Republic of Iraq
Ministry of Higher Education and Scientific Research
Al-Nahrain University
College of Science
Department of Chemistry

Synthesis ,characterization ,antioxidant and anti-Cancer activity studies of gold (III)complexes with heterocyclic compounds

A Thesis
Submitted to the College of Science Al-Nahrain University as a Partial Fulfillment of the Requirements for the Degree of M. Sc. in Chemistry

By:
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(B.Sc. 2011)

Supervised by:
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(2014)

Chapter one

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((أَلَمْ نَشْرَحْ لَكَ صَدْرَكَ (1) وَوَضَعْنَا عَنْكَ
وِزْرَكَ (2) الَّذِي أَنْقَضَ ظَهْرَكَ (3) وَرَفَعْنَا لَكَ
ذِكْرَكَ (4) فَإِنَّ مَعَ الْعُسْرِ يُسْرًا (5) إِنَّ مَعَ الْعُسْرِ
يُسْرًا (6) فَإِذَا فَرَغْتَ فَانْصَبْ (7) وَإِلَىٰ رَبِّكَ
فَارْغَبْ(8)))

صَدَقَ اللهُ العَظِيم

(سورة الانشراح)

Chapter Two

Ayah

Chapter Three

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I wish to express my great thanks to **ALLAH** for uncountable gifts and for helping me to present this thesis .

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Thanks to the staff of the chemistry department, college of science, Al-Nahrain University.

Also, I am very grateful to all the people who helped me during my work.

Ayah

Supervisor Certification

I certify that this thesis was prepared under my supervision at the Department of Chemistry, College of Science, Al-Nahrain University as a partial requirements for the Degree of Master of Science in Chemistry.

Signature:

Assistant Professor

Dr. Firas A. Hsaan

In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

Signature:

Assistant Professor

Dr. Nasreen R. Jber

Head of Chemistry Department

College of science

Al Nahrain university

Committee Certification

We, the examining committee, certify that we have read this thesis and have examined the student "Ayah J. Abdul hameed " in its contents and that in our opinion it is adequate with (Excellent) standing as a thesis for the degree of Master of Science in chemistry.

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Date: / /2014

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Date: / /2014

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Name: Assistant Professor

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Date: / /2014

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Name: Assistant Professor

Dr. Firas A. Hsaan

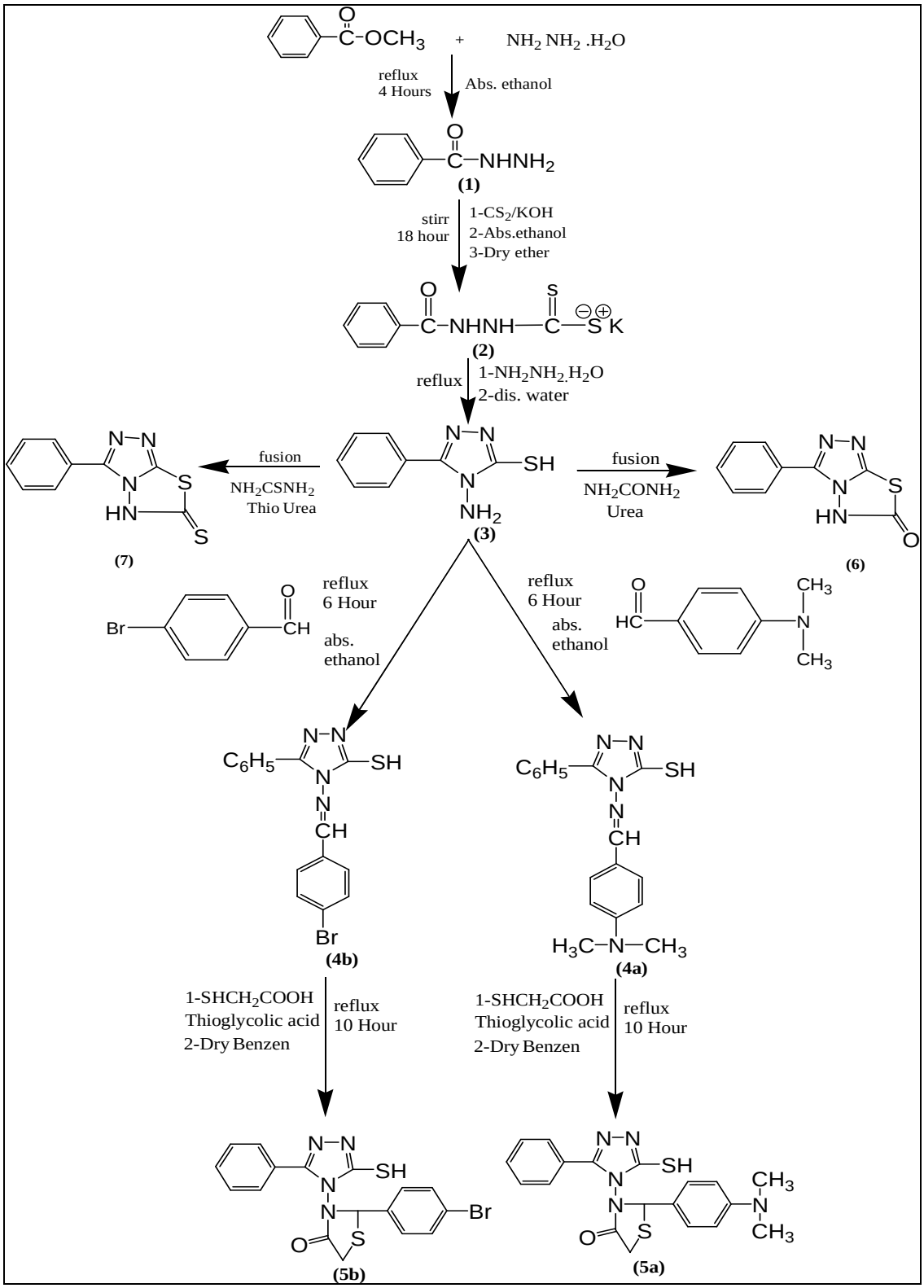
Date: / /2014

Approved for the College Committee of Graduate Studies

Signature:
Name: Dr. Hadi M. A. Abood
Scientific Degree: Assist. Prof.
Title: Dean of the College of Science
Date: / /2014

Abstract:

- A series of 1,2,4-triazole derivatives were synthesized by cyclization reaction, the benzoic acid hydrazide **(1)** was synthesized by reaction of methyl benzoate with hydrazine hydrate then compound **(1)** was reacted with CS_2 in solution of alkali ethanol to give potassium dithiocarbazinate salt **(2)** , the basic nucleus 4-amino-5-phenyl-1-4H-1,2,4-triazole -3-thiol **(3)** was prepared by cyclization of potassium salt **(2)** with hydrazine hydrate using water as solvent under reflux condition . compound **(3)** was subjected to addition reaction with different aromatic aldehydes to synthesize Schiff bases **(4a,b)** which were cyclized by treating with thioglycolic acid to prepare compounds **(5a,b)**.compounds **(6)** and **(7)** obtained by cyclization reaction of compound **(3)** with urea and thiourea .
- Also in this research, 1,3,4- thiadiazole derivatives were synthesized by cyclization of thiosemicarbazied with substituted carboxylic acid and sulphuric acid, to yield 2-amino-5-R-1,3,4-thiadiazole **(8)**. Schiff bases formation **(9a,b)** were by reflux of aromatic aldehyde with 2-amino-5-R-1,3,4-thiadiazole **(8)** in the presence of absolute ethanol. Compounds **(10a,b)** were prepared by cyclization reaction of compounds **(9a,b)** with thioglycolic acid.
- The Synthesized compound were confirmed by their melting point ,FTIR ,U.V-visible ,^{1}HNMR spectra and evaluated for their antioxidant activity by using stable free radical 1,1-diphenyl-2-picryl-hydrazyl DPPH. Of all tested compounds. compound **(5b)** was the most active in all concentrations compared to standard Ascorbic acid with an IC_{50} value 5.84 µg/ml .


NH2 NH2 .H2O
reflux
4 Hours
Abs. ethanol
(1)
stirr
18 hour
1-CS2/KOH
2-Abs.ethanol
3-Dry ether
(2)
reflux
1-NH2NH2.H2O
2-dis. water
(3)
fusion
NH2CSNH2
Thio Urea
(7)
fusion
NH2CONH2
Urea
(6)
reflux
6 Hour
abs.
ethanol
reflux
6 Hour
abs.
ethanol
(4b)
(4a)
1-SHCH2COOH
Thioglycolic acid
2-Dry Benzen
reflux
10 Hour
1-SHCH2COOH
Thioglycolic acid
2-Dry Benzen
reflux
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(5b)
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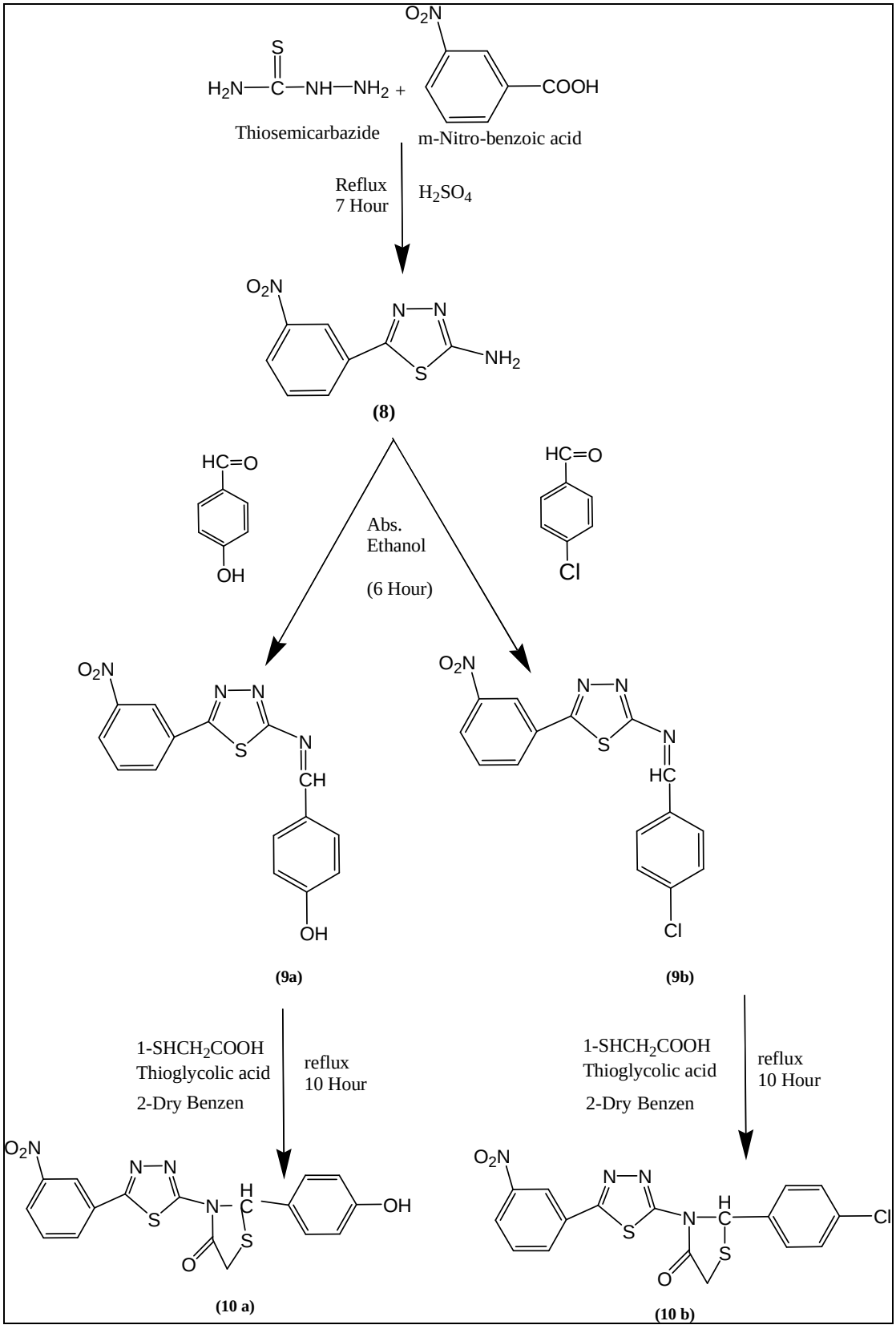
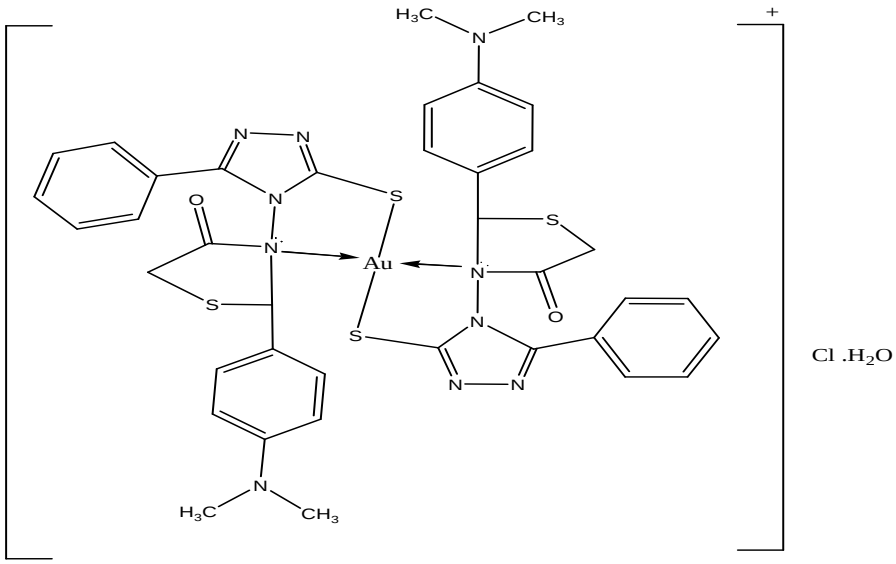

Thiosemicarbazide
m-Nitro-benzoic acid
Reflux
7 Hour
H2SO4
(8)
Abs.
Ethanol
(6 Hour)
(9a)
(9b)
1-SHCH2COOH
Thioglycolic acid
2-Dry Benzen
reflux
10 Hour
(10 a)
(10 b)

- In this study, the cytotoxic effects for compounds **(5a),(5b),(6),(7),(10a),(10b)** were studied in one cultured cellular models (MCF7 cell line) breast cancer (at different concentration) compared to doxorubicin as positive control by cell viability assay **(MMT assay),** compound **(5b)** showed the highest cytotoxicity effect with an IC_{50} value =56.98µg/ml .
- Also, we examine the cytotoxic effects of gold III complex **(AuL_2)** of bi-dentate ligand **(5a)** in one cultured cellular models (MCF7 cell line) by High Content Screening and analysis **(HCS).** The inhibitory effect of AuL_2 on breast cancer cell growth was due to induction of apoptosis as evidenced by Annexin V staining and cell shrinkage. We found that AuL_2-mediated lead to disruption of mitochondrial membrane potential (MMP), cell membrane permeability, and release of cytochrome c from the mitochondria into the cytosol. suggesting **(AuL_2)** as a potential MCF7 inhibitor. Thus, we suggest that **(AuL_2)** may have therapeutic value in breast cancer treatment worthy of further development.



