeutic agent, based on observations from WWI survivors exposed to mustard gas **1** [20]. Thus, the gas **1** has an effect on cancer cells, just as it affects rapidly growing white blood cells. When this drug was given to many cancer patients intravenously instead of inhaled in the 1940s, they improved clearly. It was a temporary improvement [21].

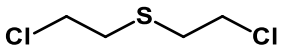

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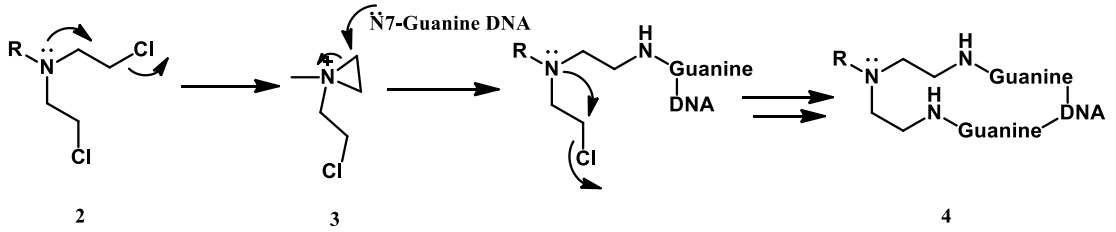
Figure 1.3: Chemical structure of mustard gas

Nitrogen mustard gas **2** appeared during the Second World War. It is considered as mustard gas derivative. It is a form of a cancer treatment [22]. In 1946, it was demonstrated that nitrogen mustard **2** reduced the growth of

tumors in mice through a mechanism, where two strands of DNA were attached by a molecule of nitrogen mustard **2**. It has been proven that the sensitivity of mice bone marrow to mustard gas **2** is similar to that of humans. Nitrogen mustard gas **2** has become part of modern chemotherapy, as it is used mainly in treating cancer of the lymph glands (Hodgkin's disease) [23].

By intramolecular displacement of the chloride by the amine nitrogen, nitrogen mustards **2** form cyclic aminium ions **3** (aziridinium rings). When the N-7 nucleophilic center on the guanine base attacks this aziridinium ring, it alkylates DNA. After the second chlorine is displaced, the second alkylation step occurs, resulting in the formation of interstrand cross-linking **4** (scheme 1.1). Thus, preventing the cell division, which leads to apoptosis [24].



Scheme 1.1: The mechanism action of Nitrogen mustard gas

1.2.1 Chemotherapy Mechanism

There are several differences between cancer cell and normal cell that must be known. Normal cells stop growing when enough cells are reached but the cancer cell continues to grow leading to the formation of tumor [25]. Normal cells react to the signals sent by neighboring cells while cancer cells do not [26]. The apoptosis occurs in normal cell, in cancer cell it does not [27].

Normal cell does not spread in different parts of the body but the cancer cell spreads because it does not contain adhesion molecules (CAMs) [28]. The environment of a normal cell is an alkaline, while the cancer cell is an acidic or slightly alkaline [28]. Oxygen is required in a normal cell [29]. The figure 1.4 explains the shape of the normal cell and the cancer cell.

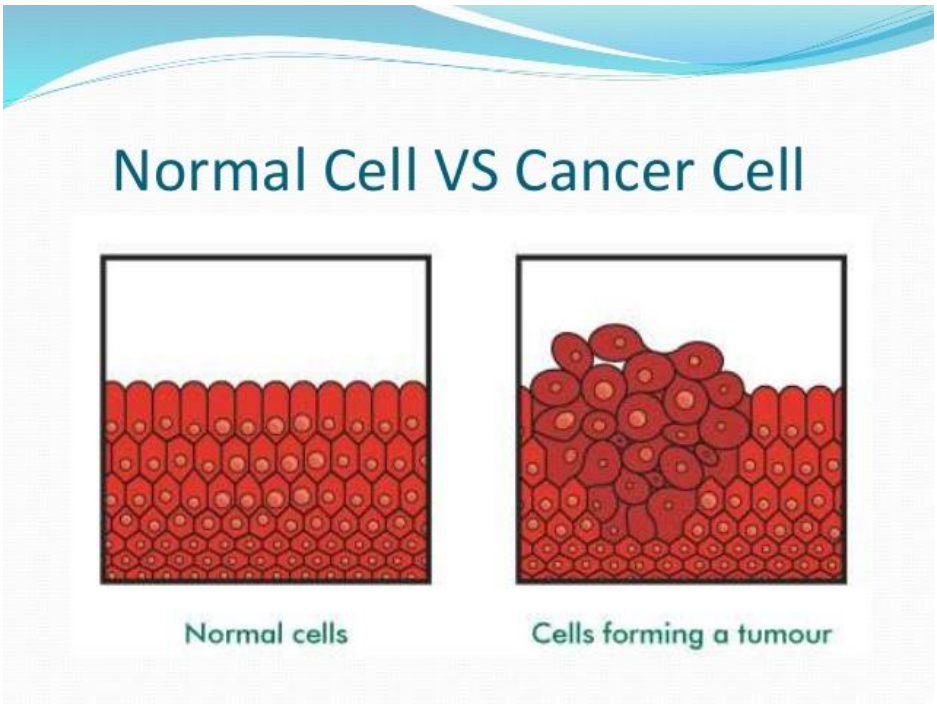


Figure 1.4: Normal cell vs cancer cell structure

Chemotherapy's cytotoxic agents work by interrupting the cell cycle. Cancer cells lose their ability to detect inhibitory signals for growth and die as a result [30]. Chemotherapy drugs cause cell death by apoptosis through interfering with DNA directly or by targeting the proteins that control cell division [30]. It may also be toxic to normal dividing cells, especially cells with a high turnover, such as the bone marrow and mucous membranes [31].

Chemotherapy drugs are divided into two categories: those that affect the cell cycle (phase specific agents) and those that are categorized based on

their biochemical properties [30]. Chemotherapy with similar biochemical properties has the same mechanism of action and can be divided into Alkylating agents such as Nitrogen Mustards (Melphalan **5**), Anti-metabolites such as Pyrimidine analogues (Gemcitabine **6**), Anti-tumour antibiotics such as Anthracyclines **7**, and Topoisomerase inhibitors such as TOP1 (Topotecan **8**) [30].

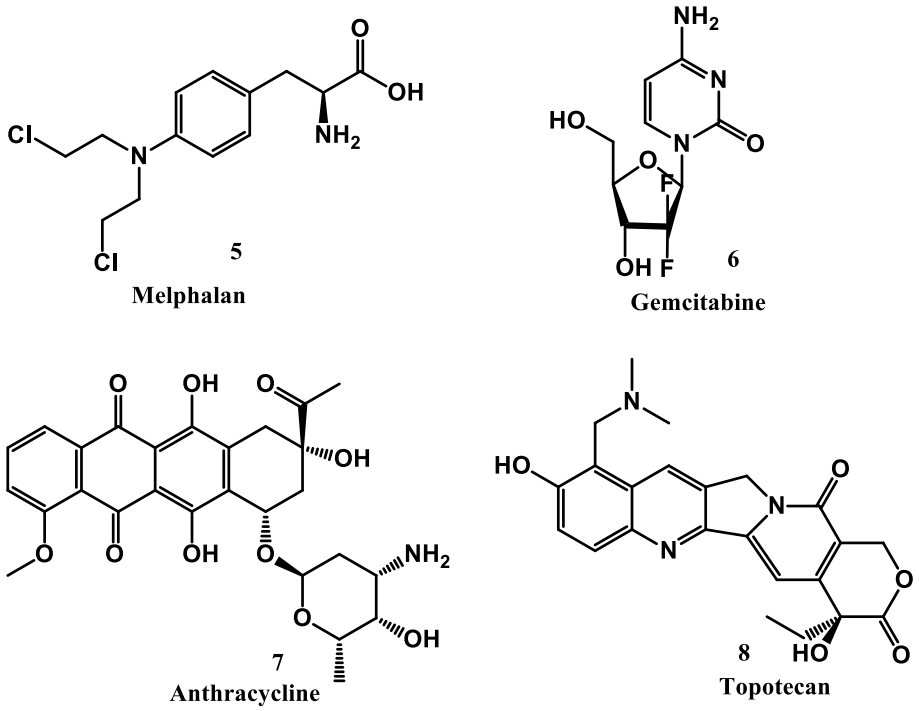
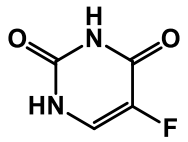


Figure 1.5: Chemical structure of alkylating agent drugs

Chemotherapy drugs are classified by cell cycle (phase specific agent) since this influence how related drugs are scheduled and combined to achieve the best possible effect [30]. The 5-Fluorouracil (5-FU) **9** is an example of a phase specific agents. The 5-FU 7 metabolites have the ability to inhibit the manufacture of thymidylate, one of the enzymes involved in DNA synthesis, or cause it to be incorrectly incorporated during synthesis [30]. This medication must be administered to cancer cells during the DNA replication stage and for

a longer period of time [32]. In contrast to cisplatin **10**, which damages DNA at any stage of the cell cycle during a normal treatment period [30].



9
5-Fluorouracil

Figure 1.6: Chemical structure of 5-Fluorouracil

1.2.2 Combination chemotherapy that reduced drug resistance

Combination chemotherapy is simply the use of more than one drug to treat cancer. Most of the current treatments for many types of cancer use two or more drugs at the same time. Chemotherapy drugs affect cancer cells at different points in the cell cycle. The use of more than one drug increases the chance of eliminating most of cancer cells. Despite this, using more than one drug has the disadvantages of increasing the number of chemical reactions that may cause it, making it impossible to determine which drug caused it [33]. Combination of chemotherapy is used to treat a variety of solid tumors, such as treating non-small cell lung cancer with a combination of cisplatin and vinorelbine (NVB) [34, 35], as shown in figure 1.7, which resulted in tumor shrinkage.

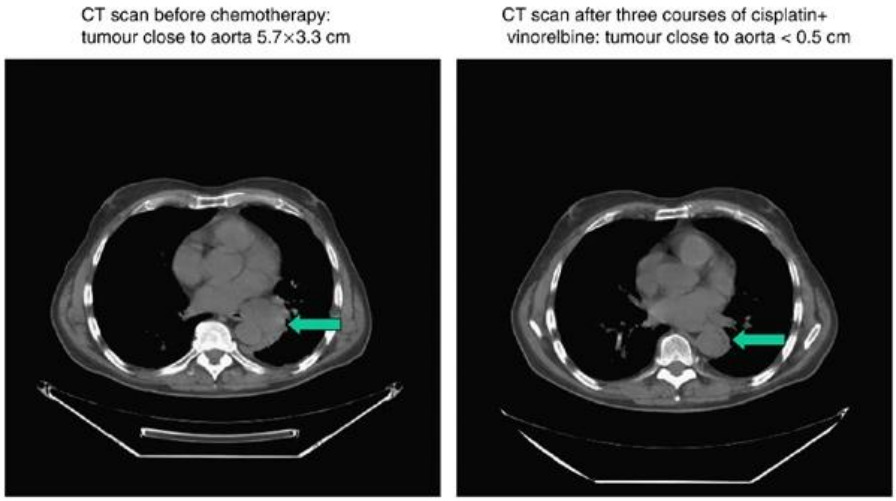


Figure 1.7: Computed tomography (CT) scan before and after three courses of cisplatin and vinorelbine in MPM with shrinkage of tumour close to aorta.

More recently, chemotherapy has been used in combination with type of an immunotherapy to make the immunotherapy drugs more effective. When chemotherapy is used in a combination with the immunotherapy, the benefits may exceed the use of a combination of drugs. The immune system medications work with the help of the immune system to recognize and attack cancer cells [36]. When cancer cells are destroyed with the chemotherapy drugs, the immune system helps to recognize these cells as abnormal [36]. This phenomenon is known as the abscopal effect. One of the advantages of using combination of drugs than using single drug treatment is to reduce the chance of tumors to resist the treatment [37].

1.3 Common drugs that are used in chemotherapy

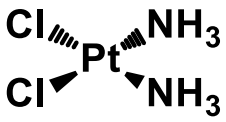
Chemotherapy is used to treat cancer in a variety of ways, either alone or in combination with other treatments. Each drug has its own chemical

composition, therapy method, level of contribution to cancer treatment, and potential side effects. The following sections present different types of them.

1.3.1 The Cisplatin and medical use

The cisplatin **10** used as chemotherapy for different types of cancer [38], such as lung, breast, ovarian, head, neck, bladder, esophageal, brain, neuroblastoma and other types of tumors [39]. Cisplatin **10** enters the body through an intravenous (IV) injection [39]. Its success is notable and successful for testicular cancers, as it works to raise the recovery rate from 15% to 85% [40]. Cisplatin **10** was discovered in 1845 and granted a medical license in 1979 [41]. The World Health Organization (WHO) has worked to ensure that drugs are used safely [42].

Cisplatin is cis-diamminedichloroplatinum(II) **10**, a metallic coordination compound having square planar geometry [43], which is soluble in water, the dimethylprimanide and N,N-dimethylformamide (DMF) [44].



10

Figure 1.8: The chemical structure of cisplatin

The cisplatin **10** gained its importance when scientist Rosenberg was working in electrolysis at Michigan State University in 1965, he found that certain electrolysis products of the platinum network electrodes worked to inhibit cell division in coliform bacilli [45]. Based on that, they worked on using products

of electrolysis in cancer chemotherapy. And then show that the agent (cis-dichlorodiammineplatinum (II) was responsible for this distinctive activity (the cell inhibition). And thus, became interested in the noble elements such as platinum and palladium in the treatment of cancer [45].

1.3.1.1 Side effects of the cisplatin

Most medicines can have side effects, so cisplatin **10** has many effects that limits its use, including the nephrotoxicity (kidney toxicity). When taking dose of it, the adequate hydration must be used to prevent or mitigate the damage of the kidney [46]. The Neurotoxicity (nerve damage) which is the perception of insight and hearing upset, and these symptoms occur shortly after taking the treatment [47]. The Nausea and vomiting that appear significantly and treated with antibiotics [48], ototoxicity (hearing loss) or ringing in the ears [49] and hemolytic anemia after take several doses of the cisplatin **10** [50]. Also, the hair loss, loss of appetite, pallor of the face and the weight loss may occur [51].

1.3.1.2 Mechanism of action of cisplatin and its resistance

The cisplatin **10** mechanism of action has been comprehensively investigated. It is assumed that cisplatin **10** enters cells by both passive diffution and active transport with the aid of copper transporter 1, where it displaces its two chlorides and bind to purines in the genomic DNA. Platinum binding to DNA cause structural alteration of the double-stranded structure, leading to inhibition of DNA replication and transcription, and the

subsequent DNA damage response leads to apoptosis [52] as show in the figure 1.9.

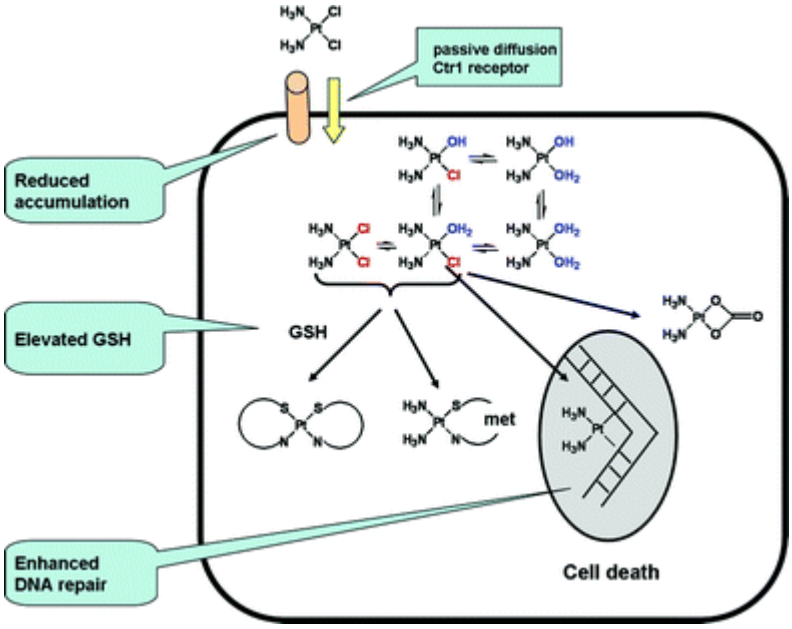


Figure 1.9: The mechanism of action of platinum anticancer agents

It was proposed that cisplatin **10** resistance emerges from multifactorial cellular modifications that affect drug uptake and metabolism at the cellular level, including reduced cellular accumulation of platinum, detoxification of platinum by thiol-containing molecules, and elevated levels of DNA damage repair [53, 54]. These factors all result in fewer Pt-induced DNA lesions in the genomic DNA, leading to cancer cell survival [54].

1.3.1.3 The Cisplatin analogues

Considerable work has been made on the clinical and pre-clinical development of the cisplatin **10**, after it was shown having cytotoxic properties in the 1960s [55]. Thousands of analogues have been discovered and manufactured that improve and enhance its role in treatment. About 13

of these analogues have been evaluated through some clinical trials, but it has found that one of these analogues provided advantage over the ciplatin in reducing the toxicity, which is the carboplatin **11** [55]. Other analogue of the cisplatin **10** is oxaliplatin **12**, the effectiveness oxaliplatin is shown in the treatment of the colon and rectal cancer. Sometimes it is used with another drug when the tumor is in its advanced stage [56].

