Abstract

This research is divided into two parts*:*

Part one:

This part involved in the synthesis of some pyrimidine derivatives which play an important role in the medicinal chemistry because they possess promising cytotoxic activity.

The synthesized compounds:

1. 5,6-diaminouracil-2,4-diol hydrochloride.
2. 5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride.
3. 2-mercapto-6-methylpyrimidin-4(3H)-one.
4. 2-hydrazinyl-6-methylpyrimidin-4(3H)-one
5. 2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidin-4(3H)-one.

Pyrimidine derivatives were identified by using spectroscopy (FTIR and UV-Visible) and by measuring their melting points.

Part two:

This part involved the affectivence of the synthesized pyrimidine derivatives on different cell lines in vitro, using two cell lines (HepG2 and MCF7), and by using Neutral red and MTT assays respectively, different concentrations of pyrimidine derivatives were prepared. The results showed an inhibitory effect on the growth of the two cell lines, 600µg/ml concentration showed the best concentration for the best inhibition (IR) and gave the lowest percentage growth (Cell Viable) on two cell lines, also regression showed a significant negative relationship between pyrimidine derivatives and the cell lines growth, high concentration showed significant effect on cell lines growth.

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List of Abbreviations

Abbreviation	Full name
Abs	Absorbance
ATP	Adenosine triphosphate
C^o	Celsius
CD28	Cluster of differentiation 28
Conc	Concentration
Cm^{-1}	1/centimeter
DNA	Deoxyribonucleic acid
DMSO	dimethylsulfoxide
dTMP	deoxythymidine 5'-monophosphate phosphate
D.W	Distilled water
ER	Estrogen receptor
ELISA	Enzyme-linked immonosorbent assay
F.T.IR	Fourier transforms infrared spectrophotometer
FCS	Fetal calf serum
g	Gram
HepG2	Hepatocellular carcinoma
hrs	Hours
I.R	Inhibition rate
IU	International unit
$IC_{50\%}$	half maximal inhibitory concentration
M	Molar
MCF7	Michigan cancer foundation - 7
MEM	Minimum essential media
µg	Microgram
µl	Microliter
ml	mililiter
m.p.	Melting point
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NRU	Neutral red uptake
nm	nanometer
NADH	nicotinamide adenine dinucleotide hydrogen

O.D.	**Optical density**
P	**Probability**
PC	**Picometer**
PBS	**Phosphate buffered saline**
PH	**logarithm of the reciprocal of the hydrogen ion activity**
R^2	**coefficient of determination**
RNA	**Ribonucleic acid**
SE	**Standard error**
SDS	**Sodium Dodecyl sulfate**
TLC	**Thin Layer Chromatography**
tRNA	**Transfer RNA**
UV-VIS spectrophotometer	**Ultra violate-Visible spectrophotometer**

الخلاصـــة

يقسم هذا البحث الى جزئين:

الجزء الأول:

تم تحضير البعض من مشتقات البرميدينات التي تلعب دورا هاماً في الكيمياء الطبية لأنها تمتلك النشاط السام أتجاه الخلايا السرطانية.

وفيمايلي المركبات المُحضرة:

1. 5,6-diaminouracil-2,4-diol hydrochloride.
2. 5,6-diamino-2-mercaptopyrimidine-4-ol Hydrochloride.
3. 2-mercapto-6-methylpyrimidin-4(3H)-one.
4. 2-hydrazinyl-6-methylpyrimidin-4(3H)-one.
5. 2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidin-4(3H)-one.

وتم تشخيص المركبات المحضرة بواسطة الطرق الطيفية (طيف الأشعة تحت الحمراء F.T.IR وطيف الاشعة فوق البنفسجية Ultra violet) وتم قياس درجة الأنصهار لهذه المركبات المحضرة.

الجزء الثانـي:

خضعت المركبات المحضرة (مشتقات البريميدنات) الى نوعين من الخلايا السرطانية البشرية (سرطان الكبد (HepG2) و سرطان الثدي (MCF7)) خارج الجسم الحي وذلك بأستخدام فحوصات مختلفة منها (متعادل الصبغة الحمراء (neutral red) و (MTT)) وأظهرت النتائج أن هذه المركبات ذات فعالية سُمية أتجاه خلايا الخطين السرطانيين وكانت هذه الفعالية بتأثير متباين بين انواع الخلايا المختلفة وبين التراكيز المختلفة،وأن التركيز ٦٠٠ غم /مل يعتبر التركيز الافضل لمعدل التثبيط والذي يعطي أقل نسبة نمو لكلا الخطين السرطانيين ،ويؤكد هذه النتيجة العلاقة المعنوية السالبة للانحدار، وزيادة تركيز المادة المحضرة يكون اكثر تأثيراً في نمو الخط السرطاني .

Committee Certification

We, the examining committee, certify that we have read this thesis entitled **"Synthesis and Cytotoxic Activity of Some Pyrimidine Derivatives"** and examined the student **"Tamara Sami Naji"** in its contents and that in our opinion; it is accepted for the Degree of Master of Science in/ chemistry.

Signature:
Name: Dr.Perry H. Saif-Allah
Scientific Degree:
Date:
(Member)

Signature:
Name: Dr.Shatha A. Abd-Alrahman
Scientific Degree:
Date:
(Member)

Signature:
Name: Dr.Firas A. Hassan
Scientific Degree:
Date:
(Member)

Signature:
Name: Dr.Salman A. Ahmed
Scientific Degree:
Date:
(Supervisor)

I, hereby certify upon the decision of the examining committee.

Signature:
Name: Dr. Khulood Waheeb AL-Samarrae
Scientific Degree: Professor.
Title: Dean of the College of Science
Date:

Supervisor Certification

I certify that this thesis entitled "Synthesis and Cytotoxic Activity of Some Pyrimidine Derivatives" was prepared by "**Tamara Sami Naji**" under our supervision at the College of Science/Al-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Chemistry.

Signature:

Name: **Dr. Salman Ali Ahmed**

Scientific Degree: Assist Prof.

Date:

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

Signature:

Dr. Hadi M. A. Abood

Title: Head of Chemistry Department

Date:

Al-Nahrain University

Committee Certification

We, the examining committee, certify that we have read this thesis entitled **"Synthesis and Cytotoxic Activity of Some Pyrimidine Derivatives"** and examined the student **"Tamara Sami Naji"** in its contents and that in our opinion; it is accepted for the Degree of Master of Science in/ chemistry.

Signature:
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Scientific Degree:
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Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
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(Supervisor)

I, hereby certify upon the decision of the examining committee.

Signature:
Name: Dr. Khulood Waheeb AL-Samarrae
Scientific Degree: Professor.
Title: Dean of the College of Science
Date:

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

Synthesis and Cytotoxic Activity of Some Pyrimidine Derivatives

A Thesis Submitted to the College of Science Al-Nahrain University in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry

By

Tamara Sami Naji

B.Sc. Chemistry–Al-Nahrain University-2010

Supervised by

Salman Ali Ahmed

2013 **1434**

بِسْمِ اللهِ الرحْمن الرحِيم

﴿قالوا سُبحانكَ لا عِلمَ لنا الا ما علمتنا انك أنت العليمُ الحكيمُ﴾

صَدَقَ اللهُ العَظِيم

سورة البقرة / الآية (٣٢)

Acknowledgement

I wis[illegible]untable gifts and for helping me to present this thesis. The honor is mine to express my sincere thanks and gratitude to my supervisor ***Dr.Salman Ali Ahmed*** *for his guidance and sustain efforts throughout this work.*

A special thanks to the head and staff of the chemistry department, College of Science, Al-Nahrain University for their assistance to me in stage of study courses.

The preparation of the organic compound would have been much more difficult without the help of ***Dr.Nisreen Raheem, Dr.Ahmed Abd Alrazaq and Dr. Mahdi Salh*** *my great thanks to them.*

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Finally, I'm deeply indebted to my family for their support and patience during the years of my study.

✍ *TaMaRa SaMi*

الإهداء

إلى من ضحى لأجل أن أكون

إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم

الى من غرس في نفسي الطموح...وساندني في مسيرتي العلمية

وكان لي أباً و صديق اً... إلى القلب الكبير((والـدي العزيز))

الى التي حملتني وهناً على وهن

إلى من أرضعتني الحنان والأيمان وهي في قلبي أروع ما خلق الرحمن

إلى القلب الناصع بالبياض ((والدتي العزيزة))

إلى من اشد به أزري في هذه الحياة..إلى مقلة عيني ((أخـي العزيز))

إلى كل من بذل جهداً لمساعدتي ... عرفاناً بالجميل..((أساتذتي الاعزاء))

إلى رفاق الدرب الطويل ... إلى بواعث بهجتي وزينة حياتي((صديقاتي العزيزات))

إلى كل من ساعدني في إخراج هذا البحث المتواضع ... زملائي

اهدي ما وفقني إليه ربي ثمرة جهدي

تهاوة سامي

٢٠١٣

الشكـر والتقديـــر

الشكر والحمد لله عز وجل

بسم الله والصلاة والسلام على محمد رسول الله وعلى اله وصحبه أجمعين. يطيب لي وأنا على اعتاب نهاية هذا البحث أن أقدم شكري وتقديري الخالصين الى أستاذي الدكتور سلمان علي احمد، لأقتراحه موضوع البحث واشرافه المباشر عليه، ولما قدمه لي من النصائح والارشادات التي كان لها الدور الكبير في اعداد هذه الرسالة لتكون على مستوى الطموح والجهود الثمينة التي انارت الطريق لهذا البحث.

كما أتقدم بالشكر الجزيل الى رئيس وكادر قسم الكيمياء- كلية العلوم- جامعة النهرين لمساعدتهم لي في اكمال البحث وأخص منهم د.نسرين رحـــيم،د.احمدعبـــد الـــرزاق،د.مهـــدي صـــالح،ســـت رشاسعد،د.ضياءالربيعي.واخص بالذكر د.فاروق أبراهيم ،د.أحمد فاضل و د.بتول عمران من مركز التقنيات الاحيائية ومع جزيل الشكر والامتنان لجميع أساتذتي الافاضل..

وأتقدم بشكري واحترامي وحبي الكبير لعائلتي الكريمة لما قدموه لي من دعم وتشجيع ورعاية لأكمال الدراسة فجزاهم الله عني خير الجزاء.

تمارة سامي

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

تحضير عدد من مشتقات البريميدنات ودراسة فعاليتها السمية

رسالة

مقدمة إلى كلية العلوم- جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
تـمارة سامي ناجي
بكالوريوس ٢٠١٠ ((علوم كيميـاء / جامعة النهرين))

أشـــراف

د.سلمـــان علي أحمد

١٤٣٤هـ ٢٠١٣

Synthesis, Characterization and Cytotoxic Activity of Some Pyrimidine Derivatives

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Abstract:

Synthesis of some pyrimidine derivatives which plays an important role in the medicinal chemistry because it possesses promising cytotoxic activity. The synthesized compounds were characterized by UV-Visible and FT-IR spectral data. Some of the new compounds were evaluated for their potential cytotoxicity against two different human cancer cell lines HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma) in vitro, using Neutral Red and MTT assays. The synthesized compounds were active against two cell lines under study and a toxic effect was clear with a significant difference at the level of probability ($p < 0.0001$) and this effect was contrasted among different concentrations for each synthesized compound.

Keywords: Pyrimidine derivatives, Cytotoxic activity, Human cancer cell lines.

Introduction:

Pyrimidine derivatives are well known for their pharmacological activities. Various drugs containing pyrimidine nucleus were synthesized and used as anticancer agents like 5-Fluorouracil (5-FU), Tegafur and Thioguanine[1] (Fig.1).

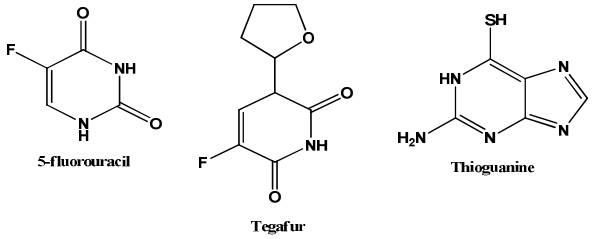


Fig. 1: Pyrimidine derivatives as anticancer agents.

An interest in pyrimidine derivatives as anticancer agents has led to the preparation and anticancer activity evaluation of hundreds of such molecules. For example, 2-cyanopyrimidines [2], hydrazino pyrimidine-5-carbonitriles [3], 1,3-dialkylated-pyrimidin-2,4-diones[4] and 4-anilino-2-(2-pyridyl). Pyrimidines were evaluated as a new class of potent anticancer agents [5]. Pyrimidines and their derivatives have been found to possess a broad spectrum of biological activities such as antimicrobial, anti-inflammatory, analgesic, antiviral and anticancer activities [6-13]. Quantitative methods for in vitro cytotoxicity have been described and recommended in the literature, although these methods present available cannot be easily automated. A range of assays based on different aspects of cellular activity can be applied for the assessment of biocompatibility. In the present study, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and neutral red (NR) assays for quantitative evaluation which have been adapted for human inactivated allografts are presented [14]. The first group of assays measures the ability of viable cells to reduce a water-soluble yellow dye, MTT, to a water-insoluble purple formazan product. This is converted by intracellular dehydrogenase to colored formazans. A second group of assays monitors' cell membrane integrity, and is based on the spectro-photometric determination of NR (3-amino-2-methylphenazine hydrochloride) taken up by viable cells and stored in their lysosomes.

In view of these facts, we aimed to investigate these uracil derivatives for their anti-cancer activities. Four compounds were subjected to 2

human tumor cell lines and all of these compounds are active against two cell lines.

Instrumental

Method:

Synthetic methods for the preparation of pyrimidine derivatives are summarized in (scheme1). Melting points were measured by using (Gallen Kamp) melting point (table1). Infrared spectra were recorded on F.T.IR-8300 Fourier transforms infrared spectrophotometer SHIMADZU as potassium bromide disc in the (600-4000) cm^{-1} spectral range (table 2). The electronic spectra of the compounds were obtained using (SHIMADZU UV-Vis. 160A) ultraviolet spectrophotometer (table 2). The reactions were monitored by TLC and purification of the compounds were carried out by recrystallization method using suitable solvent. The cytotoxic effect of the synthesized compounds was evaluated by Neutral Red and MTT assays.

Synthesis of 5,6-diaminopyrimidine-2,4- diol hydrochloride (a): [15]

Three-necked flask equipped with a reflux condenser and an efficient stirrer was placed. To 203ml. of absolute ethanol (99.99%), (8g, 0.34 g. atom) of sodium, (18.5 ml, 0.17 mole) of ethyl cyanoacetate , and (10.4 g, 0.17 mole) of urea were added. The mixture was heated under reflux for 4 hours. (203ml) of hot (80°) water was added; the stirred mixture was heated at 80° for 15 minutes and then neutralized to litmus with glacial acetic acid. Additional glacial acetic acid (15.2 ml.) was added, followed by cautious addition of a solution of (13.1 g, 0.19 mole) of sodium nitrite which was dissolved in (14.1ml) of water. The nitroso compound was removed by filtration and washed twice with a small amount of ice water. The moist material was transferred back to the flask, and (87.3 ml) of warm water (50°) was added. Then an additional (6.1g) of sodium hydrosulfite was added. The dense diaminouracil bisulfite was filtered from the cooled solution, washed well with water, and partially dried. Then concentrated hydrochloric acid was added until the consistency of the resulting mixture was such as to permit mechanical stirring (20 to 40 ml. of acid). The slurry was heated on a steam bath with stirring for 1 hour. Tan diaminouracil hydrochloride was filtered on a sintered glass funnel, washed well with acetone.

Synthesis of 5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride (b): [15]

Three-necked flask equipped with a reflux condenser and an efficient stirrer was placed. To 203ml. of absolute ethanol (99.99%), (8g, 0.34 g. atom) of sodium, (18.5 ml, 0.17 mole) of ethyl cyanoacetate , and (13.29 g, 0.17 mole) of thiourea were added. The mixture was heated under reflux for 4 hours. (203ml) of hot (80°) water was added; the stirred mixture was heated at 80° for 15 minutes and then neutralized to litmus with glacial acetic acid. Additional glacial acetic acid (15.2 ml.) was added, followed by cautious addition of a solution of (13.1 g, 0.19 mole) of sodium nitrite which was dissolved in (14.1ml) of water. The nitroso compound was removed by filtration and washed twice with a small amount of ice water. The moist material was transferred back to the flask, and (87.3 ml) of warm water (50°) was added. Then an additional (6.1g) of sodium hydrosulfite was added. The dense diaminouracil bisulfite was filtered from the cooled solution, washed well with water, and partially dried. Then concentrated hydrochloric acid was added until the consistency of the resulting mixture was such as to permit mechanical stirring (20 to 40 ml. of acid). The slurry was heated on a steam bath with stirring for 1 hour. Tan diaminouracil hydrochloride was filtered on a sintered glass funnel, washed well with acetone.

Synthesis of 2-mercapto-6-methylpyrimidin-4(3H)-one (c): [16]

0.1 mole, 3.9 g of sodium hydroxide in (2.4 ml) water was added to the mixture of the (0.04 mole, 3 g) of thiourea and (0.04 mole, 5.0ml) of ethylacetoacetate in (4 ml) of ethanol in round bottomed flask and the mixture was refluxed for (2 hours), then hot solution was added (8 ml) of concentrated hydrochloric acid in (4 ml) of

water to the product. The product was filtered and washed with cold distilled water.

Synthesis of 2-hydrazinyl-6-methylpyrimidin-4(3H)-one (d): [17]

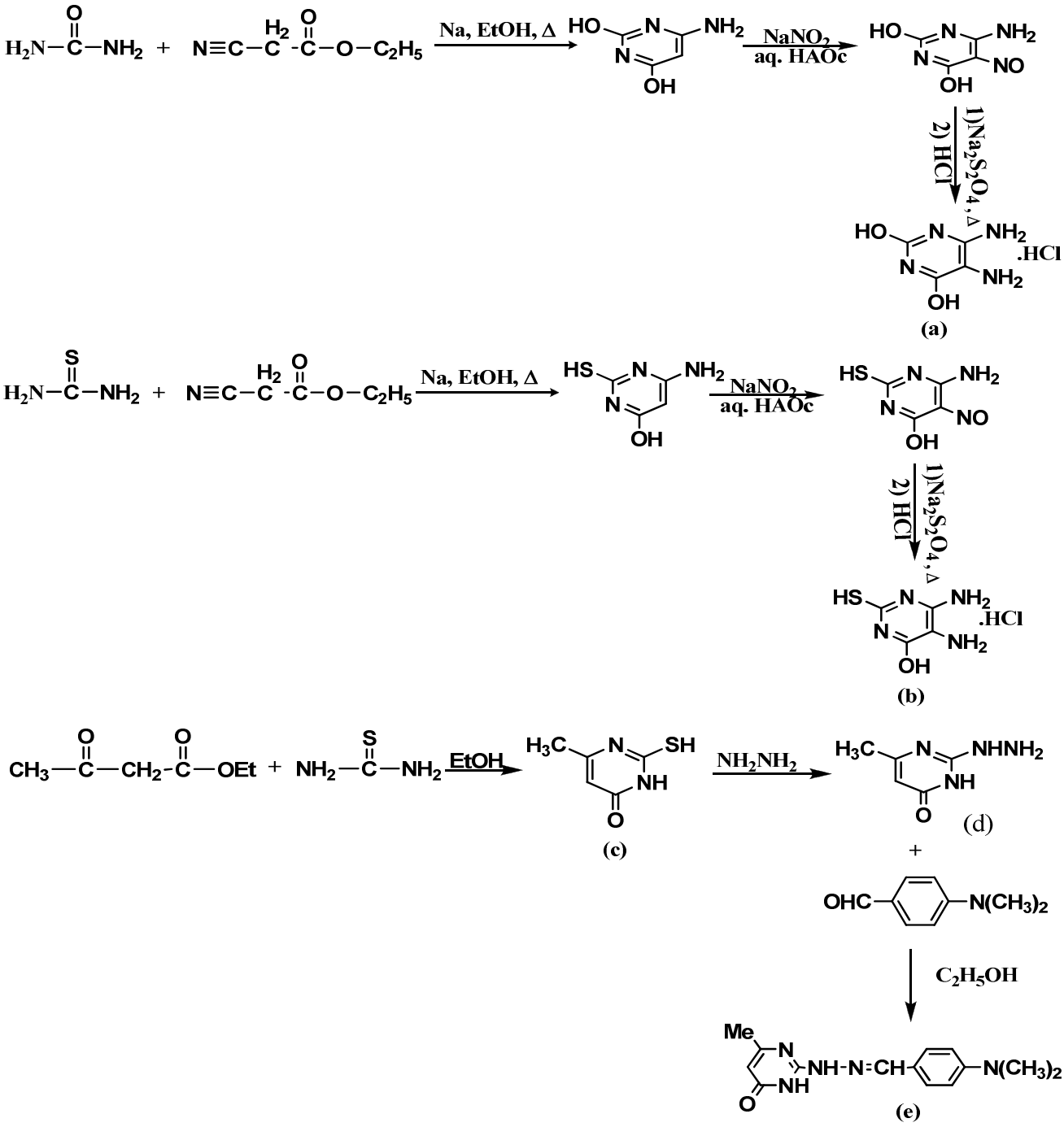
A mixture of 0.02 mole, 3.63 g of 2-mercapto-6-methylpyrimidin-4(3H)-one and (12.7 ml) of hydrazine hydrate (99%) was refluxed in around bottomed flask for (3 hours). The product was filtered and washed with cold distilled water.