Bis(2-(4-Dimethylamino-phenyl)-3-(3-mercapto-5-phenyl-[1,2,4]triazol-4-yl)-thiazolidin-4-one)gold(III) chloride. monohydrate

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Symbols & Observations

Symbol	Full meaning
DNA	Deoxyribonucleic acid
MCF-7	Michigan Cancer Foundation - 7
ER	Estrogen receptor
ATP	Adenosine triphosphate
NADH	Nicotinamide adenine dinucleotide
HCS	High content screening
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
DPPH	2,2-Diphenyl-1-picrylhydrazyl
TFA	Tri fluoro acetic acid
SB	Schiff bass
FTIR	Fourier Transform Infrared
NMR	Nuclear Magnetic Resonance
CNS	Central nervous system
CFTR	Cystic fibrosis transmembrane conductance regulator
HIV	Human immunodeficiency virus
IC_{50}	Inhibitory concentration 50
MMP	Mitochondrial membrane potential
NADPH	Nicotinamide adenine dinucleotide phosphate
TGA	Thermo gravimetric analysis
FADD	Fas-associated death domain protein
Caspases	Cysteine acid proteases
TrxR	Thioredoxin Reductase
COX	Cyclooxygenases
LOX	Lipoxygenases
DNase I	Deoxyribonuclease I
RNase A	Ribonuclease A
Bcl-2	B-cell lymphoma 2
Bcl-xl	B-cell lymphoma extra large
P53	53-Kilodalton protein
CD95	Cluster of differentiation 95
KDa	Kilo Dalton
Apaf-1	Apoptotic protease activating factor 1
SOD	Superoxide dismutases
GSH	glutathione
CAT	Chloramphenicol acetyltransferase
GPx	Glutathione peroxidase
DMSO	Dimethyl sulfoxide
SDS	sodium dodecyl sulfate

References

جمـــــــــــــــــــهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعـــــــــــــــــة النهريـــــــن
كليـــــــــــــــــة العـــــــــلوم
قـــــــــــــــــسم الكيميـــــــــاء

تحضير ،تشخيص، دراسة فعاليه مضاده للاكسده ومضاده للسرطان لمعقدات الذهب (III) مع مركبات حلقيه غير متجانسه

رسالة
مقدمة الى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

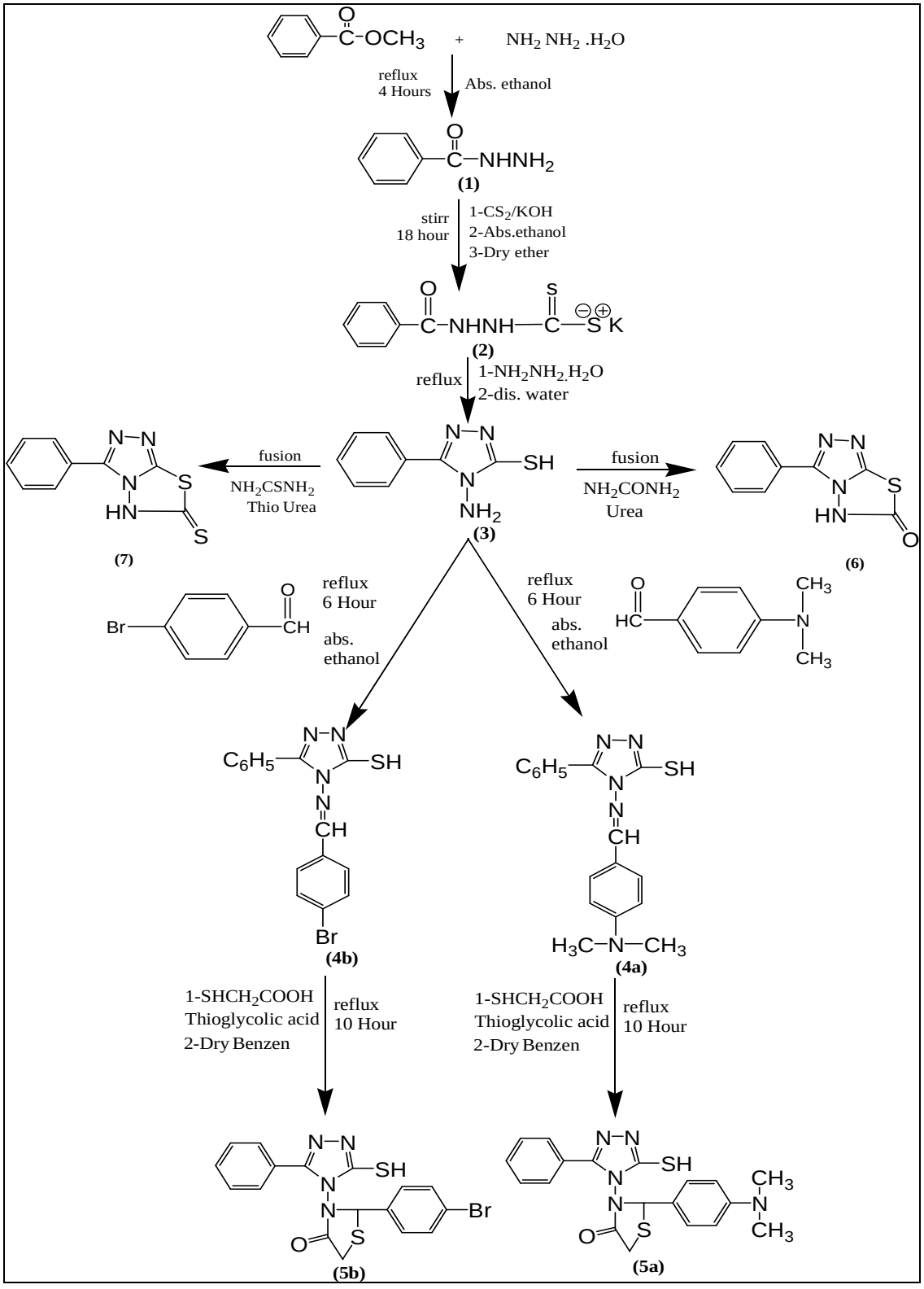
من قبل
اية جمال عبد الحميد
(بـكـالـوريـوس ٢٠١١)

المشرف
الاستاذ المساعد الدكتور فراس عبد الله حسن

(2014)

الخلاصـــــــــة

- تم تحضير سلسلة من مشتقات ١، ٢، ٤- ترايزول بواسطة تفاعلات الغلق ،حضر حامض البنزويك هيدرازايد **(١)** بواسطة تفاعل مثيل بنزويت مع الهيدرازين ثم تفاعل المركب **(١)** مع CS_2 في محلول كحولي قاعدي ليعطي ملح البوتاسيوم **(٢)**. حضر المركب **(٣)** بواسطة غلق ملح البوتاسيوم **(٢)**مع الهيدرازين باستخدام الماء كمذيب.استخدم المركب **(٣)** في تفاعل اضافة مع الديهايدات مختلفة لتحضير قواعد شف **(٤ أ،ب)** التي عوملت بواسطة حامض الثايوكلايكول لتحضير مركبات **(٥ أ ،ب)** ،اما مركبات ٦و٧ حضرت بواسطة تفاعلات غلق لمركب **(٣)** مع اليوريا والثايويوريا .
- كذلك في هذا البحث تم تحضير مشتقات ١، ٣، ٤-ثايودايزول بواسطه غلق الثايوسميكاربزايد مع حوامض كاربوكسيلية معوضه بالاضافه الى حامض الكبريتيك ليعطي المركب **(٨)**،وتم تحضير قواعد شف **(٩أ،ب)** بواسطة تفاعل المركب **(٨)** مع الالديهايدات الاروماتيه بوجود الايثانول ، اما مركبات **(١٠ أ،ب)**حضرت عن طريق تفاعلات غلق للمركبات **(٩أ،ب)** مع حامض الثايوكلايكول .
- تم تشخيص المركبات المحضره بواسطة درجة الانصهار ،FTIR ،U.V-visible ،^{1}HNMR ، وقيّمت لفعاليتها المضاده للاكسده باستخدام الجذر الحر المستقر DPPH . من بين المركبات المختبره المركب **(٥ ب)** كان الاكثر فعالية في كل التراكيز المستخدمه مقارنة بحامض الاسكوربك القياسي وكانت قيمة IC_{50}=٥,٨٤ µg/ml.
- كذلك تم دراسة التأثير السمي للمركبات **(٥أ)،(٥ب)،(٦)،(٧)،(١٠أ)،(١٠ب)** على خلايا الخط السرطاني**(MCF7)** لسرطان الثدي في تراكيز مختلفه مقارنة مع الدكسوربسين باستخدام تقنية **(MTT)** وقد اظهر المركب **(٥ب)** اعلى تأثير سمي وكانت قيمة IC_{50}=٥٦،٩٨ µ g/ml.


+ NH2 NH2 .H2O
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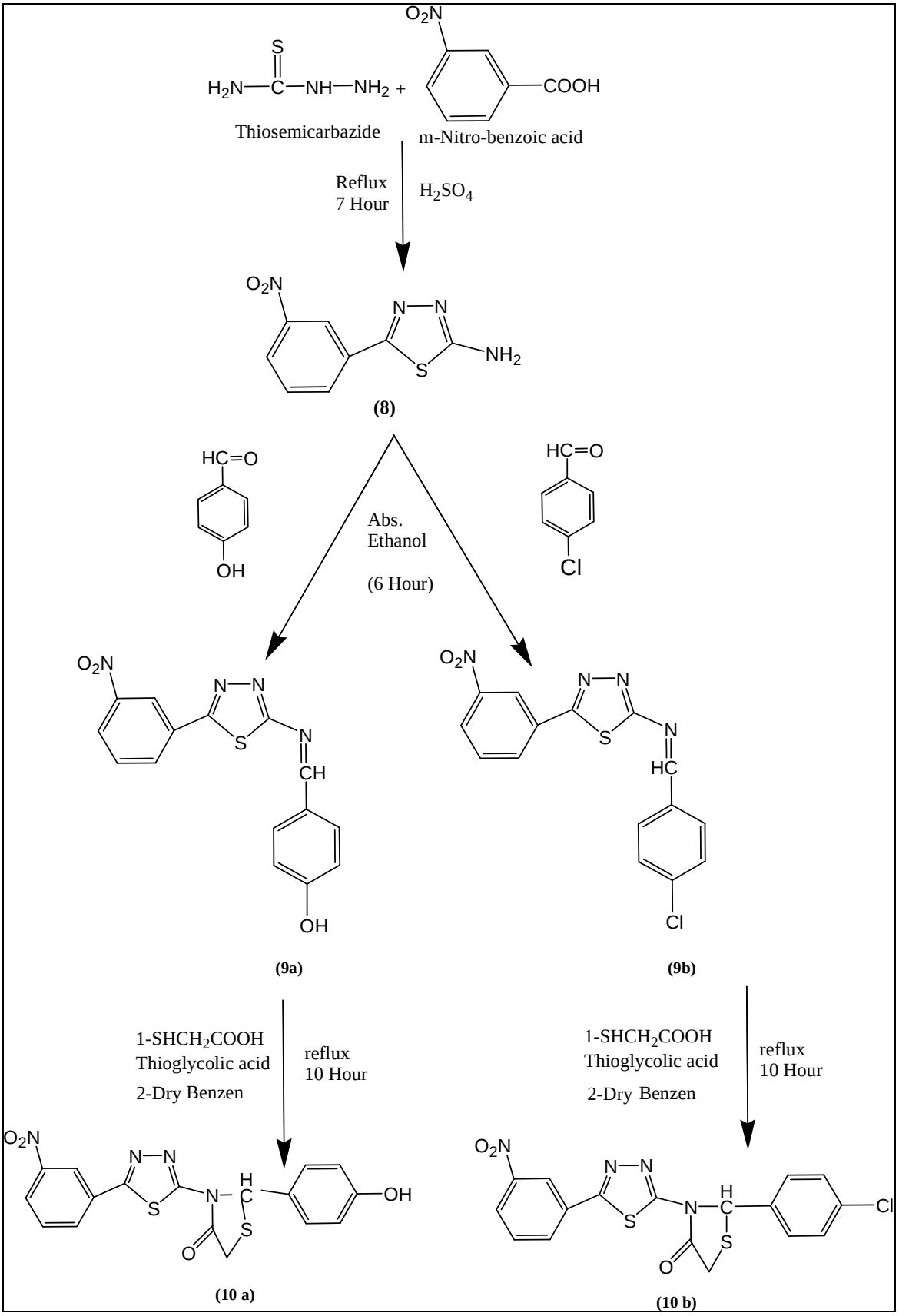
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Thioglycolic acid
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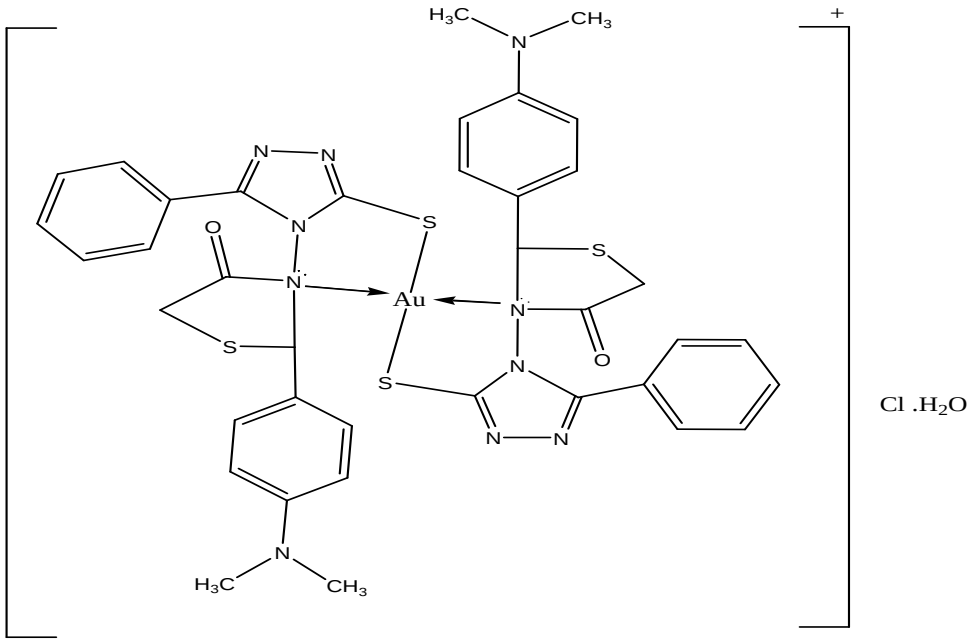
reflux
10 Hour
(10 a)
(10 b)

كذلك تم تحضير ودراسة الفعاليه السميه لمعقد الذهب الثلاثي **(AuL_2)** ثنائي الليكند **(٥ أ)**على خط الخلايا السرطانيه**(MCF7)** لسرطان الثدي بواسطة تقنية الفحص العالي للمحتوى **(HCS)**.و كان التأثير التثبيطي للمعقد الذهب الثلاثي على نموالخلايا السرطانيه عن طريق استحداث الموت المبرمج للخلايا وقد وجد ان المعقد يؤدي الى اضطراب في نفاذية غشاء المايتوكندريا (MPP) ،نفاذية غشاء الخلية و تحرير السايتوكروم c من المايتوكندريا الى السايتوسول .بالتالي اقترح ان معقد الذهب الثلاثي يمتلك فعالية تثبيطيه لخلايا الخط السرطاني **(MCF7)** ويمتلك فعالية علاجية لسرطان الثدي تستحق التطوير .



1.1. Cancer

Cancer is the second leading cause of death in the world after cardiovascular diseases. Today, millions of cancer people extend their life due to early identification and treatment [1].

Most of cells are specialized, they have a specific form and function that suits them to the role they play in the body .normal cells are growing under controlled mechanisms ,contact inhibition, one organized layer and differentiated cells, The basic different between cancer cells and normal cells are uncontrolled cell proliferation ,decreased cellular differentiation ,ability to invade surrounding tissue, and ability to establish new growth at ectopic sites [2].

Normal cells can enter the cell cycle for about 50 times and then they die , while cancer cell can enter the cycle repeatedly, the nuclei of cancer cells are enlarged and there may be an abnormal number of chromosomes. in the body , cancer cell divides to form an abnormal mass of cell called a (tumor) which invades and destroys neighboring tissue .

there is two type of tumor ,benign tumor is disorganized usually encapsulated mass but dose not invades adjacent tissue, second type is malignant tumor which consist of an abnormal uncontrolled proliferation of cells with partial or often complete lack of organization ,malignant tumors invade surrounding tissues and often in the later stages of the disease [3].

Cancer is a complex genetic disease that is caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to [4].

Any chemical that cause a change in the DNA sequence is called a mutagen, mutagens are also carcinogens (cancer-causing chemicals), most cancers result from mutation in a single normal cell , mutation also can arise from mistakes made by DNA polymerase during DNA replication [5].

Bishop et. al. [6] previously reported that cancer occur in different forms in different tissues and organs and often develop in different forms even in a single tissue , the first stage in the development of cancer is the transformation of normal cell to cell that differentiates abnormally , through cell division population of cancer cells are formed .

The second stage is the metastasize of cancer cells to the other organs of body , making difficult to deal with one cell begin to grow elsewhere in the body [7].

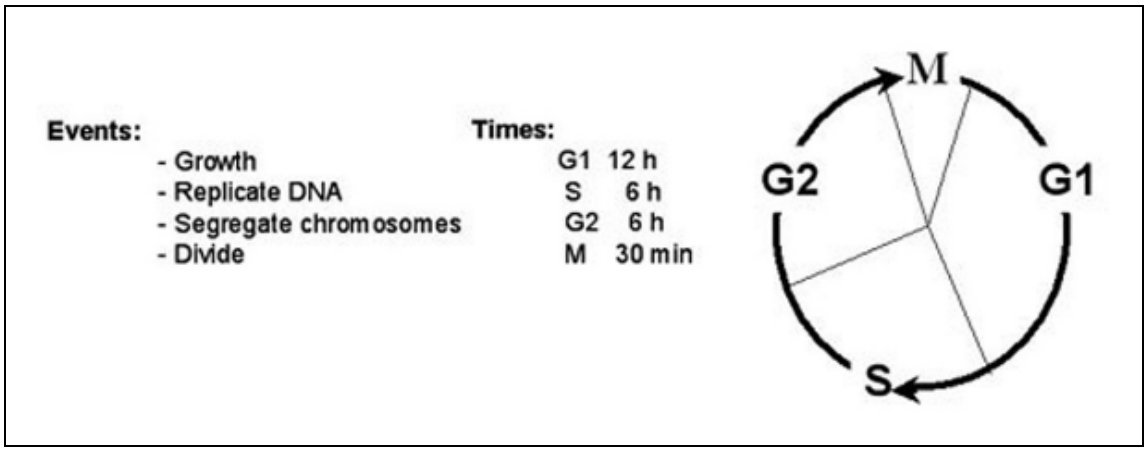
Kundson et. al. [8] studied cellular growth and division are controlled by biochemical pathways using signals from inside and outside the cell, disrupted control can be caused by genetic alterations of growth controlling genes , viral infection ,increased stimulation by growth factors , or a combination of these factors .