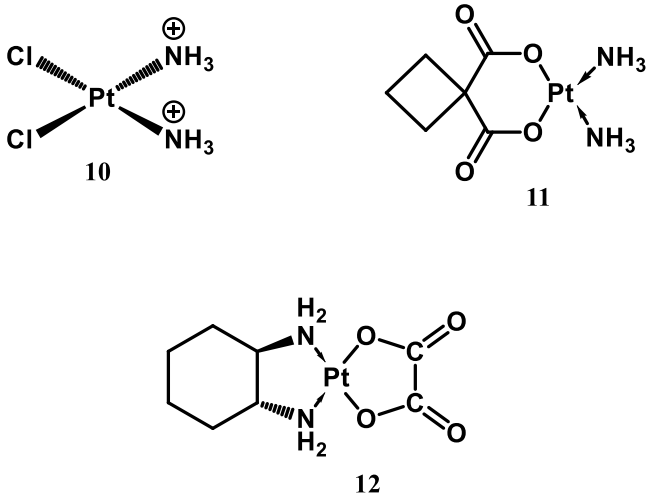


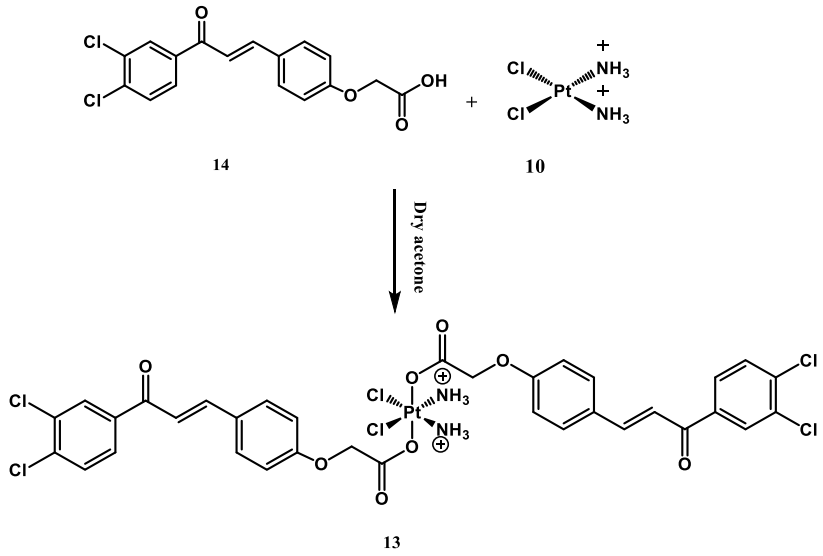
Figure 1.10: Chemical structure of platinum drugs

The cisplatin **10** and its analogues consist of a platinum ion carrying +2 charge and surrounded by four bonds (ligands). The figure 1.10 shows that cisplatin analogues modified and corrected some defects of cisplatin. Among these modifications, the chloride groups were altered in the carboplatin **11**, which led to a better conduction in the required cells and reduced side effects [57]. In the oxaliplatin **12**, the amino groups are presented to form larger cross-link allowing greater number of cells to bind [57].

1.3.2 Pt(IV) prodrugs based on chalcone

A Pt(IV) anticancer complexes are prodrugs of their Pt(II) derivatives. They are complexes with improved stability and a low-spin d^6 octahedral geometry [58]. They are some of the most promising methods for overcoming the cisplatin **10** resistance and side effects, not only in dealing with cancer cells but also in the tumor microenvironment [59]. The development of these prodrugs has relied on several different modifications, including combination with lipid chains to increase water resistance (hydrophobicity), and coupling with short peptide chains or nanoparticles to improve drug delivery, and addition of bioactive ligands to the axial positions of Pt(IV) complexes to exert dual-function effects [60]. The axial ligand have been modified to enhance the pharmacological properties and reduce drug resistance and side effects [61].

Recently, Ma et al. developed a novel dual-targeting Pt(IV) anticancer prodrug. Chalcoplatin **13**, based on cisplatin **10** and chalcone **14** (scheme 1.2). Compared to cisplatin **10**, chalcoplatin **13** revealed a significantly higher cytotoxicity (10-fold) for wild-type p53 [62, 60]. These results indicated that the chalcone moiety in chalcoplatin **13** played a crucial role in killing cancer cells, possibly by activating p53.



Scheme 1.2: Synthesis of chalcoplatin

1.3.3 Curcumin and Its Derivatives as Potential Therapeutic Agents

The curcumin **15** (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione diferuloylmethane) is a phenolic compound extracted from the turmeric plant and is a well-known spice and food coloring agent [63]. Curcumin and its derivatives have received great attention due to their vital functional properties, as it is considered an anti-cancer, anti-oxidant, anti-inflammatory, antimicrobial, antihepatotoxic and anti-Alzheimer's [64]. All of these characteristics are attributed to the main parts of its structure [65]. The structural composition of curcumin is responsible for its biological effect [65]. Curcumin **15** is known as a symmetric compound consisting of two rings of phenyl linked to two groups of hydroxyl and methoxyl, diketo functional group, two double bonds and an active methylene part [66]. Each of these parts is able to make some adjustments to improve curcumin's

efficiency. Also, curcumin is used in both keto **15** and enol **15a** forms as shown in figure 1.11 [66].

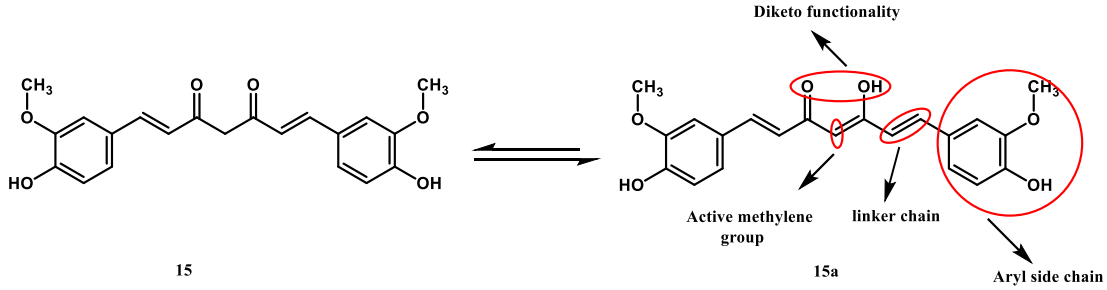


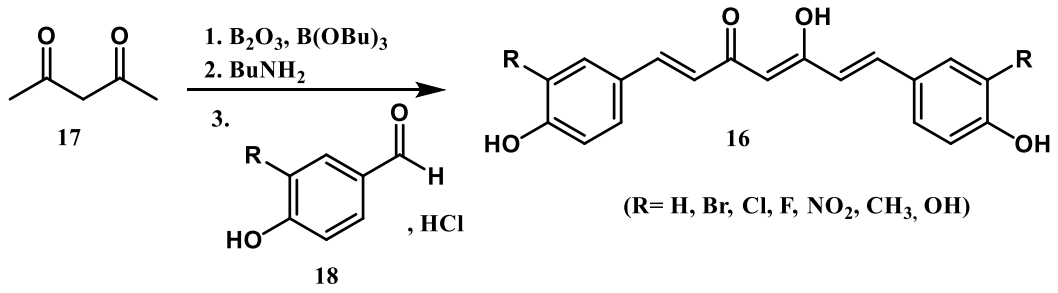
Figure 1.11: Keto –enol tautomerism of curcumin

The unique anti-cancer activity of curcumin **15** is represented by inhibiting cell proliferation and apoptosis by overcoming a variety of cellular signals [67]. Several studies have emerged confirming curcumin's anti-cancer activity for different types of cancer, including: the breast, lung, and prostate cancer and brain tumors [68]. The biological activity of curcumin is related to two main reactions namely the oxidation reaction of curcumin **15** and Michael addition reaction. Curcumin react with reactive oxygen species (ROS) by single electron transfer, and could react as Michael acceptor [69, 70].

However, its application in clinical medicine is limited due to its low level of solubility in water, which leads to difficulty in oral availability and a decrease in its chemical stability [71]. Its hydrophobicity, leads to the weak ability of cells to absorb it, as it works to bind to the fatty acyl chains of fats through hydrogen and lipid interactions leading to its unavailability within the cytoplasm [72]. To overcome this disadvantage and improving its bioavailability, several strategies were used such as preparation of curcumin nanoparticles and synthesis of curcumin derivatives [73, 74].

1.3.3.1 Synthesis of derivatives and analogues of curcumin based on β-dicarbnoyl by modification of diaryl moieties

Studies have shown that the significant activities of curcumin **15**, such as the antioxidant activities, were caused by the presence of a diaryl group that contains the phenolic OH group. Therefore, various studies have been conducted on changing and replacing the methoxy group (OCH_3) or hydroxy groups (OH) present in curcumin **15**, and studying their effect on biological activities [75]. A group of curcumin analogues **16** were synthesized with different substituents (R= H, Br, Cl, F, NO_2, CH_3, OH) in place of the methoxy group (scheme 1.3) by treating of diketone **17** with suitable aldehyde **18** under acidic condition. The results showed that the methoxy group in curcumin reduces the anti-cancer effects of curcumin. Therefore, the presence of these alternatives substituents improves the biological activities of curcumin significantly [76, 77].

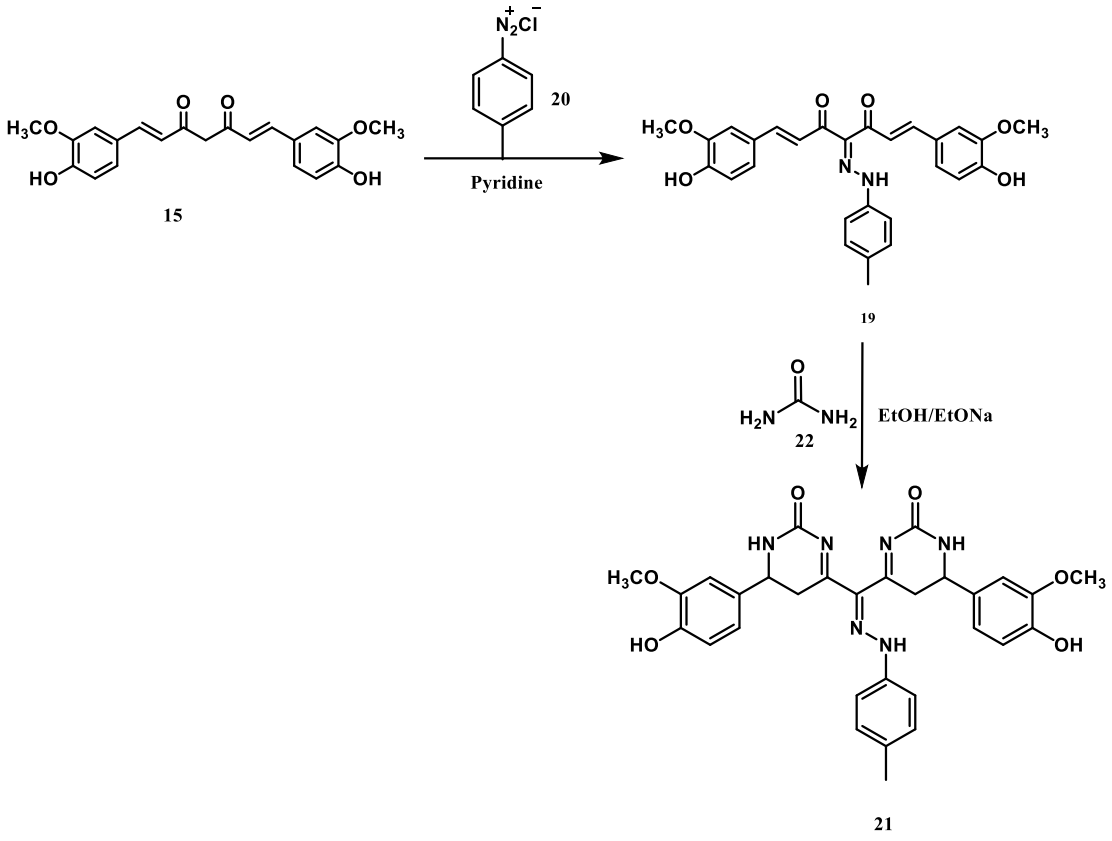


Scheme 1.3: Synthesis of curcumin derivative with different substituents at diaryl moieties.

1.3.3.2 Synthesis of derivatives and analogues of curcumin based on β-dicarbnoyl by arylhydrazone substituents at C-4 of curcumin

The arylhydrazones **19** was synthesized by diazo coupling reaction of curcumin **15** with diazonium salts of *p*-toluedine **20** in a pyridine solution.

The synthesis of imine analouges **21** were carried out by the intermolecular cyclization of **19** with urea **22** in sodium ethoxide and ethanol (scheme 1.4). Arylhydrazone analogue **19** has moderate antioxidant activities but the hydrazone **21** had good antioxidant activity compared with ascorbic acid. Antibacterial activity of **21** decrease because existence of a highly electronegative atom [78].

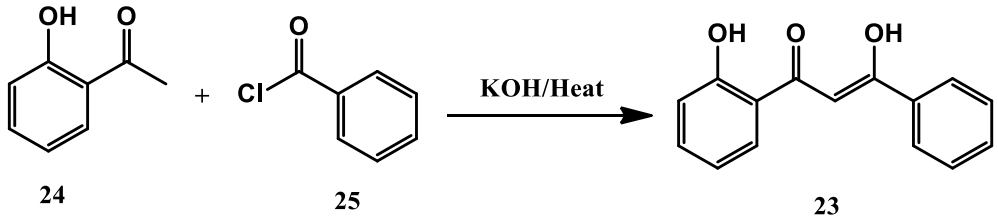


Scheme 1.4: Synthesis of curcumin analogues with arylhydrazone at C-4 position.

1.3.3.3 Synthesis of derivatives and analogues of curcumin based monocarbonyl by 3- carbon linker and 5-carbon linker

The presence of β-diketone in the chemical structure of curcumin **15** is one of the reasons for its instability and lack of good bioavailability. The

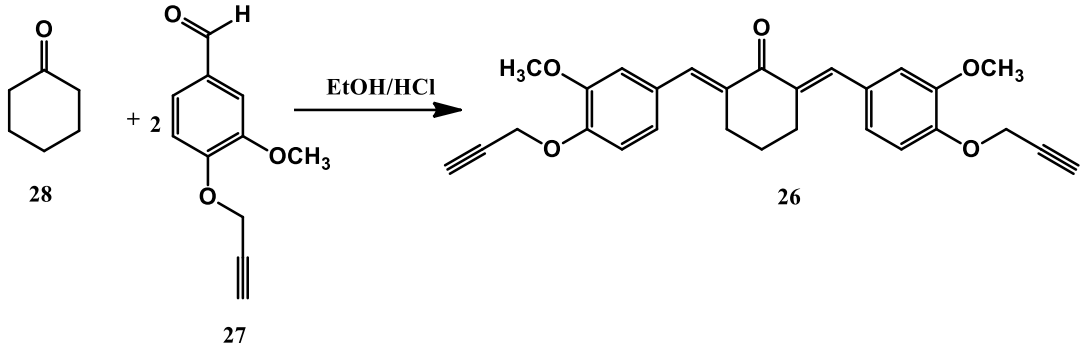
monocarbonyl curcumin analogues were synthesized to overcome these disadvantages [79]. The monocarbonyl curcumin analogues (MCACs) differ according to the number of carbon inserted between the two phenyl rings. The heptadienone bridge was replaced by a shorter carbon bridge. Compound **23**, which was generated by combining 2-hydroxyacetophenone **24** and benzoyl chloride **25** (scheme 1.5), is one of the analogues with a 3-carbon linker carbon between two phenyl rings. Its anti-cancer property was studied against MCF-7 breast cancer cells using MTT assay. It was discovered, that **23** has a more selective toxic effect against breast cancer cells than curcumin **15**, due to the direct apoptosis and G2/M cell cycle arrest [80].



Scheme 1.5: Synthesis of analogue 23 based on monocarbonyl by 3-carbon linker

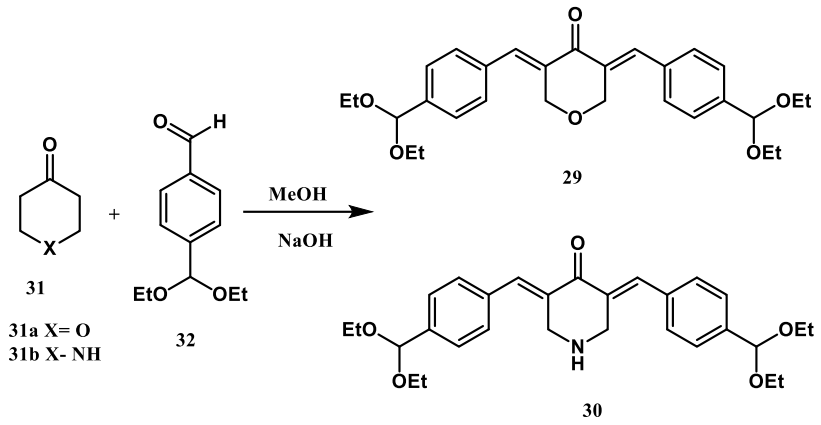
A large number of five-carbon linker containing cycloalkanone derevatives were synthesized in order to assess their biological and medicinal properties. Some studies showed that the introduction of two functional groups with less polarity than OH in the position of the two OH groups in curcumin increase the antiproliferative properties such as synthesis of benzylidine cyclohexanone analogues **26** with the non-polar moieties, methoxy, ethoxy and propoxy groups. These analogues were prepared by the reaction of proper ratios of aromatic aldehydes **27** with cyclohexanone **28** in ethanol in the presence of

hydrochloric acid gas (scheme 1.6). All the synthesized analogues **26** showed superior activity, more ability in apoptosis induction and cell cycle arrest at G1 phase than curcumin in vitro studies on gastric and esophageal cancer cells. The bispropoxy analogue **26** showed higher inhibitory activity more than curcumin by 17 fold after 48 h incubation [81, 82].