Synthesis of 2-(2-(4-(dimethylamino) benzylidene)hydrazinyl)6-methylpyrimidin-4(3H)-one (e):

A mixture of 0.002 mole, 0.28g of 2-hydrazinyl-6-methylpyrimidin-4(3H)-one and (0.002 mole, 0.29g) of 4-N,N-dimethylamino benzaldehyde in around bottomed flask with (5ml)ethanol, 1-2 drops of glacial acetic acid were added, and the mixture was refluxed for 5 hours, ice bath was used to separate the product, and the mixture was then filtered and precipitate was isolated.

Statistical Analysis:

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using SAS computer program version 7.5[18] Differences in results were considered significant at probability value equal or less than 0.0001.


Na, EtOH, Δ
NaNO2
aq. HAOc
1)Na2S2O4, Δ
2) HCl
.HCl
(a)
Na, EtOH, Δ
NaNO2
aq. HAOc
1)Na2S2O4, Δ
2) HCl
.HCl
(b)
EtOH
(c)
NH2NH2
(d)
+
C2H5OH
(e)

Scheme 1

Table (1)

Physical Data of The Prepared Compounds

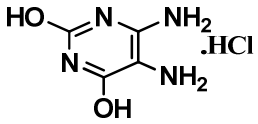
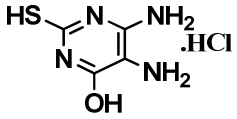
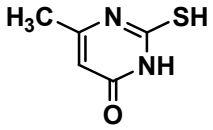
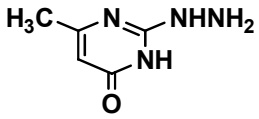
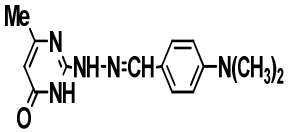
Name of compound.	Structural Formula	M.wt g.mol^{-1}	M.P.	Yield %	Color
5,6-diaminopyrimidine-2,4- diol hydrochloride (a)		178.58	298-300C°	59.4%	Light tan
5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride (b)		194.64	300-303C°	30.3%	Light Yellow
2-mercapto-6-methyl pyrimidin-4(3H)-one (c)		142.02	330C° (sublime)	64.2%	Yellowish-white
2-hydrazinyl-6-methyl pyrimidin-4(3H)-one (d)		140.07	212-214C°	42.8%	white
2-(2-(4-(dimethylamino) benzylidene)hydrazinyl)-6-methylpyrimidin -4(3H)-one (e)		271.14	≥300C°	55.5%	orange

Table (2)

Spectral Data of The Prepared Compound

Compounds	IR spectral data $\upsilon(cm^{-1})$	UV-Visible λmax (nm)
5,6-diaminopyrimidine-2,4- diol hydrochloride (a)	3406.1-3300(NH_2), 1712.7(C=O), 1668.3(C=N), 1618.2(C=C), 1195.8(C-N)	253nm
5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride (b)	3390-3400(NH_2), 2300.9(S-H), 1631.7(C=N), 1548.7(C=C), 1225(C=S), 1176.5(N-C), 763.8(C-S)	271nm
2-mercapto-6-methyl pyrimidin-4(3H)-one (c)	3112.9(N-H), 3010arm 2887.2alph(C-H), 2580.6-2370.4(S-H), 1631.7(C=O), 1556.4(C=N), 1240.1-1197.7(C=S), 740(C-S)	292nm, 271nm
2-hydrazinyl-6-methyl pyrimidin-4(3H)-one (d)	3211.3-3250(N-H), 2925.8alph 3010arm(C-H), 1643.2(C=O), 1593.1(C=N), 1157.2(C-N)	404nm, 251nm
2-(2-(4-(dimethylamino) benzylidene)hydrazinyl)-6-methylpyrimidin -4(3H)-one (e)	3209.3-3290(N-H), 3010arm 2927.7 alph(C-H), 1641.3(C=O), 1600broad(C=N), 1164.9(C-N)	264nm, 355nm

Method of cytotoxicity assay

A-Neutral red assay:

Single cell suspension was prepared by treating 25 cm^3 tissue culture flask with 2 ml trypsin solution incubated for 2 min at 37°C in an incubator supplemented with (5%) CO_2 after detachment of the cells from the flask surface single cell suspension by gently taping of the flask followed by the addition of 20 ml of growth medium supplemented with 10% fetal calf serum then the viability test of the cells was made by using trypan blue dye which stains the dead cells. Cells suspension was well mixed followed by transferring 200 µl/well to the 96 well flat bottom micro titer plate using automatic micropipette containing ($1x10^5$ cell/well). Plates were incubated at 37°C in an incubator supplemented with (5%) CO_2 until 60-70% confluence of the internal surface area of the well for HepG2 cell line, the cells were then exposed to different concentration(600,300,150,75and 37.5 µg/ml) of new synthesized compounds, each compound was added to the cells in triplicate form

of each concentration, only cells incubated with culture media represented the negative control, then the 96-well cell culture plate incubated at 37°C in an incubator supplemented with (5%) CO_2 for 48 hrs. After elapsing the incubation period, 50 µl/well of neutral red dye freshly prepared were added to each well and incubated again for 2 hrs, viable cells will uptake the dye and the dead not, the plates washed by PBS to remove the excess dye, then 100µl/well of eluent solution were added to each well to draw out the dye from the viable cells. Optical density of each well was measured by using *ELISA* reader at a transmitting wave length on 492nm [19].

MTT based cytotoxicity assay:

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cytotoxicity assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of surviving cells is directly proportional to the level of the formazan created. Cell proliferation assay was carried out by MTT cell proliferation assay kit (Roche applied sciences, Germany). Equal number of (MCF7) cells were seeded in each well of 96-well micro plate and incubated at 37C°, in presence of 5% CO_2. The cells were treated with synthesized compounds at various concentrations (100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml and 3.125µg/ml) for 24 hrs. In vehicle control culture wells, a maximum of 0.5% DMSO was added. After 24 hrs. treatment, 5 µl of MTT reagent(R&D systems USA) along with 45µl of phenol red and FBS free DMEM (sigma Life Science, USA) was added to each well and incubated for 4 hrs. at 37C° in presence of 5% CO_2. Thereafter 50 µl of solublization buffer (R&D systems, USA) was added to each well to solubilize the coloured formazan crystals produced by the reduction of MTT. The optical density was measured at 570 nm using micro plate reader (Hidex Chamelon plate reader). The results (mean O.D.± SD) obtained from quadruplicate wells were used in calculation to determine the cytotoxicity (50% inhibitory concentration, IC50) of the test compounds.

Results and discussion:

Some new pyrimidine derivatives were synthesized in good yields (scheme 1). The antitumor activity results indicated that all the tested compounds are active against two cell lines (HepG2 and MCF7). The results of treated of cancer cell lines under study showed a toxic effect is clear with a significant differences at the level of probability ($p< 0.0001$) and this effect was found among different concentrations of the compounds prepared.

Cytotoxic effect of synthesized compounds on HepG2 cell line:

Results indicated in (table 3) showed that diamino uracil hydrochloride (a) has a cytotoxic effect on HepG2 cell line with a significant differences ($P<0.0001$) started at the lower concentration and continued to the higher concentrations where the growth inhibition of HepG2 cell line was increased gradually with the increased treatment with Diamino uracil hydrochloride concentration, when compared with the negative control (the same cell line without any treatment), Maximum inhibitory effect of Diamino uracil hydrochloride was reached 61.3% when the cell culture of HepG2 was treated with 600µg/ml of 5,6-diaminouracil-2,4-diol hydrochloride while the inhibitory effect decreased to 9% after treatment with 37.5% µg/ml of diaminouracil hydrochloride.

Cytotoxic effect of synthesized compounds on MCF7 cell line:

(Table 4) showed that all compounds exhibited growth inhibition activity on the tested tumor panel breast cancer cell line (MCF7) between 100-3.12µg/ml in comparison to the negative control. And the synthesized compounds having IC50 values in the range of 20.9–23.2µg/mL can be classified as possessing mild cytotoxic activity against MCF7 cell line**.** The compounds **b** and **c** (with nitrogen heterocyclic ring in the molecule) showed moderate anticancer activity against MCF7 cell line while compound **d** showed high selectivity for MCF7 cell lines. Compound **a** exhibited high anticancer activity against HEPG2 and MCF7 cell lines.

Table (3):

Cytotoxicity Effect of Synthesized Compounds (at Different Conc.)on HepG2 Tumor Cell Line after, Incubation for 48 Hours Measured at 492nm.

compounds	Concentration µg/ml	Absorbance of compounds	Inhibition Rate% ±SE	$IC_{50\%}$
5,6-Diaminouracil-2,4diol hydrochloride (a)	37.5	1	9.00±0.57 e	432.6141
	75	0.968	12.00±0.00 d	
	150	0.788	28.30±0.34 c	
	300	0.599	45.50±0.28 b	
	600	0.425	61.3±0.17 a	
5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride(b)	37.5	0.978	11.00±0.57c	447.9962
	75	0.967	12.00±1.15 c	
	150	0.967	12.00±0.00 c	
	300	0.703	36.00±0.57b	
	600	0.368	66.5±0.28 a	
2-mercapto-6-methyl pyrimidin-4(3H)-one (c)	37.5	1.099	0.09±0.00d	531.6179
	75	1.097	0.27±0.00d	
	150	1.056	4.00±0.57 c	
	300	1.037	5.7±0.11b	
	600	0.371	66.2±0.11 a	
2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one (e)	37.5	0.997	9.30±0.17c	451.5608
	75	1.075	2.20±0.20e	
	150	1.03	6.30±0.00d	
	300	0.765	30.40±0.40b	
	600	0.422	61.60±0.11 a	

** ($P<0.0001$), different letters= significant differences between mean.

ʌ

Table (4):

Cytotoxicity Effect of Synthesized Compounds (at Different Conc.)on MCF7 Tumor Cell Line after, Incubation for 24 Hours Measured at 570nm.

compounds	Concentration µg/ml	Absorbance of compounds	Viable cell% ±SE	$IC_{50\%}$
5,6-diaminouracil-2,4-diol hydrochloride(a)	3.125	0.646	96.12±0.22 a	21.9
	6.25	0.540	80.35±0.30 b	
	12.5	0.463	68.89±0.34 c	
	25	0.318	47.31±0.22 d	
	50	0.190	28.32±0.26 e	
	100	0.084	12.54±0.30 f	
5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride(b)	3.125	0.645	95.97±0.30 a	23.2
	6.25	0.558	83.03±0.30 b	
	12.5	0.467	69.53±0.34 c	
	25	0.335	49.84±0.30 d	
	50	0.202	30.10±0.52 e	
	100	0.090	13.38±0.39 f	
2-mercapto-6-methyl pyrimidin-4(3H)-one (c)	3.125	0.661	98.36±0.17 a	22.6
	6.25	0.559	83.18±0.17 b	
	12.5	0.478	71.17±0.21 c	
	25	0.342	50.98±0.48 d	
	50	0.175	26.13±0.26 e	
	100	0.074	11.00±0.25 f	
2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one(e)	3.125	0.653	97.16±0.17 a	20.9
	6.25	0.566	84.27±0.17 b	
	12.5	0.447	66.56±0.21 c	
	25	0.320	47.71±0.48 d	
	50	0.169	25.14±0.26 e	
	100	0.055	8.27±0.25 f	

Structural-activity relationship (SAR).

From the obtained results (table 3,4**)**, we can conclude that the anticancer activity is due to:

(i) The presence of nitrogen heterocyclic rings.

(iii) The presence of 2-thiouracil moiety is essential for inhibition activity.[20]

Conclusion:

Due to the presence of sulphur and nitrogen in the heterocyclic compounds skeleton, they show diverse biological activities. Pyrimidines are the important heterocyclic compounds which show promising pharmacological activities i.e. anticancer agent. The present results suggests that 4 compound (a, b, c and e) induced cytotoxicity on HepG2 and MCF7 cell lines.

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الخلاصة:

تضمن هذا البحث تحضير عدد من (مشتقات البريميدنات) التي تلعب دوراً هاماً في مجالات الكيمياء الطبية وذلك لانها تمتلك فعالية حياتية (فعالية سُمية أتجاه الخلايا)، و تم تشخيص المركبات المحضرة بواسطة الطرق الطيفية (طيف الاشعة تحت الحمراء F.T.IR) و الاشعة فوق البنفسجية (UV-VISIBLE) و قياس درجة الانصهار لهذه المركبات، وخضعت هذه المركبات الى نوعين من خطوط الخلايا السرطانية البشرية (سرطان الكبد (HepG2) و سرطان الثدي (MCF7)) خارج الجسم الحي وذلك بأستخدام فحوصات مختلفة (متعادل الصبغة الحمراء (neutral red) و (MTT))، و وجدَ أن هذه المركبات ذات فعالية اتجاه كلا الخطين السرطانيين وبفروق معنوية عند مستوى احتمالية ($P<0.0001$) وكان هذا التأثير متباين بين التراكيز المختلفة لكل مركب من المركبات المحضرة .

INTRODUCTION

1. *Nitrogen bases:*

There are two kinds of nitrogen containing bases – purines and pyrimidines, figure (1-1). Purines consist of a six – member and a five – member nitrogen – containing ring, fused together. Adenine and guanine are purines type, found in DNA and RNA. Pyrimidine has only a six – member nitrogen – containing ring, cytosine, uracil and thymine are pyrimidine type. Cytosine is found in both DNA and RNA. Uracil is found only in RNA. While thymine is normally found in DNA. Sometimes tRNA contain thymine as well as uracil.

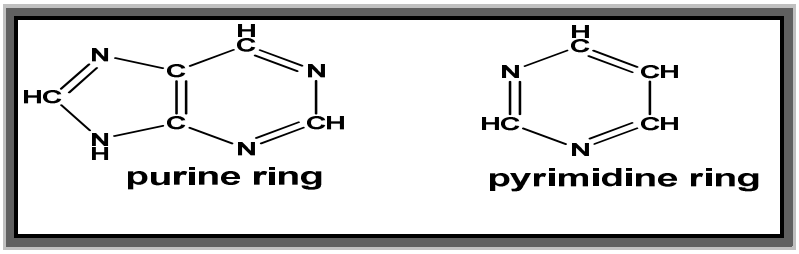


Figure (1-1) Base pairs

These bases considered as aromatic molecules forming (lactam) or lactim which are tautomers. Figure (1-2)

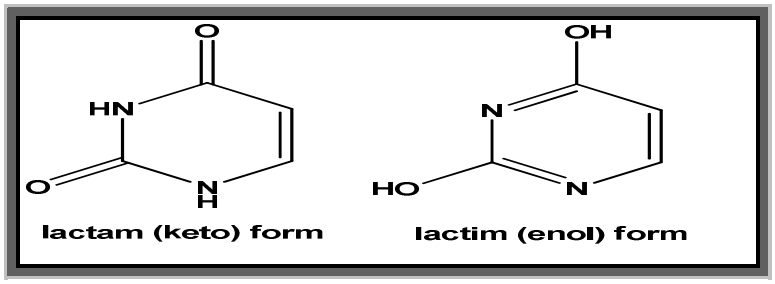


Figure (1-2) Tautomers of pyrimidine

Nitrogen base having the chemical properties of a base. It is an organic compound that owes its property as a base to the one pair of electrons of a nitrogen atom. Both pyrimidines and purines resemble pyridine and are thus weak bases and relatively unreactive towards electrophilic aromatic

substitution. Their flat shape is particularly important when considering their roles in nucleic acids as nucleobases (building blocks of DNA and RNA): adenine, guanine, thymine, cytosine, and uracil. These nitrogenous bases make hydrogen bonds between opposing DNA strands to form the rungs of the "twisted ladder" or double helix of DNA or a biological catalyst that is found in the nucleotides. Adenine is always paired with thymine, and guanine is always paired with cytosine. Uracil is only present in RNA replacing thymine and pairing with adenine [(1)].

1.1.1 ***Purine:***

A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring. Purines, including substituted purines and their tautomers, are the most widely distributed kind of nitrogen-containing heterocycle in nature. Two of the four deoxyribonucleotides and two of the four ribonucleotides, the respective building-blocks of DNA and RNA, are purines [(2)].

1.1.2 ***Synthesis of purine :***

In addition to in vivo synthesis of purines in purine metabolism, purine can also be created artificially. Purine is obtained in good yield when formamide is heated in an open vessel at 170 Cº for 28 hours. As shown in the following reaction. Figure (1-3).

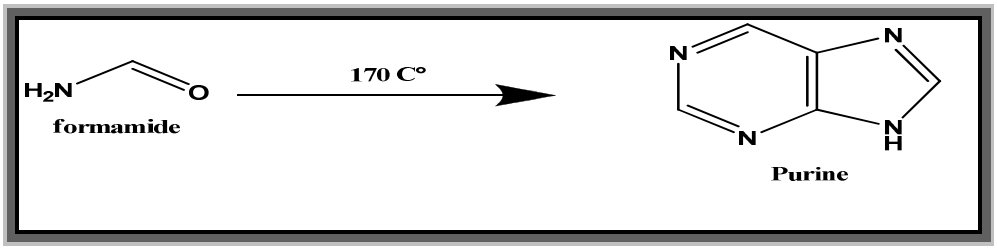
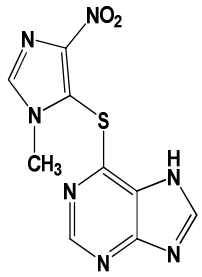
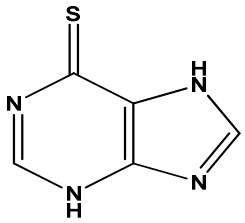
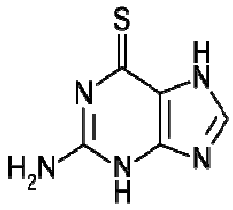
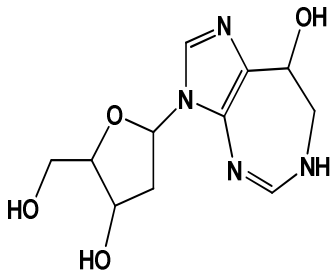


Figure (1-3) Synthesis of purine

Purine analogues are antimetabolites that mimic the structure of metabolic purines. As shown in Table (1-1) and Figure (1-4).