1.1.1.Cell Division cycle

Cell division occurs by an elaborate series of events, whereby chromosomes and other components are duplicated and evenly distributed into two daughter cells. It is a highly ordered and tightly regulated process that causes an irreversible and unidirectional change in cell state.

Under appropriate environmental conditions, unicellular organisms like yeast exist perpetually in the cell cycle, constantly growing and dividing. Although the same occurs with some cancer cells, most cells in multicellular organisms are not cycling. A significant fraction of these cells, including those that have already differentiated, are no longer (generally) capable of proliferation .

cells could be divided into four main stages. S phase, the period of DNA synthesis, is separated from mitosis by an interval of several hours, called G_2. Similarly, the period between the end of mitosis and the beginning of S was called G_1 . The eukaryotic cell cycle can be divided into two fundamental stages: Interphase, comprising Gap 1 (G1),Synthesis (S) phase and Gap 2 (G2), and M phase, composed of two major events, nuclear division (Mitosis) and cytoplasmic events. As shown in scheme (1-1) [9].



Scheme (1-1): Organization of the cell cycle.

1.1.2. Apoptosis

Apoptosis is a term coined by Kerr ,Wyllie and Currie in 1972, characterized by an ordered series of physical and biochemical reactions that are controlled by variety of genes such as P53 and Bcl-2 [10].

Remarkably cellular dehydration is an early event for apoptosis , resulting in cytoplasmic condensation and changes in cell shape and size ,which is followed by condensation of nuclear chromatin. Nuclear fragmentation then occurs and DNA droplets of different sizes evenly distributed throughout the cytoplasm are formed.

The nuclear fragments and other intracellular components,like mitochondria ,are then packed and enveloped by the cell membrane and these resultants called (apoptotic bodies) are shed from the apoptotic cell (Figure 1-1),apoptotic cells are phagocytized by macrophages [11].

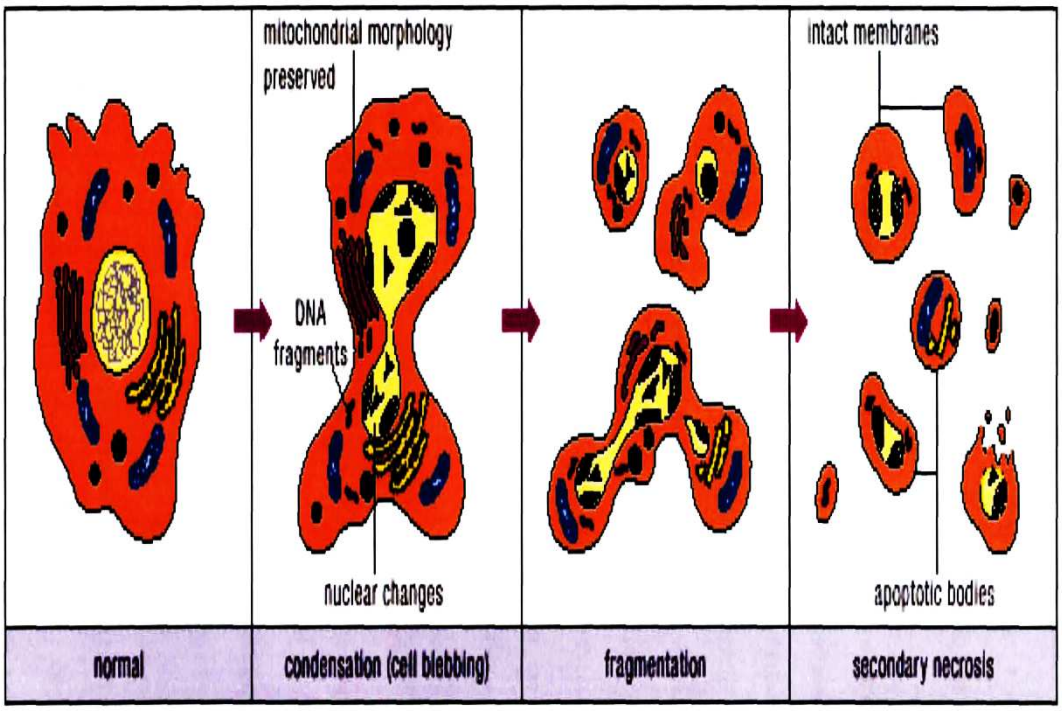


Figure (1-1).cellular change during apoptosis[11].

1.1.3.Molecular mechanism of apoptosis

- Death –receptor pathway and mitochondrial pathway

There are two major cell-intrinsic pathways for inducing apoptosis ,one that begins with the ligation of cell surface death receptors (death –receptor pathway) and another involves the mitochondrial release of cytochrome C (mitochondrial pathway).the death-receptor pathway is triggered by members of death receptor subfamily (such as CD95) [12].

Binding of CD95 induces receptor clustering formation of a death inducing signaling complex .this complex recruits via the adaptor molecule FADD (Fas-associated death domain protein), multiple procaspase-8 molecules and results in caspase-8 activation.

Another pathway , the mitochondrial pathway is triggered extensively in response to extracellular cues and internal insults. These diverse response pathways converge on mitochondria ,often through the activation of a pro-apoptotic member of the Bcl-2 family such as Bax and

Bid .Pro- and anti-apoptotic Bcl-2 family members meet at the surface of mitochondria ,where they compete to regulate cytochrome C exit by a mechanism that is still debated .if the pro-apoptotic camp wins an array of molecules is released from the mitochondrial compartment. among the released molecules ,cytochrome C , which associates with Apaf-1 and then procaspase-9 to form the apoptosome which in turn activate apoptosis [13].

- Important regulated proteins

Caspase. Apoptosis is a regulated physiological process leading to cell death (figure 1-2) Caspases , a family of cysteine acid proteases , are the central regulators of apoptosis. Initiator caspases (including 8,9,10 and 12) are closely coupled to pro-apoptotic signals .once activated ,these caspases cleave and activate downstream effector (including 3 ,6 and 7) , which cleave cytoskeletal and nuclear proteins .cytochrome C released from mitochondria is coupled to the activation of caspases-9 ,a key initiator [14].

Bcl-2 family. Apart from caspases ,members of the Bcl-2 family of proteins are important regulators of programmed cell death pathways with individual members that can suppress (e.g. Bcl-2 ,Bcl-$_{XL}$) . Bcl-$_{XL}$ exists as a 26 KDa integral membrane protein , its blocks apoptosis and thereby may contribute to tumor genesis by prolonging cell survival rather than by accelerating the rate of cell proliferation .down-regulation of Bcl-2 may be an important step during the induction of apoptosis .

Similar to Bcl-2, overexpression of Bcl-$_{XL}$ which can be induced by various condition and agents also inhibits apoptosis [15].

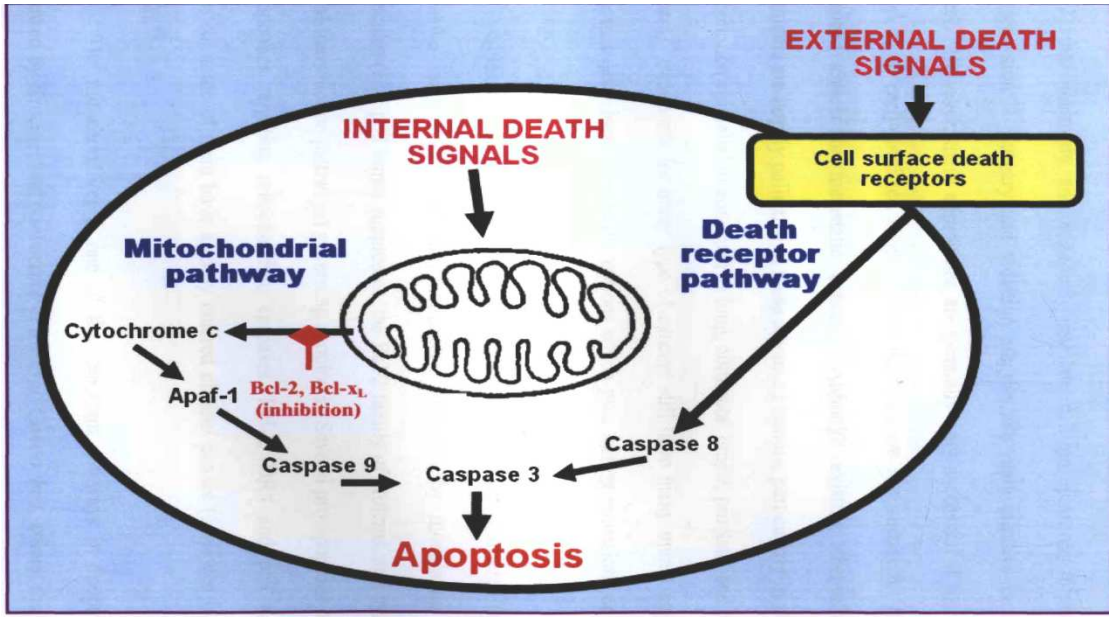


Figure (1-2) schematic diagram of two apoptotic pathways of caspase activation .

1.1.4. Tumor cell line

The first human cell line was established in a Baltimore laboratory over 50 years ago by George Gey . This cell line was HeLa – named after Henrietta Lacks, the lady from whom the cell line was derived, who had cervical carcinoma. Gey's vision paved the way for cell culture as we know it today, allowing its widespread development into an important experimental tool in cancer research. One of the major benefits of using cultured cell lines in cancer research is that they offer an infinite supply of a relatively homogeneous cell population that is capable of self-replication in standard cell culture medium [16].

A number of Factors Influencing Drug Responses in Tumor Cell Lines , such as solubility, chemical or metabolic stability, protein binding, and cellular uptake, can limit drug-induced inhibition of cell growth, an understanding of the relationship between such factors and drug structure facilitates the prediction of activity of new analogues in a series [17].

1.1.4.1.breast cancer

Breast cancer is one of the most common and serious malignancies worldwide. Despite intensive cancer control efforts, it remains the second-leading cause of cancer death among women [18].

MCF-7 is the acronym of Michigan Cancer Foundation -7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers [19].

MCF7 is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Cancers originating from ducts are known as ductal carcinomas, while those originating from lobules are known as lobular carcinomas. Breast cancer occurs in humans and other mammals. While the overwhelming majority of human cases occur in women, male breast cancer can also occur [20].

MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen, in the form of estradiol, via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line [21].

1.2. Chemotherapy

Chemotherapy may be defined as the use of chemical agents in the treatment of diseases. Chemicals, that employed are referred to be chemotherapeutic agents, the most essential feature of good chemotherapeutic agents must show a high degree of selective toxicity towards a microorganism, so that, it can be given in sufficient doses to inhibit or kill the microorganism throughout the body without harming the body cells.

The resistance of tumor cells to chemotherapeutic agents is a major problem in the clinical treatment of cancer; so a wide array of selective and potent compounds is needed to match the growing problems associated with cancer [22].

Anticancer agents are classified into several broad groups, which are usually defined according to their different mechanisms of action Scheme (1-2).

Most chemotherapeutic agents have the capacity to induce, either directly or indirectly, potential lethal damages to tumor cells [23].

These agents are classified into:-

(1) Alkylating agents and related compounds such as: Cisplatin, chlorambucil.

(2) Antimetabolites such as: methotrexate and nucleoside analogues.

(3) Anti-tumor antibiotics ((purine & pyrimidine base which are blind block of DNA, so, they prevent there substance of bowing in corporation DNA during sphere (of cell got) stopping normal development and dividing.

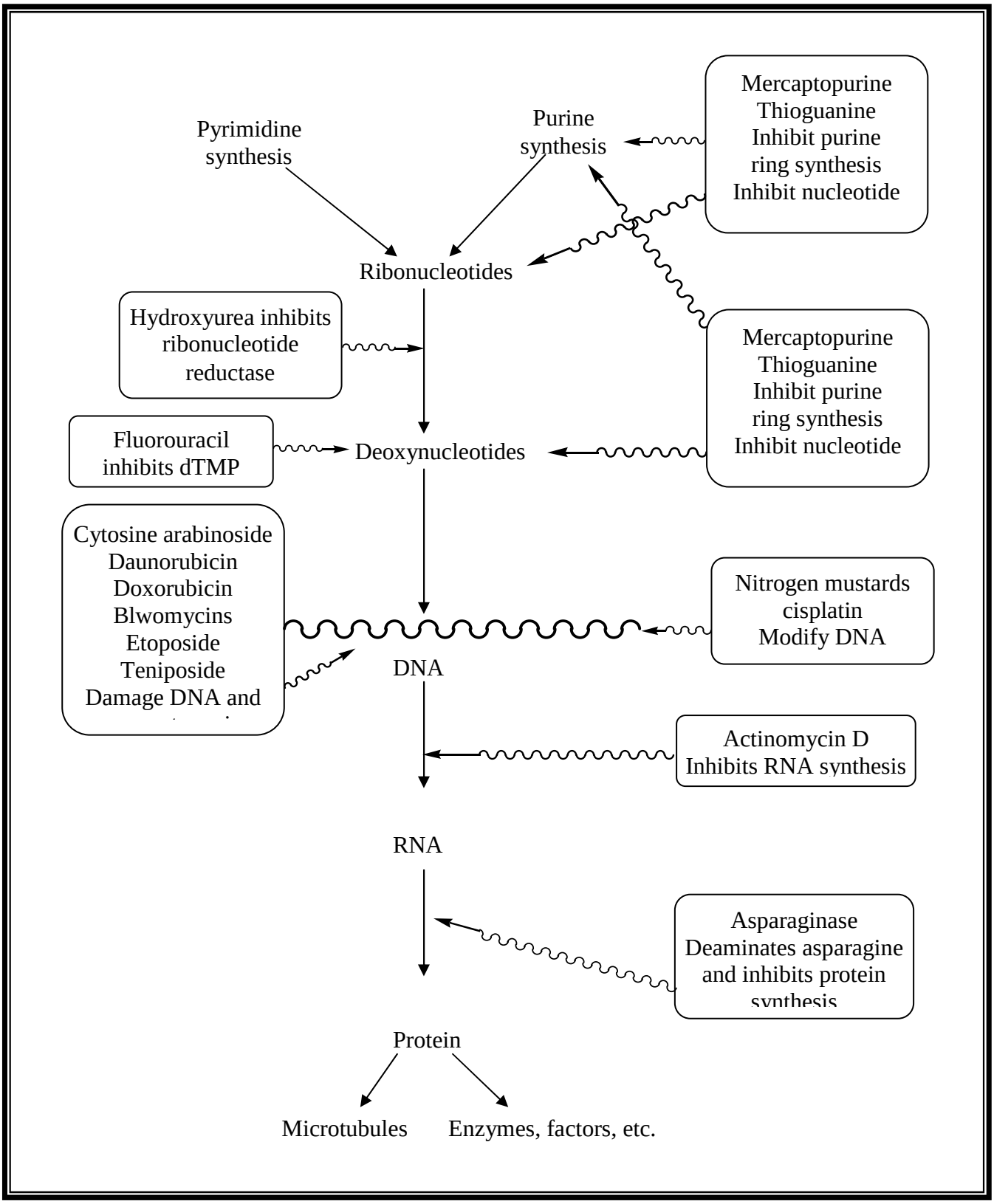
(4) Topoisomerase inhibitors.

(5) Mitotic inhibitors.

(6) Corticosteroids.

(7) Miscellaneous chemotherapy drugs.

(8) Other types of cancer drugs.



Scheme (1-2): Sites of action of selected drugs used in the treatment of cancer[24]

1.3. Cell viability assays(cytotoxicity)[25]

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. Figure (1-3) indicates various reagents used for cell viability detection. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, Co-enzyme production and nucleotide uptake activity. Many have established methods such as colony formation method, crystal violet method, tritium labeled thymidine uptake method, MTT and WST methods which are used for counting the number of live cells.