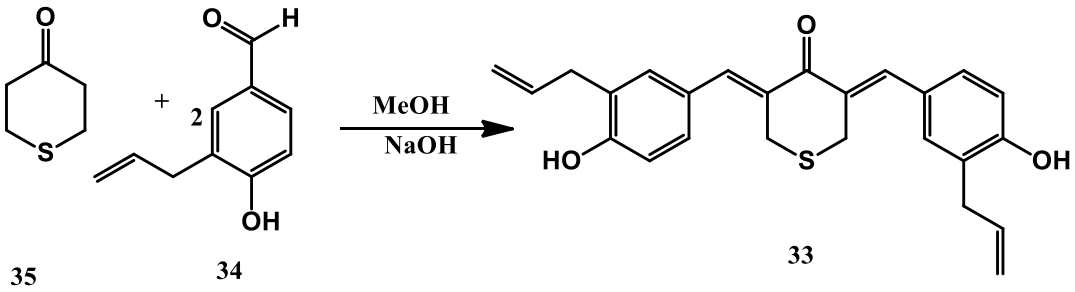
Scheme 1.6: Synthesis of the bispropoxy analogue

The inhibition of some types of enzymes and cytokines that bind to the anti-inflammatory effects was evaluated by synthesis of many strong anti-inflammatory agent. Different reports indicated that many curcumins containing α,β-unsaturated carbonyl moiety are considered as a good starting point to synthesize it. Compounds **29** and **30** prepared by reaction of suitable ketone **31** with benzaldehyde **32** in methanol under basic condition (scheme 1.7), which inhibited secretory phospholipase A_2 ($sPLA_2$) more effectively than curcumin and also had higher inhibition activities against cyclooxygenases (COX-1) [83]. The existence of N-methyl-4-piperidone and 4- piperidone moieties improved the ability of the enzyme and cytokine inhibitors [83].



Scheme 1.7: Synthesis of cycloalkanone curcumin analogues with N, O heteroatom

Also allyl monocarbonyl analogues that contain cyclohexa-, piprazine, morpholine and pyrrole moieties showed cytotoxic activities against human cholangiocarcinoma cell lines (HUCCA, QBC-939 and RBE) [84]. It was reported that compound **33** possess potent antiproliferative activity against all the tested cancer cell lines [84], which was prepared by reaction of benzaldehyde **34** with tetrahydro-thiopyran **35** in methanol under basic condition (scheme 1.8).



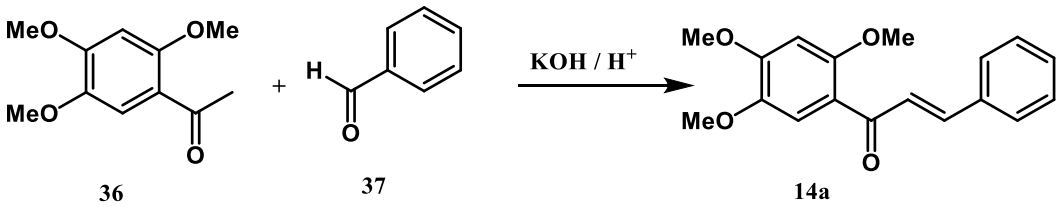
Scheme 1.8: Synthesis of allylated monocarbonyl curcumin analogues

1.4 Different methods of chalcone synthesis

Several methods and techniques were used to synthesize chalcone derivatives. Some of which were used in the synthesis of our compounds.

1.4.1 Claisen–Schmidt reaction

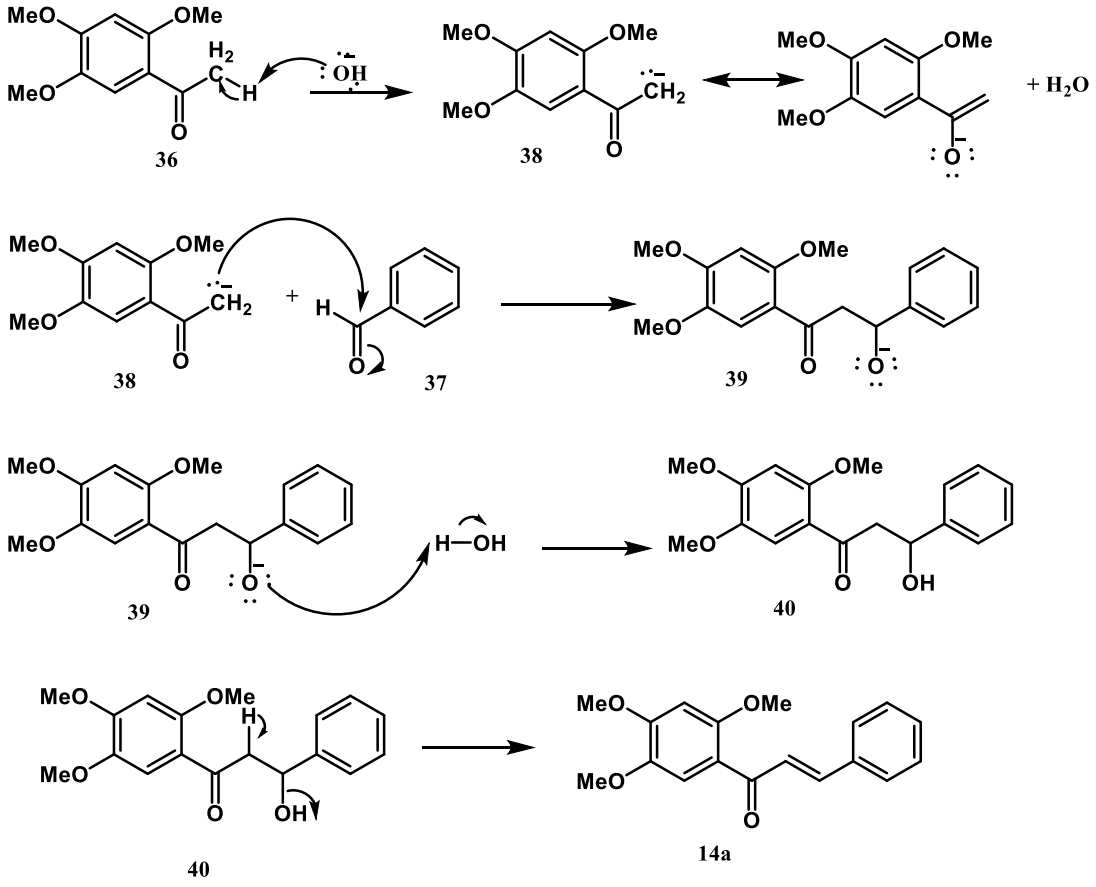
The claisen–schmidt reaction is an easy procedure for the synthesis of chalcone **14a** and its derivatives in which acetophenone **36** and aldehyde derivatives **37** undergo condensation in the presence of acid, or base catalysts in polar solvents at 50–100 °C for several hours as shown in scheme 1.9 [85]. The general bases that are used for this condensation are NaOH, KOH and NaH. The main disadvantages of this method are slow reaction rate and some of by-products are produced [86]. However, Claisen–Schmidt condensation is the most generally used procedure for the synthesis of chalcones due to its simplicity and high yields compared with other traditional methods [87].



Scheme 1.9: Claisen–Schmidt reaction in the presence of base/acid catalyst.

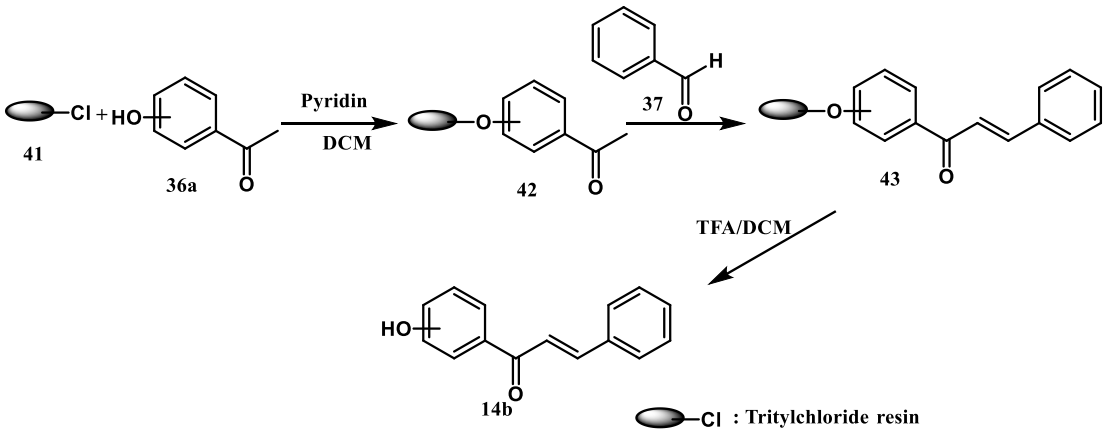
The hydroxide attacks the acidic proton in the acetophenone **36**, forming enolate **38** that is stabilized by resonance. The enolate attacks the carbonyl group of benzaldehyde **37** forming the alkoxide **39**. Alkoxide ions take proton from H_2O to yield β-hydroxyketone (aldol) **40**. The β-hydroxyketone

compound has α-proton to the carbonyl group easily released causing formation of chalcone **14a** (scheme 1.10) [88].



Scheme 1.10: Claisen–Schmidt reaction mechanism in the presence of base catalyst

Generally, Claisen–Schmidt condensation reaction could be executed in the liquid phase or solid-phase [89]. In the solid-phase condensation reaction, the acetophenone derivative **36a** is fixed to the resin **41**, and then treated with derivatives of benzaldehydes **37**. Finally, the chalcones **43** were released from the resin by treating it with trifluoroacetic acid as shown in scheme 1.11 forming chalcone **14b**. The complex of Co (II) cross-linking 4-vinyl pyridine styrene and tritylchloride **41** are the best and most used resins [89].



Scheme 1.11: Claisen–Schmidt reaction in solid-phase medium

1.4.2 Grinding method

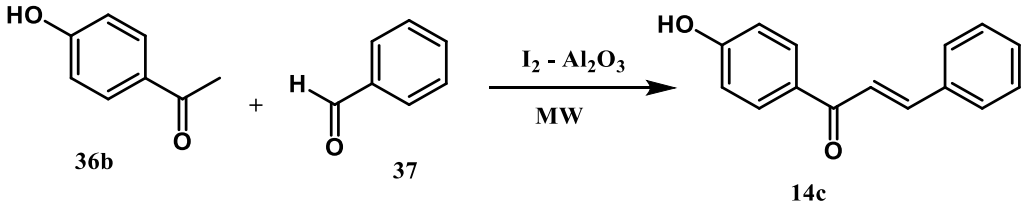
The grinding method is very simple, enviromentally friendly and high yielding. This kind of synthesis occurs at a room temperature. This method was employed in the synthesis of diversed chalcones by grinding the mixture of methyl ketones, aldehydes and sodium hydroxide using a pestle in open mortar [90].

1.4.3 Microwave irradiation condition

The microwave irradiation technique is now a famous technique in organic synthesis. This method has more advantages over other traditional methods because it minimizes the reaction time, by-products, and evaporation of solvents. Good yields were obtained [91].

The microwave irradiation reaction of acetophenone derivatives **36b** with aldehydes **37** catalyzed by the alumina, impregnated by iodine molecular and solvent-free. The neutral alumina works as a catalytic surface, and the molecular iodine act as Lewis acid to stimulate the carbonyl group of aldehydes

for the nucleophilic attack with a hydroxy aryl ketone forming chalcone **14c** as given in scheme 1.12 [91].

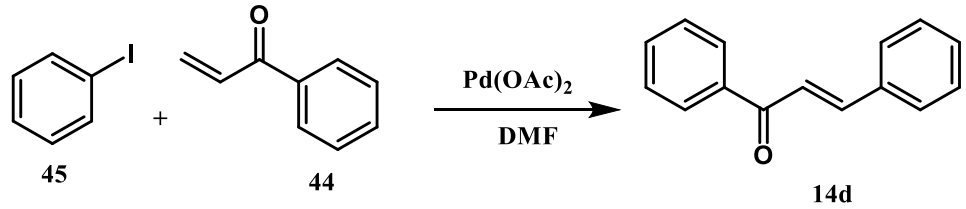


Scheme 1.12: Synthesis of chalcones through solvent-free microwave irradiation condition

1.4.4 Coupling reactions:

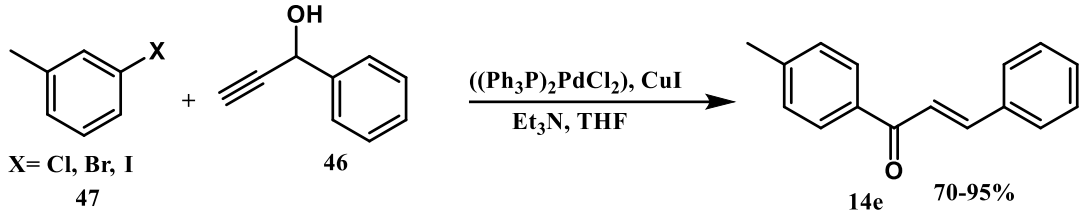
The synthesis of a new derivatives of chalcones with different substituents by traditional methods leading to formation of undesirable products. Presently, new methods such as cross-couplings with transition metal catalysts, Julia–Kocienski olefnation, Wittig, Friedel–Crafts acylation, Heck coupling and other type of coupling reaction have been used for the synthesis of active pharmaceutical molecules including chalcones **14d** [92].

The metal-catalyzed Heck coupling reaction is a significant methodology for the synthesis of chalcones. Coupling of aryl vinyl ketones **44** with aryl iodide **45** lead to the formation of a C-C bond in the presence of $Pd(OAc)_2$, Ph_3P or K_2CO_3 in DMF as shown in the scheme 1.13 [92].



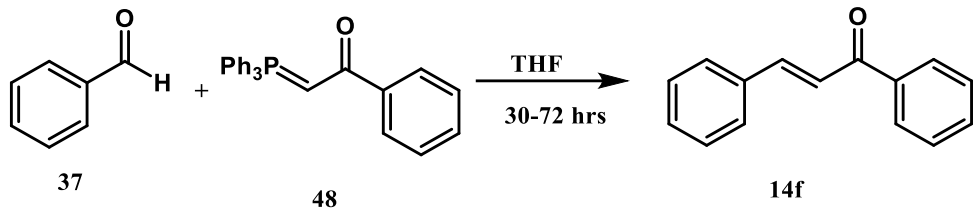
Scheme 1.13: Chalcone synthesis through Heck coupling

Sonogashira coupling has also been used in preparation of chalcone. Coupling of terminal alkynes **46** with aryl halides **47** in the presence of palladium catalyst with a co-catalytic quantity of CuI in a boiling mixture of trimethylamine and THF, under inert atmosphere for 16–24 h afforded the chalcone **14e** as depicted in scheme 1.14 [93]. Several chalcones were prepared with moderate to excellent yields using this procedure. Sonogashira coupling has some drawbacks such as long reaction times, a completely excess of base and need of electron withdrawing group in the aryl halides. To overcome these drawbacks, qualified microwave-assisted a coupling isomerization reaction (MACIR) was developed for the preparation of chalcones with less than a half-hour reaction time in notable yields [94].



Scheme 1.14: Sonogashira isomerization coupling for the synthesis of chalcones

Chalcones are also synthesized through Wittig olefination by taking into account the key function of chalcones as α,β-unsaturated carbonyl derivatives as shown in scheme 1.15. Chalcones **14f** were obtained in moderate yields by treatment of triphenylbenzoylmethylene phosphorane **48** with benzaldehyde **37** in THF for 30-72 hrs [95]. Microwave-assisted synthesis of chalcones in high yields using Wittig olefnation was achieved in 5–6 min. This advanced trial minimize the reaction time with increasing the reaction rates to obtain excellent yields [96].



Scheme 1.15: Synthesis of chalcones using Wittig olefnation reaction

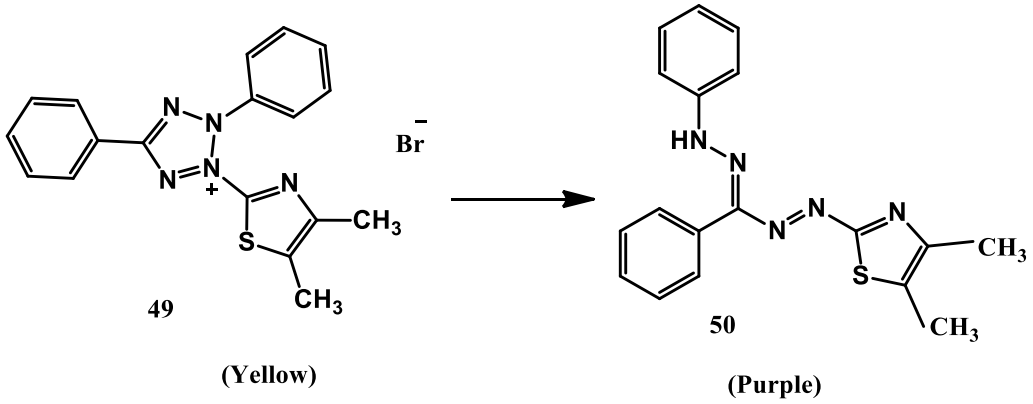
1.5 Cell Proliferation Assays

There are many types of cell proliferation assays that are usually used in drug discovery to estimate a compound's capacity to cause or block a biologic activity without having toxic effects on cells. Some of these assays will be discussed briefly.