Table (1-1) purine analogues:

Compounds	*Structure*	*Definition*
Azathioprine		Azathioprine is a pro-drug**,** it is metabolized into the active 6-mercaptopurine, it is impedes DNA synthesis and thus inhibits the proliferation of cells, Azathioprine blocks the downstream effects of CD28 costimulation [(3)].
Mercaptopurine		Mercaptopurine (also called thiopurine.) Is an immunosuppressive drug.It is used to treat leukemia, polycythemia vera, psoriatic arthritis and inflammatory bowel disease such as Crohn's disease and ulcerative colitis [(4)].
Thioguanine		Thioguanine: it is classified as an antimetabolite, and it is used for treatment of cancer such as (an acute and chronic myelogenous leukemia) [(5)].

Pentostatin		It is classified as a purine analog, which is a type of antimetabolite. It mimics the nucleoside adenosine and thus inhibits the enzyme adenosine deaminase, interfering with the cell's ability to process DNA [6].

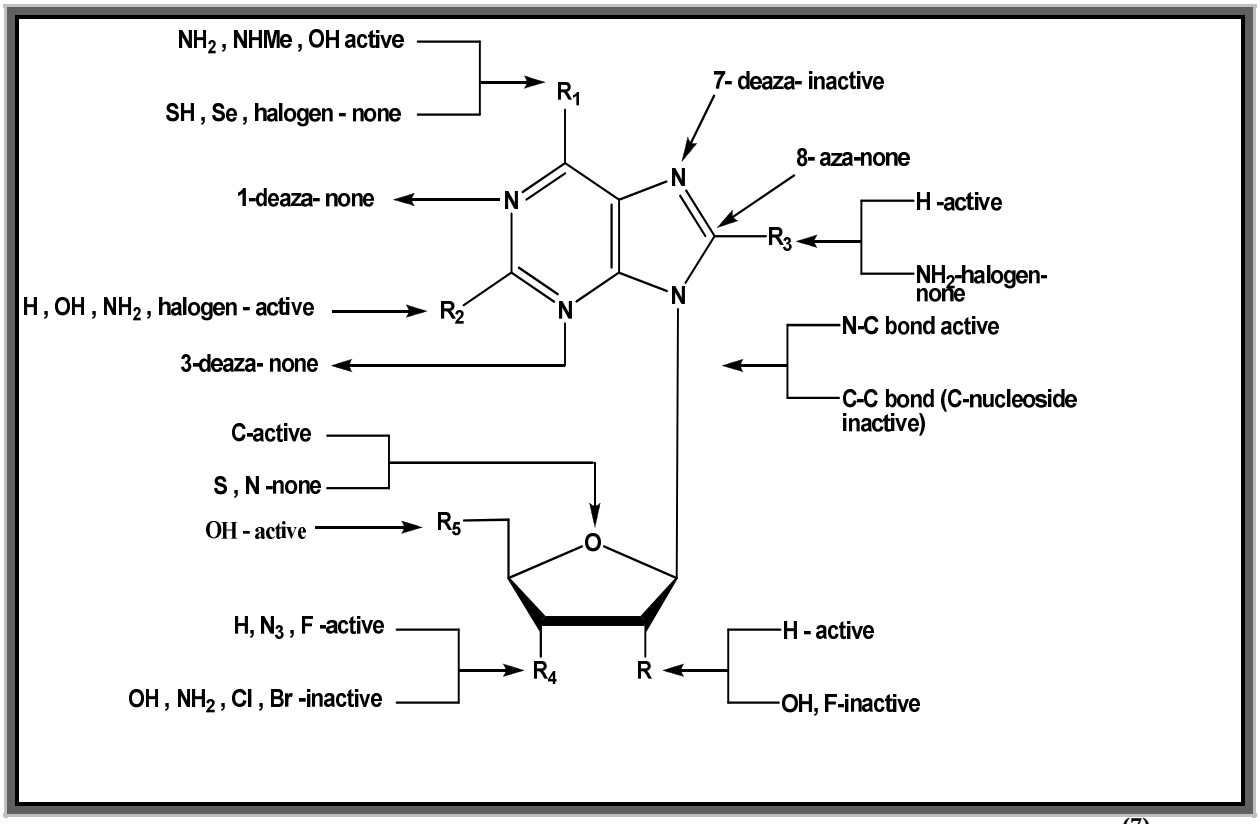


Figure (1-4) Structure-activity relationship of purine analogues [7]

1.2.1 *Pyrimidine:*

Pyrimidines also known as m-daizine, which is the parent substance of large group of heterocyclic compounds that have attracted much attention for a long time. These compounds which belong to this group where known as breakdown products of uric acid at a very early date of the history of organic chemistry, but systematic study of this ring system

began with work Pinner[8] who first applied the name pyrmidine to the unsubstantiated parent body.

*Pyrimidine derivatives play an important role in many biological processes, the ring being present in nucleic acid, several vitamins coenzymes, uric acid and other purine. Pyrimidine is also found in meteorites, but scientists still do not know its origin.

*Many drugs (barbituric acid derivatives) and chemotherapeutic agents (Sulfadiazine) contain pyrimidine ring.

*Pyrimidine can be regarded as cyclic amidine and the chemical behavior of its derivatives is dominated by this fact.

*According to X-ray diffraction studies pyrimidine exist as distorted hexagon.

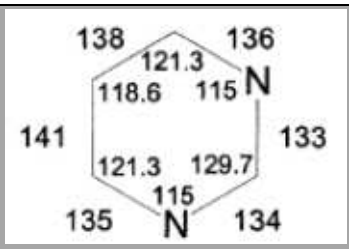


Figure (1-5) Bond parameters of pyrimidine (bond lengths in pm, bond angles in degrees)

1.2.2 Pyrimidine Bases:

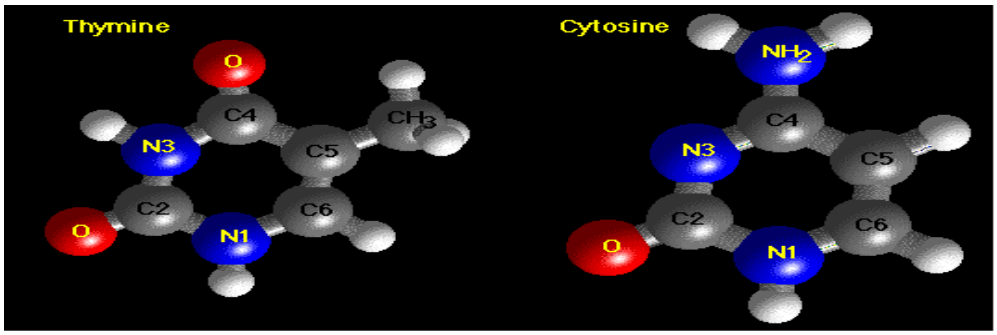


Figure (1-6) Chemical structure of pyrimidine bases

The 6 atoms (4 carbons, 2 nitrogen) are numbered 1-6. Like purines, all pyrimidine ring atoms lie in the same plane [9].

1.2.3 Synthesis of pyrimidine analoques:

Pyrimidine is a colorless liquid with characteristic pyridine like odor, it has been prepared by the reduction of di or tri chloropyrimidines. It does not normally serve as starting point for the preparation of substituted pyrimidine.

There are three methods for the preparation of pyrimidine according to the fundamental nature of the fragments which combined together to form pyrimidine nucleus. Figure (1-7)

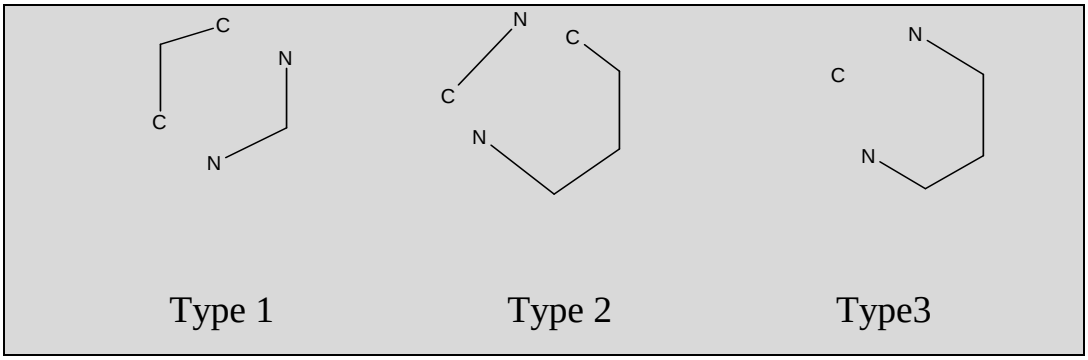


Figure (1-7) methods for the preparation of pyrimidine

1.2.4*Types of pyrimidine synthesis basic methods:-*

1.2.4.1*Type one [10]:-*

The commonest pyrimidine synthesis are belonging to type 1. The stylization usually involves a double condensation with elimination of water, alcohol or hydrogen halide between amino and carbonyl, carboxyl ester, acyl chloride and enol ether, or condensation by addition of amino group to cyano groups or to polarized double bonds without an elimination reaction.

- Urea condensed with malonic acid in the presence of phosphoryl chloride and produce barbituric acid.

- Then malonic ester is used instead of malonic acid in the presence of sodium alkaloid as catalyst. As shown in the following reaction figure (1-8).

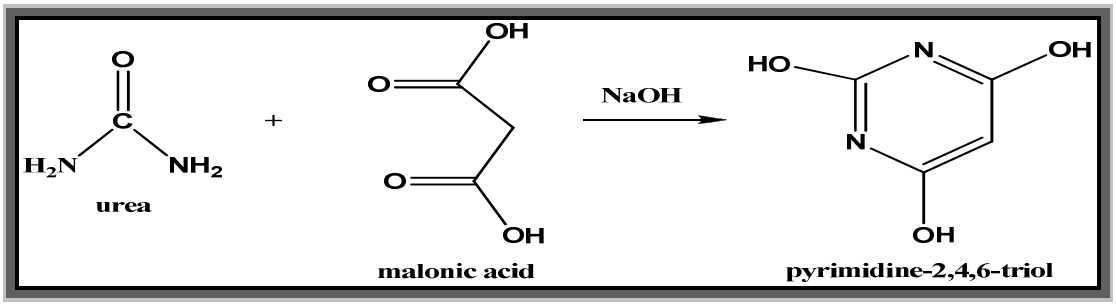


Figure (1-8) synthesis of pyrimidine-2,4,6-triol

The applications of this process especially with ester of dialkyl malonate are used in the prepareation of barbiturate drugs.

- β-ketoesters and there enol form are suitable for condensation with urea to form 6-methyluracil(pyrimidine derivatives). As shown in the following reactions. Figures (1-9) and (1-10).

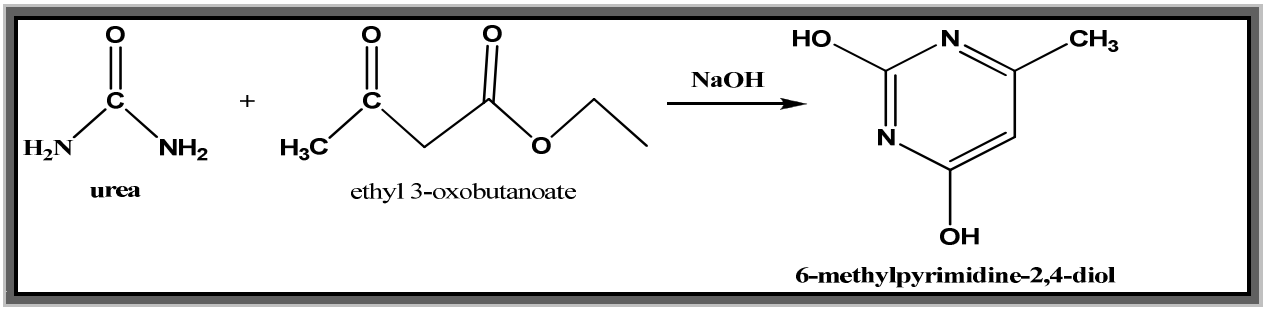


Figure (1-9) synthesis of 6-methylpyrimidine-2,4-diol

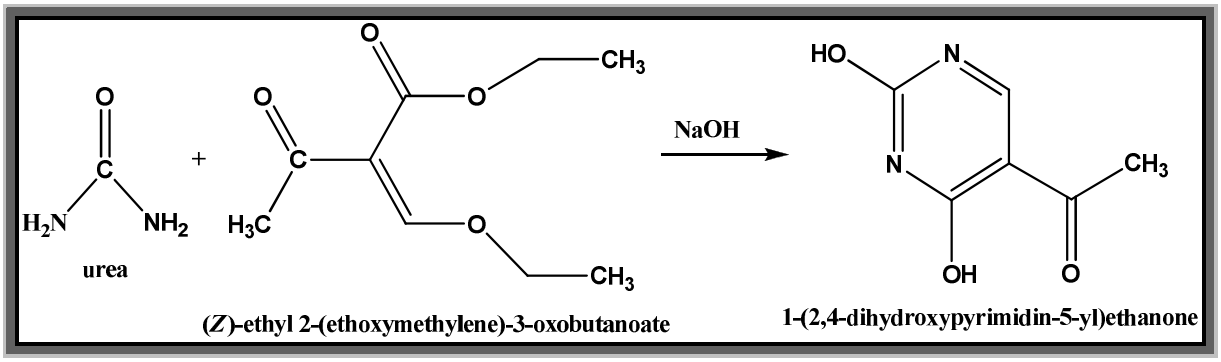


Figure (1-10) synthesis of 1-(2,4-dihydroxypyrimidine-5-yl)ethanone

An important example is the prepareation of uracil from urea and formylacetic acid (prepared in situ by the action of sulfuric acid on malonic acid). As shown in the following reaction. Figure (1-11).

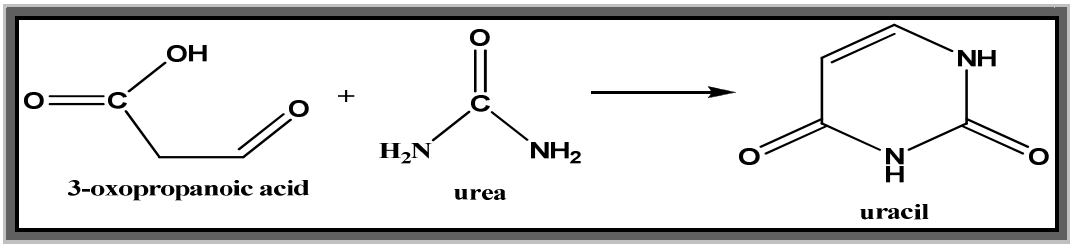


Figure (1-11) synthesis of uracil

- Acetone dicarboxylic acid (prepared from citric acid) reacts with urea similarly. As shown in the following reaction. Figure (1-12).

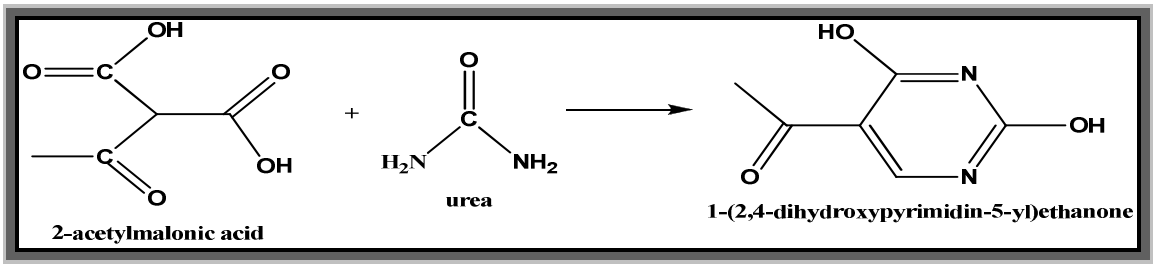


Figure (1-12) synthesis of 1-(2,4-dihydroxypyrimidine-5-yl)ethanone

- Mitchell [(11)] *et al* have shown that orotic acid can be synthesized (uracil-4- carboxylic acid) from urea and oxaloacetic ester. As shown in the following reaction. Figure (1-13).

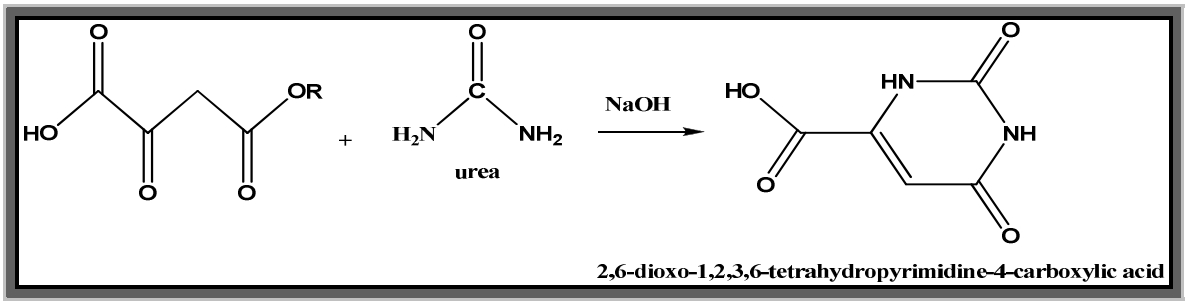


Figure (1-13) synthesis of 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid

1.2.4.2*Type Two:-*

This method require an amino ethylene intermediate which may be obtained from the corresponding ethoxy methylene compounds

with ammonia but it can also result from the reaction of imino ether or amidine with reactive methylene compounds. As shown in the following reaction. Figure (1-14).

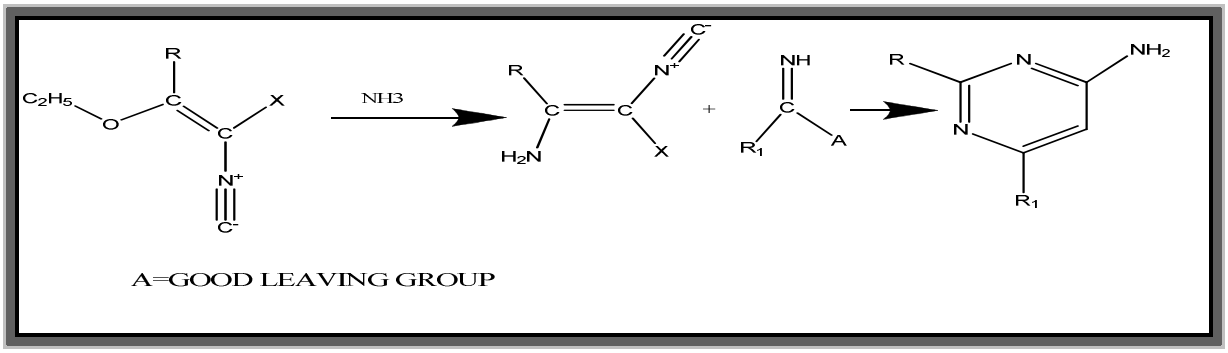


Figure (1-14) Reaction between ethoxy methylene compounds with ammonia

- β-Amino acids and β-amino ketones may be employed to obtain dihydropyrimidine closer being effected with acetyl chloride in the presence of ammonia. As shown in the following reaction. Figure (1-15).