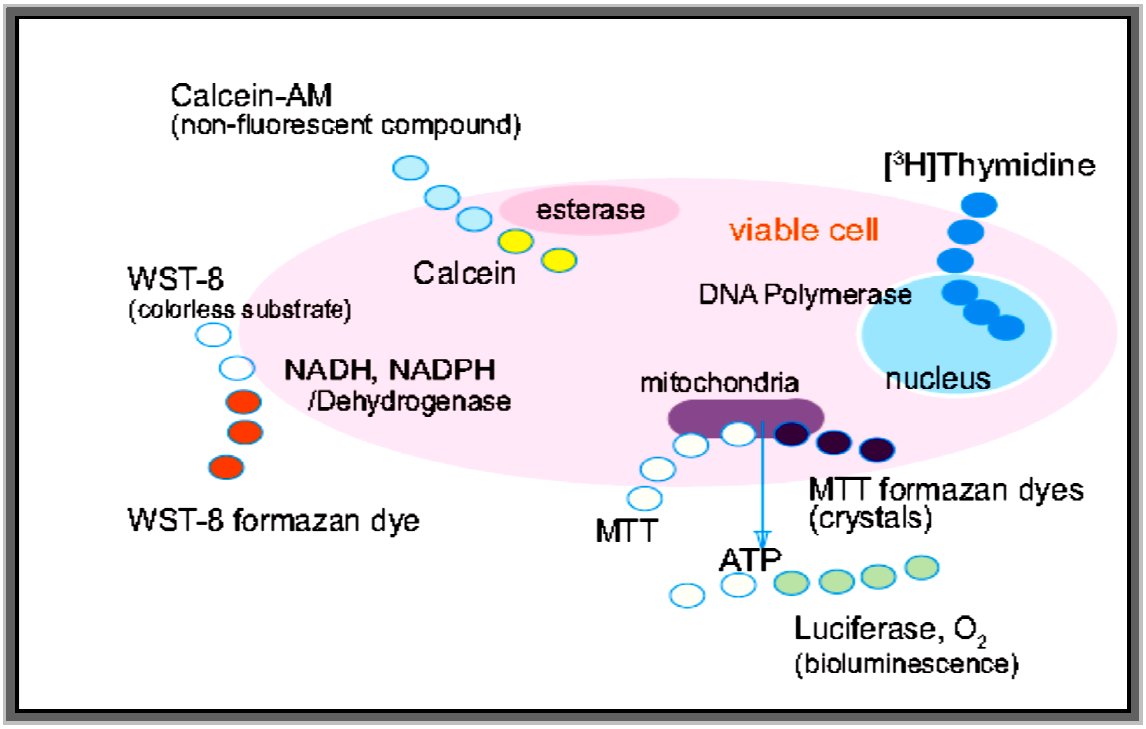


Figure (1-3) Reagents and methods for cell viability detection[25].

1.3.1. MTT cell proliferation assay

Enzyme-based methods using MTT rely on a reductive coloring reagent and dehydrogenase in a viable cell to determine cell viability with a colorimetric method. This method is far superior to the previously mentioned methods because it is easy-to-use, safe, has a high reproducibility, and is widely used in both cell viability and cytotoxicity tests. Therefore, this method is suitable for those who are just beginning such experiments. Among the enzyme-based assays, the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole)is reduced to a purple formazan by NADH, Figure (1-4). Reduction occurs outside the cell via plasma membrane electron transport [26].

However, MTT formazan is insoluble in water, and it forms purple needle shaped crystals in the cells. Therefore prior to measuring the absorbance, an organic solvent is required to solubilize the crystals. Additionally, the cytotoxicity of MTT formazan makes it difficult to remove cell culture media from the plate wells due to floating cells with MTT formazan needles, giving significant well-to-well error.

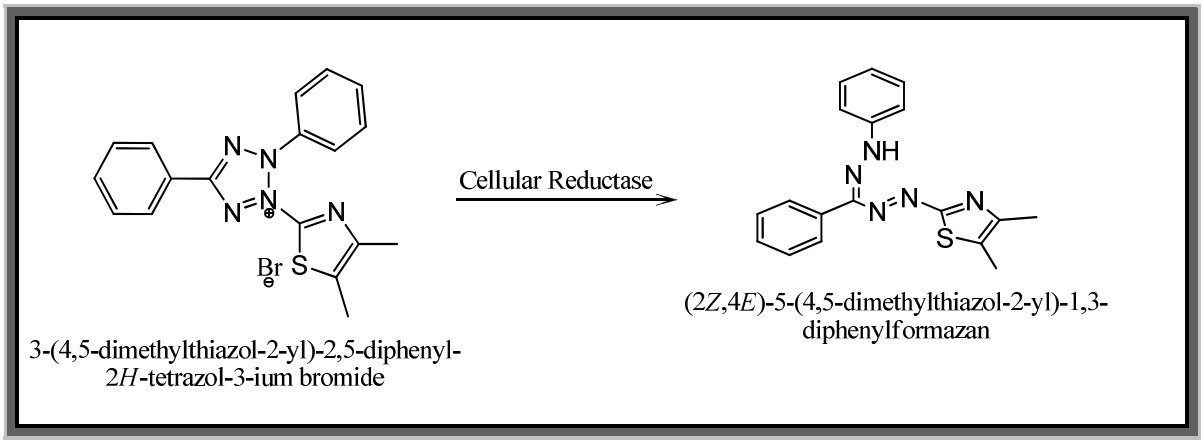


Figure (1-4) Reduction of MTT reagent.

A soluble solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate (SDS) in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum is dependent on the solvent employed [27].

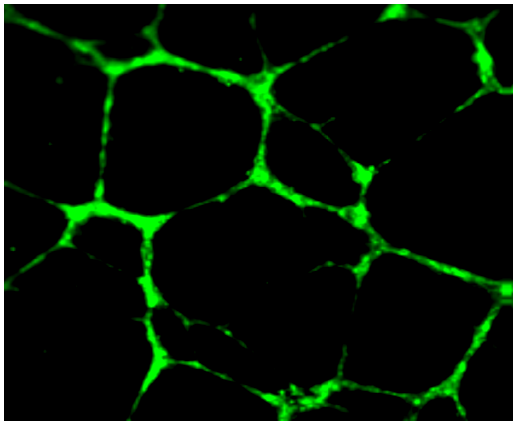
Absorbance detection has been available in micro plate readers for more than 3 decades, and is used for assays such as MTT assay for cell viability. A light source illuminates the sample using a specific wavelength (selected by an optical filter, or a monochromator), and a light detector located on the other side of the well measures how much of the initial (100%) light is transmitted through the sample, the amount of transmitted light will typically be related to the concentration of the molecule of interest [28].

1.4. High content screening (HCS)

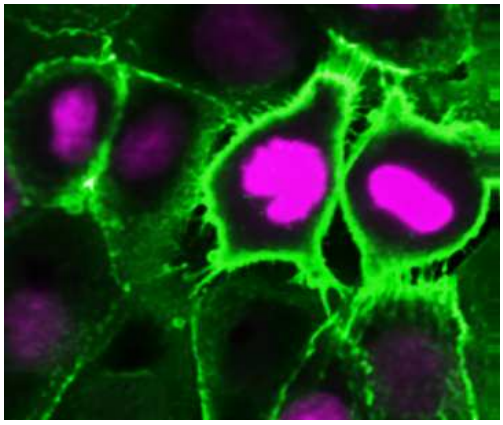
High content screening (HCS) was created in 1996 to offer a new platform that could be used to permit relatively high-throughput screening of cells, in which each cell in an array would be analyzed at a subcellular resolution using multicolored, fluorescence-based reagents for both specificity and sensitivity [29].

High-throughput and high-content analysis of cultured cells or even small organisms are of imminent importance in drug discovery, toxicology and in individualized medicine. In medicine, the identification of tailored patient-specific therapy is important in cancer, chronic inflammation, infection and other diseases .Substances that are screened are traditional drugs like antibiotics or cytostatic but also new small molecule drug classes bioactive peptides, or RNA Frequently, analysis is done after treatment of the cells by end-point measurement of changes in the cell's

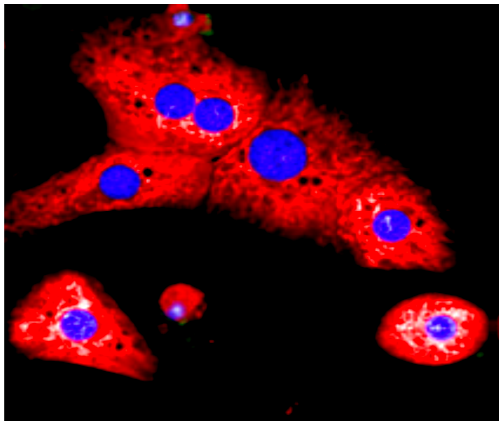
function and morphology. Flow cytometry is, by nature, the high-content technology as it measures a multitude of markers simultaneously. Nevertheless, imaging and image cytometry are standard for high content-screening in many drug companies and in medicine because imaging needs lower amounts of biological material, is well suited for high throughput and yields more information on the cell's morphology as shown in Figure (1-5) [30] .



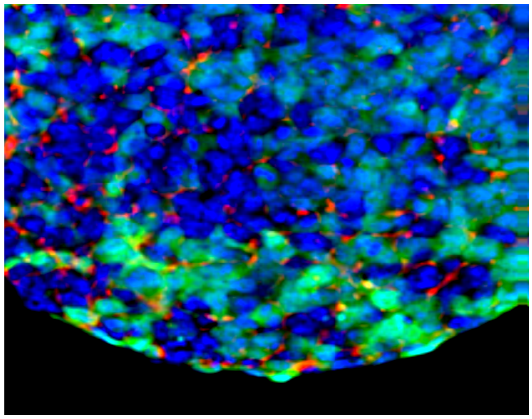
Angiogenesis



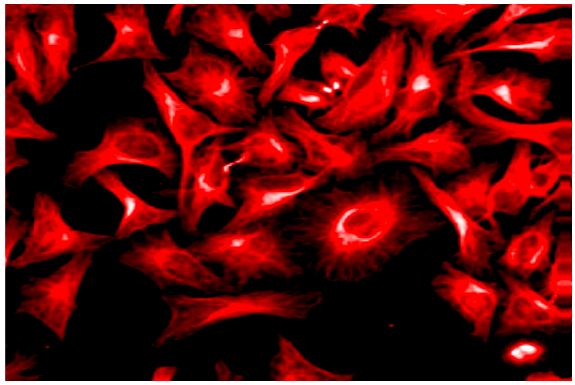
Cell membrane and nuclear stain



Primary hepactocytes



Embryoid body



HeLa cells stained with anti-Tublin

Figure (1-5) some high quality images of morphological parameters in cellular systems.

High Content Screening was developed with the perspective of the history of the development of the automated DNA sequencers that revolutionized the field of genomics. Furthermore, HCS was based on a history of important developments in modern cytology.

HCS integrates the instrumentation, application software, reagents, sample preparation, and bioinformatics required to rapidly flow from producing data, generating information, and ultimately creating new cellular knowledge. The HCS platform is beginning to have an important impact on early drug discovery, basic research in systems cell biology, and is expected to play a role in personalized medicine [31].

High Content Screening (HCS) technology offers a major opportunity to improve the drug discovery and development process ,HCS enables the evaluation of multiple biochemical and morphological parameters in cellular systems and enables characterization of the subcellular distribution of fluorescent signals with labeled reagents. By combining the automated imaging of cells in micro titer plates with validated detection reagents and powerful image analysis algorithms, scientists can now acquire deeper knowledge of multiple morphological or biochemical pathways at the single-cell level, usually in a single assay, at an early stage in the development of new drugs [32].

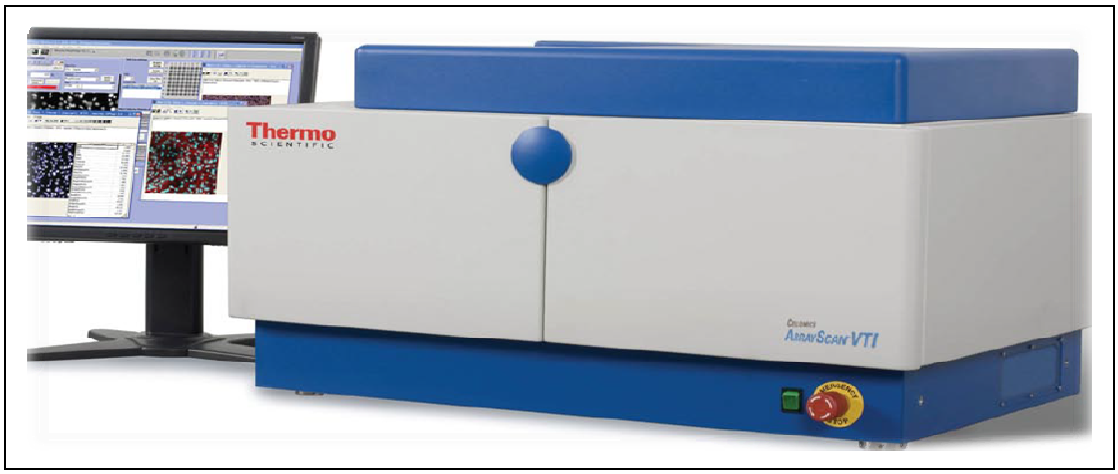


Figure (1-6) Array Scan (Thermo Fisher Scientific).

HCS platforms such as the IN Cell Analyzer (GE Healthcare), Array Scan (Thermo Fisher Scientific)(Figure 1-6), or Opera (Perkin Elmer), can be used to deliver detailed profiles of cellular systemic responses .Successful HCS assays rely on high quality reagents . With the commercial availability of thousands of immune reagents and fluorescent probes, large numbers of fixed-endpoint HCS assays are possible. However, incompatibility of reagents when integrated into a single assay can lead to a significant drop-off in assay performance [33].

1.5. Antioxidants

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases [34].

Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized,

another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being [35] .

1.5.1. Oxidative stress and Reactive Oxygen Species

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

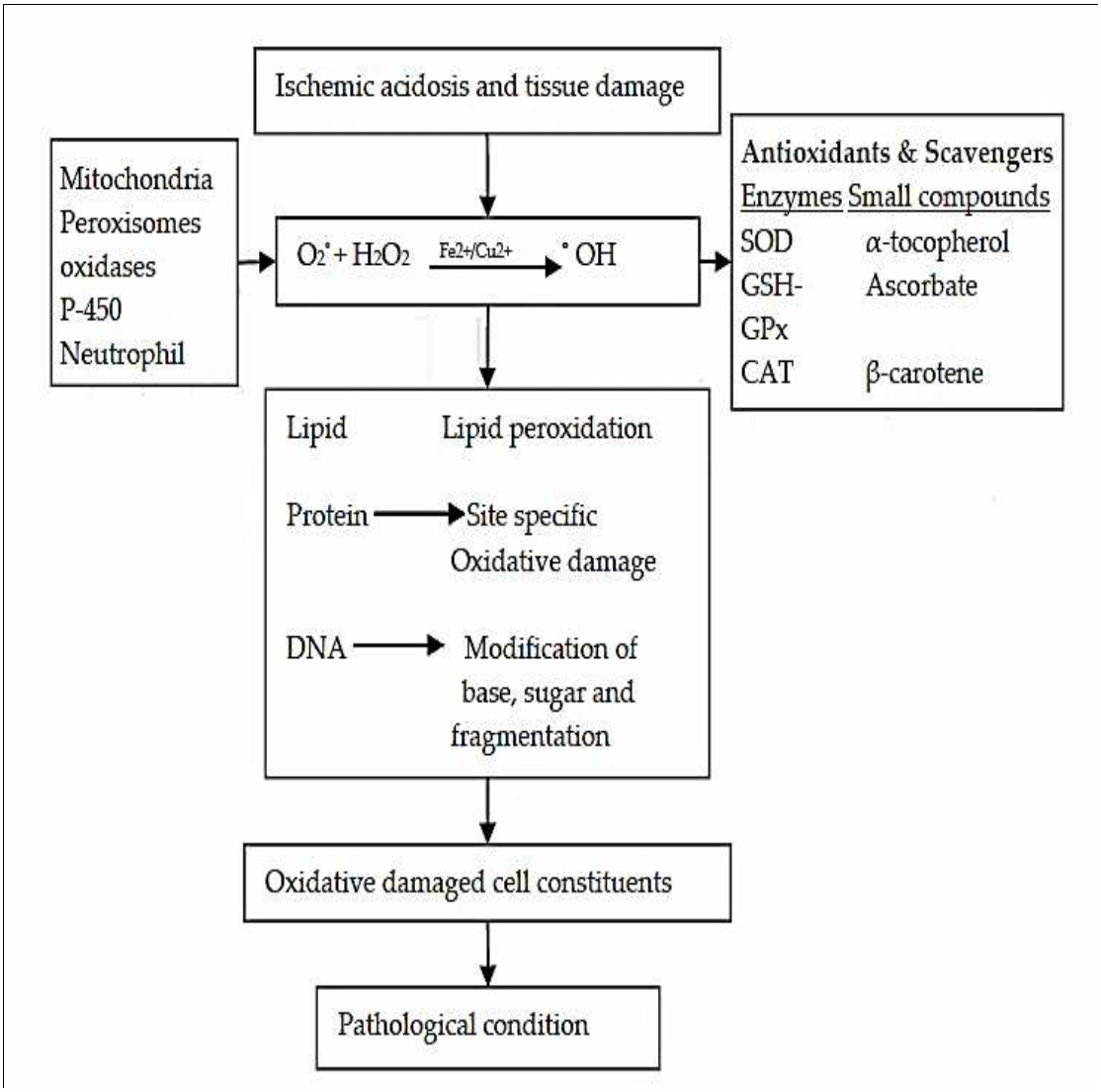
Oxidative process that is regularly going on in cell is essential for life and death of a cell. All aerobic organisms are susceptible to oxidative stress simply because semi-reduced oxygen species, super- oxide and hydrogen peroxide, are produced by mitochondria during respiration . The exact amount of ROS produced is considered to be about 2% of the total oxygen consumed during respiration, but it may vary depending on several parameters [36].