1.5.1 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay (MTT)

The MTT assay is used to evaluate the cellular metabolic activity as a signal of cell viability, cytotoxicity and proliferation. This colorimetric assay is dependent on the reduction of yellow tetrazolium salt (3-(4,5 -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **49** or the MTT) to purple formazan **50** crystals by metabolically active cells (scheme 16) [97]. NAD(P)H-dependent cellular oxidoreductase enzymes may show the number of viable cells availble. These enzymes are having the ability of lowering the tetrazolium dye (MTT) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **49** to its insoluble formazan **50** [98]. The insoluble formazan crystals are dissolved in a solubilization solution that result colored solution that absorbed at 500-600 nanometers using multi-well

spectrophotometer. If the darker the solution is present, the number of viable, metabolically active cells are great.



Scheme 1.16: The principle of MTT assay

1.5.2 BrdU Cell Proliferation Assay

5-Bromo-2-deoxyuridine **51**(BrdU) is used to examine cell proliferation, because of its ease integration into DNA during the S-phase of the cell cycle. BrdU **51** promotes cellular differentiation and maturation in leukemia cell lines while inhibiting erythroleukemia cell differentiation. In studies of BrdU integration, BrdU-specific antibodies with fluorescent tags are often used to detect the integrated BrdU using flow cytometry or fluorescence microscopy [99].

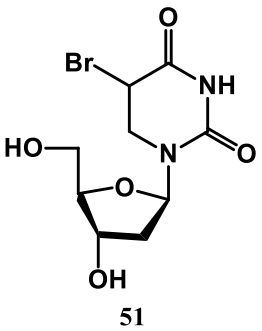


Figure 1.12: Chemical structure of the BrdU

1.5.3 EdU Proliferation Assays

EdU proliferation assays provide an active tool for observing replicating DNA in fluorescence. EdU, a modified nucleoside, is applied to live cells and is involved in DNA replication. Rapid link of fluorescent probes to the EdU is allowed by Cu-induced click chemistry [100] as shown in the figure 1.13. This provides a quantitative method to observe cells that are proliferating. The assays are available in various designs for the microscopic imaging, flux cytometry and for in vivo studies [100].

Figure 1.13: Edu-Click cell proliferation kits incorporate EdU (5-ethynyl-2'-deoxyuridine) into DNA during active DNA synthesis and are measured using a click-chemistry fluorescent detection method.

1.6 Antioxidant Capacity Assays:

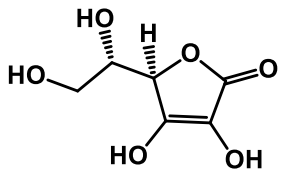
Presence of radical caused various of diseases in living organism. It has the potential to trigger the evolution of toxic compounds within cells. Sources of radical species (ROS, RNS) include cellular respiration, biomolecule interactions with ionizing radiation, and dedicated cellular pathways for ROS, RNS formation [101]. There are several mechanisms that are responsible for conversion of ROS and RNS species into harmless byproducts. Such as:

1.6.1 Oxygen Radical Antioxidant Capacity (ORAC)

The ORAC assay depends on free radical damage to fluorescent probe. A loss of fluorescent intensity through time that is caused by an oxidizing reagent [102]. The resultant damage of fluorescent probe can then be linked with the amount of an oxidant existent. On the contrary, the inhibition of oxidative damage to the fluorescent probe can be linked with the antioxidant ability of compound performing as free radical scavenger [102].

1.6.2 Ascorbic Acid Assay (FRASC)

The Ascorbic Acid Assay Kit (colorimetric) supplys quick, easy, and sensitive means of detecting the ascorbic acid **52** in various types of samples such as biological samples (a serum, a tissue and cell extracts, cancer drugs). This assay is dependent on the Ferric Reducing/Antioxidant Ascorbic Acid (FRASC) chemistry. Fe^{3+} is reduced to Fe^{2+} by any antioxidants available. The ferrous iron forms a chelate with the colorimetric probe to give product with strong absorbance band at 545 - 600 nm. The insertion of ascorbate oxidase to samples removes any ascorbate present leaving surroundings amount that is subtracted from the total to give ascorbate content [103].

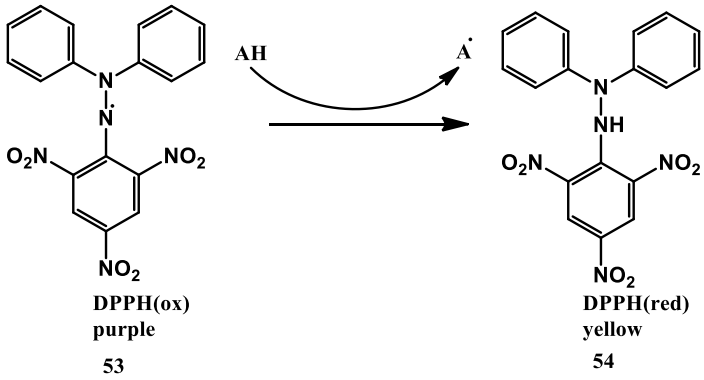


52

Figure 1.14: Chemical structure of ascorbic acid

1.6.3 2,2-diphenyl-1-picrylhydrazyl radical assay (DPPH)

DPPH **53** is known as radical and scavenger for other radicals. Therefore, the reduction rate of chemical reaction when adding of DPPH **53** is used as an indicator of the radical nature of that reaction. The DPPH radical has a dark violet color in solution, and it becomes colorless or light yellow when neutralized **54** (scheme 1.17). This property allows optical controlling of the reaction, and the number of initial radicals can be counted from the variation in the optical absorption at 520 nm [104].



Scheme 1.17: The principle of DPPH assay

1.7 Objectives of this study

The main objectives of this current study:

1. To synthesize a series of monocarbonyl curcumin analogues (MCACs) based on chalcone derivatives and derivatives of daidzain.

2. To study its effect on inhibition of cancer cells and to find the IC_{50} for each and compare with curcumin and other derivatives.

3. Also, to study its effect as antioxidants for DPPH and find IC_{50} and compare with Trolox and curcumin.

Chapter Two

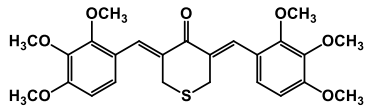
Experimental section

2.1 Chemistry

All reagents and materials were obtained from commercial suppliers Sigma-Aldrich. The reaction progress were monitored by thin-layer chromatography (TLC) using silica gel, visualized under UV light at 365nm. All the samples were evaporated by using Hiedolph, Rotary Evaborator, and the identified by Thermo Scientific Nicolet (IR).

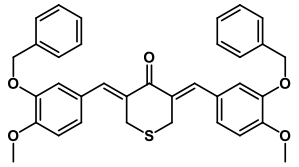
2.1.1 Synthetic procedures

3.5-Bis(2,3,4-trimethoxybenzlidene) dihydro-2H-thiopyran-4(3H)-one (56):



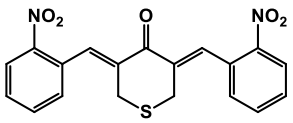
To a solution of tetrahydro-4H-thiopyran-4-one **55** (0.5 g, 4.3 mmol) in methanol (10 mL), 2,3,4-trimethoxy benzaldehyde (2.1 g, 10.7 mmol, 98%) and 10% aqueous solution of KOH (1 mL) were added. The mixture was refluxed for 2h at 70^{o}C. The progress of the reaction was monitored by TLC. Water (20 mL) and diluted HCl were then added. The yellow precipitate was filtered, washed with cold water and recrystallized from methanol to obtain desired yellow product (1.91g, 4 mmol, 94%), (m.p. 85-86.5^{o}C) and IR spectra was shown in Figure a1 (Appendix).

3-(3-(benzyloxy)-4-methoxybenzylidene)-5-(4-methoxy-3-(-2-methylpenta-2,4-dien-1-yl)oxy)benzylidene)dihydro-2H-thiopyran-4(3H)-one (57):



To a solution of tetrahydro-4H-thiopyran-4-one **55** (0.1 g, 0.86 mmol) in methanol (10 mL), 3-(Benzyloxy)-4-methoxybenzaldehyde (0.45 g, 1.89 mmol) and 10% aqueous solution of KOH (1 mL) were added. The mixture was heated at 70^{o}C for 24h. The progress of the reaction was monitored by TLC. Water (20 mL), and diluted HCl were then added. The organic compounds extracted with ether (30 mL x2), and the combined organic layers were dried with $MgSO_4$, filitered and the solvent was removed under reduced pressure. Then the crude product was subjected to silica gel column chromatography (R_f = 0.28; CH_2Cl_2: ether 9:1) (0.24 g, 0.42 mmol, 49%), (m.p.98-98.5^{o}C) and IR spectra was shown in Figure a2 (Appendix).

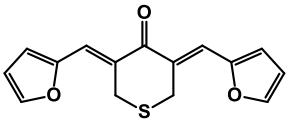
3,5-Bis(2-nitrobenzylidene)dihydro-2H-thiopyran-4(3H)-one (58):



To a solution of tetrahydro-4H-thiopyran-4-one **55** (0.1 g, 0.86 mmol) in methanol (10 mL), 2-Nitrobenzaldehyde (0.28 g, 1.89 mmol) and 10% aqueous solution of KOH (1 mL) were added. The mixture was heated for 2.5h at 70^{o}C and monitored by TLC until completion. Water (20 mL) and diluted HCl were then added, and the organic compounds were extracted

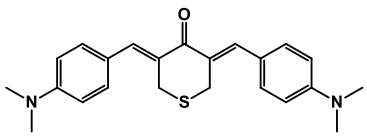
with ethyl acetate (15 mL x2). The combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was subjected to silica gel column chromatography (R_f = 0.3; Hexane: Ether 7:3) to afford orange solid product (0.19g, 0.49 mmol, 58%), (m.p.90-91^oC) and IR spectra was shown in Figure a3 (Appendix).

3,5-Bis(furan-2-ylmethylene)dihydro-2H-thiopyran-4(3H)-one (59):



To a solution of tetrahydro-4H-thiopyran-4-one **55** (0.1 g, 0.86 mmol) in methanol (5 mL), 2-Furaldehyde **60** (0.18 g, 1.89 mmol) and 10% aqueous solution of KOH (1 mL) were added. The mixture was heated at 70^oC for 2h and monitored by TLC until completion. Water (20 mL) and diluted HCl were then added, the formed precipitate was filtered and subjected to silica gel sub column chromatography (R_f = 0.3; CH_2Cl_2:ether 9.5: 0.5) to afford dark yellow solid product **59** (0.08g, 0.29 mmol, 34%), (m.p. 85-85.5^oC) and IR spectra was shown in Figure a4 (Appendix).

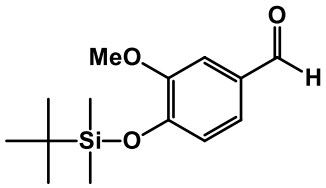
3,5-Bis(4-(dimethylamino)benzylidene)dihydro-2H-thiopyran-4(3H)-one (61):



To a solution of tetrahydro-4H-thiopyran-4-one **55** (0.1 g, 0.86 mmol) in methanol (5 mL), 4-dimethylamine benzaldehyde (0.28 g, 1.89 mmol) and 10% aqueous solution of KOH (1 mL) were added. The mixture was heated at 70^oC for 24h. The progress of the reaction was monitored by TLC. Water

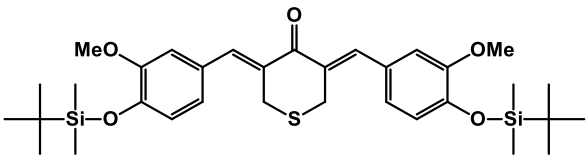
(20 mL) was then added, the precipitate was filtered and purified by preparative TLC putting in large champer (R_f = 0.27; CH_2Cl_2 :Ether 9.7: 0.3) to obtain brown product **61** (0.18g, 0.47 mmol, 55%), (m.p.90-91°C) and IR spectra was shown in Figure a5 (Appendix).

4-(tert-butyldimethylsilyloxy)-3-methoxybenzaldehyde (64):



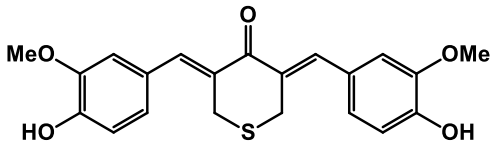
To a solution of vanillin **62** (2.0 g, 13.1 mmol) in chloroform (50ml), tert-butyldimethylsilyl chloride **63** (TBDMSCl) (2.3 g, 15.7 mmol) and aqueous solution of triethyl amine (3 mL) were added. The mixture was stirred at room tempreture for 24h under nitrogen and the completion of the reaction was monitored by TLC. Water (15 mL) and CH_2Cl_2 were added, the organic solution (80 mL) was extracted with CH_2Cl_2 (30 mL x3). The combined organic layers were dried with Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was subjected to silica gel column chromatography (R_f = 0.3; Hexane: Ethyl acetate 9:1) to obtain silylated aldehyde **64** (3.1g, 11.6 mmol, 89%).

3,5-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxybenzylidene)dihydro-2H-thiopyran-4(3H)-one (65):



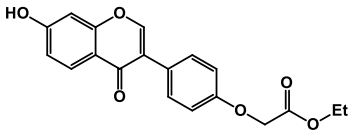
To a solution of tetrahydro-4H-thiopyran-4-one **55** (0.3 g, 2.58mmol) in methanol (8 mL), 20% aqueous solution of NaOH (0.3g, 7.7 mmol, 0.30 ml) and silylated aldehyde **64** (1.49g, 5.6 mmol) were added. The mixture was heated at 70^{o}C for 24h and the completion of the reaction was monitored by TLC. Water (20 mL) was added, and the organic solution was extracted with ethyl acetate (20 mL x3). The organic combined layers were dried with $MgSO_4$, filtered and the solvent evaporated. Then the crude product was purified by sub column chromatography (R_f = 0.25 Hexane: Ethyl acetate 1:9) to obtain product **65** (0.7g, 1.84 mmol, 47%).

3,5-Bis(3-hydroxy-4-methoxybenzylidene)dihydro-2H-thiopyran-4(3H)-one (66):



To a solution of tetrabutylammonium floride **66a** (TBFA) (0.167 g, 0.64 mmol) in THF (5 mL) and methanol (10 mL), compound **65** (0.2g, 0.32 mmol) was added. The mixture was stirred at room tempreture for 24h and the reaction was monitored by TLC. Water (15 mL) was added and the organic solution was extracted with ethyl acetate (15 mL x3). The combined organic layers were dried with $MgSO_4$, filtered and the solvent evaporated. The organic product was purified by preparative TLC (R_f = 0.3; Hexane: Ethyl acetate 0.5:9.5) to obtain the desired product (**66**) (0.18 g, 0.46 mmol, 18%), (m.p.110-110.5^{o}C) and IR spectra was shown in Figure a6 (Appendix).

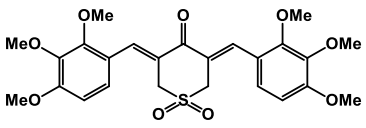
Ethyl-2-(4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenoxy)acetate (67):



4,7-dihydroxylioflavone **67a** (0.5 g, 1.96 mmol) and 2 equiv of NaH 60% (0.157 g, 3.93 mmol) were stirred in dry DMF(20 mL) under N_2 for 2h. Ethyl chloroacetate **67b** (0.24 g, 1.96 mmol, 0.21 mL) was then added, the mixture was stirred at room tempreture for 24h and the reaction completion was monitored by TLC. Water (15 mL) and diluted HCl were added, and the organic compounds were extracted with ethyl acetate (20 mL x3). The combined organic layers were dried with $MgSO_4$, filtered and the solvent evaporated under reduced pressure. Organic product was washed with brine (15mL x3). Then the crude product was subjected to silica gel column chromatography (R_f = 0.27; CH_2Cl_2: ether 9.7:0.3) to afford a solid product **67** (0.09 g, 0.26 mmol, 13%), (m.p.95-96^{o}C) and IR spectra was shown in Figure a7 (Appendix).

2.1.2 Oxidation reaction procedures

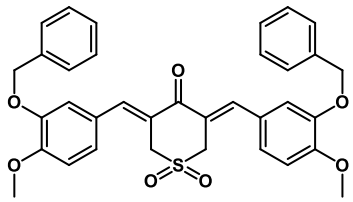
3.5-Bis(2,3,4-trimethoxybenzlidene)dihydro-2H-thiopyran-4(3H)-one 1,1-dioxide (56a):



To a solution of preparing compound **56** (0.2 g, 0.423 mmol) in DCM (5ml) a solution of 71.5% *m*CPBA **68** (0.35 g ,255 mmol) in DCM (10ml) was

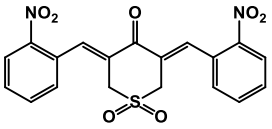
added at 0°C. The mixture was stirred at room temp for 24h and monitored by TLC until completion. It was washed using 10% of aqueous solution of $NaHCO_3$ (100 mL), and extracted with DCM (15 mL) and evaporated the organic part to obtain the desired product (0.18 g, 0.35 mmol, 84%).

3-(3-(Benzyloxy)-4-methoxybenzylidene)-5-(4-methoxy-3-(-2-methylpenta-2,4-dien-1-yl)oxy)benzylidene)dihydro-2H-thiopyran-4(3H)-one 1,1-dioxide (57a):



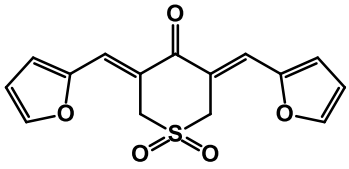
A solution of the compound **57** (0.1 g, 0.17 mmol) with 71.5% *m*CPBA **68** (0.35 g, 255 mmol) using similar condition of compound **56a** and gave a product (0.07 g, 0.11 mmol, 66%).