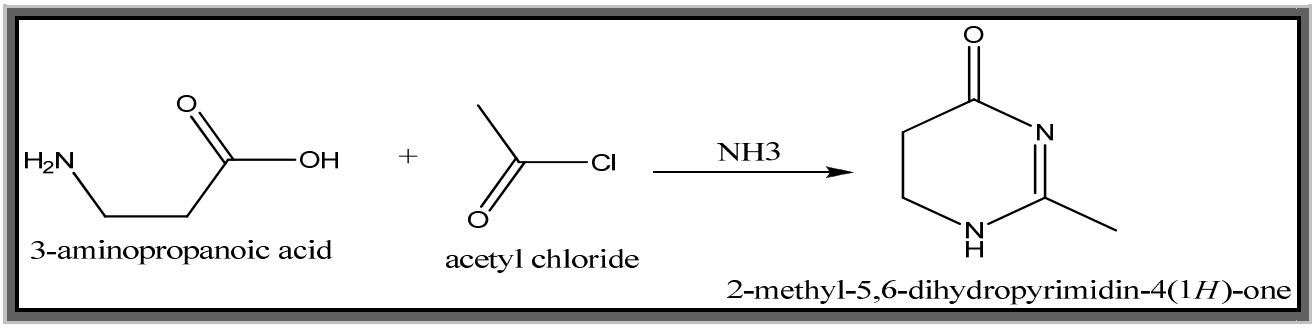


Figure (1-15) synthesis of 2-methyl-5,6-dihydropyrimidine-4(1H)-one

1.2.4.3 ***Type Three:-***

The insertion of a single carbon atom between nitrogen of 1,3 diamines to obtain hydrogenated pyrimidine may be achieved by a number of conventional process, for instance treatment with diethyl carbonate, phosgene or aldehyde. The amide derived from carboxylic acid also can be cyclized to terahydropyrimidine

- The amide of β-amio acid may be converted into 4-hydroxy 5,6-

dihydropyrimidine with derivatives of carbonic acid .

- Malonic ester or malonyl chloride with malonamide yield 4,6-dihydroxy-2- methyl pyrimidine, with hypobromate or permanganate, methylene asparagine undergoes an oxidative cyclization followed by dehydrogenation yielding pyrimidines. As shown in the following reaction. Figure (1-16).

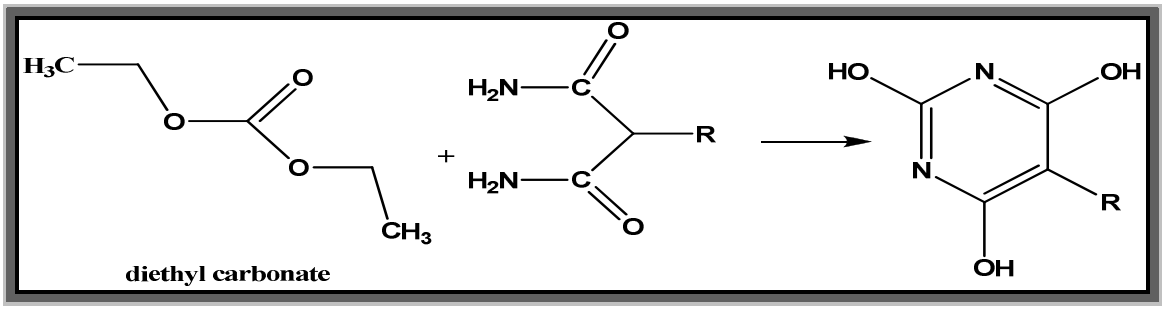


Figure (1-16) reaction between diethyl carbonate with malonamide

1.2.4.4 *Unclassified methods :*

A few pyrimidine synthesis method which do not fall into any of the three main types and they are not important.

Malic diamide is converted by sodiumhypochlorate into uracil. As shown in the following reaction [12]. Figure (1-17).

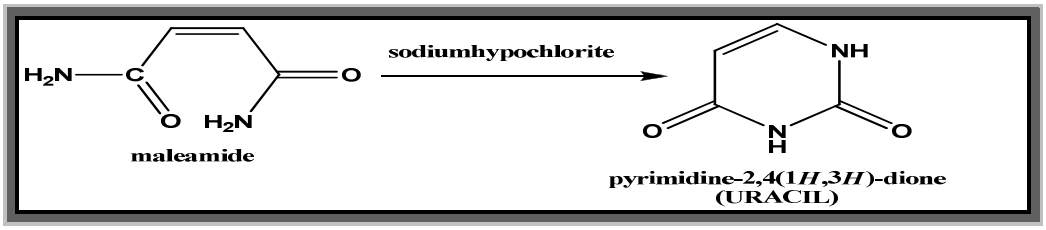


Figure (1-17) synthesis of pyrimidine-2,4(1H,3H)-dione

- β-urido acid derivatives on heating with HCl give mixture of dihydropyrimidine Pyrimidines. As shown in the following reaction. Figure (1-18).

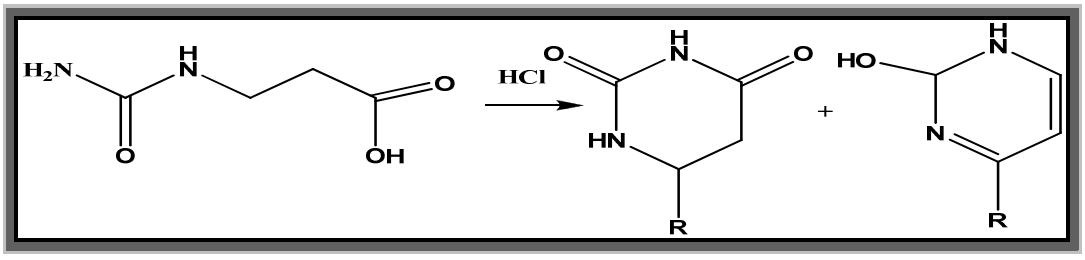


Figure (1-18) synthesis of dihydropyrimidine derivatives

Mathes and Swedish [13] have prepare pyrimidine derivatives from mesityloxide ammoniumthiocyanate and primary amine. As shown in the following reaction. Figure (1-19).

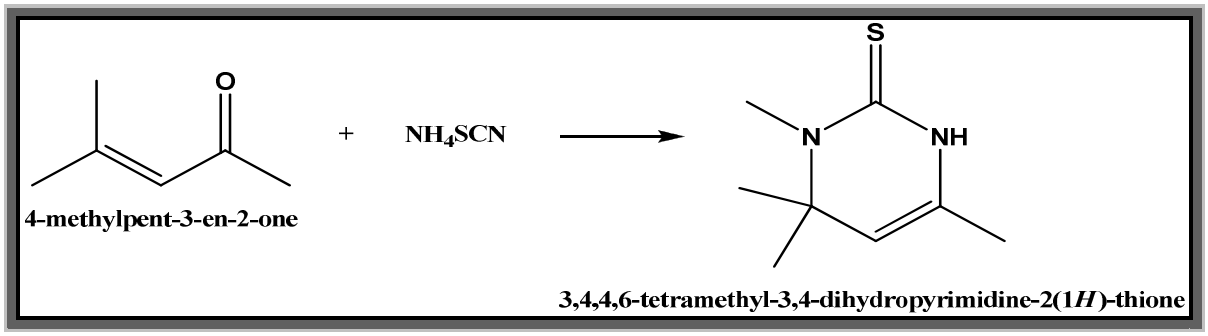


Figure (1-19) synthesis of 3,4,4,6-tetramethyl-3,4-dihydropyrimidine-2(1H)-thione

- Alejandro *et,al* [14] have prepared some azolo pyrimidines,

The method involved the reaction of N-protected bromomethylazoles and tosylmehylisocyanide derivatives in non-hydrous media. Figure (1-20).

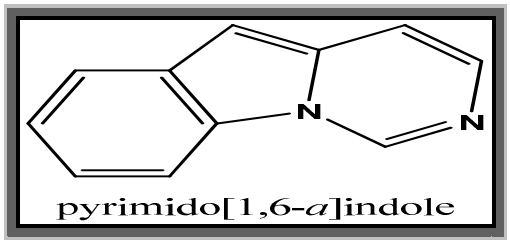


Figure (1-20) structure of pyrimido(1,6-a)indole

- Gibson *et,al* [15] have prepared pyrimidine derivatives which have inhibition on gunosine triphosphate cyclohydrolasel. Figure (1-21)

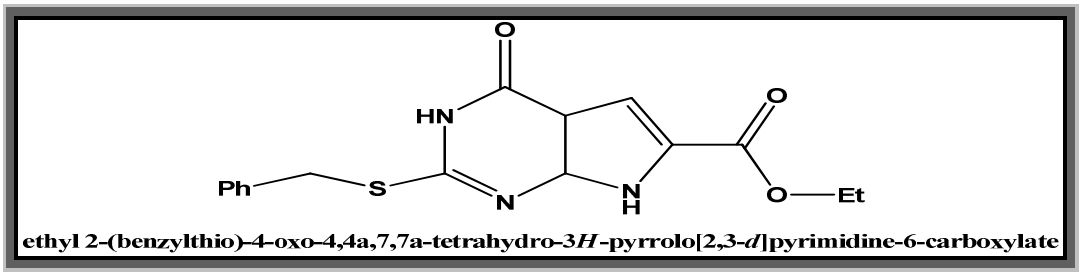
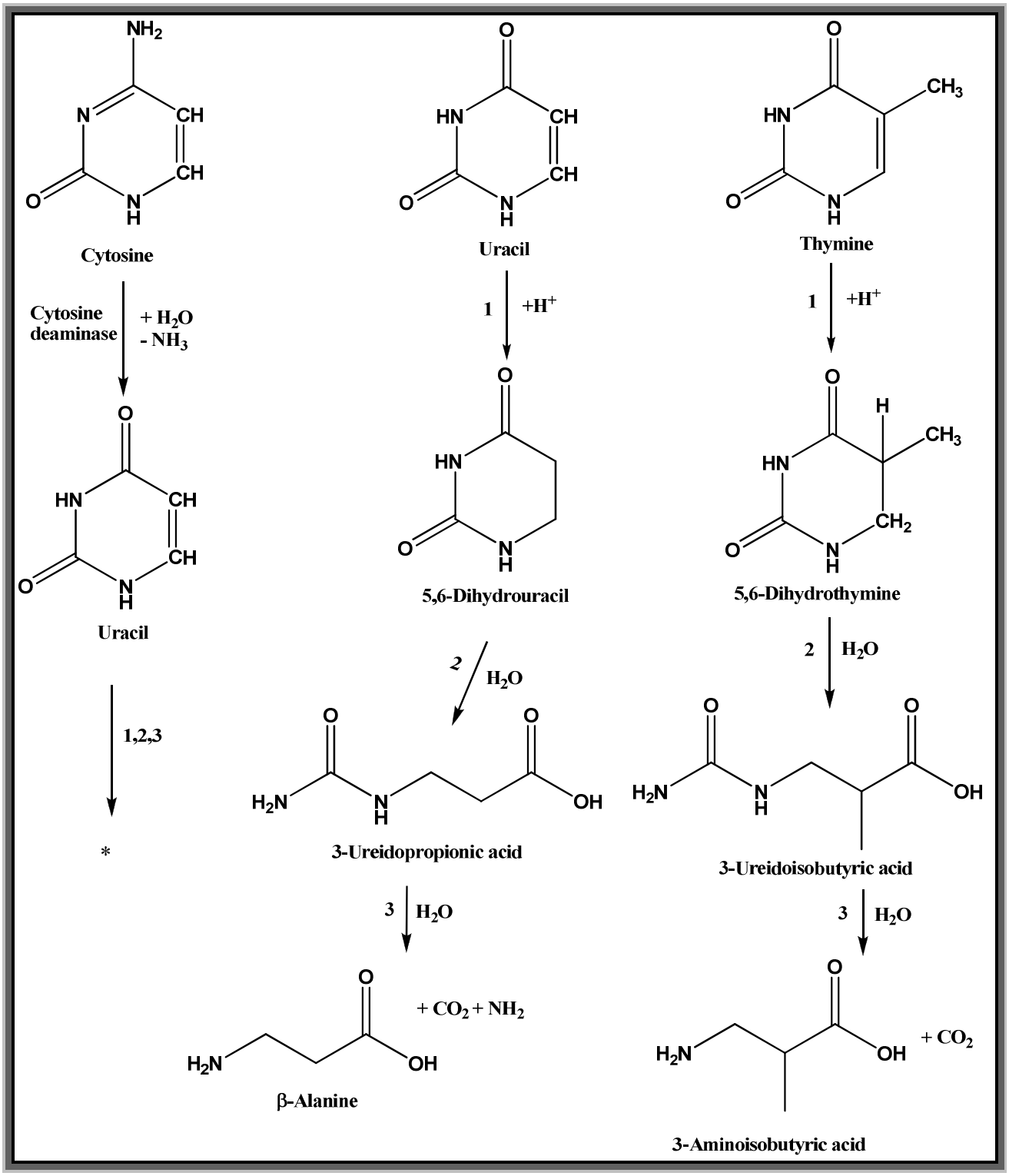


Figure (1-21) structure of ethyl2-(benzylthio)-4-oxo4,4a,7,7a-tetrahydro-3H-pyrrolo(2,3-d)pyrimidine-6-carboxylate

1.2.5 ***Metabolism of pyrimidine:***

The metabolism of uracile and thymine is initiated by reduction reactions to give the dihydro compounds (5,6-dihydrouracil and 5,6-dihydrothymine). These are then hydrolyzed by hydropyrimidine hydrase to the ureido compounds (3-ureidopropionic acid, 3-ureidoiso-butyric acid). Further hydrolysis yields the amino acids (β-Alanine, 3-Aminoisobutyric acid) respectively, while the metabolism of cytosine is initiated by reduction reactions to give the uracil compound. The stages of pyrimidine degradation from cytosine, uracil and thymine are summarized in the scheme (1) **[16].**



Scheme (1)

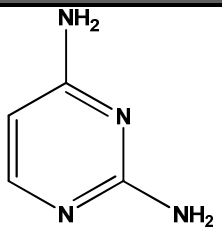
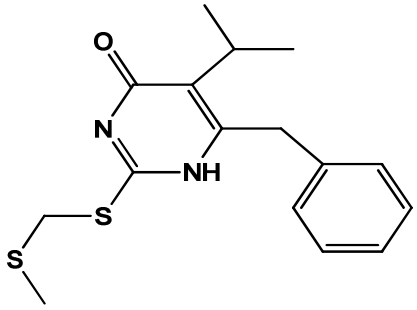
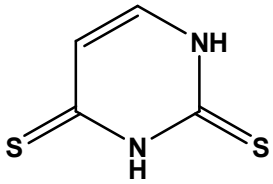
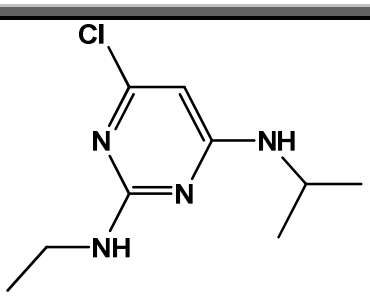
The relevant metabolic pathways of pyrimidine metabolism

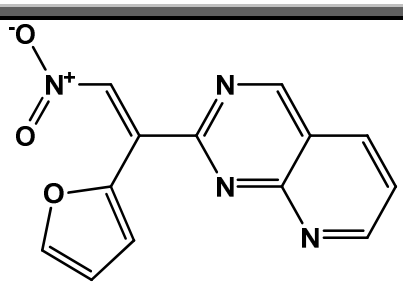
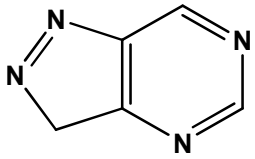
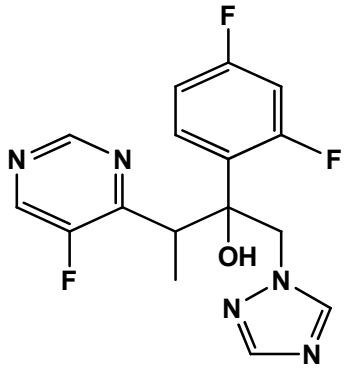
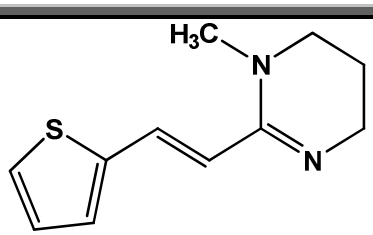
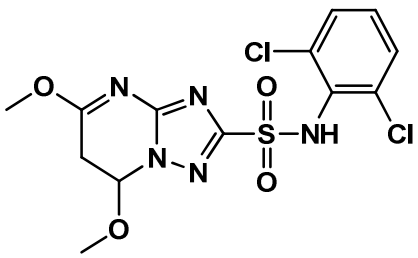
Enzymes involved are: dihydropyrimidine dehydrogenase (1) dihydropyrimidinase (2), and β-ureidopropionase (3). * The same degradation in the secondary column.