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage [37].

ROS are generated by a number of pathways as shown in scheme (1-3), Most of the oxidants produced by cells occur as:

- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.

- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.
- Xenobiotic metabolism, i.e., detoxification of toxic substances. Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load [38].



Scheme (1-3). An overall picture of the metabolism of ROS and the mechanism of oxidative tissue damage leading to pathological conditions [39] .

1.5.2. Free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay.

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical 2,2- Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors ,and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample [40].

The molecule of 2,2-Diphenyl-1-picrylhydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimers, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm.

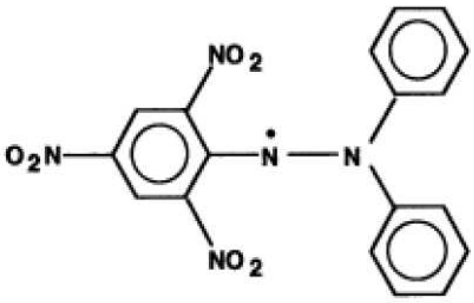
When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (**2**) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by $Z^{\cdot}$ and the donor molecule by AH, the primary reaction is:

$$Z^{\cdot} + AH = ZH + A^{\cdot} \qquad (1)$$

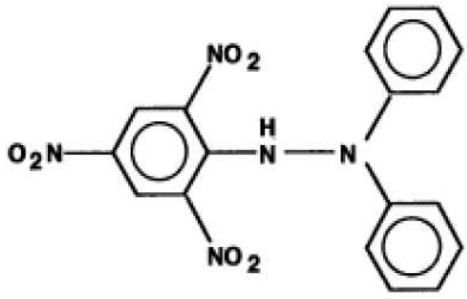
Where ZH is the reduced form and $A^{\cdot}$ is free radical produced in this first step.

This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reactant.

The reaction (1) is therefore intended to provide the link with the reactions taking place in an oxidizing system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule $Z^{\cdot}$ is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH [41].



1: Diphenylpicrylhydrazyl (free radical)

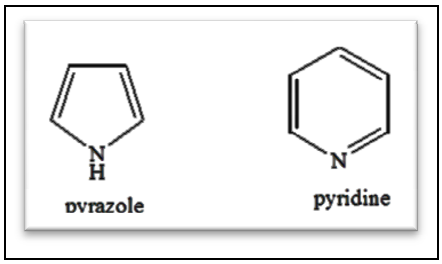


2: Diphenylpicrylhydrazine (nonradical)

1.6. Heterocyclic compounds

A heterocyclic compound is one which contains a ring made up of more than one kind of atom, when the ring of cyclic compound is made up only of carbon atoms such compounds are called homo cyclic compounds **.** If at least one ring atom is a carbon atom, then the molecule is an organic heterocyclic compound. In this case, all the ring atoms which are not carbon are called heteroatoms. Nitrogen, oxygen and sulfur are considered the most hetero atoms known. Many heterocyclic compounds are of great biological importance, and several are of significance in environmental engineering and science [42,43].

In principle, all elements except the alkali metals can act as ring hetero atoms. Along with the type of ring atoms, their total number is important since this determines the ring size. The smallest possible ring is three-membered, The most important rings are the five- and six membered heterocyclic [44] .



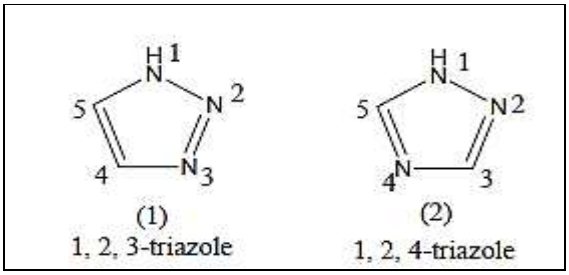
Organic compounds containing five membered heterocyclic ring like; triazole, thiadiazole and thiazolidinone have occupied unique place in the field of medicinal chemistry due to their diverse biological activities such as antifungal [45], antimicrobial [46], antiflammatory [47], cytotoxicity [48], antioxidant [49],antihistaminic [50], antituberculor [51] , anticonvulsant [52].

1.6.1. Triazole

Triazole are five membered heterocyclic compound containing three nitrogen and two carbon atoms. There are two type of triazole, and each has one pyrrole-like nitrogen and two pyridine-like nitrogen.

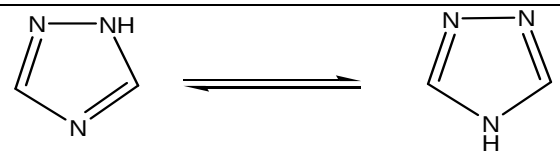
The name triazole was first given to the carbon nitrogen ring system $C_2N_3H_3$ by Bladin who described its derivatives in early 1885, although the structures reported slightly incorrect [53].

Both triazole have the possibility of tautomerism (in 1, 2, 3-triazole the tautomer's are identical) [54].

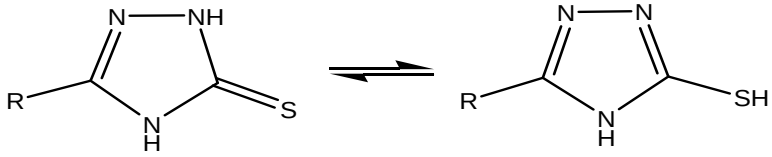


The stability of 1,2,4-triazole nucleus is an inherent property of its aromatic nature. An aromatic sextet is formed by contribution of one π electron from each atom joined by double bonds and the remaining two electrons from a nitrogen atom. Such a system is stabilized by resonance and though the triazole nucleus may be represented by tautomeric forms, The different isomers are characterized by the position of the nascent hydrogen. Thus 1,2,4-triazoles are exist in two forms i.e. 1*H* and 4*H* [55].

In this work 1,2,4triazol ligand has been prepared . 1, 2, 4-Triazoles exhibit two tautomeric forms namely [4*H*]-1,2,4-triazoles and [1*H*]-1,2,4-triazoles .



Among the substituted 1,2,4-triazoles, 3-mercapto-1,2,4-triazoles exist in two tautomeric forms, because the labile hydrogen may be attached either to the nitrogen or the sulfur atom. It exhibits thione-thiol tautomeric forms shown below. This compound exists predominantly in thione form [56].



Out of its two possible isomers of triazole , 1, 2, 4- triazole is (wonder nucleus) which posses almost all types of biological activities. 1, 2, 4-triazole have drawn great attention to medicinal chemists from two decades due to its wide variety of activity, low toxicity and good Pharmacokinetic and Pharmacodynamics profiles [57].

Literature survey reveals that 1, 2, 4-triazole derivatives exhibit wide range of biological activities including Antibacterial [58], Antifungal [59] , Antitumor [60], Anti-inflammatory [61], Anti-tubercular [62], Antidepressant [63], Ant mycobacterial [64], Antimalarial [65] ,Antiviral [66], Antioxidant [67].

Several compounds containing 1,2,4-triazole rings are well known as drugs as shown in figure (1-7) For example, fluconazole is used as an antimicrobial drug [68], while vorozole, letrozole and anastrozole are non-steroidal drugs used for the treatment of cancer [69] and loreclezole is used as an anticonvulsant [70].

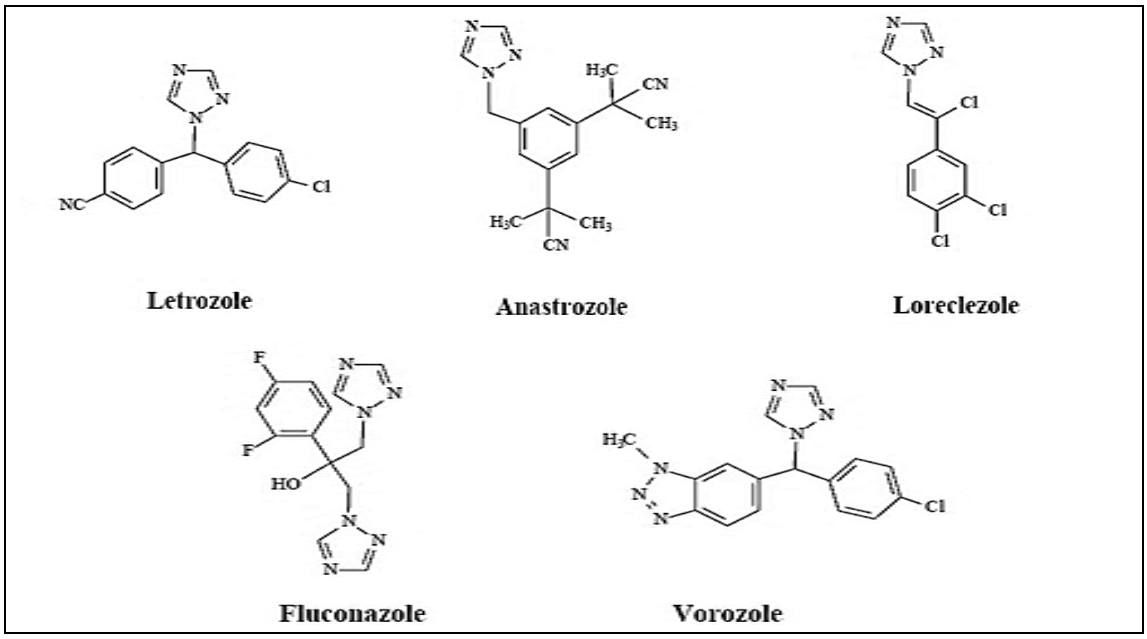
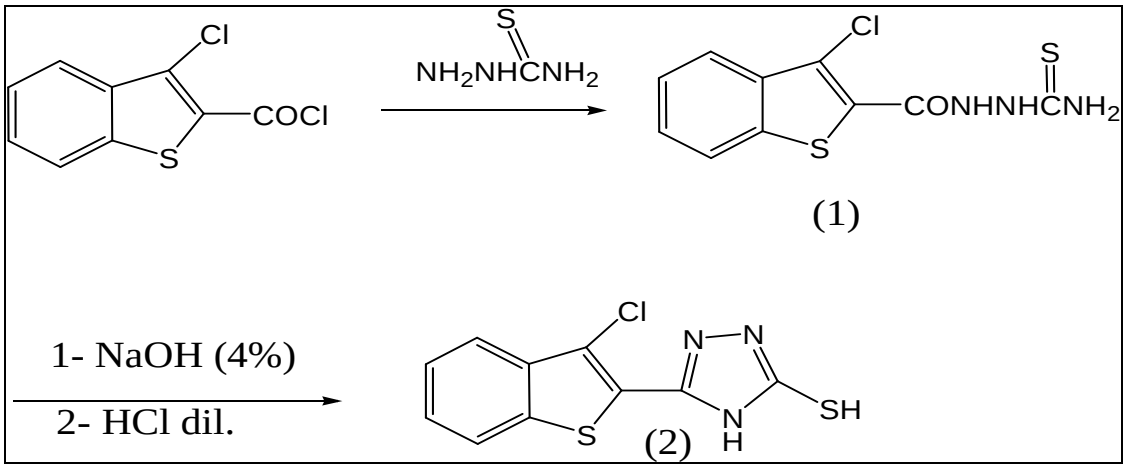


Figure (1-7) drugs containing 1,2,4-triazole rings.

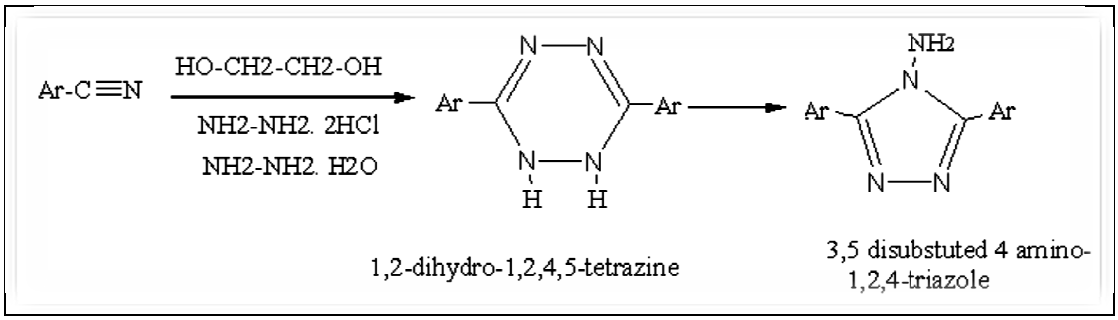
1.6.1.1.Synthetic Aspect

Several methods have been reported for the preparation of 1,2,4-triazole . some procedures for synthesizing 1,2,4-triazole have been described as under.

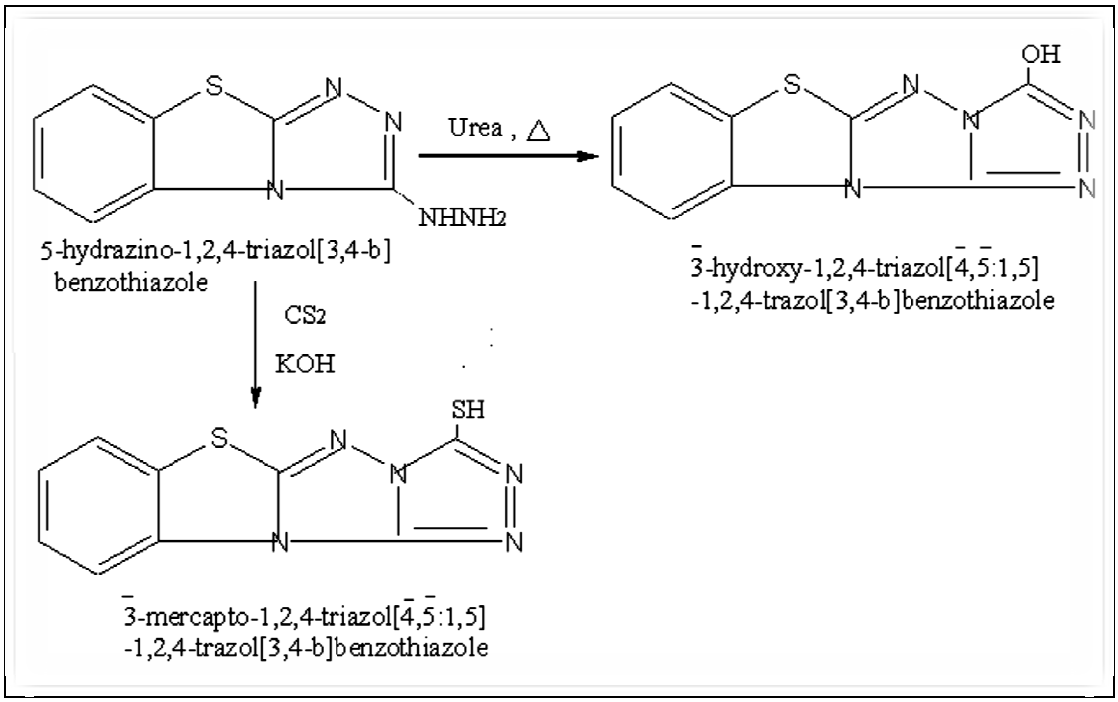
1- **Sharba et al.** [71], prepared 1, 2, 4-triazole derivative (2) by cyclization of compound (1) with dil. NaOH, the Compound (2) was obtained by acidification with dil. HCl.



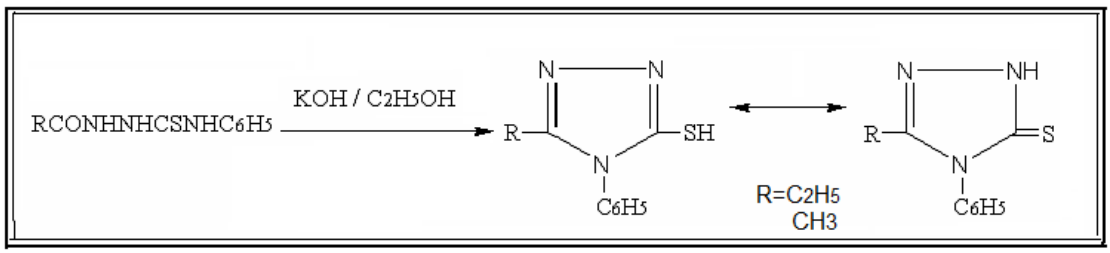
2- **Bentiss *et al*** [72] in 2000 reported the reaction of aromatic nitriles with hydrazine di-hydrochloride in the presence of hydrazine hydrate in ethylene glycol under microwave irradiation, give 3,5- di-substituted 4-amino-1,2,4-triazoles.