3,5-Bis(2-nitrobenzylidene)dihydro-2H-thiopyran-4(3H)-one 1,1-dioxide (58a):



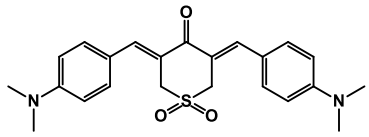
A solution of the compound **58** (0.08 g, 0.20 mmol) with 71.5% *m*CPBA **68** (0.35 g, 255 mmol) using similar condition of compound **56a** and gave a product (0.055 g, 0.13 mmol, 63%).

3,5-Bis(furan-2-ylmethylene)dihydro-2H-thiopyran-4(3H)-one 1,1-dioxide (59a):



A solution of the compound **59** (0.05 g, 0.18 mmol) with 71.5% *m*CPBA (0.35 g, 255 mmol) using similar condition of compound **56a** and gave a product (0.032 g, 0.10 mmol, 58%).

3,5-Bis(4-(dimethylamino)benzylidene)dihydro-2H-thiopyran-4(3H)-one 1,1-dioxide (61a):



A solution of the compound **61** (0.08 g, 0.21 mmol) with 71.5% *m*CPBA (0.35 g, 255 mmol) using similar condition of compound **56a** and gave a product (0.066 g, 0.16 mmol, 76%).

2.2 Biology

DMSO 10% was used in the cytoxcity of cancer cells was purchased from Sigma-Aldrich. DPPH **69** (2,2-Diphenyl-1-prcrylhydrrazyl), Trolox **70** (6 hydroxy-2,5,7,8- tetramethylchroman-2- carboxylic acid) and Methanol were also purchased from Sigma-Aldrich.

Balance maximum capacity 4500 g (boeco, Germany), UV-Vis (Ultraviolet-Visible) Spectrophotometer (Jen WAY 7315, UK) was utilized for assessment the antioxidant and anticancer cytoxicity.

2.2.1 Procedure of Cell proliferation assay

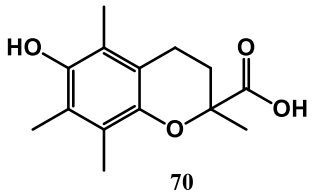
Cervical (HeLa), breast (MCF-7) and two types of human liver cancer cell lines (HepG2, Hep3B) will be cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin antibiotics and 1%

l-glutamine. Cells have been grown in a humidified atmosphere with 5% CO_2 at 37°C. Cells were seeded at 2.6 x 10^4 cells/well in a 96-well plate. After 48 h, cells have been confluented, media was changed and cells were incubated with 1000, 500, 250, 125 and 62.5 µg/ml of the synthesized compounds (**56, 57, 58, 59, 61, 66, 67, 56a, 59a and 61a**) for 24 hr. Cell viability have been assessed by CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's instructions (Promega Corporation, Madison, WI). Briefly, at the end of the treatment, 20 µL of MTS solution per 100 µL of media was added to each well and incubated at 37°C for 2 hours. Absorbance was measured at 490 nm [105].

2.2.2 Procedure of DPPH radical method for in vitro evaluation of antioxidant activity

Stock solutions of monocarbonyl analogues of curcumin (MCACs) compounds (**56a, 57a, 58a, 59a, 61a, 66, 67**) and Trolox **70** (the reference compound) were prepared in methanol at a concentration of 0.1 mg/mL (10 mg of sample in 100 mL methanol). Working solutions with the following concentrations: 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80 and 100 µg/mL were prepared by taking volumes of (0.1, 0.2, 0.3, 0.5, 0.7, 1.0, 2.0, 3.0, 4.0, 5.0, 8.0 and 10.0 mL) respectively of stock solution (MCACs and Trolox) and bringing them up to 10 mL with methanol, using 10 mL volumetric flask (VF). The DPPH **69** (2,2-diphenyl-1-picrylhydrazyl) solution was prepared freshly at concentration (0.002% w/v), 2 mg of DPPH **69** was dissolved in 100 mL of methanol using 100 mL VF. A mixture of DPPH, methanol and

the compounds (MCACs and Trolox) of each of the above mentioned working solutions was prepared at 1:1:1 ratio. A blank solution was prepared by mixing the DPPH solution with methanol at 1:1 ratio. After that, all of those solutions were incubated at room temperature in a dark cabinet for 30 minutes. The absorbance of those solutions was measured after the incubation period by UV-Vis spectrophotometer at 517 nm wavelength, and methanol was used to zero the spectrophotometer [106].



70

Figure 2.8: Chemical structure of Trolox

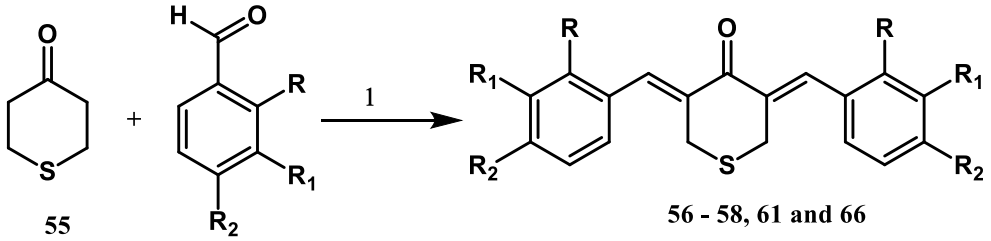
Chapter Three

Result

3.1 Design and synthesis

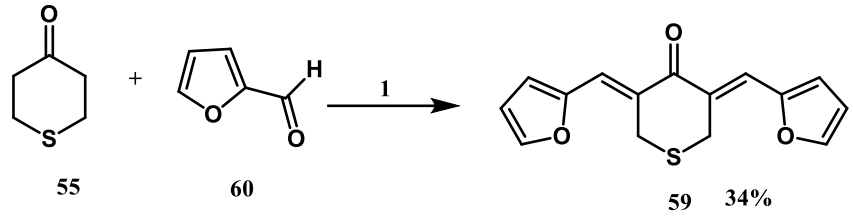
A series of monocarbonyl analogues of curcumin were synthesized by a base–catalyzed aldol condensation reaction as shown in scheme 4.2. Additionally, 4- ethoxy carbonyl methyl-7-hydroxyisoflavone **67** was synthesized in two steps as indicated in scheme 4.4. Yield value of each compound was calculated as:

= (weight of product/theoretical yield)*100% **Eq.1**



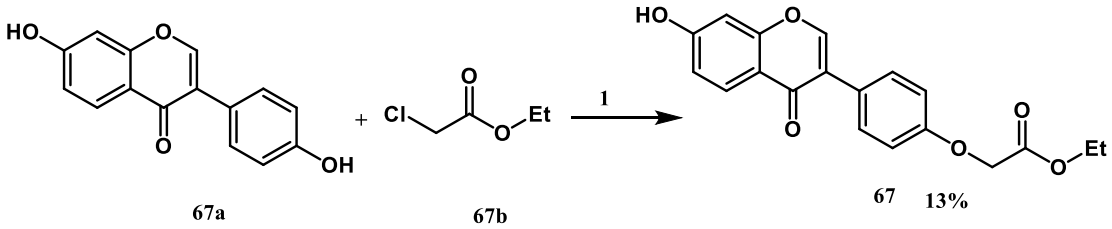
compound	R	R_1	R_2	Yield %
56	OCH_3	OCH_3	OCH_3	94
57	H	Benzyloxy	OCH_3	49
58	NO_2	H	H	58
61	H	H	H_3CNCH_3	55
66	H	OMe	OH	18

Fig 3.1: Synthesis and structure of MCACs. Reagents and conditions: (1) KOH/CH_3OH, 80°C.



Compound	Yield%
59	34

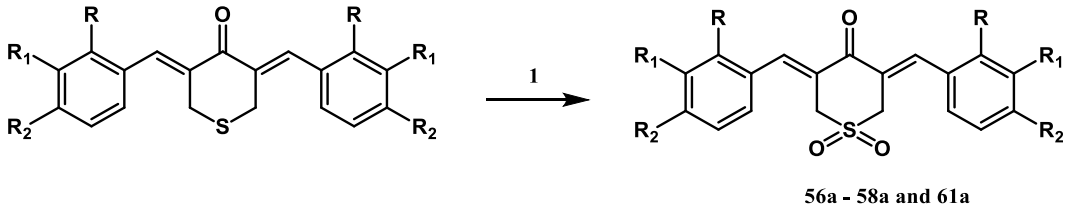
Fig 3.2: Synthesis and structure of MCACs using Furaldehyde as aldehyde. Reagents and conditions: (1) KOH/CH_3OH, 80°C.



Compound	Yield%
67	13

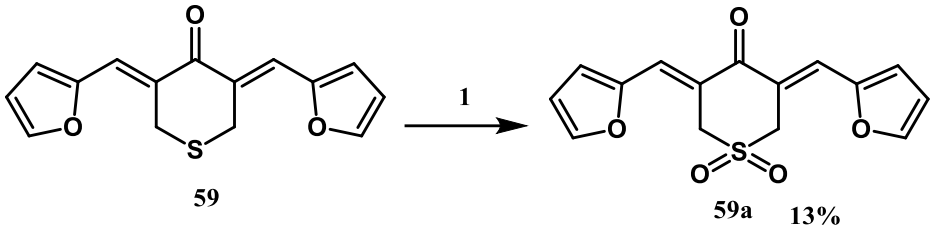
Fig 3.3: Synthesis and structure of isoflavone. Reagents and condition: (1) NaH,THF, at room temperature

3.2 Oxidation of synthesized compounds



Compound	R	R_1	R_2	Yield %
56a	OCH_3	OCH_3	OCH_3	84
57a	H	Benzyloxy	OCH_3	66
58a	NO_2	H	H	64
61a	H	H	$-N(CH_3)_2$	77

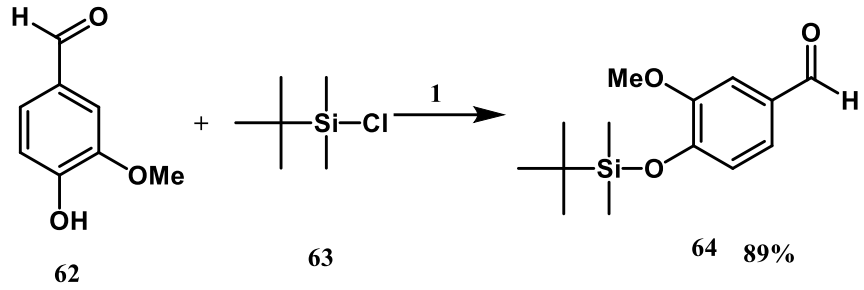
Fig 3.4: Oxidation of MCACs. Reagents and conditions: (1) m-CPBA/DCM, at room temperature.



Compound	Yield%
59a	13

Fig 3.5: Oxidation of compound 59 (MCACs). Reagents and conditions: (1) m-CPBA/DCM, at room temperature.

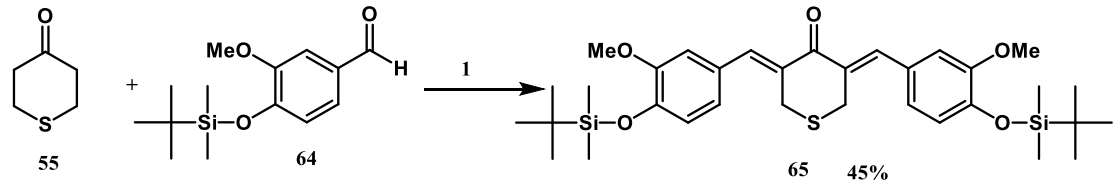
3.3 Protection of 4-hydroxy-3-methoxybenzaldehyde with TBDMSCl



Compound	Yield%
64	89

Fig 3.6: Protection of 4-hydroxy-3-methoxybenzaldehyde. Reagents and conditions: (1) $NEt_3/HCCl_3$, at room temperature.

3.4 Reaction of silylated aldehyde with tetrahydro-4H-thiopyran



Compound	Yield%
65	45

Fig 3.7: Synthesis and structure of compound 65. Reagents and conditions: (1) KOH/CH_3OH, 80°C.

3.5 Antioxidant sensitivity test

The inhibition percentage of 2,2-diphenyl-1-picrylhydrazl **69** (DPPH) activity was measured to determine the antioxidant activity of synthesized compounds by using the following equation:

In% = ((A Blank-A Sample)/A Blank)*100% **Eq.2**

Where A blank represented the absorption of the control reaction (all Reagent without the sample) and A sample represented the absorbance of the sample.

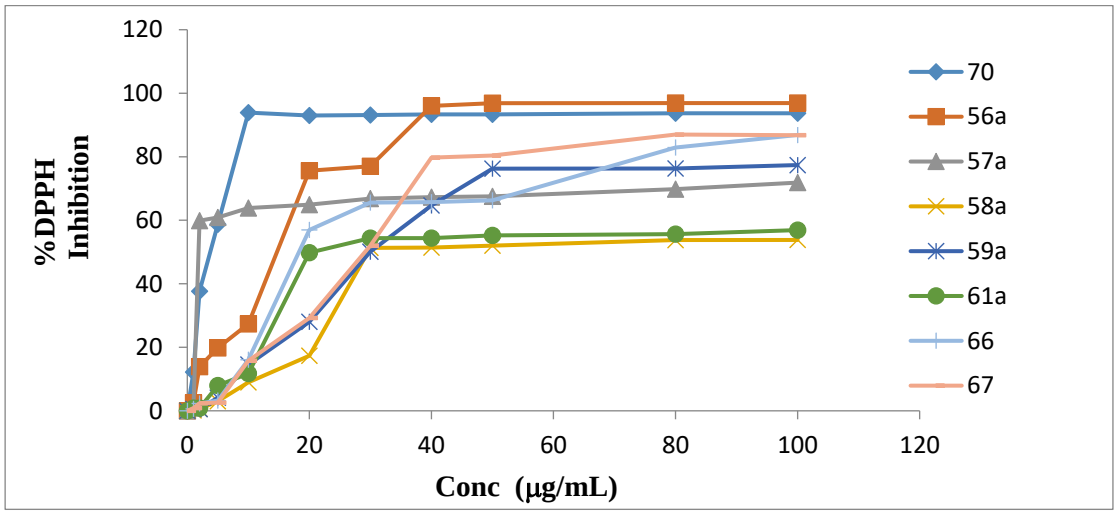


Figure 3.8: Percentage Inhibition of DPPH free radical by Trolox and synthesized compounds at 517 nm

The antioxidant half-maximal inhibitory concentration (providing 50% inhibition, IC_{50}) for each of the studied compounds and Trolox (positive control) as well as their standard deviations as shown in table 3.1 was calculated from the graph plotted of the inhibition percentage against Log concentration, using Microsoft Office Excel 2007.

Table 3.1: IC_{50} values of MCACs and Trolox against DPPH radical

Compound	IC_{50} µg/ml
70	3.4 ± 0.015
56a	11.8 ± 0.28
57a	15.8 ± 0.01
58a	66 ± 0.15
59a	25.2 ± 0.11
61a	44 ± 0.1
66	18.07 ± 0.01
67	20.9 ± 0.011

3.6 Anticancer activity test

The cancer cells inhibitory activity was expressed as percent inhibition and was calculated using equation 3. The % cancer cell inhibition was plotted against the synthesized compounds concentration and the IC_{50} values was obtained from the graph.

% cancer cell inhibition:

= ((Abs of control-Abs sample)/Abs of control)*100% **Eq.3**

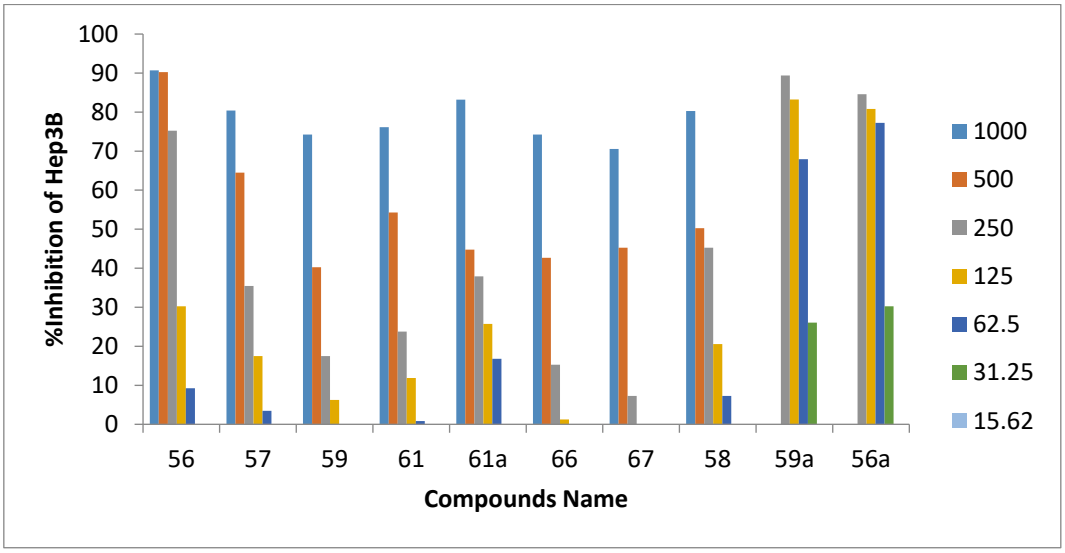


Figure 3.9: Percentage Inhibition of Hep3B cancer cell by synthesized compounds at 460 nm