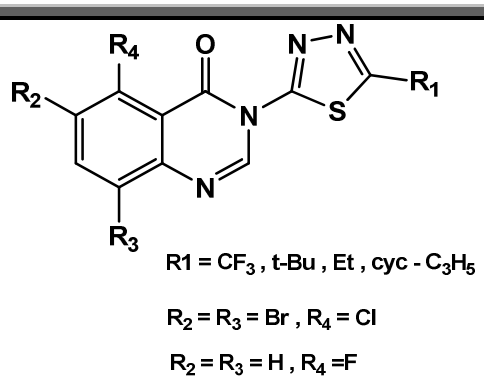
1.2.6 *Uses of Pyrimidine derivatives:*

Pyrimidines and their derivatives have been found to possess a broad spectrum of biological activities such as antimicrobial, anti-inflammatory, analgesic, antiviral and anticancer activities [17-24]. As shown in table (1-2)

Table (1-2): ***Pyrimidine and their derivatives uses***

Compound	*Structure*	*Activity*
2,4 diaminopyrimidine		**Antifolate[25]**
5-isopropyl-2-[(methylthiomethyl)thio]-6-(benzyl)-pyrimidin-4-(1H)-one		**Anti-Human Immunodeficieny Virus Activity[26]**
Dithiouracil		**Anti –leukemia[27]**
4-chloro-2-(ethylamino)-6(2propylamino)pyrim-idine		**Herbicidal[28]**

Nitrofurylvinylpyrido [2,3-d] pyrimidine		**Antibacterial[29]**
3H-pyrazolo[4, 3-d] pyrimidine		**antileukemic and antitumor[30]**
Voriconazole		**antifungal medication[31]**
(*E*)-1-methyl-2-(2-(thiophen-2-yl)vinyl)-1,4,5,6-tetrahydropyrimidine		**Anthelmintic[32]**
N-(2,6-dichlorophenyl)-5,7-dimethoxy-6,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide		**Herbicidal and as plant growth regulator agent[33]**

Cyril		**antibacterial and anticancer**[34]

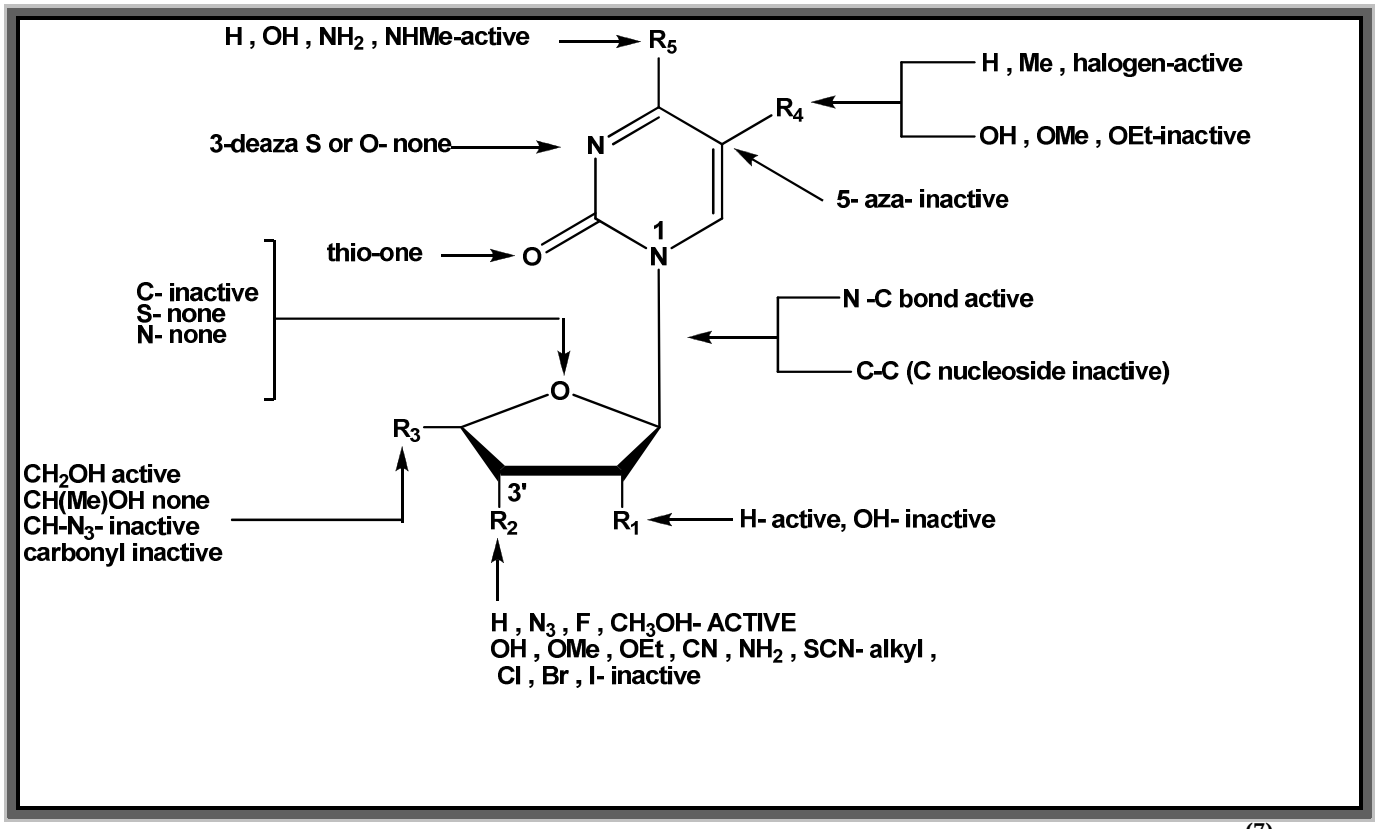


Figure (1-22): Structure-activity relationship of pyrimidine analogues [7]

1.3 *Nucleosides, Nucleotides And Nucleic Acids*

The term "nucleoside" was originally coined by Leven and Jacobs [35]. For some time referred to compounds isolated from nucleic acids that contained a carbohydrate attached through nitrogen to either a purine or pyrimidine base. The evolution of the subject has brought to the point that considered a nucleoside to be a compound of natural or synthetic origin

that has a carbohydrate attached to a nitrogen heterocyclic through either a carbon-nitrogen bond as is commonly found, or a carbon-carbon bond, as found in C-nucleosides, a class of compounds discovered and developed relatively [36a] .

- Esterfication of their 5-hydroxyl group with phosphoric acid leads to nucleotides which are the building blocks of the nucleic acids (DNA and RNA) [37] Fig. (1-23).

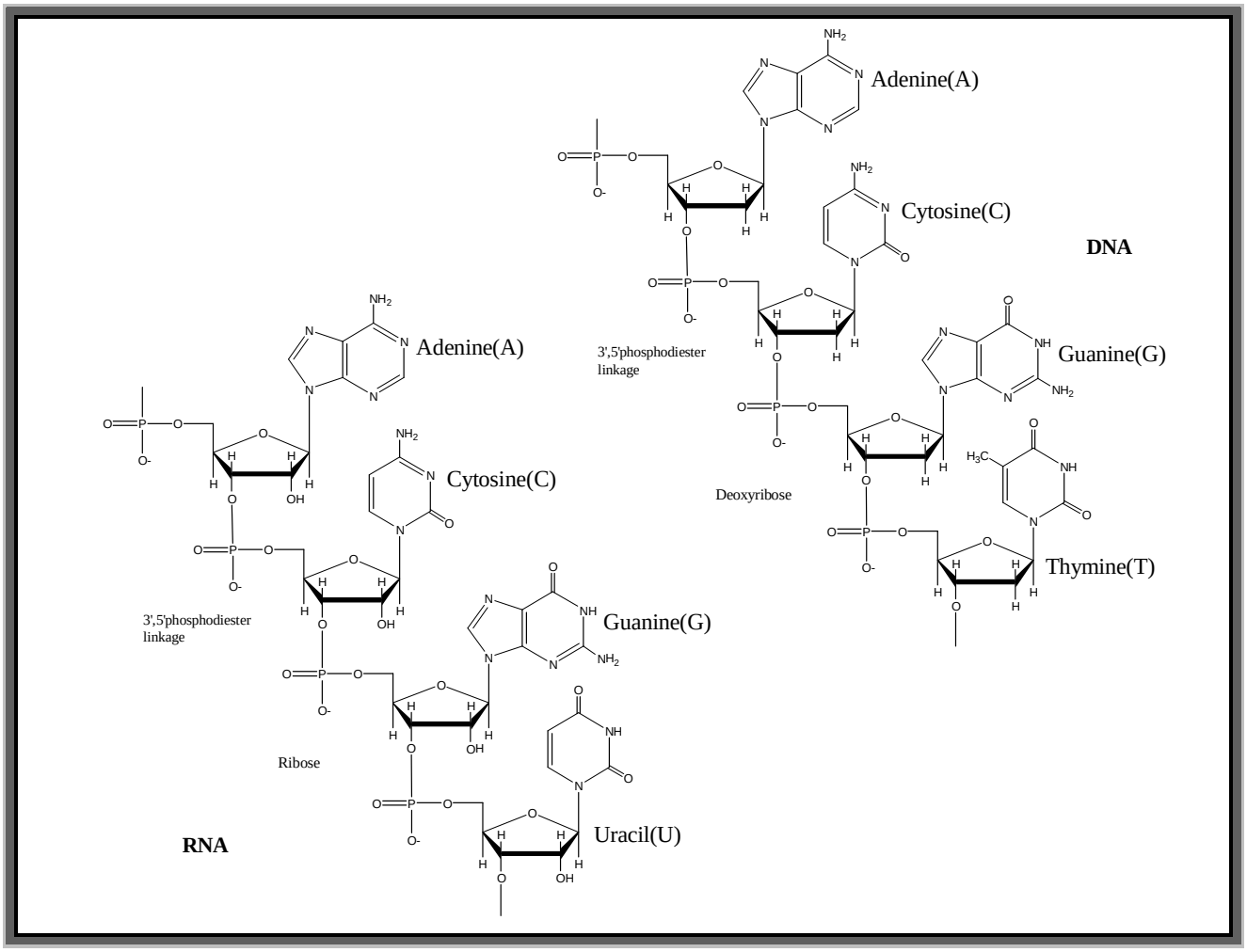


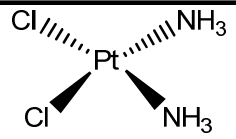
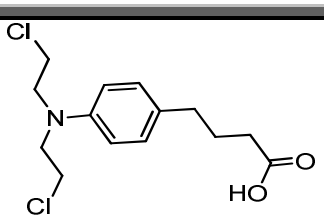
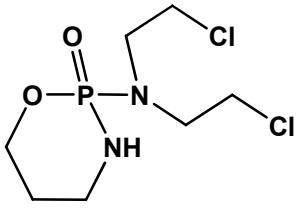
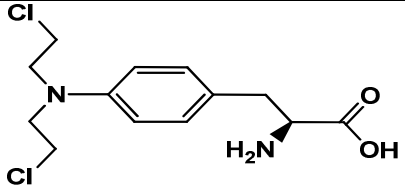
Figure (1-23): Part of chemical structures of the strands of DNA and RNA. The sequence of nucleosides differs for each naturally occurring type of DNA or RNA

1.4 ***Anticancer Drugs*** [38]:

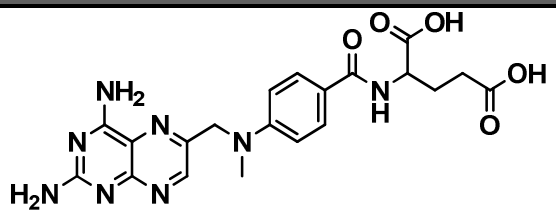
Cancer is a class of diseases in which a group of cells display the traits of uncontrolled growth, invasion, and sometimes metastasis. The resistance of tumor cells to chemotherapeutic agents is a major problem in the clinical treatment of cancer; so a wide array of selective and potent compounds is needed to match the growing problems associated with cancer. Anticancer agents are classified into several broad groups, which are usually defined according to their different mechanisms of action Scheme (2). Most chemotherapeutic agents have the capacity to induce, either directly or indirectly, potential lethal damages to tumor cells.

These agents are classified into:-

(1)Alkylating agents and related compounds such as:

Name	Structure
Cisplatin	
Chlorambucil	
Cyclophosphamide	
Melphalan	

(2)Antimetabolities such as: methotrexate and nucleoside analogues

Name	Structure
Methotrexate	

(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.

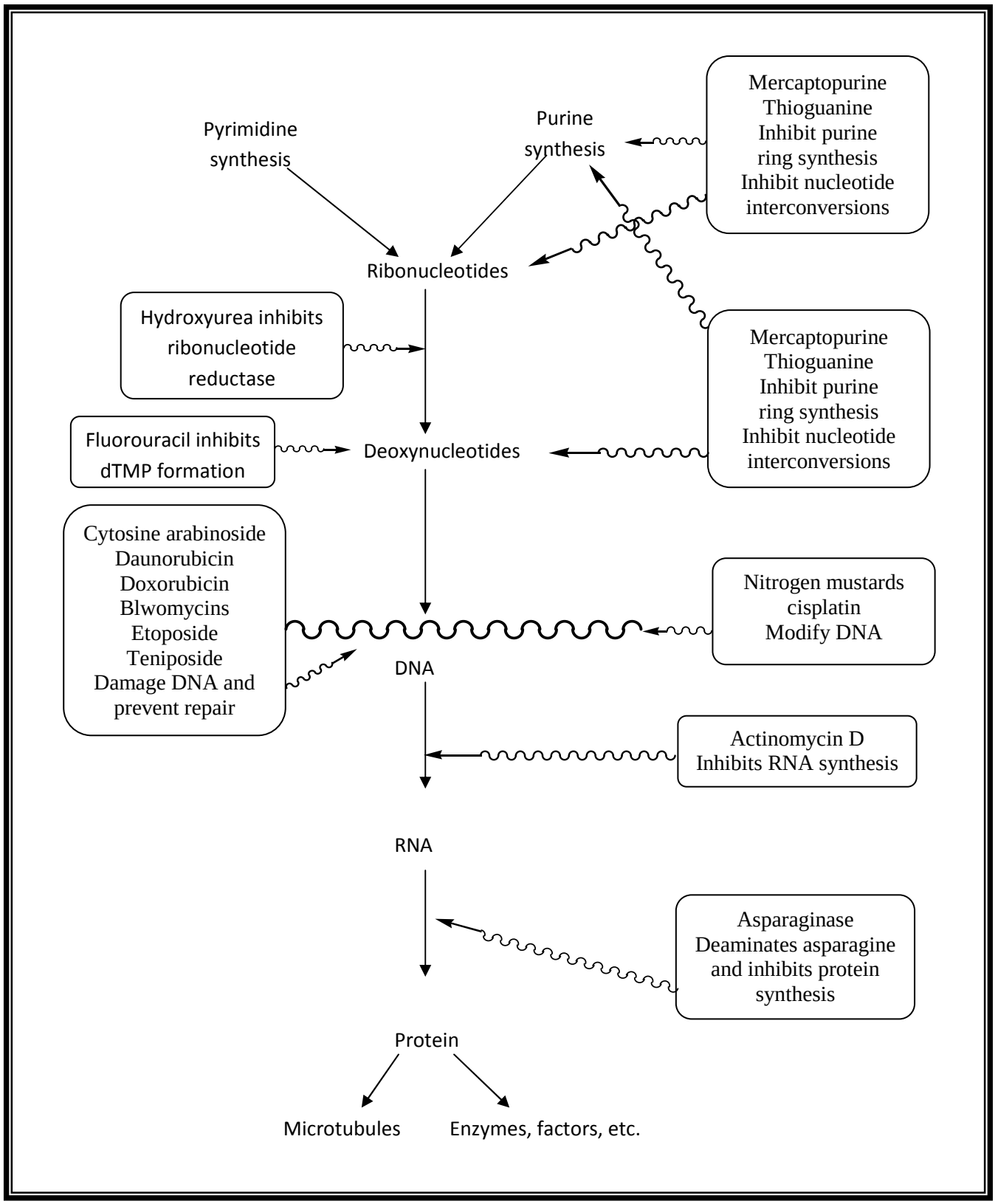
(4) Topoisomerase inhibitors.

(5) Mitotic inhibitors.

(6) Corticosteroids.

(7) Miscellaneous chemotherapy drugs.

(8) Other types of cancer drugs.



Scheme (2): Sites of action of selected drugs used in the treatment of cancer [(39)]

1.5 Cell viability (cytotoxicity):

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. Fig (1-24) 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.

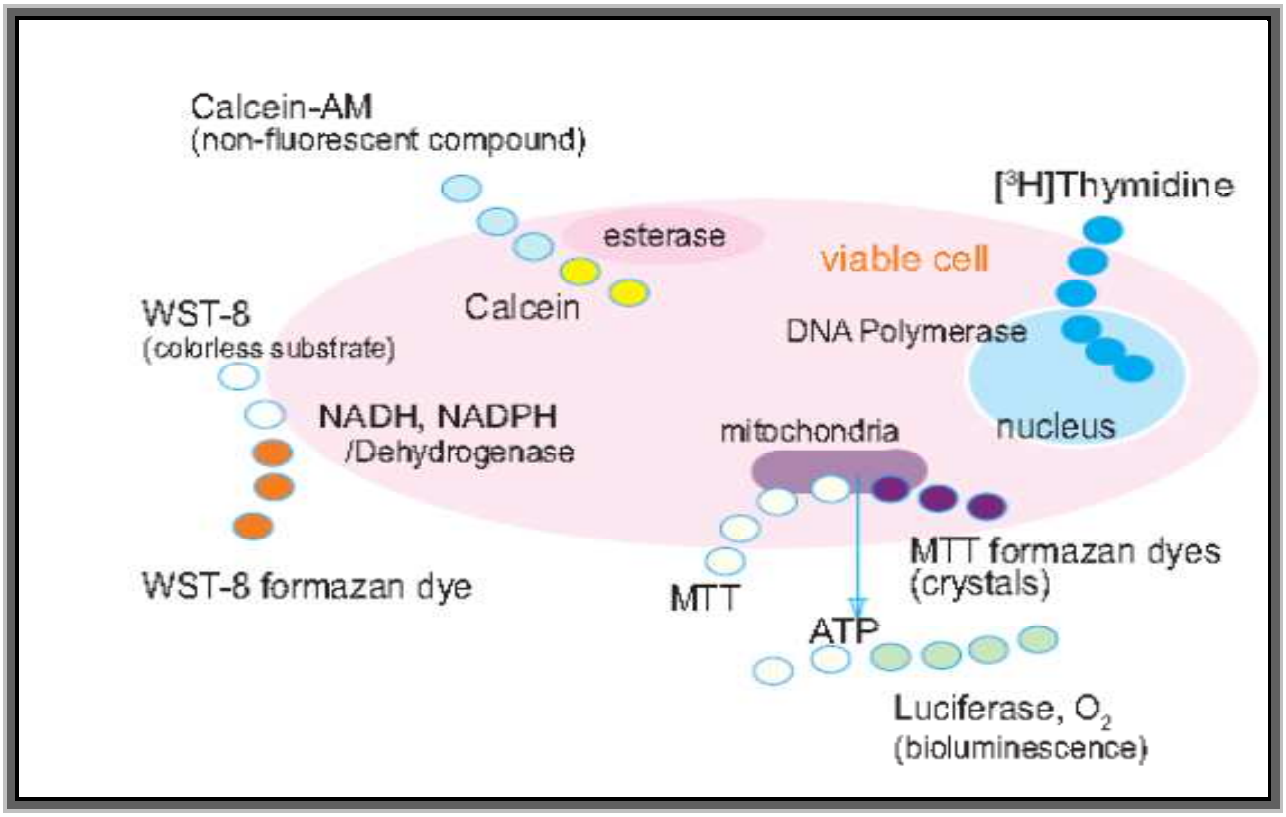


Figure (1-24) Reagents and methods for cell viability detection [40]

1.6 *Biological Assay of Virulence factor Using Animal Tissue Culture technique* [41]*:*

The advantages of tissue culture technique can be achieved as cytotoxic test of new drugs, cosmetics, food additives involves a large number of animal experiment, which are very costly and raise

considerable public concern, therefore much pressure, both human and economic; perform at least part of cytotoxicity testing *in vitro*.

The choice of the assay will depend on: the agent under study, the nature of the response, and the target cell. The assay can be divided into two major classes:

A- An immediate or short term response, such as an alteration in membrane permeability or a perturbation of a particular metabolic pathway.

B- Long- term survival, usually measured by the retention of self-renewal capacity, or survival in altered state e.g.: expressing genetic mutation or, malignant transformation.

A- Short Term Assay – Viability:

Assay of this type are used to measure the proportion of viable cell following a potentially traumatic procedure, such as primary disaggregation, cell separation, or freezing and thawing. Most viability test rely on break down in membrane integrity determined by the uptake of a dye to which the cell is normally impermeable or release of a dye or isotope normally taken up and retained by viable cells. While short – term tests are convenient and usually quick and easy to perform; they only reveal cells that are dead (i.e. permeable) at the time of the assay frequently, cell subjected to toxic influences e.g. antineoplastic drug, will only show an effect several hours or even days later.

B- Long – Term Assay – Survival

Long-term tests are often used to demonstrate the metabolic or proliferation capacity of cells after, rather than during, exposure to toxic influences.

The objective is to measure survival rather than short term toxicity, which may be reversible. Definition of cytotoxicity will tend to vary depending on the nature of the study, whether cells are killed or simply have their metabolism altered or an alteration in cell- cell signalling such as might give rise to an inflammatory or allelic response.

All of the *in vitro* assays advantages are

- Over simplify the events , cheap , easily qualified and reproducible
- Control the environmental (pH, temp., osmotic pressure, CO_2, O_2)

1.7 ***Neutral Red Uptake Assay (NRU):***

The Neutral Red Uptake Assay is a cytotoxicity test which was developed in the 1980s by Bohrenfreund & Puerner (1985) **(42)**. It can be used to test the cytotoxic effects of chemical substances and environmental samples on cell membranes. It is basing on a dyeing reaction which allows distinguishing between dead and live cells. Usually the test is performed with the adherent permanent cell line HEP-G2 (human hepatocyte cells). But also several other cell lines like human keratinocytes or fish cells can be used.In the microtitre plate version a monolayer cell culture, which has been grown over a period of 24 hours, is exposed over a defined period of time (usually between 2 and 24 hours) against different concentrations of the substance to be tested. The metabolic activation of toxic substances in the organism can be simulated. At the end of the exposure period the cells are washed and the Neutral Red dye is added. It passes the cell membrane and binds to intracellular phosphate and carboxyl groups. The accumulation of the dye takes place mainly in the lysosome. Dead cells or such with membrane damage cannot accumulate the dye, so that during the following washing and fixation steps the dye stuff is not retained intracellularly. Wells with

dead or damaged cells or with a reduced number of cells due to growth inhibitive effects are less coloured than those with vital cells. The evaluation of the test is done by resolving the intracellular bound dye and measuring the intensity photometrically in comparison to an untreated control sample. The toxicity is expressed as percent of rate of inhibition [43].