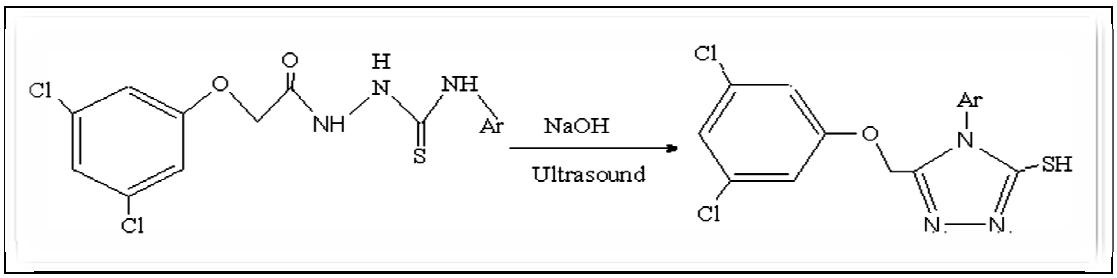
3- **Baheti *et al*** [73] in 2005 synthesized 3-hydroxy and 3-mercapto- 1,2,4-triazolo [4,5:1,5] -1,2,4-triazolo [3,4-b] benzothiazole by heating 5-hydrazino-1,2,4-triazolo [3,4-b] benzothiazole independently with urea and carbon disulphide in the presence of alkali.



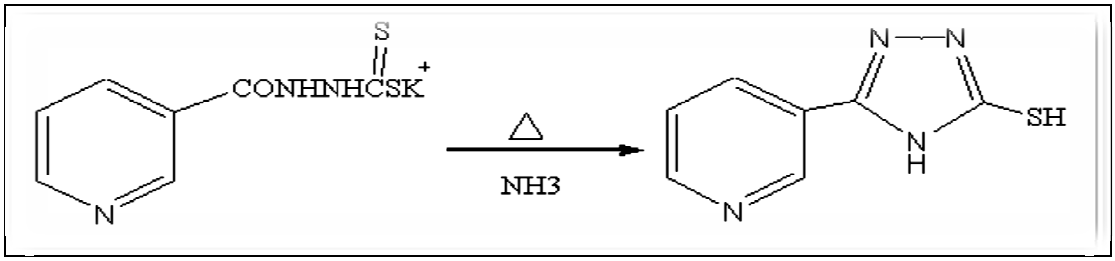
4-Toliwal [74] reported synthesis (5-alkyl-[1,2,4]-triazole- 3-mercapto)-4-Phenyl from Phenyl thiosemicarbazide in the presence of KOH/C_2H_5OH.



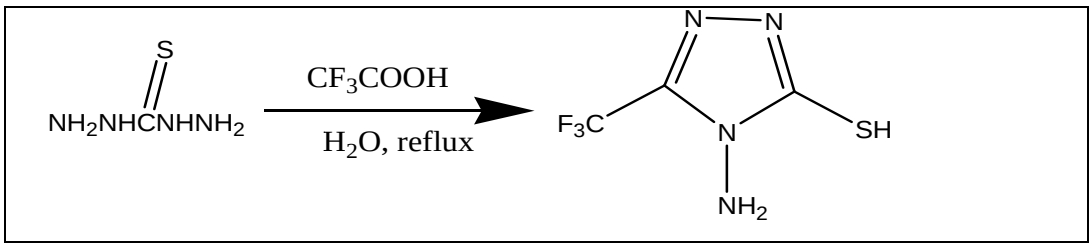
5-Randhavane, et. al [75] reported the synthesis of triazole derivative from aryl thiosemicarbazide and using ultra sound irradiation method.



7-Mali, et. al [76] in 2009 synthesized 3-(3'-pyridyl)-1, 2, 4-triazole-5-thiol in moderate to higher yields by refluxed of potassium 3-pyridyldithiocarbazate salt with ammonia for 4-6 hrs.

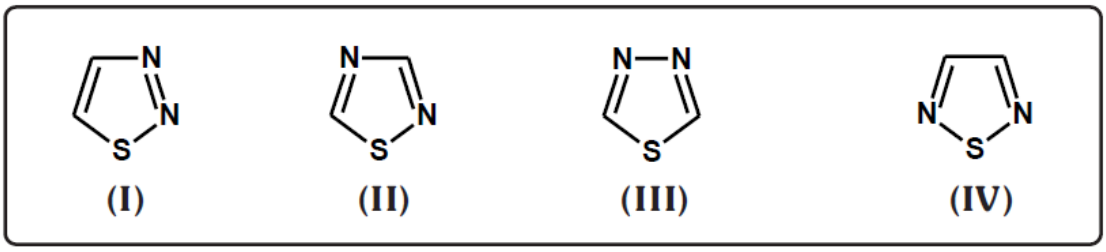


8-Chen M. et al. [77], prepared (4-amino-5-(trifluoromethyl)-4H-1,2,4-triazole-3-thiol) by reaction of trifluoroacetic acid (TFA) with thiocarbohydrazide in refluxing water for 5 hrs.



1.6.2.Thiadiazole

Thiadiazole is five-membered ring composed of two nitrogen atoms and one sulfur atom .According to their position, thiadiazole system are classified as 1,2,3-thiadiazoles (I) ,1,2,4-thiadiazoles (II),1,3,4-thiadiazoles (III)and 1,2,5-thiadiazoles(IV).



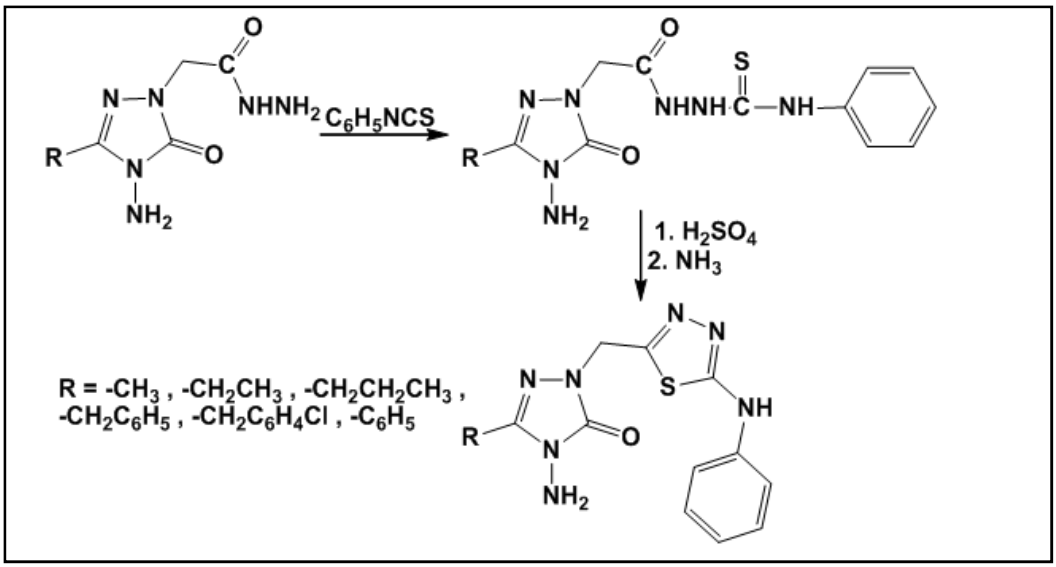
Thiadiazole moiety acts as “hydrogen binding domain” and “two-electron donor system”, It also acts as a constrained pharmacophore [78].

In recent years 1,3,4-thiadiazole derivatives have received significant attention and have been increasingly investigated due to their diverse range of biological properties. They exhibit antimicrobial [79], anti-mycobacterial [80],anticancer [81],anti-inflammatory [82], carbonic anhydrase inhibiting effect [83], antianxiety, antidepressant [84] ,antioxidant properties [85]. These biological activities possibly due to the presence of the –N=C-S moiety that acts as two-electron donor system [86].

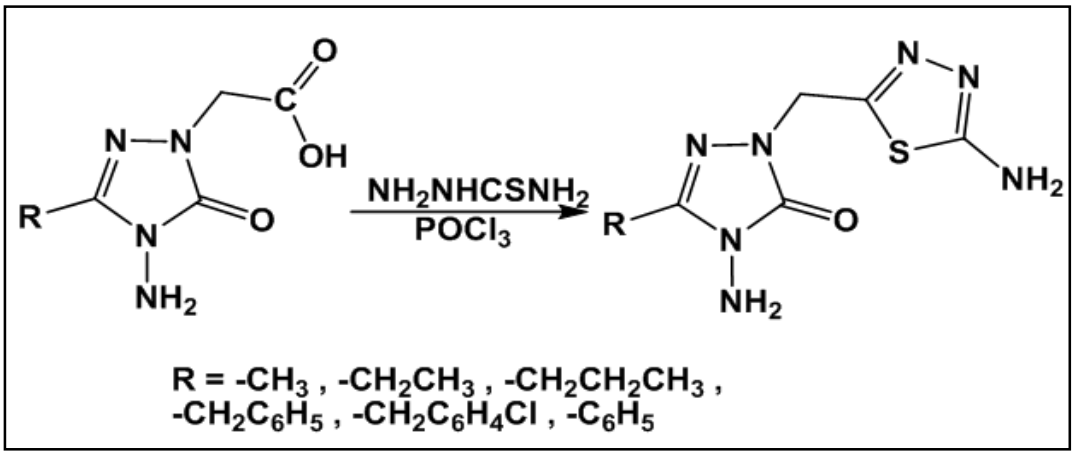
1.6.2.1.Synthetic Aspect

Several methods have been reported for the preparation of 1,3,4-thiadiazoles. some procedures for synthesizing 1,3,4-thiadiazoles have been described as under.

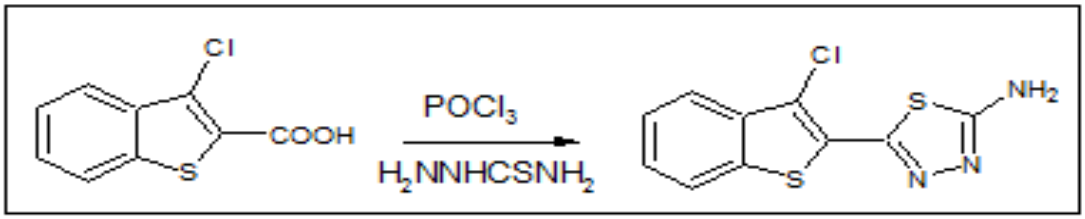
1- **N. Demirbas** [87] synthesized derivatives of 1,3,4-thiadiazole from the reaction of (4-amino-3-substituted-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl) acetic acid hydrazide with phenyl isothiocyanate and the resulting thiosemicarbazide derivatives were cyclized using sulfuric acid.



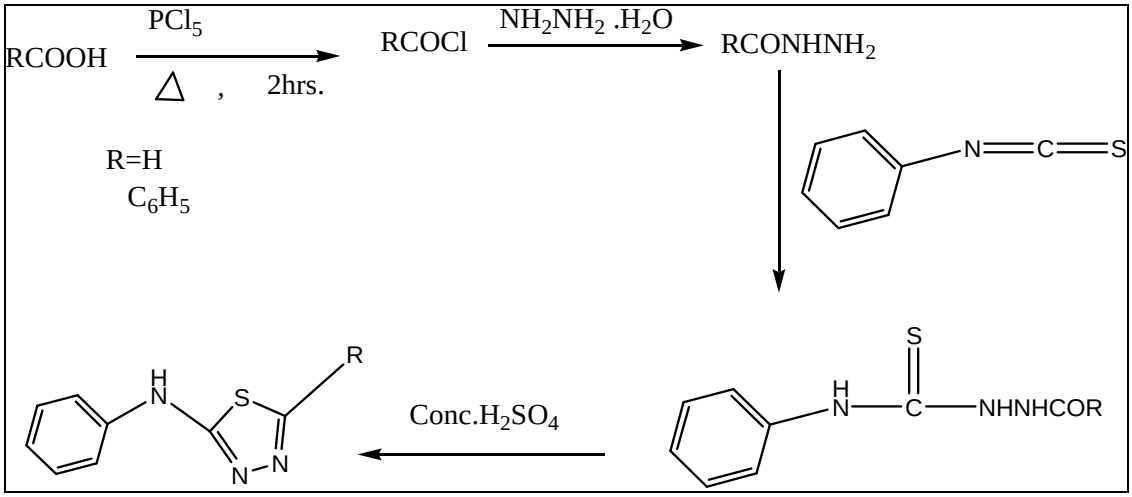
2- **N. Demirbas** [88] also synthesized derivatives of 2-amino-1,3,4-thiadiazole from the reaction of (4-amino-3-substituted-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl) acetic acid with thiosemicarbazide in phosphorus oxychloride to give 1,3,4-thiadiazole ring.



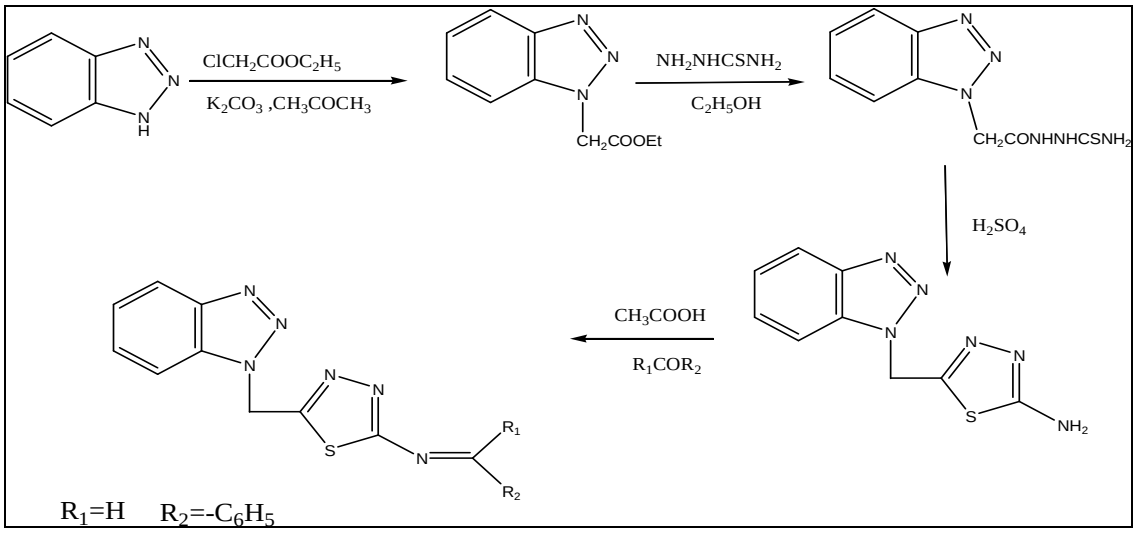
3- **Aly and El-Sayed** [89] have synthesized 2-amino-5-(3-chlorobenzo [b]thiophen-2-yl)-1,3,4-thiadiazole through condensation of 3-chlorobenzo[b]thiophene-2-carboxylic acid with thiosemicarbazide by using phosphorous oxychloride as condensing agent:



4- **Muthukumar A. et al.** [90], prepared 2-[phenyl] amino-5-substituted-1,3,4-thiadiazoles by cyclization of substituted thiosemicarbazides with sulfuric acid .



5- Singh T. et al. [91], Synthesized 1, 3, 4-thiadiazole derivative by cyclization of N-Benztrizole acetyl thiosemicarbazide with H_2SO_4.



1.6.3. Schiff bases (SB)

Hugo Schiff was the first scientist who described Schiff bases in 1864,A Schiff base (or azomethine) is a functional group that contain a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, but not hydrogen. Schiff base are of the general formula $R_1R_2C=N-R_3$, where R_3 is an aryl or alkyl group that makes the Schiff base a stable imine. A Schiff base derived from aniline, where R_3 is a phenyl or substituted phenyl, can be called an anil [92,93].

The preparation of Schiff bases involves a variety of conditions and is brought about by mixing carbonyl compounds and primary amines in various proportions and employing a range of solvents. The formation of Schiff bases is generally favored by making use of dehydrating agents[94,95].

The acid/base catalysis or heating is employed for the synthesis of Schiff bases as their reactions are mostly reversible. The Schiff bases are formed by the reaction of amines with carbonyl compounds but it does not follow simple nucleophilic addition, but give an unstable addition compound called carbinolamine as shown in figure (1-8) . The compound thus obtained is unstable and loses water molecule. The dehydration step during formation of Schiff base is actually the rate determining step. The removal of product or separation of water from the reaction mixture assists the formation of product [96].