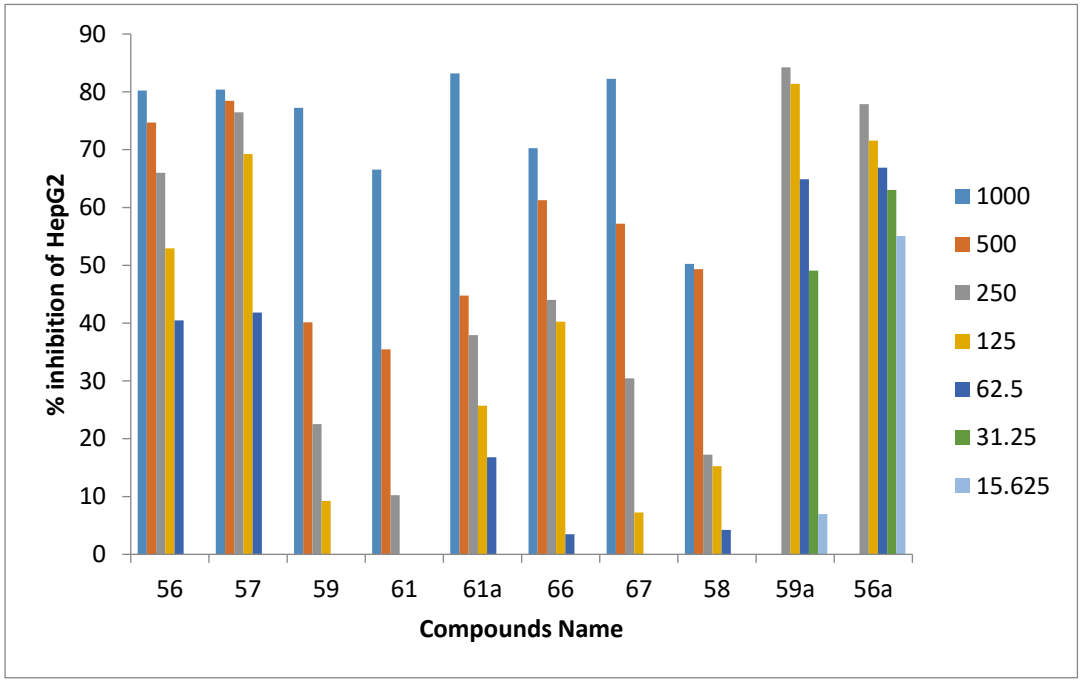


Figure 3.10: Percentage Inhibition of HepG2 cancer cell by synthesized compounds at 490 nm

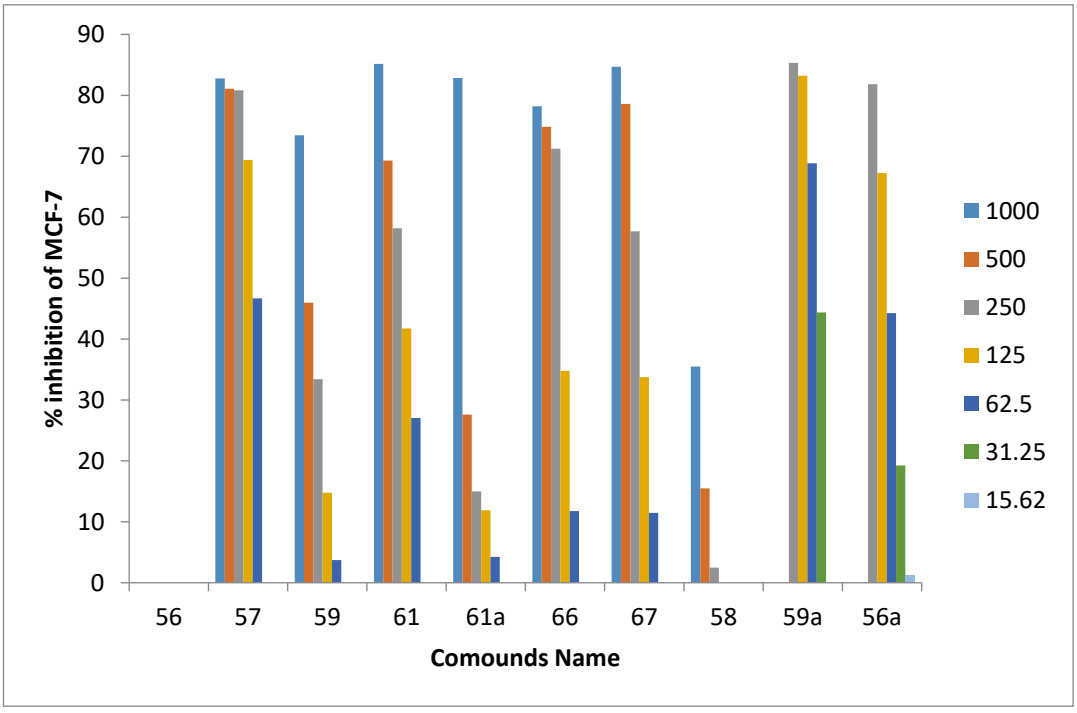


Figure 3.11: Percentage Inhibition of MCF-7 cancer cell by synthesized compounds at 490 nm

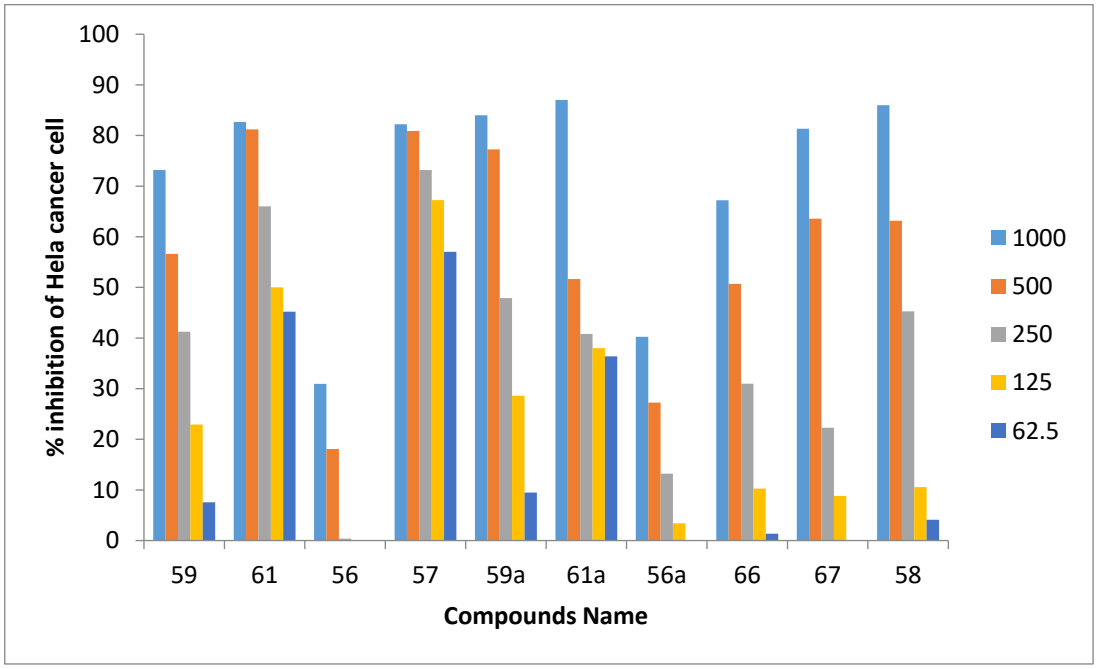


Figure 3.12: Percentage Inhibition of Hela cancer cell by synthesized compounds at 490 nm

The half inhibitory concentration (IC_{50}) of these compounds were calculated from the graph plotted of inhibition percentage against Log concentration, using Microsoft Office Excel 2007 (Table 3.2).

Table 3.2: IC_{50} values of MCACs against HeLa, HepG2, Hep3B and MCF-7

Compound/cell line	IC_{50} µg/ml			
	HepG2	Hep3B	HeLa	MCF-7
56	102.3± 2.5	186.2± 1.4	7943± 0.95	NI
57	50.11± 1.4	346.7± 2.5	22.38± 1.5	39.8± 2.1
58	870± 1.9	354± 0.99	323.5± 1.6	NI
59	524.8± 0.7	583.4± 1.4	363± 1.1	467.7± 1.4
61	758.5± 1.4	446.6± 3.1	251± 1.1	181.9± 1.1
66	349.9± 2.7	575± 0.98	512.8± 2.4	208.9± 1.7
67	398.1± 2.2	630± 2.2	398.1± 1.6	218.7± 1.3
56a	7.194± 2.6	53.7± 1.7	2238.7±1.4	NI
59a	46.77± 2.2	56.2± 2.1	251.1± 2.6	50.11± 2.4
61a	363.1± 2.7	363.1± 3.5	234.4± 1.4	562.34±2.1

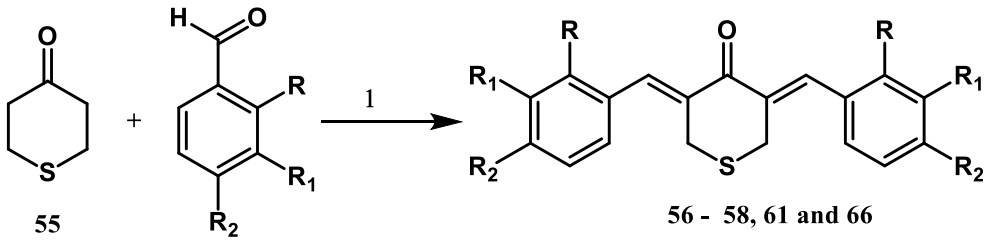
Chapter Four

Discussion

4.1 Synthesis of compounds

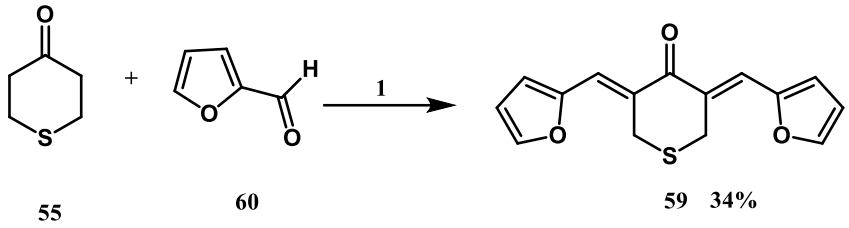
In the present study, monocarbonyl analogues of curcumin were synthetized. Tetrahydro-4H-thiopyran-4-one **55** (ketone) embedded in two benzene rings conjugated with flanking C=C bonds was used to displace the central keto-enol curcuminoid moiety. And the synthesis of it was determined by IR-spectra. The C=C peak appeared at about 1600 cm^{-1} in **56,57,58,59,61** and **66** compounds. And also, the C=O peak was appeared at 1731 cm^{-1} indicate the formation of ester in compound **67**.

Compounds **56, 57, 58, 59, 61,** and **66** were obtained by treating the necessary aromatic aldehydes with tetrahydro-4H-thiopyran-4-one **55** under basic conditions, as previously described. Compounds with electron denoting groups at three positions in the aldehyde have higher yields, such as compound **56**, which yields 94% compared to compound **58**, which has a strong electron withdrawing group only at one position in the aldehyde and yields 58%.



Compound	R	R_1	R_2	Yield %
56	OCH_3	OCH_3	OCH_3	94
57	H	Benzyloxy	OCH_3	49
58	NO_2	H	H	58
61	H	H	H_3CNCH_3	55
66	H	OMe	OH	18

Fig 4.1: Synthesis and structure of MCACs. Reagents and conditions: (1) KOH/CH_3OH, 80°C.



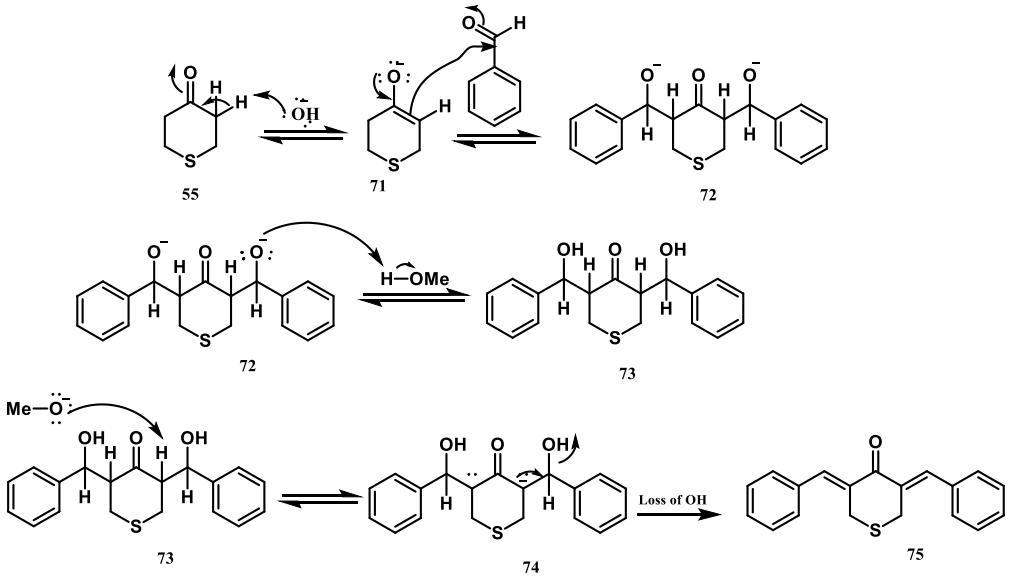
Compound	Yield%
59	34

Fig 4.2: Synthesis and structure of MCACs using Furaldehyde as aldehyde. Reagents and conditions: (1) KOH/CH_3OH, 80°C.

4.2 Aldol condensation mechanism that used

Synthesis of Compound **56, 57, 58, 59, 61** and **66** were achieved by aldol condensation reaction based on chalcon derivatives. The detailed mechanism of this reactions is given in scheme 4.1. Due to the electron withdrawing property of the carbonyl group in aldehyde and ketone **55**, the protons at α-position is relatively acidic [107]. The hydroxide ion deprotonates the acidic proton that is stabilized by resonance to form enolate **71**, enolate **71** reacts with aldehyde forming the alkoxide anion **72**. The

alkoxide anion **72** is protonated to form aldol product **73**, product **73** is converted into enolate **74** by methoxide ion. Sodium methoxide catalysed removal of the C-H-acidic, followed by E_1cb mechanism afforded the chalcone **75**.



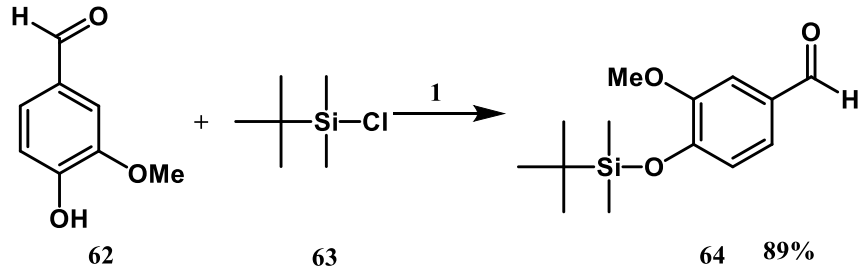
Scheme 4.1: The mechanism of aldol condensation reaction

4.3 Protection and deprotection reaction for OH functional group in 4-hydroxy-3-(methoxy) benzaldehyde that used in synthesis of compound 66

Protection reaction is a reaction that used a protecting group through it. A protecting group is a compound that temporarily change a specific functional group into another allowing for making reactions that are otherwise conflicting with that functional group [108]. Ether-protecting groups are the most using for alcohol. Ethers are considered the least reactive of the organic functional groups [108].

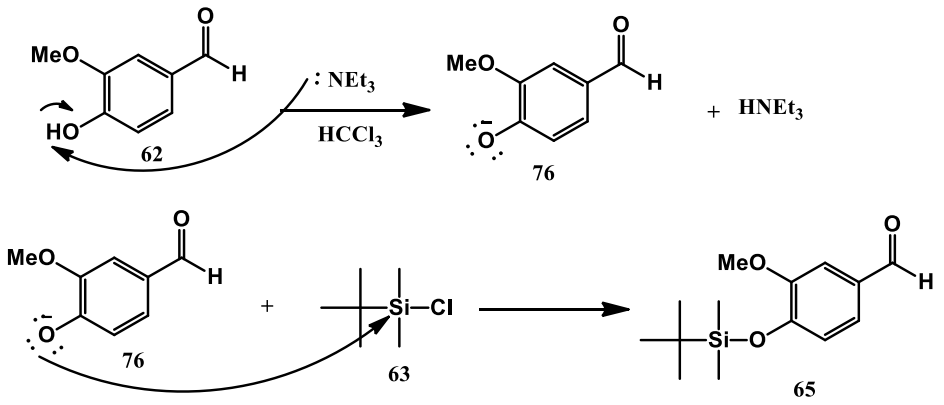
Compound **64** was obtained in 89% yield by treatment of vanilin **62** with tert-butyldimethylsilyl chloride by SN_2 reaction in the presence of

triethylamine in chloroform at room temperature before condensation reaction as shown in scheme 4.2 to protect the OH in vanillin **62** that facilitate the reaction with ketone **55** later. And finally deprotected by TBAF **66a**, deprotection reaction is carried out in the presence of triethylamine (TEA).



Compound	Yield%
64	89

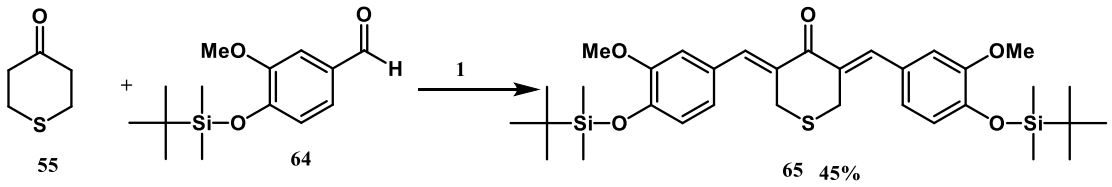
Fig 4.3: Protection of4-hydroxy-3-(methoxy) benzaldehyde. Reagents and conditions: (1) $NEt_3/HCCl_3$, at room temperature.