1.8 *MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole)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, fig.(1-25). Reduction occurs outside the cell via plasma membrane electron transport [44]. 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.

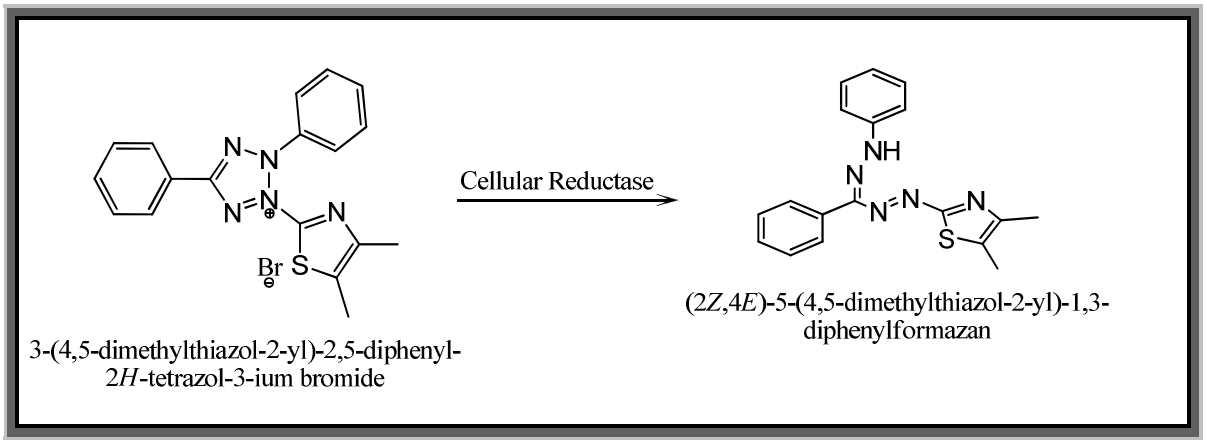


Figure (1-25) Reduction of MTT reagent

A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate 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 [(45)].

Absorbance detection has been available in microplate 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 [(46)].

1.9 ***Hepatocyte (Hep G2):***

Hep G2 is a perpetual cell line which was derived from the liver tissue of a 15 year old Caucasian American male with a well differentiated hepatocellular carcinoma. These cells are epithelial in morphology, have a model chromosome number of 55 and are not tumorigenic in nude mice. The cells secrete a variety of major plasma proteins; e.g., albumin, transferrin and the acute phase proteins fibrinogen, alpha 2-macroglobulin, alpha 1-antitrypsin, transferrin and plasminogen. They have been grown successfully in large scale cultivation systems. HepG2 cells are a suitable in vitro model system for the study of polarized human hepatocytes. Because of their high degree of morphological and functional differentiation in vitro, HepG2 cells are a suitable model to study the intracellular trafficking and dynamics of bile canalicular and sinusoidal membrane proteins and lipids in human hepatocytes in vitro [(47)].

1.10 ***breast cancer (MCF7):***

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 [(48)].

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. 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 [49].

1.11 ***The Aim of This Work:***

1) Synthesis of some of pyrimidine derivatives.
2) Screening of the synthesized compounds for their potential cytotoxicity against Hepatocyte (HepG2) and breast cancer (MCF7) cell lines *in vitro*.

EXPERIMENTAL

2.1.1 ***Instruments:***

- Melting points were measured by using (Gallen Kamp / England) melting point.

- F.T.IR-8300 Fourier transforms infrared spectrophotometer SHIMADZU the (600-4000) cm^{-1} spectral range.

- (SHIMADZU UV-Vis. 160A) ultraviolet spectrophotometer.

- Hot plate + Magnetic stirrer (Gallenkamp / England)

- Oven (Memmert / Germany)

- Balance (TP SERIES / Chinese)

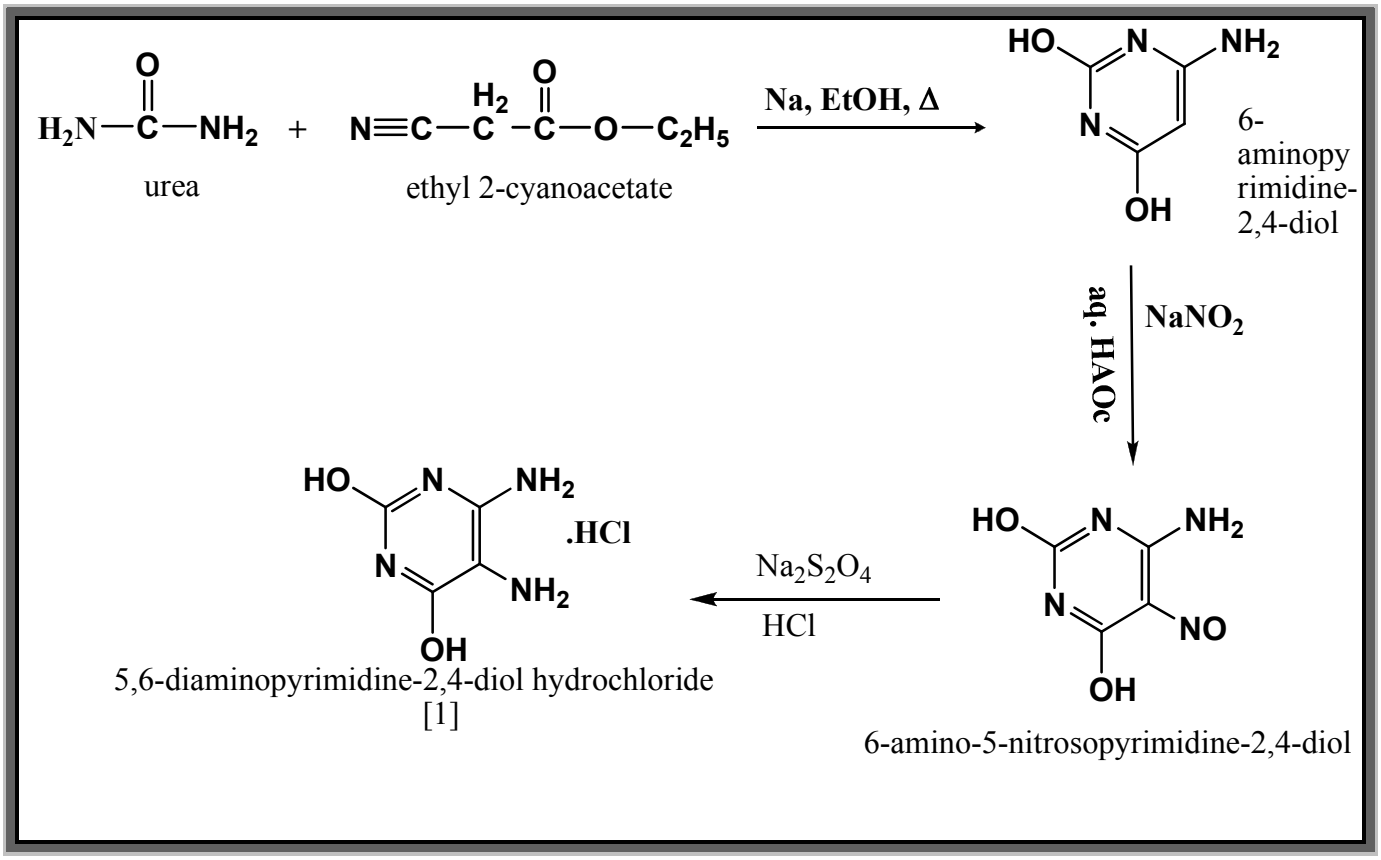
2.1.2 ***Materials:***

Table (2-1): ***Chemical materials used in the current study***

Number	***Material***	***Company***
1	Abs.Ethanol	Himedea
2	Acetone	B.D.H
3	Dimethylsulfoxide (DMSO)	B.D.H
4	Ethyl acetoacetate	B.D.H
5	Ethyl cyanoacetate	B.D.H
6	Glacial acetic acid	B.D.H
7	Hydrazine Hydrate (99%)	B.D.H
8	Hydrochloric acid	Himedea
9	n.hexane	B.D.H
10	4-N,N-dimethyl amion benzaldehyde	Merck

11	Potassium bromide for infra-red spectroscopy	B.D.H
12	Sodium dithionite (sodium hydro sulphite)	B.D.H
13	Sodium Hydroxide	B.D.H
14	Sodium nitrite	Fluka
15	Sodium(Metal)	B.D.H
16	Thiourea	B.D.H
17	Urea	B.D.H

2.1.3 *Synthesis of 5,6-diaminouracil-2,4-diol hydrochloride[1]:*[(50)]

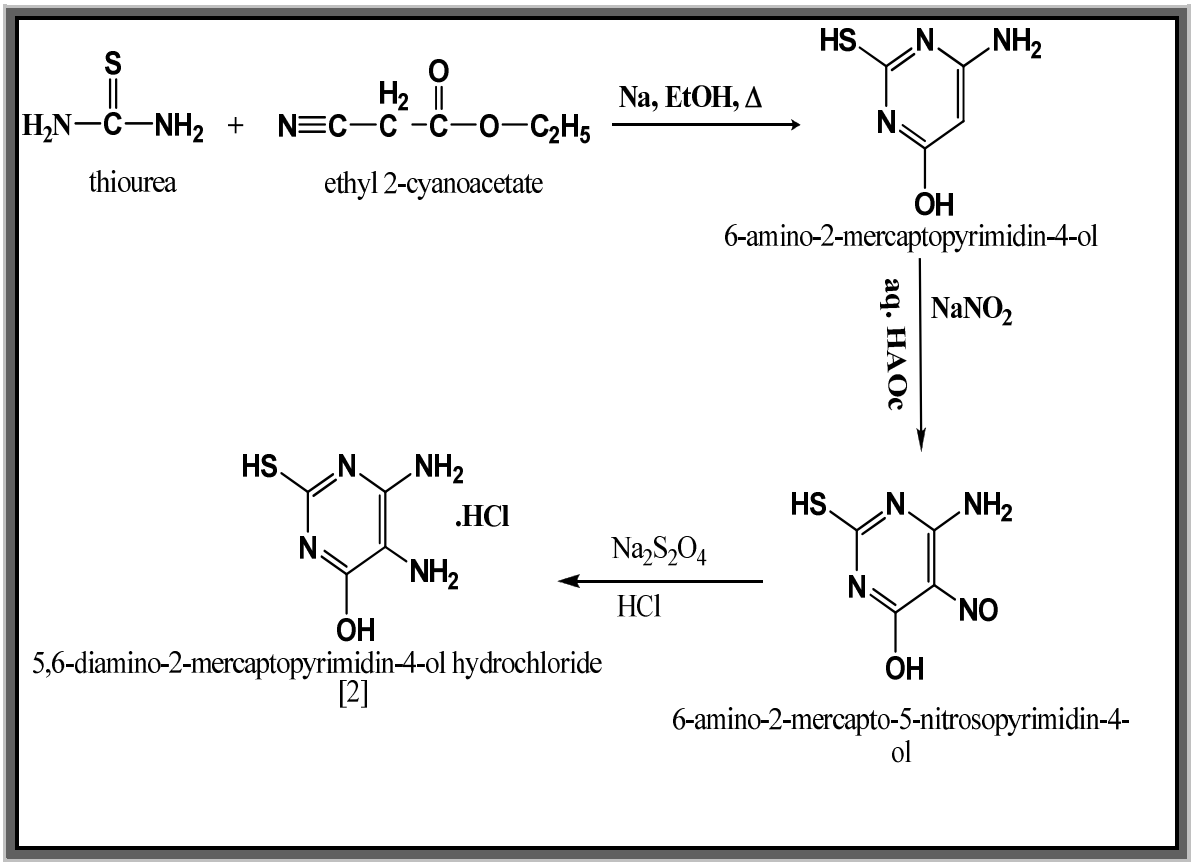


Scheme (3) Synthesis of 5,6-diaminouracil-2,4-diol hydrochloride

Three-necked flask equipped with a reflux condenser and an efficient stirrer was placed. To 203ml. of absolute ethanol (99.99%), (8g, 0.34 g. atom) of sodium, (18.5 ml, 0.17 mole) of ethyl cyanoacetate , and (10.4 g, 0.17 mole) of urea were added. The mixture was heated under reflux for 4 hours. (203ml) of hot (80°) water was added; the stirred mixture was heated at 80° for 15 minutes and then neutralized to litmus with glacial acetic acid. Additional glacial acetic acid (15.2 ml.) was added, followed by cautious addition of a solution of (13.1 g, 0.19 mole) of sodium nitrite which was dissolved in (14.1ml) of water. The nitroso compound was removed by filtration and washed twice with a small amount of ice water. The moist material was transferred back to the flask, and (87.3 ml) of warm water (50°) was added. Then an additional (6.1g) of sodium hydrosulfite was added. The dense diaminouracil bisulfite was filtered

from the cooled solution, washed well with water, and partially dried. Then concentrated hydrochloric acid was added until the consistency of the resulting mixture was such as to permit mechanical stirring (20 to 40 ml. of acid). The slurry was heated on a steam bath with stirring for 1 hour. Tan diaminouracil hydrochloride was filtered on a sintered glass funnel, washed well with acetone.

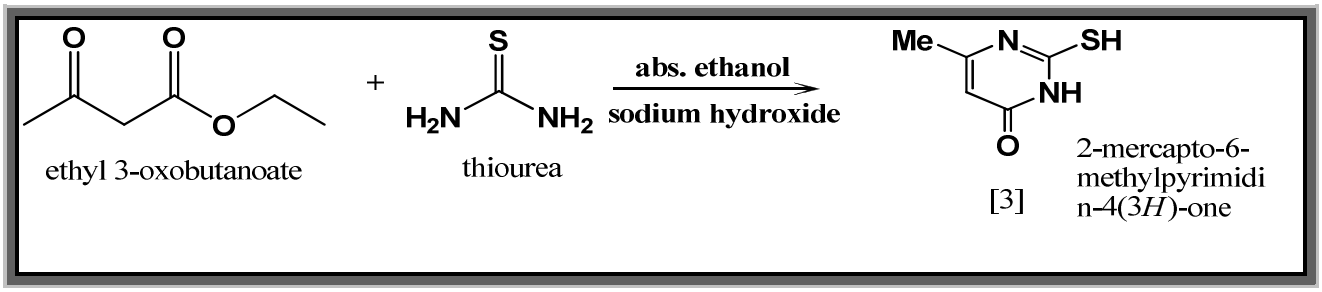
2.1.4 *Synthesis of 5,6-diamino-2-mercaptopyrimidine-4-ol Hydrochloride[2]:*



Scheme (4) Synthesis of 5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride

The same way of the previous procedure, by replacing urea rather than (13.29 g, 0.17 mole) of thiourea were added to ethylcyanoacetate in a strong basic medium.

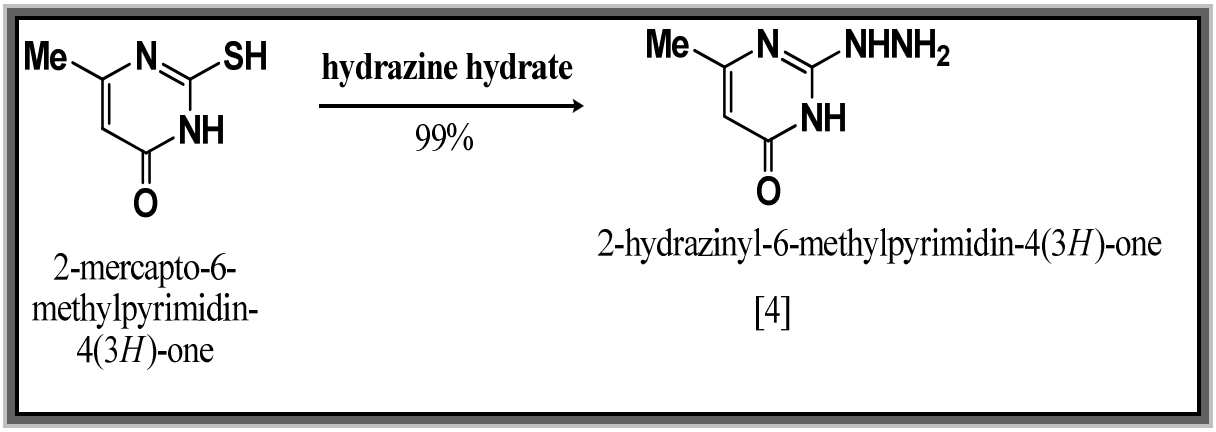
2.1.5*Synthesis of 2-mercapto-6-methylpyrimidine-4(3H)one[3]*:[51]



Scheme (5) Synthesis of 2-mercapto-6-methylpyrimidine-4(3H)one

0.1 mole, 3.9 g of sodium hydroxide in (2.4 ml) water was added to the mixture of the (0.04 mole, 3 g) of thiourea and (0.04 mole, 5.0ml) of ethylacetoacetate in (4 ml) of ethanol in round bottomed flask and the mixture was refluxed for (2 hours), then hot solution was added (8 ml) of concentrated hydrochloric acid in (4 ml) of water to the product. The product was filtered and washed with cold distilled water.

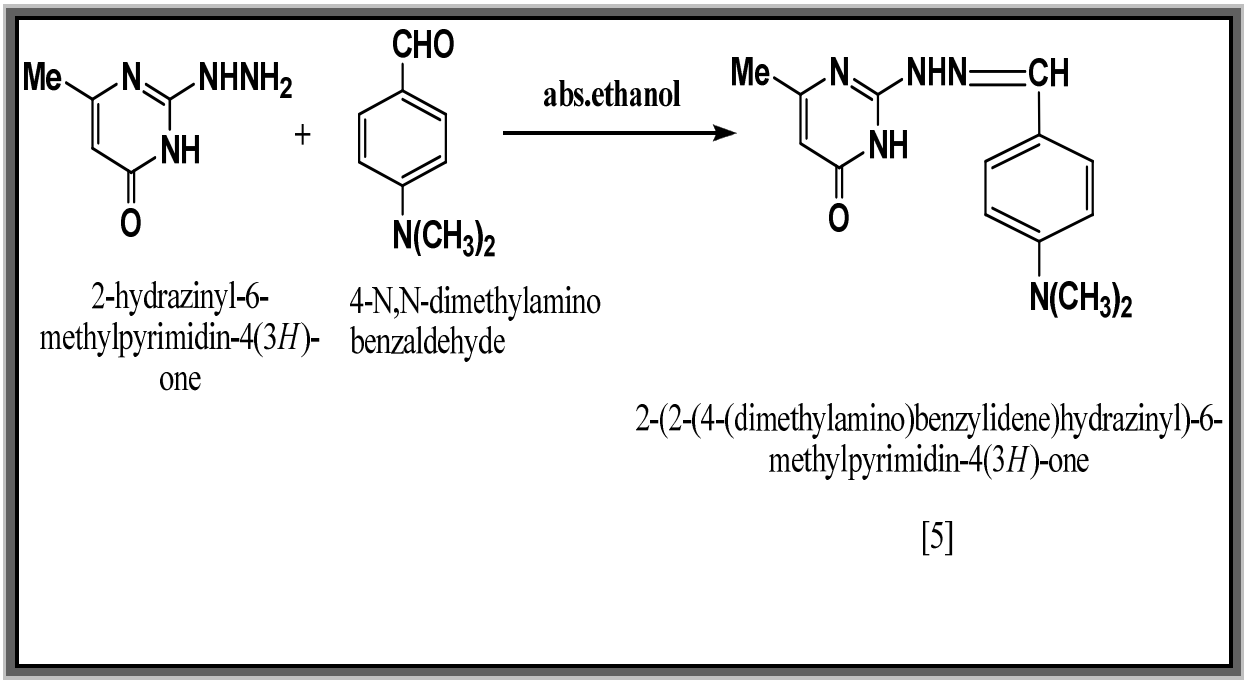
2.1.6*Synthesis of 2-hydrazinyl-6-methylpyrimidine-4(3H)one[4]:* [52]



Scheme (6) Synthesis of 2-hydrazinyl-6-methylpyrimidine-4(3H)one

A mixture of 0.02 mole, 3.63 g of 2-mercapto-6-methylpyrimidin-4(3H)-one and (12.7 ml) of hydrazine hydrate (99%) was refluxed in around bottomed flask for (3 hours). The product was filtered and washed with cold distilled water.