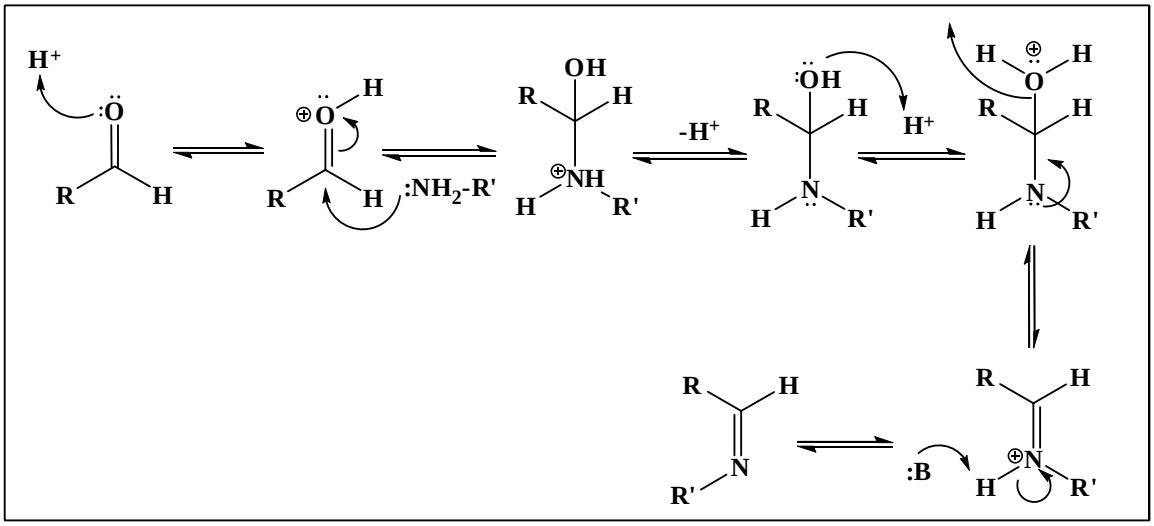
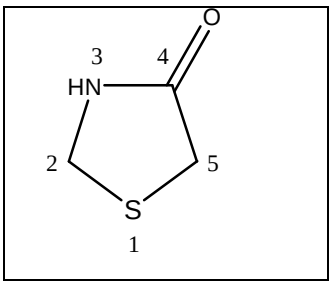


Figure (1-8). Mechanism of Schiff base

Schiff bases gain importance in medicinal and pharmaceutical field due to the most versatile organic synthetic intermediates and also showing a broad range of biological activities such as anti-tuberculosis [97] anticancer [98], analgesic and anti-inflammatory [99], anticonvulsant [100], antibacterial and antifungal activities [101].

1.6.4.Thiazolidinones

Thiazolidinones is an important group of heterocyclic compounds containing sulfur and nitrogen in a five member ring ,4-thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4 position [102]. Substitution is possible at 2, 3 and 5 position. Various optical and geometrical isomers are reported [103]. The carbonyl group of 4-thiazolidinone is highly unreactive. But in few cases 4-thiazolidinone on reaction with Lawesson's reagent gives corresponding 4-thione derivatives [104].



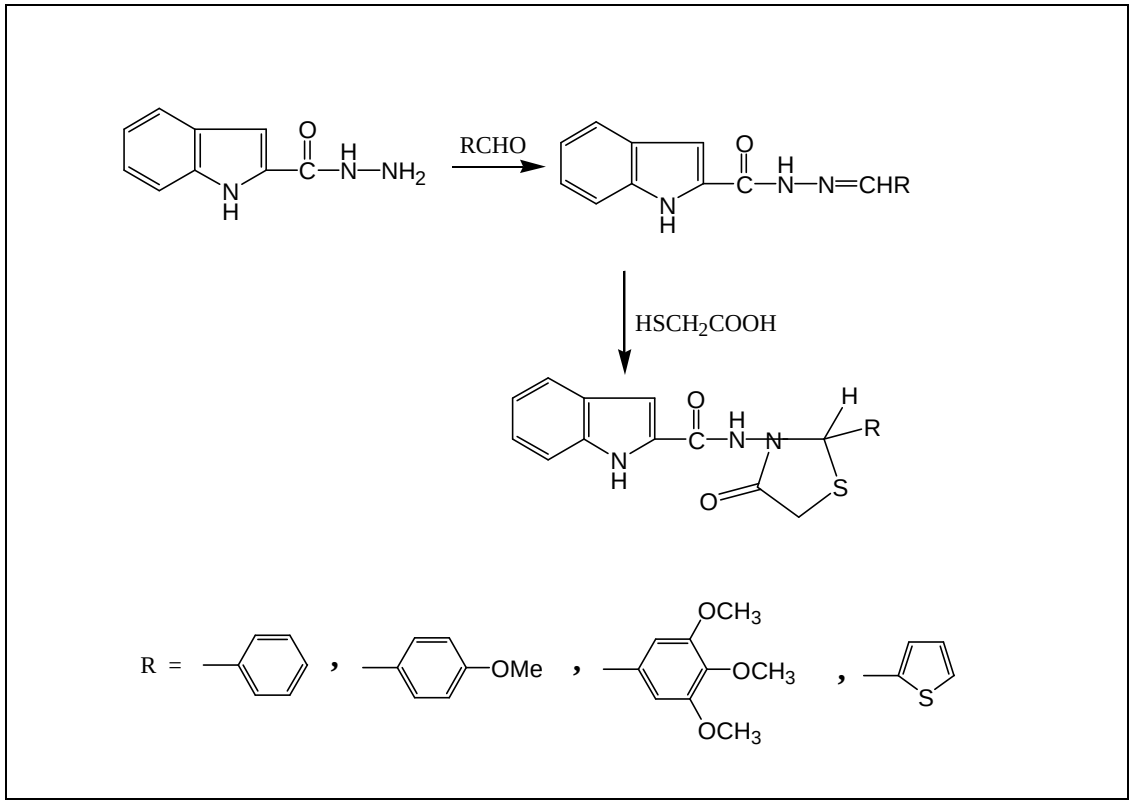
Thiazolidin -4- ones are important compounds due to their broad range of biological activities [105].

The literature survey revealed that 4- thiazolidinone and their derivatives were possessed a wide range of pharmacological activities such as anti-inflammatory , analgesic, anticonvulsant, antimicrobial (antibacterial and antifungal), local and spinal anesthetics, CNS stimulants, hypnotics, anti HIV, hypoglycemic, anticancer, FSH receptor agonist and CFTR inhibitor etc. [106].

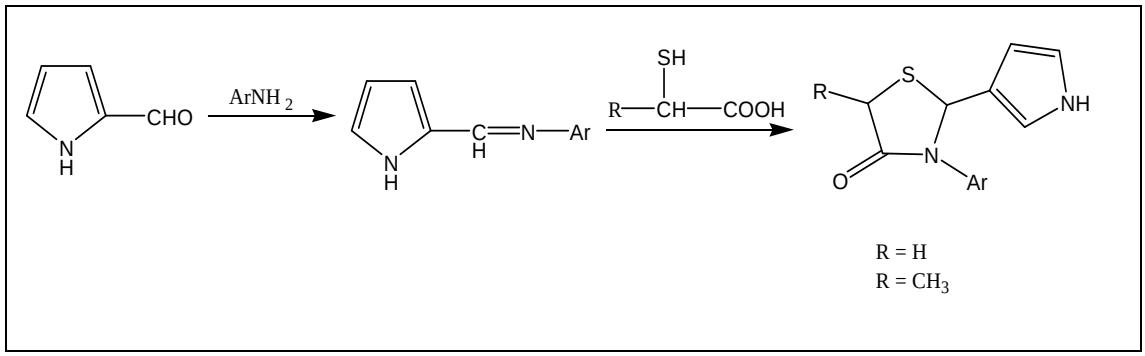
1.6.4.1.Synthetic Aspect

Several methods have been used to synthesize 4-thiazolidinones that explored the possibility of obtaining biologically useful compounds that contain the ring system.

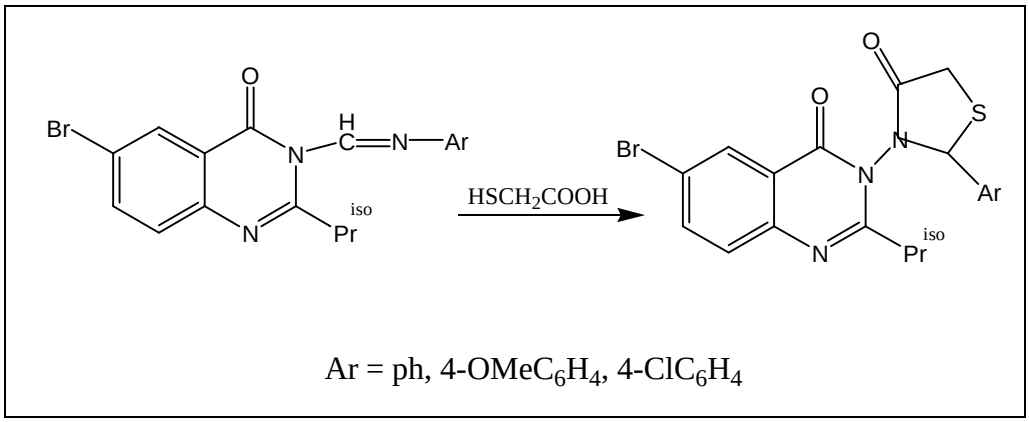
1-Fahmy et.al. [107] synthesized 2-aryl-3-(2-indolylamide)thiazolidin-4-ones from the reaction of 2-indolyl carbohydrazide with different aromatic or heterocyclic aldehydes to form 2-indolyl arylidine hydrazones that cyclocondensed with thioacetic acid to give the compound.



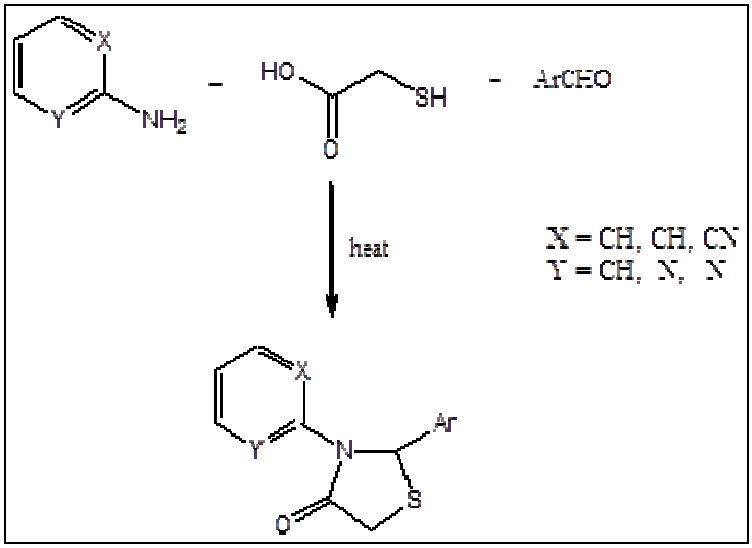
2-Aydogan et.al [108] found that 4-thiazolidinones which are heterocyclic substituted at 2-position were prepared by the reaction of mercapto acids with aldimines which were prepared by the condensation of pyrrol-2-carbaldehyde with different aromatic amines:



3-Madkour [109] synthesized 6-bromo-3-[2-(4-chloro-phenyl)-4-oxo-1,3-thiazole-3-yl]-2-isopropyl-4(3H)-quinazolinone from the reaction of 3-arylidenamino -6-bromo-2-isopropyl-4(3H)-quinazolines with thioglycolic acid in dry benzene:



4-Rao et.al [110] carried out the synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones by reacting an aromatic aldehyde with an equimolar amount of (hetero) aromatic amine in the presence of mercaptoacetic acid:



1.7.Gold Complexes

Metallic gold is quite inert, not reacting with oxygen or sulfur at any temperature; for this reason, it is considered to be the most noble of all the metals. It has the lowest oxidation potential of any metal; therefore, the preparation of positive oxidation state gold complexes requires a relatively strong oxidizing agent (*e.g.* Fe (III)) and a good ligand for gold (e.g. Cl^-). Despite the low reactivity of metallic gold, a large number of gold complexes have been successfully prepared [111].

1.7.1.History of Gold as a Therapeutic Agent

The earliest recorded medicinal use of gold was by the Chinese around 2500 BC. In 1890, Robert Koch reported that potassium aurocyanide, $KAu(CN)_2$, inhibited the growth of *tubercle bacillus*, the organism responsible for tuberculosis, which at that time was also known as the 'white plague'. This gave a biological basis for gold treatment and stimulated many studies.

Potassium aurocyanide was too toxic for clinical use, which is not surprising for a compound of cyanide, but over the next thirty years, several gold(I) thiolates were introduced for the treatment of tuberculosis; the period from 1925-1935 has been called the "gold decade" in tuberculosis treatment. In the late 1920s, Dr.K.Landé and Dr.J.Forestier, because of a belief that rheumatoid arthritis is a chronic infectious disease like tuberculosis, independently introduced gold(I) complexes for the treatment of arthritis [111] .

Recently new gold containing drugs have been prepared and tested as antineoplastic agents. A series of square planer gold (III) complexes have been synthesized by Calamai *et. al.*, all containing at least two gold chloride bonds in cis position and tested their activity *in vitro* cytotoxicity on a panel of established human tumor cell line [112].

1.7.2.Gold (III)

This is a very common and relatively stable oxidation state for gold. Gold (III) complexes are diamagnetic and most have 4- coordinate square – planar stereo chemistry with a low spin $5d^8$ electron configurations. The most common example of a gold (III) complex, $[AuCl_4]^-$, is easily prepared by dissolving gold metal in aqua regia, and is the precursor of most other gold complexes.

Five coordinate gold (III) complexes are rare but when they are found, they have a square pyramidal or a distorted square pyramidal geometry. The neutral AuF_3 is rare examples of gold (III) compound in which the gold atoms are 6- coordinate in its crystal structure . There are two criteria which can be followed to stabilized Au (III) complexes these are (i) the ligand should have more than one donor atom (multi-dentate) and (ii) contain two nitrogen atoms. These two criteria were previously reported to enhance the stability of Au (III) complexes under physiological conditions [113].

1.7.2.1. Overview of Gold Drugs

The chemical formulae of a number of gold thiol complexes, which are presently being used or have been used for medicinal purposes, are shown in Figure (1-9). Each of these are gold (I) thiol complexes, with the exception of aurol sulfide, for which the commonly represented stoichiometry indicates (a+III) oxidation state for gold, though, it is not well characterized [111].

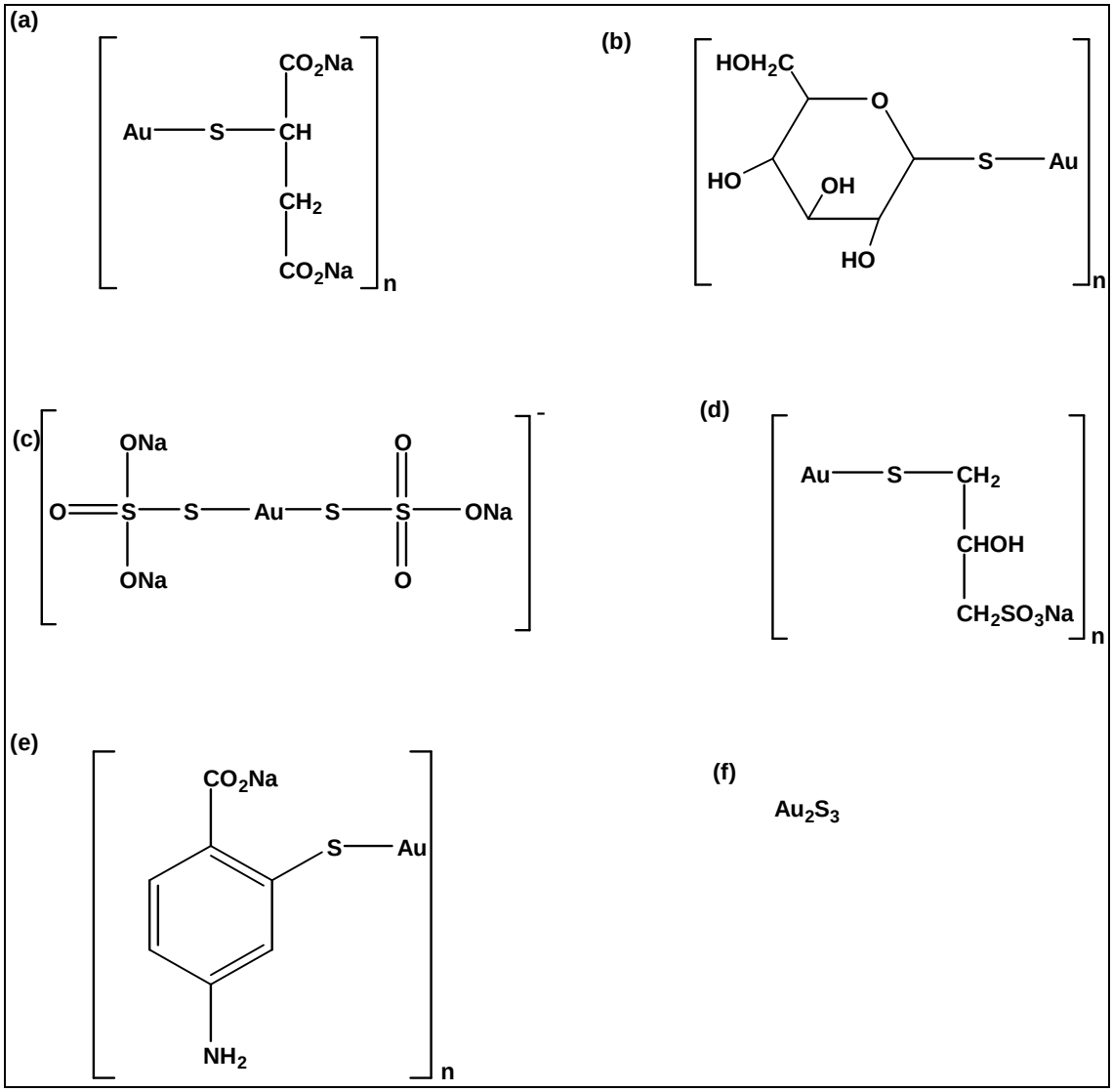


Figure (1-9):Some representative gold (I) thiol complexes of medicinal interest: (a) gold sodium thiomalate (GST;Myochrysine; Marketed in Britain, the united states (USA) and Canada) ; (b) gold B-D thioglucose (Solganal;marketed in the USA) ; (C) gold sodium thiosulfate (Sanochrysine; marketed in Europe) ; (d) gold Sodium 3- thio –2- propanol –1-Sulfonate (Allochrysine; Marketed in Europe) ; (e) gold Sodium 4- amino –2- Mercapto benzoate (Krysolgan; not currently used in medicine) ; (F) gold sulfide (Aurol Sulfide; not currently used in medicine).