Scheme 4.2: Mechanism of Protection of aldehyde by SN_2 reaction

NEt_3 moderate base removed the acidic proton of the O-H in the aldehyde **62** forming phenolate anion **76**, which attacks the strong electrophile TBDMSCl **63** and chloride departs as a leaving group in an SN_2 fashion. After that, the silylated aldehyde **64** reacts with tetrahydro-4H-thiopyran-4-

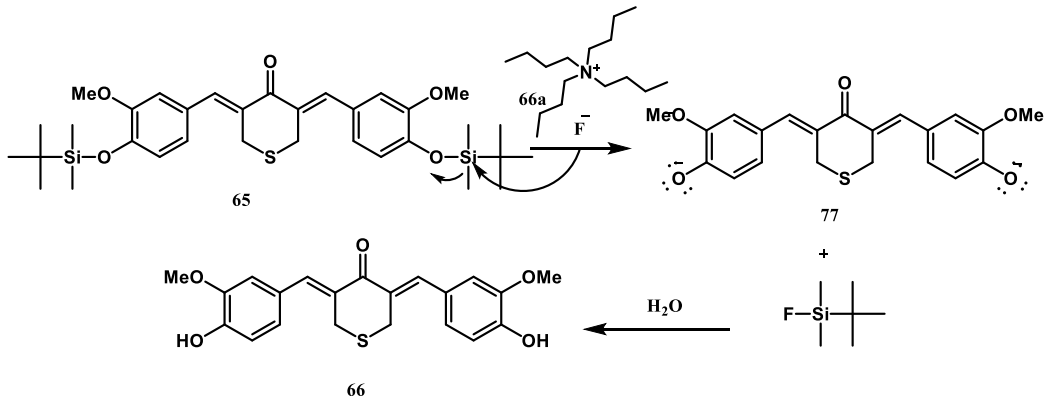
one **55** by aldol condensation reaction as shown in scheme 4.1 to yield the compound **65**.



Compound	Yield%
65	45%

Fig 4.4: Synthesis and structure of MCACs 65. Reagents and conditions: (1) KOH/CH_3OH, 80°C.

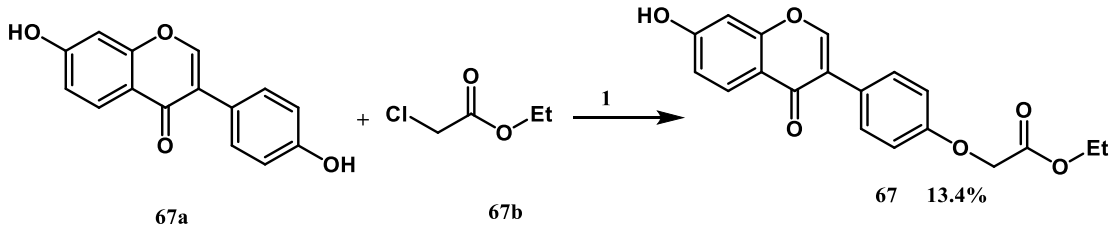
The silyl ether protecting group is removed with fluoride ion using tetrabutylammonium fluoride **66a** (TBAF) $Bu_4N^+F^-$ as shown in scheme 4.3. The four butyl groups bond to nitrogen atom to increase and enhance the solubility of the salt in organic solvents [109]. The fluoride ion acts as lewis base to make bond with Si (favorable) and an anion **77** is formed, which was neutralized by work up with water or acid.



Scheme 4.3: Mechanism of Deprotection of compound 66 using TBFA group.

4.4 The Reaction mechanism of compound 67

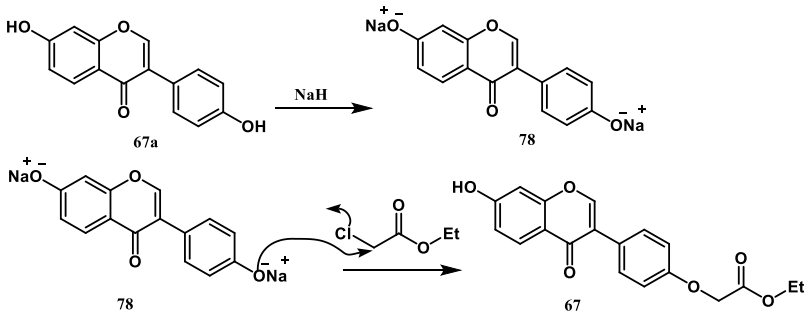
Treatment of daidzain **67a** with 2.2 equivalent of NaH in dry DMF for 2h under nitrogen condition, followed by addition of 1 equivalent of ethyl chloroacetate **67b** afforded ethoxycarbonyl methyl-7-hydroxyisoflavone **67** in 13% yield.



Compound	Yield%
67	13.4

Fig 4.5: Synthesis and structure of isoflavone. Reagents and condition: (1) NaH, THF, at room temperature.

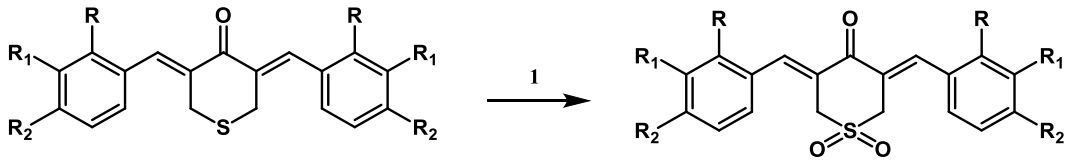
The hydroxyl group in Daidzein **67a** is deprotonated by NaH base, strong nucleophile **78** (conjugate base) is formed. It is binded directly with carbon that carry an acidic proton and Cl departs as a leaving group as shown in scheme 4.4.



Scheme 4.4: Mechanism reaction of Daidzein with ethyl chloroacetate.

4.5 Oxidation reaction for preparing compounds

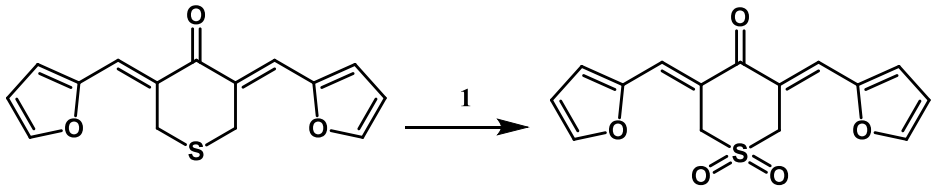
The oxidation of sulfides is a basic reaction to form sulfoxides and sulfones. Many reagents such as peracids and halogen derivatives are used of sulfoxidation reactions [110]. m-Chloroperoxybenzoic acid (m-CPBA) **68** is a peroxycarboxylic acid that is used in oxidation of our prepared compounds **56, 57, 58, 59 and 61**. It is often chosen compared to other peroxy acids because it is easy to handle [111]. Compounds **56, 57, 58, 59 and 61** were treated with excess of m-CPBA in DCM at 0°C to generate compounds **56a, 57a, 58a, 59a and 61a** with 84, 66, 63, 13 and 77% yields, respectively.



56a - 58a and 61a

Compound	R	R_1	R_2	Yield %
56a	OCH_3	OCH_3	OCH_3	84
57a	H	Benzyloxy	OCH_3	67
58a	NO_2	H	H	63
61a	H	H	$-N(CH_3)_2$	77

Fig 4.6: Oxidation of MCACs. Reagents and conditions: (1) m-CPBA/DCM, at room tempreture.

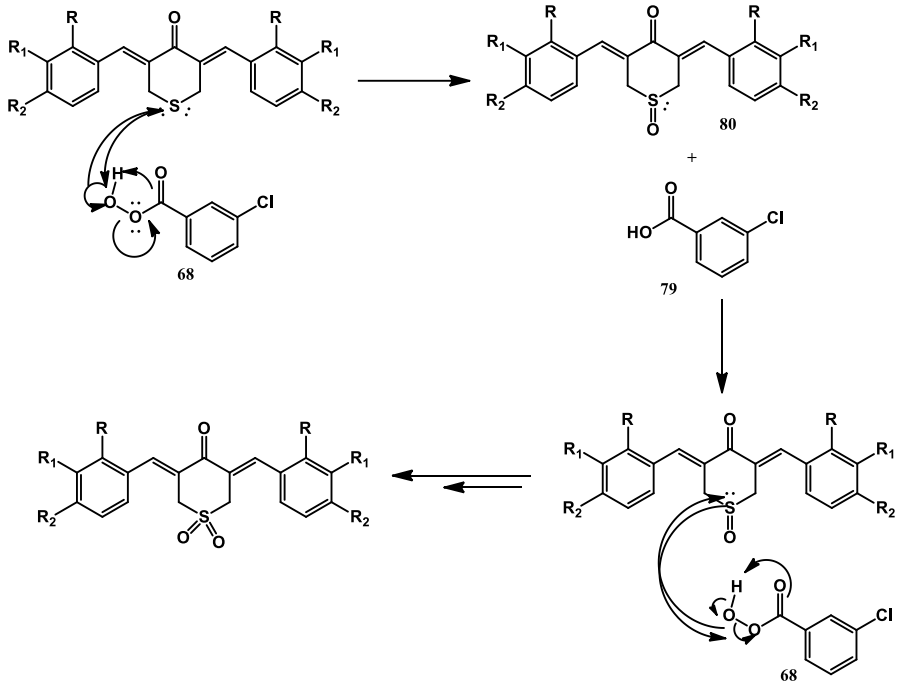


59a **13%**

Compound	Yield%
59a	13

Fig 4.7: Oxidation of compound 59 (MCACs). Reagents and conditions: (1) m-CPBA/DCM, at room temperature.

The first step of the reaction is terminal oxygen of the peroxide group **68** is attacked by the sulfer atom. Peroxide bond is broken results in the formation of m-chlorobenzoic acid **79** and a sulfoxide **80**, which undergo further oxidation with m-CPBA to afford the sulfone as given in scheme 4.5.



Scheme 4.5: Mechanism of oxidation reaction for the preparing compounds

4.6 Antioxidant evaluation

Antioxidants are a set of compounds that balance free radicals and reactive oxygen and nitrogen species (ROS, RNS) in the cell. These antioxidants give defense against harm caused by free radicals that played important roles in the devolopment of several disease which include aging, heart disease, anaemie, cancer and inflammation [112]. DPPH assay was used as in vitro approach to determine the free radical-scavenging activity and to screen for the possible antioxidant activity of the synthesized compounds. The antioxidant activities of the prepared compounds and Trolox (positive

control) was estimated by their capacity to donate a hydrogen atom or electron and was identified from changing the deep violet color of methanol solution of DPPH to colorless or pale yellow.

IC_{50} values were used to evaluate the amount of antioxidant needed to inhibit the radical (DPPH) concentration by 50%, and inversely related to their antioxidant activities.

Trolox **70** owned IC_{50} at (3.4 ± 0.015) (positive control) that described as (mean± standard deviation). The results of IC_{50} and the DPPH radical scavenging effect of the seven compounds and Trolox were evaluated as shown in table 4.1.

These results indicated that most of the compounds (**57a, 66, 67, 59a, 61a and 58a**) owned an IC_{50} greater than the Trolox. However, compound **56a** having IC_{50}=11.84 μg/ml, which is nearby to the IC_{50} of trolox **70** and curcumin **15**. Trolox **70** and curcumin **15** owned IC_{50} 3.4 and 2.3μg/ml, respectively [113]. And compound **58a** having IC_{50}= 66 μg/ml which is considered the least toxic compound when compared with trolox and curcumin. Consequently, synthesized compounds are ranked according to their activities as antioxidants:

Trolox > compound 56a > compound 57a > compound 66 > compound 67 > compound 59a >compound 61a > compound 58a.

Table 4.1: IC_{50} values of MCACs and Trolox against DPPH radical

Compound	IC_{50} µg/ml
70	3.4 ± 0.015
56a	11.8 ± 0.28
57a	15.8 ± 0.01
58a	66 ± 0.15
59a	25.2 ± 0.11
61a	44 ± 0.1
66	18.07 ± 0.01
67	20.9 ± 0.011

4.7 Cytotoxic evaluation

An MTS assay was used to determine the cytotoxic effect of curcumin derivatives on HeLa, HepG2, Hep3b and MCF-7 cancer cell lines. The half inhibitory concentration (IC_{50}) of these compounds were measured (**Table 4.2**).

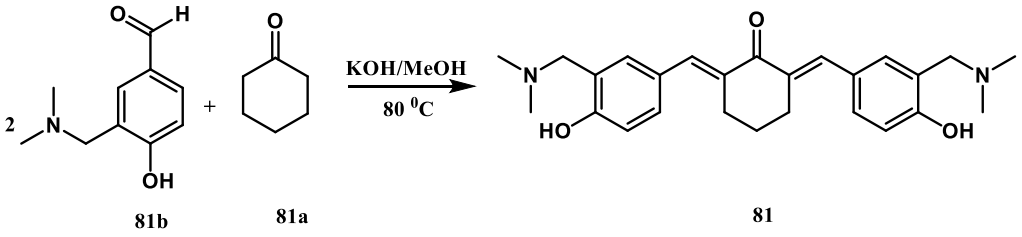
Table 4.2: IC_{50} values of MCACs against HeLa, HepG2, Hep3B and MCF-7

Compound/cell line	IC_{50} µg/ml			
	HepG2	**Hep3B**	**HeLa**	**MCF-7**
56	102.3± 2.5	186.2± 1.4	7943± 0.95	NI
57	50.11± 1.4	346.7± 2.5	22.38± 1.5	39.8± 2.1
58	870± 1.9	354± 0.99	323.5± 1.6	NI
59	524.8± 0.7	583.4± 1.4	363± 1.1	467.7± 1.4
61	758.5± 1.4	446.6± 3.1	251± 1.1	181.9± 1.1
66	349.9± 2.7	575± 0.98	512.8± 2.4	208.9± 1.7
67	398.1± 2.2	630± 2.2	398.1± 1.6	218.7± 1.3
56a	7.194± 2.6	53.7± 1.7	2238.7±1.4	NI
59a	46.77±2.2	56.2±2.1	251.1± 2.6	50.11± 2.4
61a	363.07±2.7	363.07±3.5	234.4± 1.4	562.3± 2.1

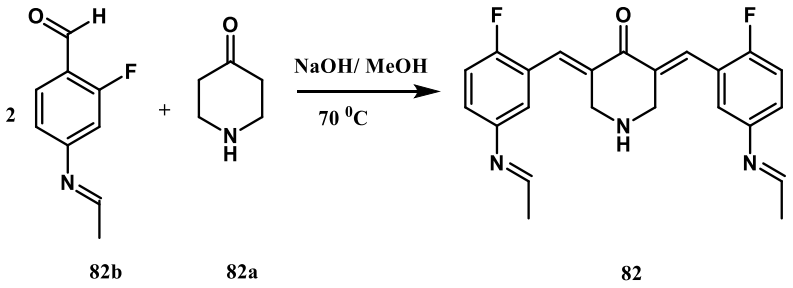
In HepG2 cell line (liver cancer cell) most of the compounds showed inhibition of cell growth at relatively high concentrations in comparison to

the IC_{50} of curcumin = 23.15 μg/ml [114], except compound **56a** with IC_{50}=7.194 μg/ml less than IC_{50} for curcumin, so it is better than curcumin for this cancer cell line. Also, oxidized compounds were shown inhibition of cell growth at law concentration better than non-oxidized one in all of cancer cell lines such as compound **59a** with IC_{50} = 46.77 μg/ml and compound **59** with IC_{50} =524.8 μg/ml.

Moreover, in HepG2 compound **56a** is better for inhibition of cell growth than other mono-carbonyl analogues of curcuminoids with cyclohexanone **81** (scheme 4.6), which obtained from the reaction of cyclohexanone **81a** and aldehyde **81b**, with IC_{50}= 25μg/ml [115]. Otherwise, monocarbonyl analogues **82** that afforded when piperidone **82a** reacts with suitable ahdehyde **82b** (scheme 4.7), which is IC_{50} (<2 μg/ml) [115] lower than IC_{50} for **56a** and better than all derevatives for inhibition.



Scheme 4.6: Synthesis of the compound 81

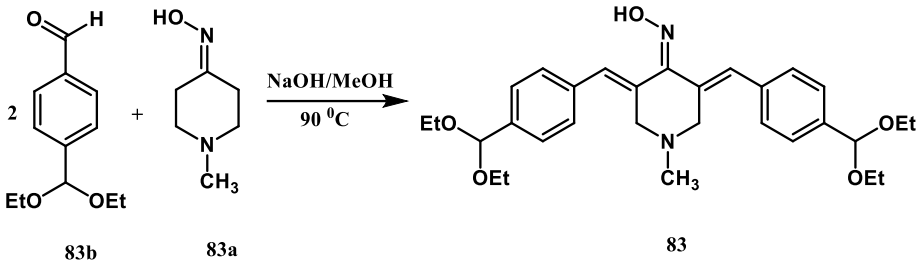


Scheme 4.7: Synthesis of the compound 82

In Hep3B cell line, all compounds displayed inhibition of cell growth at very high concentrations in a comparison to the IC_{50} of curcumin 34.99 μg/ml [116]. But the compound **56a** and compound **59a** IC_{50} for them 53.7μg/ml and 56.2 μg/ml, respectively close to IC_{50} for curcumin in this cancer cell line.

In Hela cancer cell, all synthesized compounds were shown inhibition of cell growth at very high concentrations when compared with IC_{50} for curcumin 10.46 μg/ml [117]. However, IC_{50} for compound **57** was 22.38 μg/ml is nearby IC_{50} for curcumin. Monocarbonyl analogues with piperidone **82**, which is IC_{50} (<2 μg/ml) [115] also lower than IC_{50} for compound **57** and curcumin in this cancer cell line. So the analogues with piperidone has a potent and promising anticancer activity in the most of cancer cell lines.