2.1.7 ***Synthesis of 2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidine-4(3H)-one[5]:***



Scheme (7) Synthesis of Synthesis of 2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidin-4(3H)-one

A mixture of 0.002 mole, 0.28g of 2-hydrazinyl-6-methylpyrimidin-4(3H)-one and (0.002 mole, 0.29g) of 4-N,N-dimethylamino benzaldehyde in around bottomed flask with (5ml)ethanol, 1-2 drops of glacial acetic acid were added, and the mixture was refluxed for 5 hours, ice bath was used to separate the product, and the mixture was then filtered and the precipitate was isolated.

Table (2-2): Physical properties of synthesized compounds

Name of compound	Molecular formula	Molecular Weight (g/mole)	Yield (%)	M.P (C°)	color
5,6-diaminouracil-2,4-diol hydrochloride. [1]	$C_4H_7ClN_4O_2$	178.58	59.4	298-300	Light tan
5,6-diamino-2-mercap topyrimidine-4-ol hydrochloride.[2]	$C_4H_7ClN_4OS$	194.64	30.3	300-303	Light yellow
2-mercapto-6-methyl pyrimidine-4(3H)-one.[3]	$C_5H_6N_2OS$	142.02	64.2	330	Yellowish -white
2-hydrazinyl-6-methyl pyrimidine-4(3H)-one.[4]	$C_5H_8N_4O$	140.07	42.8	212-214	white
2-(2-(4 (dimethylamino) benzylidene)hydrazinyl) -6-methylpyrimidine -4(3H)-one.[5]	$C_{14}H_{17}N_5O$	271.14	55.5	300	orange

2.2Biological Study:

2.2.1 Cytotoxicity Assay(Neutral Red method (NR)):

2.2.1.1Instruments and tools:

1. CO_2 Incubator (SANYO, Japan)
2. Inverted Microscope (MEIJI, Japan)
3. Laminar Air Flow (NAPCO, France)
4. ELISA Reader (ASYS, Austria)
5. Centrifuge (SANYO, Japan)

2.2.1.2 Materials

Minimum essential media (MEM), Fetal Calf Serum (FCS), Phosphate Buffered Saline (PBS), Penicillin G, Streptomycin, hepes and Neutral Red were obtained from Sigma. Trypsin, Na_2HPO_4, Ethanol, trypan blue and Sodium Bicarbonate $NaHCO_3$ were obtained from BDH.

2.2.1.3 Preparation of solutions

2.2.1.3.1 Minimum essential media (MEM)

Liquid MEM medium was used in this study which supplied with L-glutamine, $NaHCO_3$ and hepes. This media supplied in solution state and ready to be used.

2.2.1.3.2 Fetal calf serum (FCS)

10% FCS added to the media to support the cell growth.

2.2.1.3.3 Phosphate buffer saline (PBS)

One tablet of (PBS) was dissolved in (200) ml of distilled water sterilized by autoclave then used.

2.2.1.3.4 Antibiotic solutions

Penicillin G, sodium salt (1000000 IU) and streptomycin (1) gm were each dissolved in (5) ml of distilled water (DW) and stored at -20°C From each of these stocks, (0.5) ml was added to one liter of culture media.

2.2.1.3.5 Trypsin solution

(2.5) gm for each (100) ml of PBS dissolved then sterilized by filtering using (0.22) µm Millipore filter and stored at 4°C.

2.2.1.3.6 Neutral red dye

A quantity of (10) mg neutral red was dissolved in (100) ml of (PBS), mixed thoroughly and used immediately.

2.2.1.3.7 Eluent

One volume of Na_2HPO_4 (0.1) M was mixed with one volume of absolute ethanol.

2.2.1.3.8 Trypan blue stain

This stain was prepared by dissolving (0.1) g of trypan blue stain in (100) ml of (PBS), then filtered by using filter paper. Finally the solution was stored at 4C° until used.

2.2.1.4 Hepatocellular (HepG2) Cell line and Culture Conditions

The cells grow as a monolayer with morphology of spindle cells and multinucleated cells .HepG2 cell line which used in this study they were supplied by Animal cell culture lab, Biotechnology Research Centre / AL- Nahrain University. Cells were cultured in MEM media supplemented with 10% FCS, 50 mg/ml streptomycin and 1000U/L penicillin. Cell line was grown as a monolayer

in humidified atmosphere at 37°C with 5% CO_2. The experiments were performed when cells were healthy and at logarithmic phase of growth.

***2.2.1.5 Method of cytotoxicity assay*:**

Single cell suspension was prepared by treating 25 cm^3 tissue culture flask with 2 ml trypsin solution incubated for 2 min at 37°C in an incubator supplemented with (5%) CO_2 after detachment of the cells from the flask surface single cell suspension by gently taping of the flask followed by the addition of 20 ml of growth medium supplemented with 10% fetal calf serum then the viability test of the cells was made by using trypan blue dye which stains the dead cells. Cell suspension was well mixed followed by transferring 200 µl/well of the 96 well flat bottom micro titer plate using automatic micropipette containing ($1x10^5$ cell/well). Plates were incubated at 37°C in an incubator supplemented with (5%) CO_2 until 60-70% confluence of the internal surface area of the well for HepG2 cell line, the cells were then exposed to different concentration(600,300,150,75and 37.5 µg/ml) of new synthesis compounds, each compound was added to the cells in triplicate form of each concentration, only cells incubated with culture media represented the negative control, then the 96-well cell culture plate incubated at 37°C in an incubator supplemented with (5%) CO_2 for 48 hrs. After elapsing the incubation period, 50 µl/well of neutral red dye freshly prepared were added to each well and incubated again for 2 hrs, viable cells will uptake the dye and the dead not, the plates washed by PBS to remove the excess dye, then 100µl/well of eluent solution were added to each well to draw out the dye from the viable cells. Optical density of each well was measured by using *ELISA* reader at a transmitting wave length on 492nm. Then inhibition rate was determined for each concentration according to the formula [(53)].

Inhibition Rate = ((Abs. at 492nm of control – Abs. at 492nm of test) /Abs at 492 of control) *100

Abs. = Absorbance

2.2.2 Cytotoxicity Assay(MTT assay):

2.2.2.1 *Instruments and tools:*

- Hidex Chamelon plate Reader with a 570nm filter
- Multi-channel pipette (8 or 12 channel: 10-100µl)
- Pipette tips for 10-100µl
- CO_2 incubator
- Clean hood
- Hematocytometer or cell counter

2.2.2.2*Materials:*

- MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MW = 414), 10 vials, each containing 5 mg ((50603 Kuala Lumpur, Malaysia))
- SDS sodium dodecyl sulfate (MW = 288), 10 vials, each containing 1 gm
- phosphate-buffered saline (PBS), sterile HCl, 0.01 M solution dimethylsulfoxide (DMSO) optional

2.2.2.3Procedure:

• Cell density of 10,000 cells.

• Treatment of 1, 2, 3 and 5 on cancer cell-line for 24 hours.

• Treatment prepared in complete media followed by 2 times serial dilution (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL).

• MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] salt incubation for 2 hours

• MTT salt removed and dissolved in DMSO

• Reading at 570nm wavelength (Hidex Chamelon plate reader)

Data analysis was done in µg/ml and log values of µg/ml are being plot in graph pad prism using (cell viability) versus normalized response curve. Cell viability= (Abs. of test / Abs. of control)*100

2.3 ***Statistical Analysis:***

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using SAS computer program version 7.5.[(54)]. Differences in results were considered significant at probability value equal or less than 0.0001

RESULTS & DISCUSSION

3.1 *Synthesis of 5,6-diaminouracil-2,4- diol hydrochloride[1]:*

It was prepared from the reaction of ethylcyanoacetate with urea in a strong basic medium and the crude product (6-amino-5-nitrosouracil-2,4-diol) is readily purified by conversion to its hydrochloride salt. The following mechanism was suggested [(55)] **.** Figure (3-1)

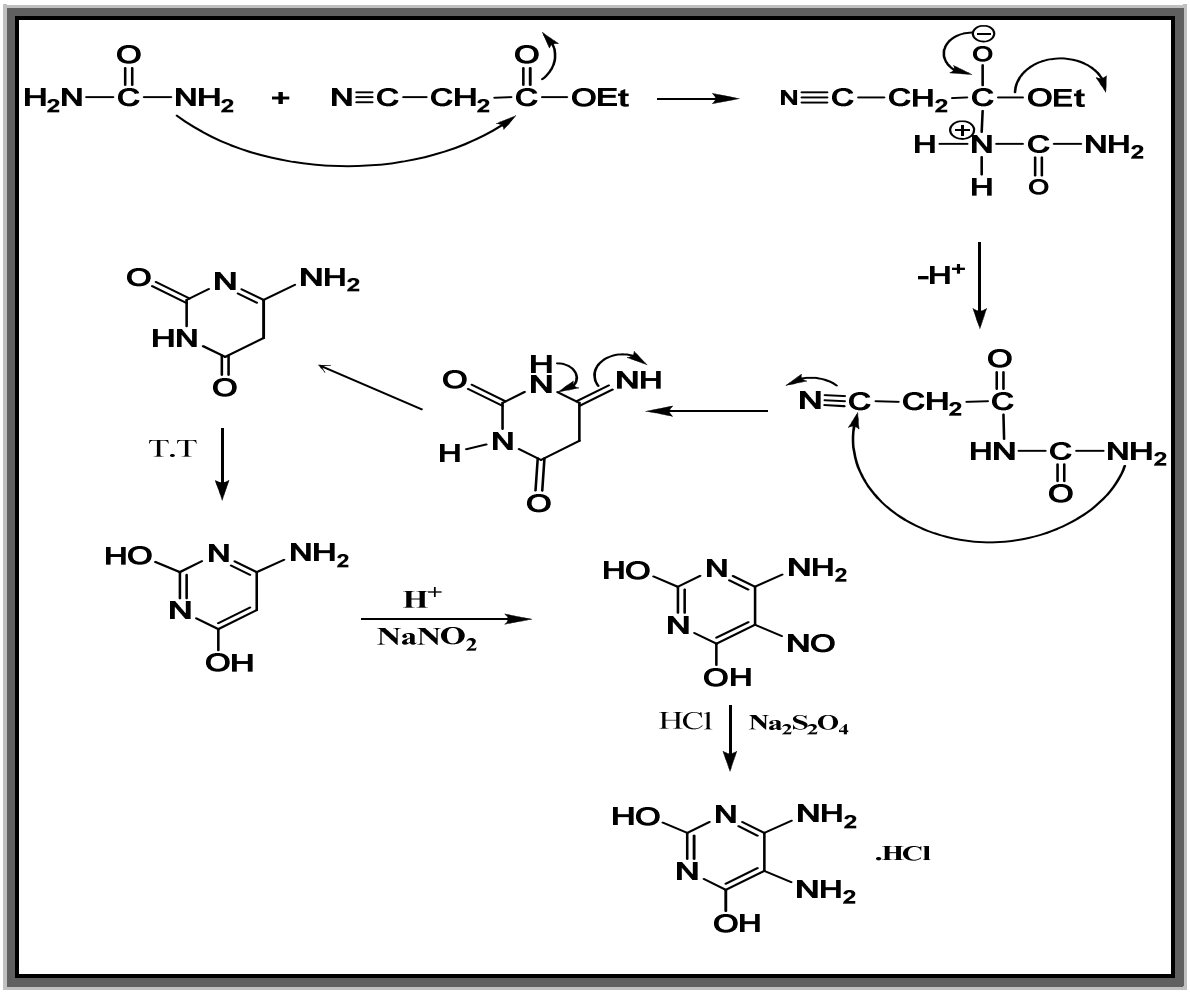


Figure (3-1) mechanism of the preparation of 5,6-diaminouracil-2,4- diol hydrochloride

F.T.IR. spectrum of (6-amino-5-nitrosouracil-2,4-diol) is shown in figure(3-1), the two bands at the 3325.0 cm^{-1} and 3138.0 cm^{-1} were due to asymmetric and symmetric stretching vibration of (NH_2) group, respectively. Beside this, the bands at 1660.6cm^{-1}, 1568.0cm^{-1} and

1340.4cm^{-1} represented the stretching vibrations of aromatic (C=N), (C=C) and (-N=O) groups, respectively.

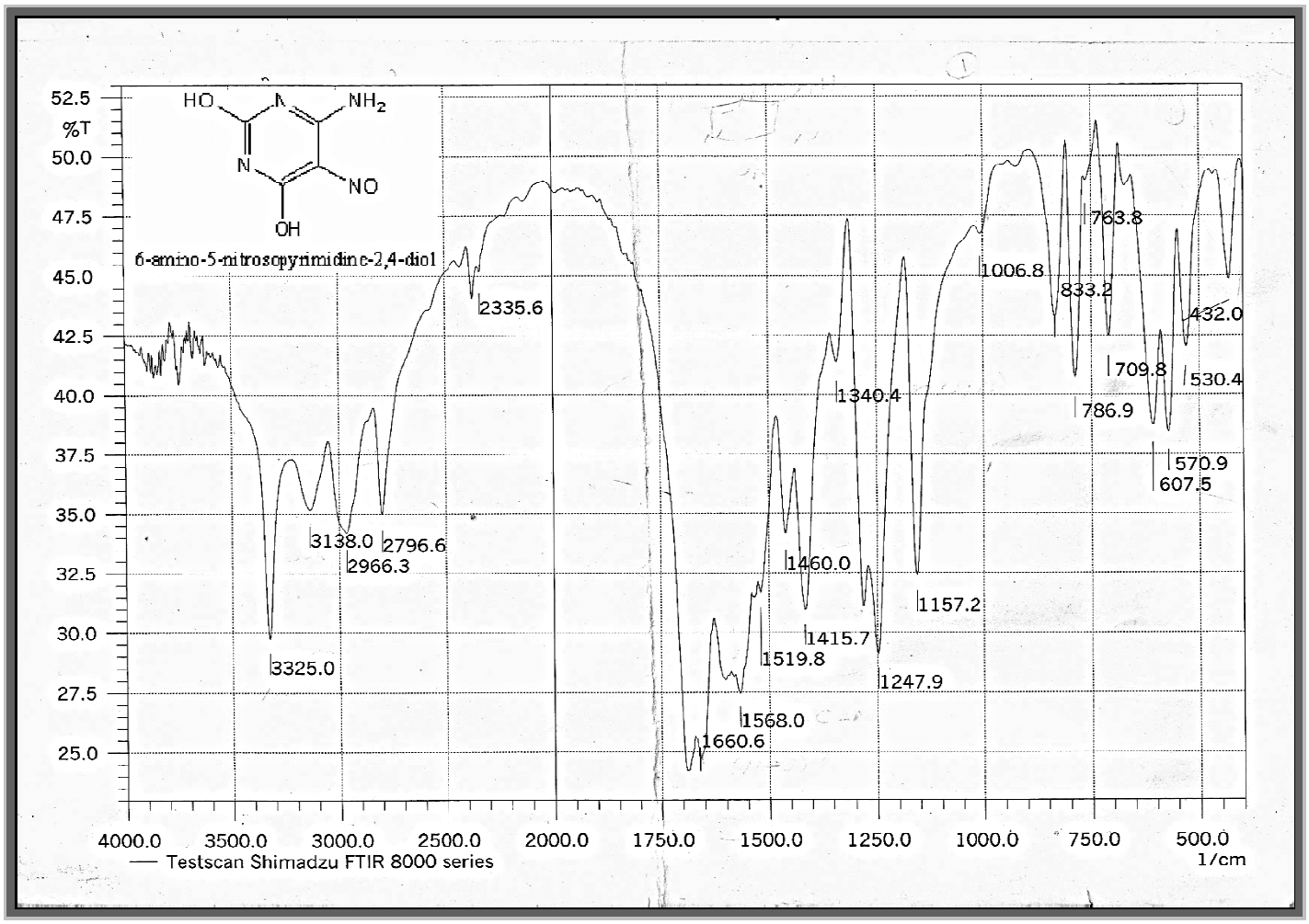


Fig. (3-2): FT-IR spectrum of 6-amino-5-nitrosouracil-2,4-diol

No.	ν NH_2 cm^{-1}	ν C=O cm^{-1}	ν C=N cm^{-1}	ν C=C cm^{-1}	ν N=O cm^{-1}	ν C-N cm^{-1}
1	3325.0-3138.0	1690	1660.6	1568.0	1340.4	1157.2

Disappearance of band at 1340.4 cm^{-1} which is attributed to (N=O) stretching frequency is good evidence for the structure given to (5,6-diaminouracil-2,4-diol hydrochloride).