Messori *et.al* .[114] and **Buckley *et.al*.** [115] used en,dien and damp as multidentate nitrogen-containing ligands to prepare gold (III) complex, which were found to be active against human cancer cell lines. Also a recent *in vitro* cytotoxicity study demonstrated promising activity of two gold(III) complexes with bipyridyl ligands, (dihydroxy(2,2'-bipyridyl)gold(III)ion)[Au(bipy)-$(OH)_2$]PF_6 and [Au(bipy-H)(OH)]PF_6 Figure (1-10) and (1-11).

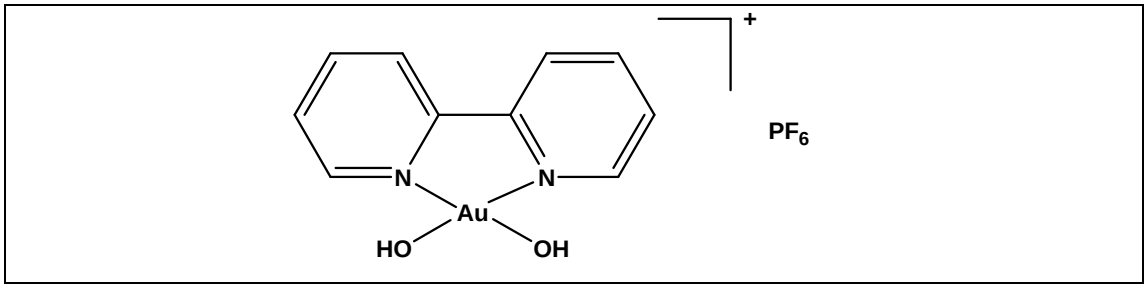


Figure (1-10): The structure of (dihydroxy(2,2'-bipyridyl) gold (III) ion) [Au(bipy) $(OH)_2$]PF_6.

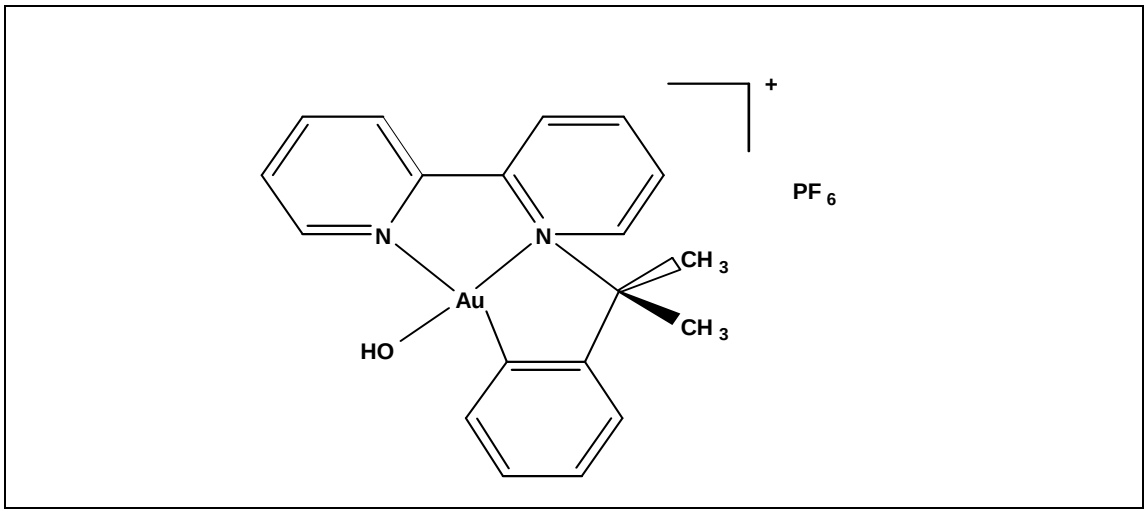


Figure (1-11): The structure of [Au(bipy-H)(OH)]PF_6.

1.7.2.2. Mechanism of Gold Complexes.

The mechanism of action of anti-proliferative gold complexes had been under question for a long time, However reports that auranofin and other gold(I) complexes potently inhibited the enzyme TrxR (Thioredoxin Reductase) have most probably answered the question on the biological main target of gold complexes, Auranofin inhibited TrxR with high potency and approximately 1000fold selectivity compared to other related enzymes (glutathione reductase and glutathione peroxidase). Based on the different ligand structures of the many gold complexes shows in (Figure 1-12), for which cell growth inhibiting properties have been observed, a unique mode of action of the agents is not likely to exist but an increasing number of reports on gold complexes with significant TrxR inhibitory properties underlines the relevance of this enzyme in the pharmacology of gold metallodrugs. In this context it is of interest to note that the inhibition of TrxR has been reported not only for different gold(I) complexes but also for various gold(III) compounds [116].

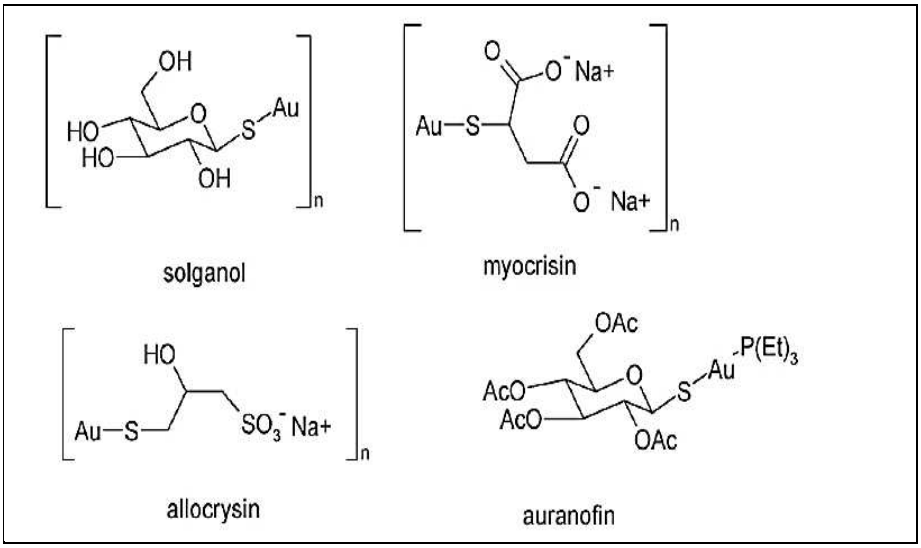


Figure (1-12) gold complexes used for chrysotherapy.

TrxR is a homodimeric protein belonging to the family of glutathione reductase like enzymes. It catalyzes the NADPH dependent reduction of (Trx) disulfide and many other oxidized cell constituents. The active site of TrxR contains a selenocysteine (Sec) containing (Gly-Cys-Sec-Gly) motif involved in the catalytic mode of action of the enzyme. During enzyme catalysis reducing equivalents are transferred from the substrate NADPH to thioredoxin by means of the FAD prosthetic group .

Based on the high affinity of the electrophilic gold center of gold(I) complexes to the nucleophilic sulfur and selenium containing residues a covalent interaction seemed likely as amode of drug action. The preferred binding of auranofin to the selenocysteine residue was suggested based on the fact that the agent inhibited glutathione reductase, an enzyme which is structurally and functionally closely related to TrxR but lacks the Sec residue in the active site, with significantly lower affinity. The inhibition of the mitochondrial form of the above mentioned enzyme TrxR by auranofin is in good agreement with early reports on anti-mitochondrial effects of gold compounds in several *in vitro* studies **[117],[118]**.

TEPAuCl ((triethylphosphine)gold(I)chloride), an analogue of auranofin containing the triethylphosphine fragment of auranofin but a chlorine ligand instead of the thiocarbohydrate moiety, caused anti-mitochondrial effects such as mitochondrial swelling or increased permeability of the inner membrane in isolated rat liver mitochondria, Auranofin itself induced the mitochondrial membrane permeability transition observed as swelling and loss of membrane potential. Both events could be completely reversed by cyclosporin A, a specific inhibitor of mitochondrial permeability transition. Cyclooxygenases (COX) and lipoxygenases (LOX) represent another class of enzymes important for the treatment of inflammation and cancer.

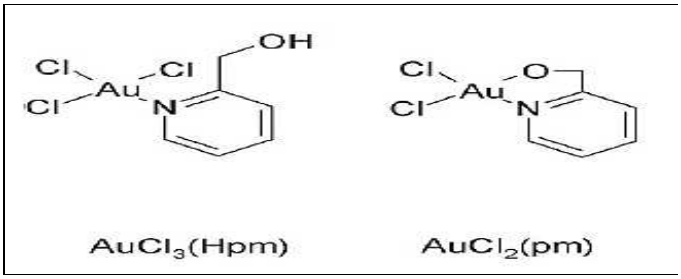
Based on molecular modeling and mutation experiments for aurothiomalate the selective targeting of Cys69 in the PB1 domain of protein kinase C iota was suggested as a possible mode of action in human lung cancer cells. For gold(III) complexes the partial reversible inhibition of ribonuclease A (RNase A) and deoxyribonuclease I (DNase I) has been reported . As an overall result these studies suggest that besides inhibition of TrxR, which may represent the most relevant target for most gold complexes, the interaction with various biomolecules seems to be important for the pharmacology of anti-proliferative gold complexes [119].

1.7.2.3.Gold(III) complexes types

Based on their structural and electronic similarity to cisplatin and cisplatin related antitumor drugs gold(III) species represent a promising class of potential anticancer agents. However, the development of gold(III) complexes as therapeutic drugs has been hampered by their low stability under physiological conditions and remains a critical parameter in the drug development of these species. Gold(III) complexes with various ligands have been prepared and biologically investigated. Most of them are complexes with Au–N bonds (eventually containing additional Au–O and Au–Cl bonds) but also some species with Au–S or Au–C bonds and their bioactivities have been described [120].

- *Gold(III) complexes with Au–N bonds* :Two gold chloride species with pyridine ligands (AuCl3(Hpm) and AuCl2(pm) (Figure 1-13)) showed good cytotoxic activity in Tlymphoblastoid and human ovarian cell lines, which was however comparable to that of NaAuCl4. Binding to the DNA was confirmed for both complexes.

Both AuCl3 (Hpm) and AuCl2(pm)were relatively stable in organic solvents but underwent hydrolysis of the chloride ligand in aqueous buffer media, a fact which might limit their practical application [121].



(Figure 1-13) AuCl3(Hpm) and AuCl2(pm) structures.

- *Gold(III) complexes with Au–S bonds* :Gold(III) dithiocarbamate complexes (see Figure 1-14 for some relevant examples) exhibited superior cytotoxic effects if compared to cisplatin, were also active in resistant cells and induced apoptosis. The compounds showed good stability under physiological conditions, bound readily to the DNA, inhibited both DNA and RNA synthesis and induced fast DNA lesions. Experiments on red blood cells indicated that hemolytic properties might contribute significantly to the bioactivity of the agents .The complexes triggered cancer cell death via apoptotic and non-apoptotic pathways, affected mitochondrial functions, generated free radicals ,increased ERK1/2 phosphorylation and inhibited TrxR [122].

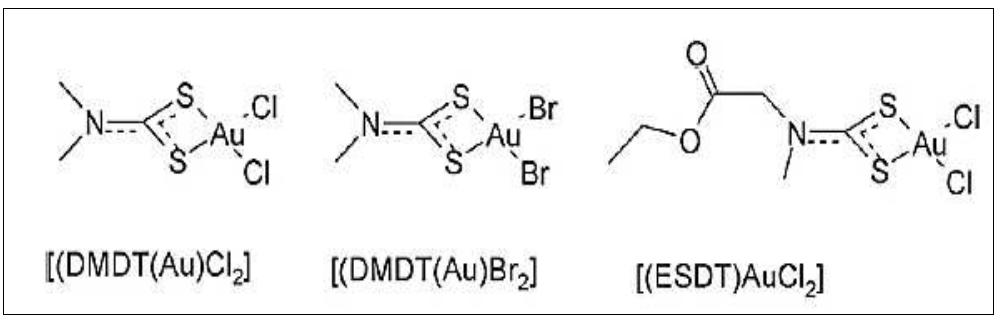


Figure (1-14) some example of Gold(III) dithiocarbamate complexes.

- *Gold(III) complexes containing Au–C bonds*:The TrxR inhibiting and antiproliferative properties of a series of gold(III) complexes including many species with gold–carbon bonds was studied. The complex depicted in (Figure 1-15) top left showed the best cytotoxicity results and inhibited TrxR activity.

  However,the extent of TrxR inhibition did not correlate with the anti-proliferative properties Gold(III) complexes with 2-[(dimethylamino)methyl]phenyl ligands were active in vitro and in in vivo .More detailed investigations on [Au(acetato)2(damp)] showed that the compound did not cause DNA interstrand crosslinks and induced only minor cell cycle alterations. Solution studies on the gold–carbon complexes AuTol, AuXyl and AuPyAcO revealed that the complexes underwent hydrolysis of the labile ligands while the gold carbon bond and the oxidation state remained intact. In cytotoxicity experiments IC_{50} values in the low micro molar range were determined. The agents also induced anti-apoptotic effects. Interestingly, this was accompanied by only modest perturbations of the cell cycle [123] .

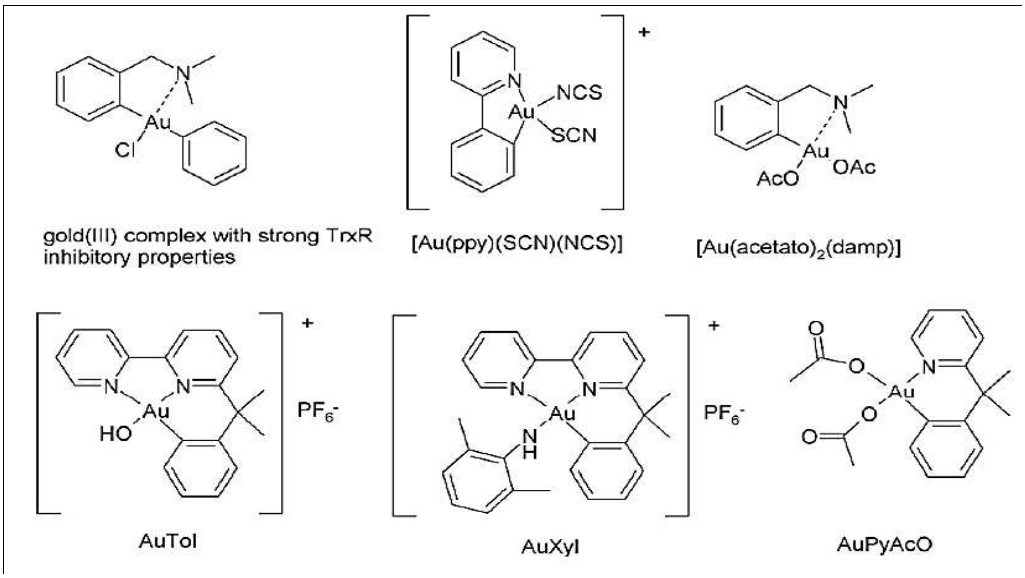


Figure (1-15) Gold(III)complexes with Au-C bond.

1.8.Aim of the work

- synthesis and characterize some 1,2,4-triazole and 1,3,4-thiadiazole derivatives .
- synthesis and characterize new gold (III) complexes.
- Evaluation of the synthesized compounds for their antioxidant activity using DPPH assay .
- Screening of the synthesized compounds for their potential cytotoxicity against breast cancer (MCF7) cell lines in vitro using Cell viability assay (MTT) and high content screening technique (HCS).