In MCF-7 cancer cell line, compound **56a, 56** and compound **58** were shown no inhibition of cell growth. Curcumin IC_{50} values of MCF-7 cells was 12 μg/ml [118] is better than all synthesized compounds. from synthesized compounds the most effective compounds that inhibit MCF-7 cells were compound **57** and compound **59a** with IC_{50} 39.8 μg/ml and 50.11μg/ml, respectively, but the monocarbonyl analogues **83** (oxime analogues) that is obtained by reaction of methyl piperidin-4-oxime **83a** with excess of aldehyde **83b** (scheme 4.8), is the best than curcumin and synthesized compound with IC_{50} =0.03 μg/ml [115].



Scheme 4.8: Synthesis of the compound 82

Conclusion

In conclusion, several compounds have been synthesized by condensation reaction under basic condition followed by oxidation reaction. And silyl ether was used to protect the hydroxyl group in aldehyde and deprotection reaction was occurred using TBAF to remove the protecting group. Seven compounds have screened against four types (HeLa, HepG2, MCF-7 and Hep3B) cancer cell lines. Monocarbonyl curcumin analogues with 3-(Benzyloxy)-4-methoxy moiety exhibiting potential cytotoxic properties. The compound **57** with IC_{50} values of 39.8± 2.10, 22.38± 1.50 and 50.11±1.40 µg/mL inhibited MCF-7, Hela and HepG2 cancer cell lines, respectively. Structure activity relationship revealed that the role of benzyloxy groups are important. Analogues with tetrahydrothio-4H- pyran series with oxidation of sulfor were found to be more selective than sulfide form. The compound **56a** exhibited inhibition against Hep3B with IC_{50} value of 53.7±1.70 better than that of compound **56** with IC_{50} value of 186.2±1.40. The compound **59a** also inhibited the growth of HepG2, Hep3B and MCF-7 with IC_{50} values of 46.77±2.20, 56.2±2.10 and 50.11±2.40, respectively. Moreover, curcumin analogues were played important role as antioxidant

agents. The compound **56a** possess excellent antioxidant activity with IC_{50} 11.8±0.28 and their antioxidant activities occur due to their presence of tri-methoxy groups that can make interaction with DPPH.

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Appendix

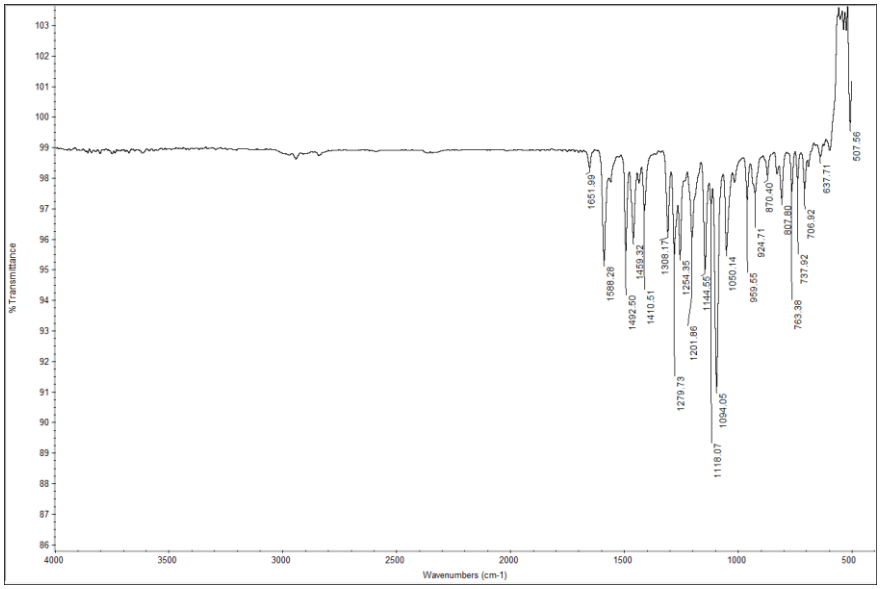

Figure 1a: IR spectra of the compound 56

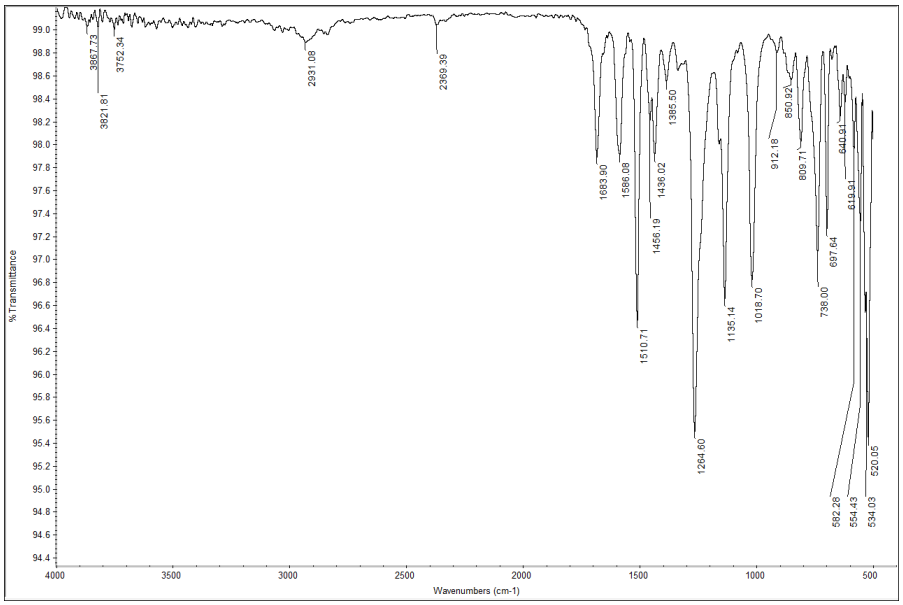

Figure 2a: IR spectra of the compound 57

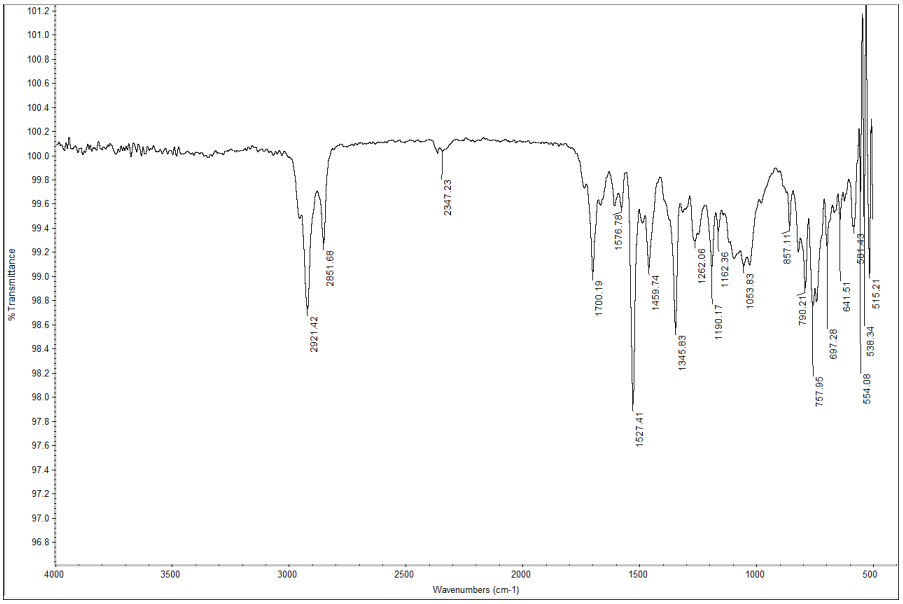

Figure 3a: IR spectra of the compound 58

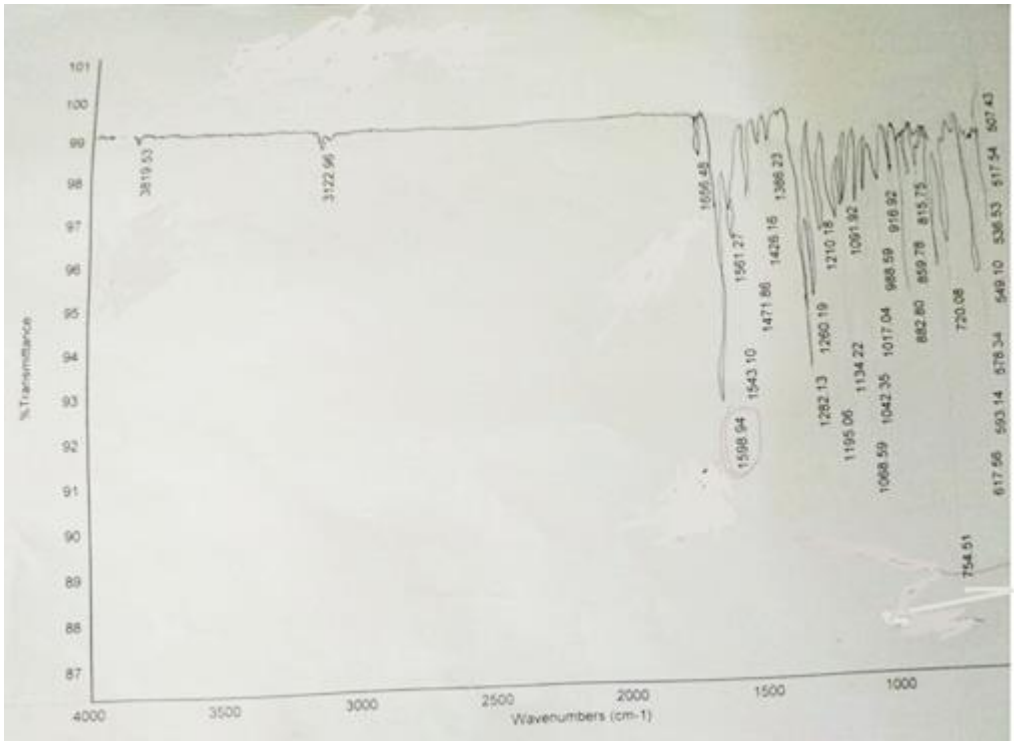

Figure 4a: IR spectra of the compound 59

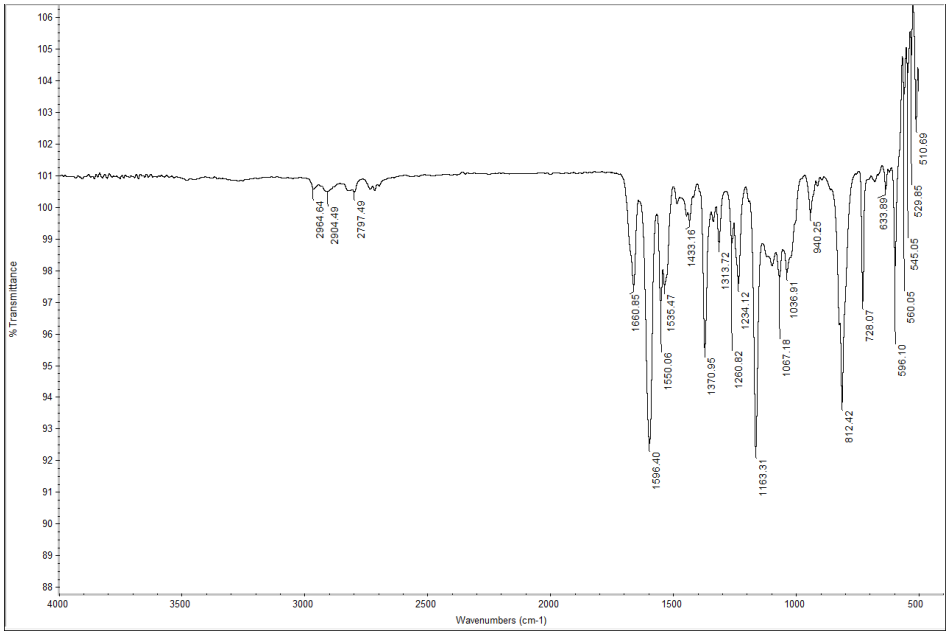

Figure 5a: IR spectra of the compound 61

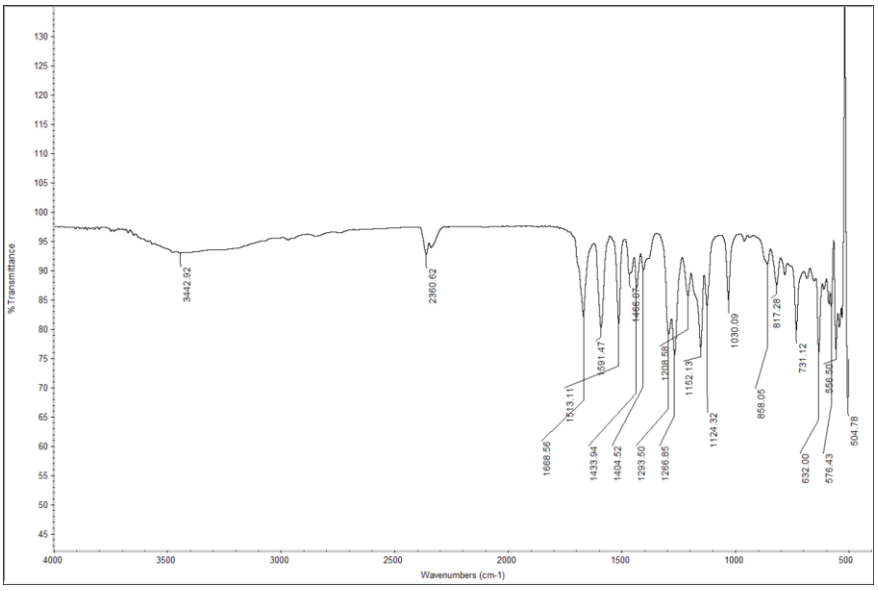

Figure 6a: IR spectra of the compound 66

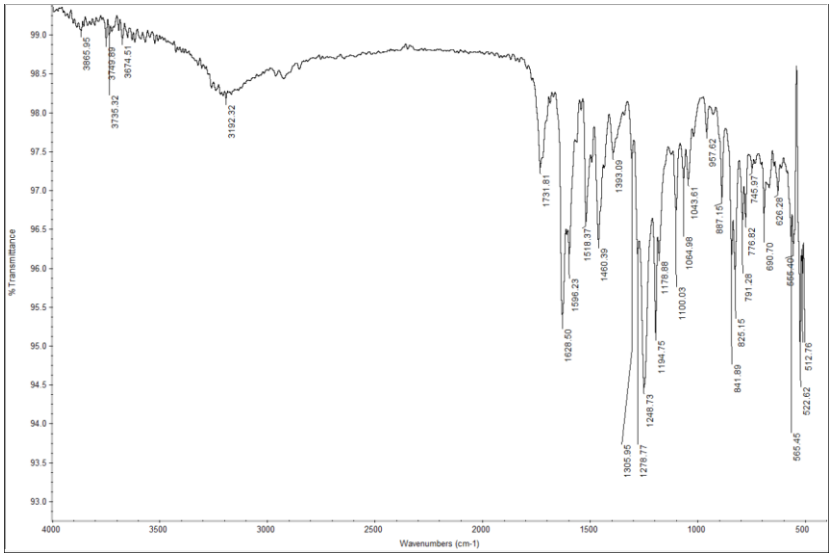

Figure 7a: IR spectra of the compound 67

جامعة النجاح الوطنية

كلية الدراسات العليا

تخليق مشتقات الكركمين الذي يحتوي على مجموعة وظيفية واحدة من الكربونيل الأحادي ودراسة تأثيرها الحيوي

إعداد

رهام نافز احمد صابر

إشراف

د. نواف المحاريق

د. نضال جرادات

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2021

تخليق مشتقات الكركمين الذي يحتوي على مجموعة وظيفية واحدة من الكربونيل الأحادي ودراسة تأثيرها الحيوي

إعداد

رهام نافز احمد صابر

إشراف

د. نواف المحاريق

د. نضال جرادات

الملخص

السرطان هو ثاني سبب رئيسي للوفاة على مستوى العالم، وهو مسؤول عن ما يقدر ب 9 مليون حالة وفاة في عام 2018 على الصعيد العالمي. كان هناك الكثير من الجهود لاكتشاف عوامل جديدة مضادة للسرطان تسمح بالعلاج باثار جانبية اقل.

تم تصنيع مجموعة من المركبات من مشتقات الكركمين وتقييم نشاطها السام للخلايا السرطانية: خلايا ضد الثدي وعنق الرحم والكبد ونشاطها المضادة للاكسدة في2,2 مقايسة ثنائي فينيل-1-بيكريل هيدرازيل عن طريق تركيز المادة الموافق للتثبيط النصفي.

تم استخدام تفاعل التكاثف للتحضير في ظروف قاعدية وبعد ذلك تم عمل تفاعل اكسدة لهم باستخدام حمض ميتا كلوربروكسي بنزويك. وتم مراقبة التفاعلات باستخام كرماتوغرافيا الطبقة الرقيقة واستخدام جهاز مطيافية الاشعة تحت الحمراء الذي يدل على حدوث تفاعل من خلال المجموعات الوظيفية الجديدة التي تظهر.

بعد ذلك، تم فحص نشاطهم السام للخلايا وقد تبين ان المركب 75 له نشاط ممتاز ضد خطوط خلايا الكبد والثدي وعنق الرحم السرطانية. وايضا تبين ان المركبات المؤكسدة لها نشاط انتقائي افضل من المركبات الغير مؤكسدة مثل المركب 65أ والمركب 65.

ولعبت ايضا بعض هذه المركبات دورا مهما كعوامل مضادة للاكسدة عند مقارنتها بالكركمين والترولوكس. حيث ان المركب 56أ كان عاملا مضادا للاكسدة جيدا.