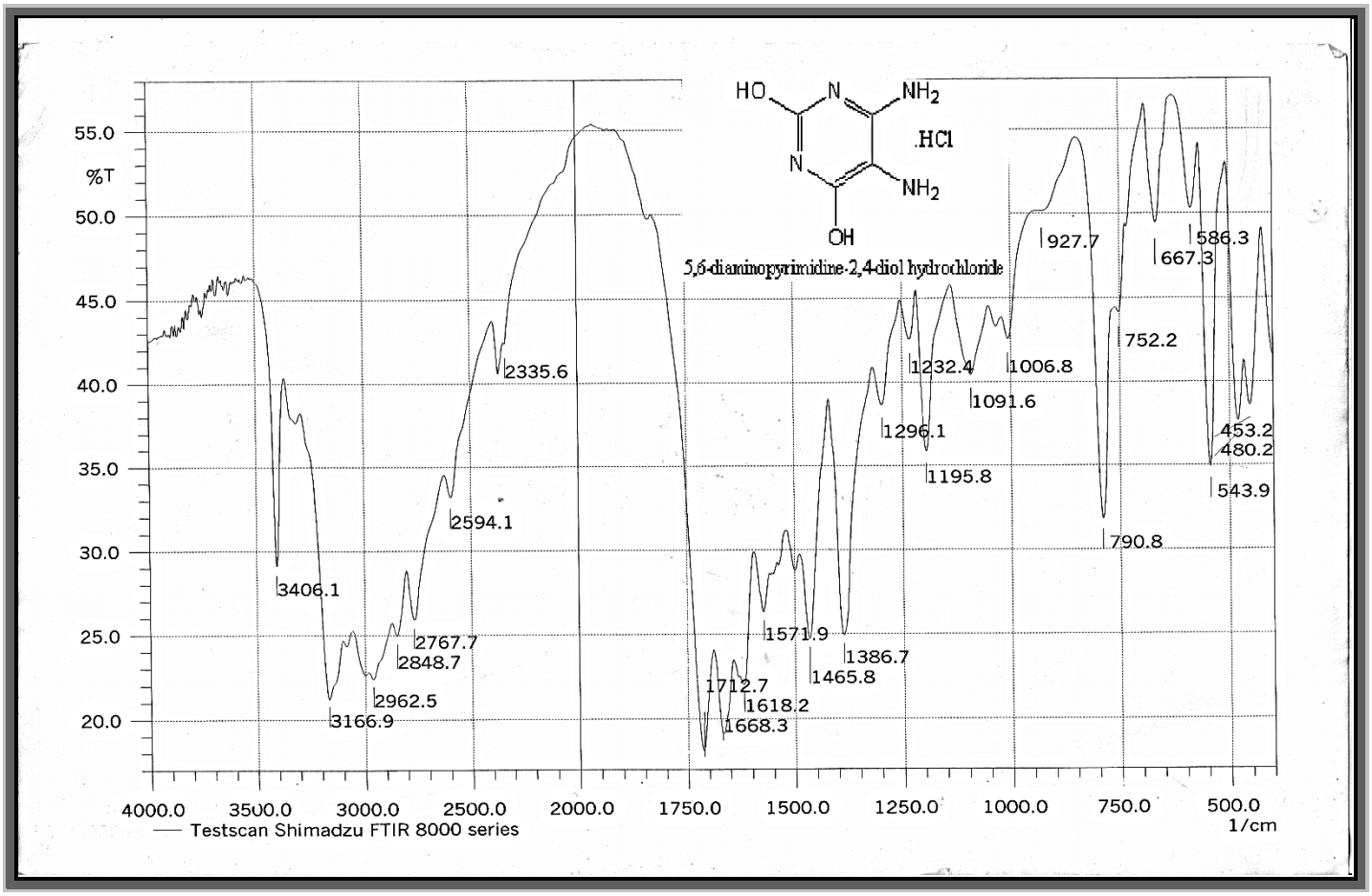


Fig. (3-3): FT-IR spectrum of 5,6-diaminouracil-2,4- diol hydrochloride

No.	ν O-H cm^{-1}	ν NH_2 cm^{-1}	ν C=O cm^{-1}	ν C=N cm^{-1}	ν C=C cm^{-1}	ν C-N cm^{-1}
2	3406.1	3166.9-3100	1712.7	1668.3	1618.2	1195.8

3.2 ***Synthesis of 5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride[2]:***

It was prepared from the reaction of ethylcyanoacetate with thiourea in a strong basic medium and the crude product (6-amino-2-mercapto-5-nitrosopyrimidin-4-ol) was readily purified by conversion to its hydrochloride salt, through the following suggestive mechanism [(55)]. Figure (3-4)

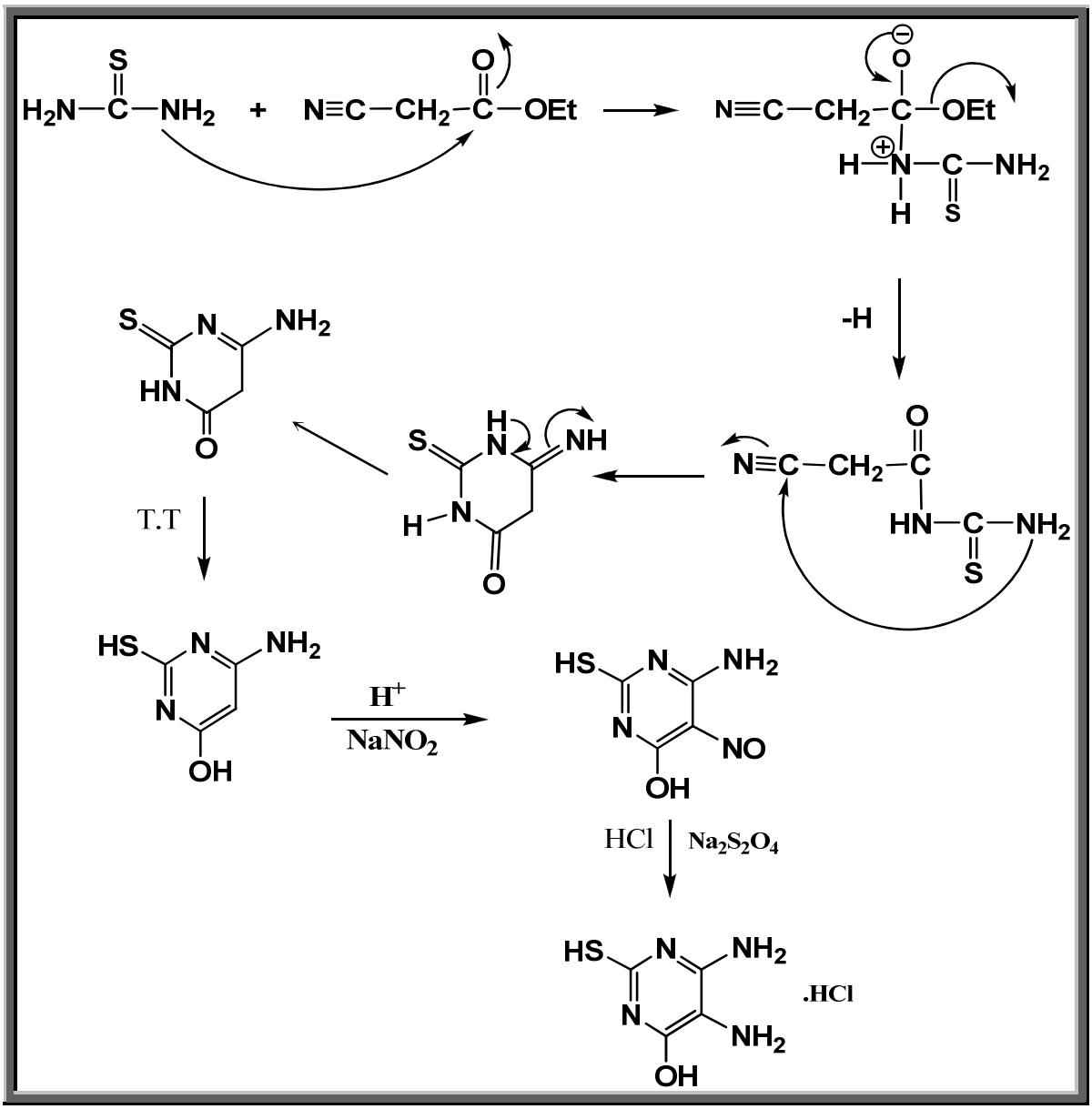


Figure (3-4) mechanism of the preparation of 5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride

F.T.IR. spectrum of (6-amino-2-mercapto-5-nitrosopyrimidin-4-ol), figure (3-3), shows the following characteristic bands: the two bands at the

3323.1cm^{-1} and 3143.8 cm^{-1} were due to asymmetric and symmetric stretching vibration of (NH_2) group, respectively. Bands at 1651.0cm^{-1} and 1251.7cm^{-1} are due to the stretching vibrations of aromatic (C=N) and (-N=O) groups, respectively.

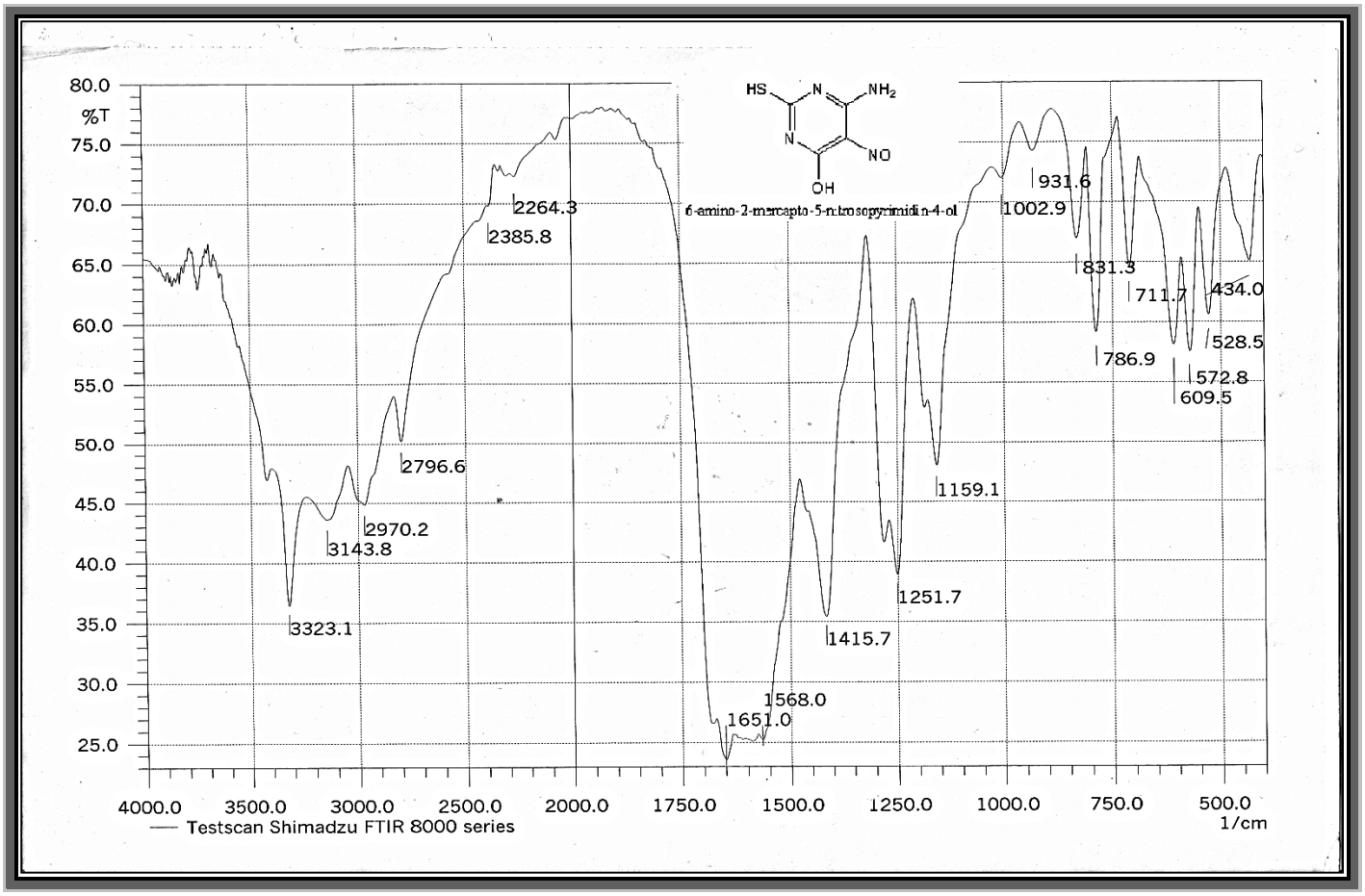


Fig. (3-5): FT-IR spectrum of 6-amino-2-mercapto-5-nitrosopyrimidin- 4-ol

No.	ν NH_2 cm^{-1}	ν S-H cm^{-1}	ν C=N cm^{-1}	ν N=O cm^{-1}	ν C=C cm^{-1}	ν N-C cm^{-1}	ν C-S cm^{-1}
3	3323.1-3143.8	2264.3	1651.0	1251.7	1568.0	1159.1	609.6

Disappearance of band at 1251.7 cm^{-1} attributed to (N=O) stretching frequency is a good evidence for the structure given to (5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride).

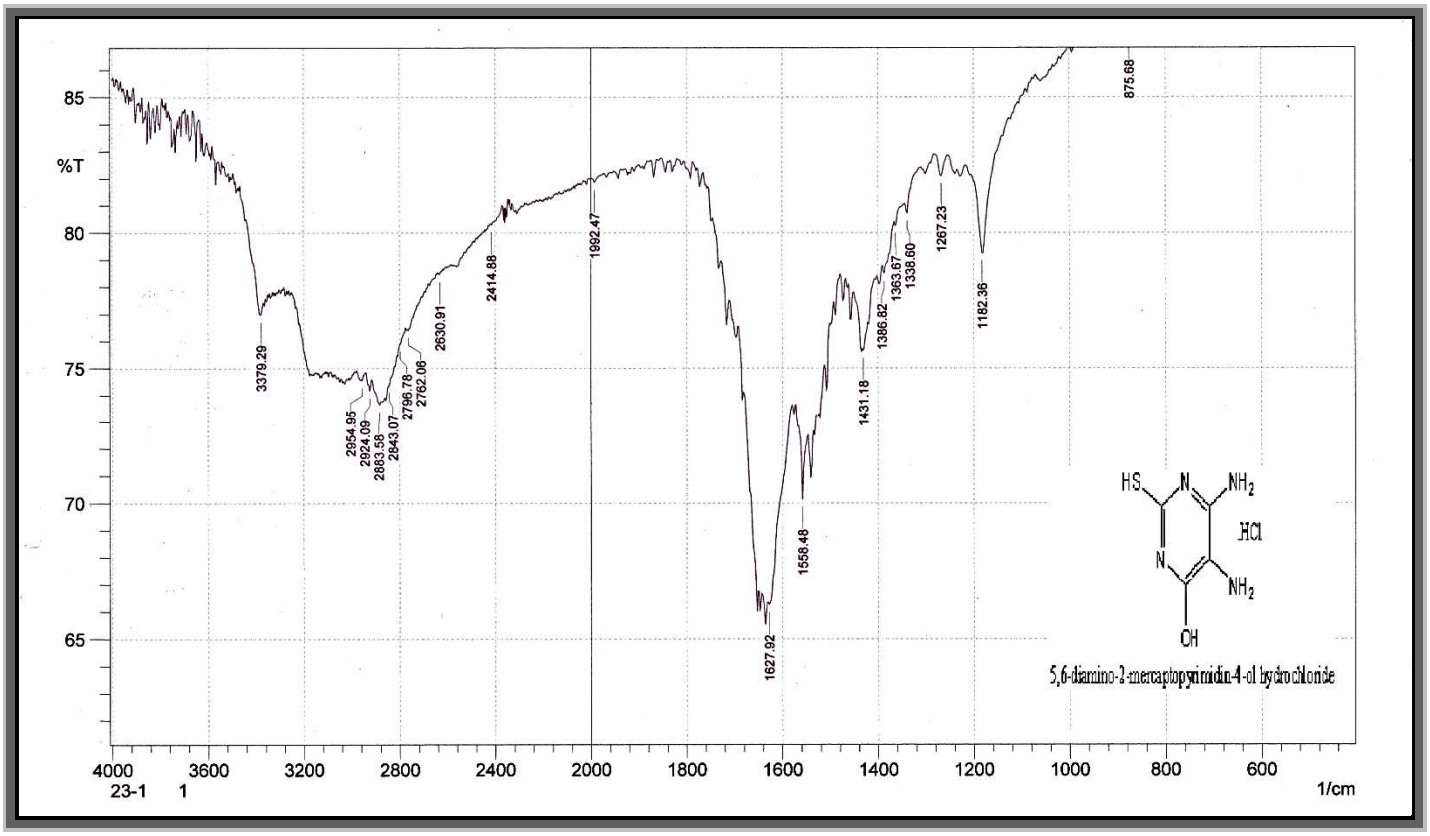


Fig. (3-6): FT-IR spectrum of 5,6-diamino-2-mercaptopyrimidin-4-ol hydrochloride

No.	ν NH_2 cm^{-1}	ν S-H cm^{-1}	ν C=N cm^{-1}	ν C=C cm^{-1}	ν C-N cm^{-1}	ν C-S cm^{-1}
4	3379.2-3330	2414.8	1627.92	1558.48	1182.36	875.68

3.3 ***Synthesis of 2-mercapto-6-methylpyrimidin-4(3H)-one[3]:***

It was prepared from the reaction of ethylacetoacetate with thiourea in basic medium, and this following suggested mechanism [(56)]. Figure (3-7)

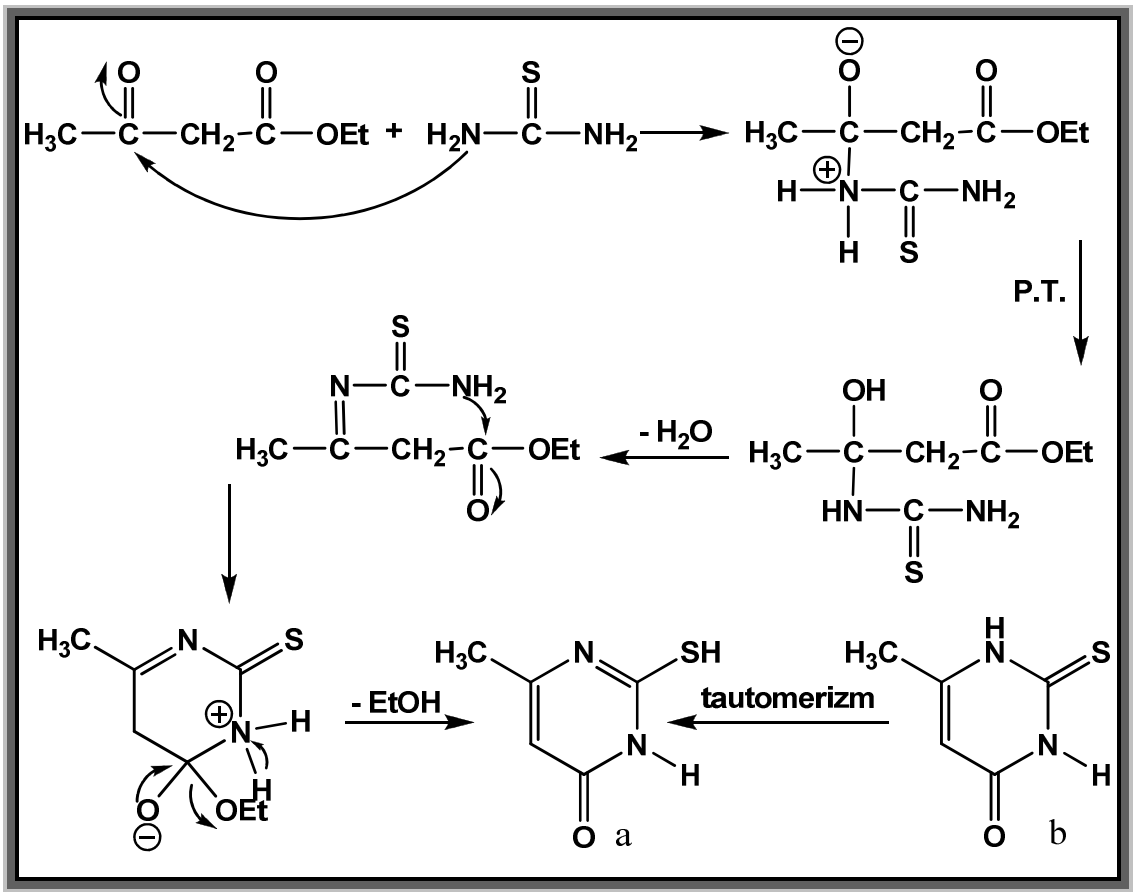


Figure (3-7) mechanism of the preparation of 2-mercapto-6-methylpyrimidin-4(3H)-one

From the suggested mechanism for this reaction, there are two structural forms (a, b) due to existing equilibrium (-SH) group and nitrogen atoms of the ring, the form (b) more stable because it was kept a resonance property. F.T.IR spectral data for (2-mercapto-6-methylpyrimidin-4(3H)-one) is shown in figure (3-5). The absorption bands of (S-H) group at 2578.8-2335.8 cm^{-1} could be attributed to stretching vibration, stretching vibration and the absorption band at 3398.5cm^{-1} could be attributed to (N-H) stretching vibration.

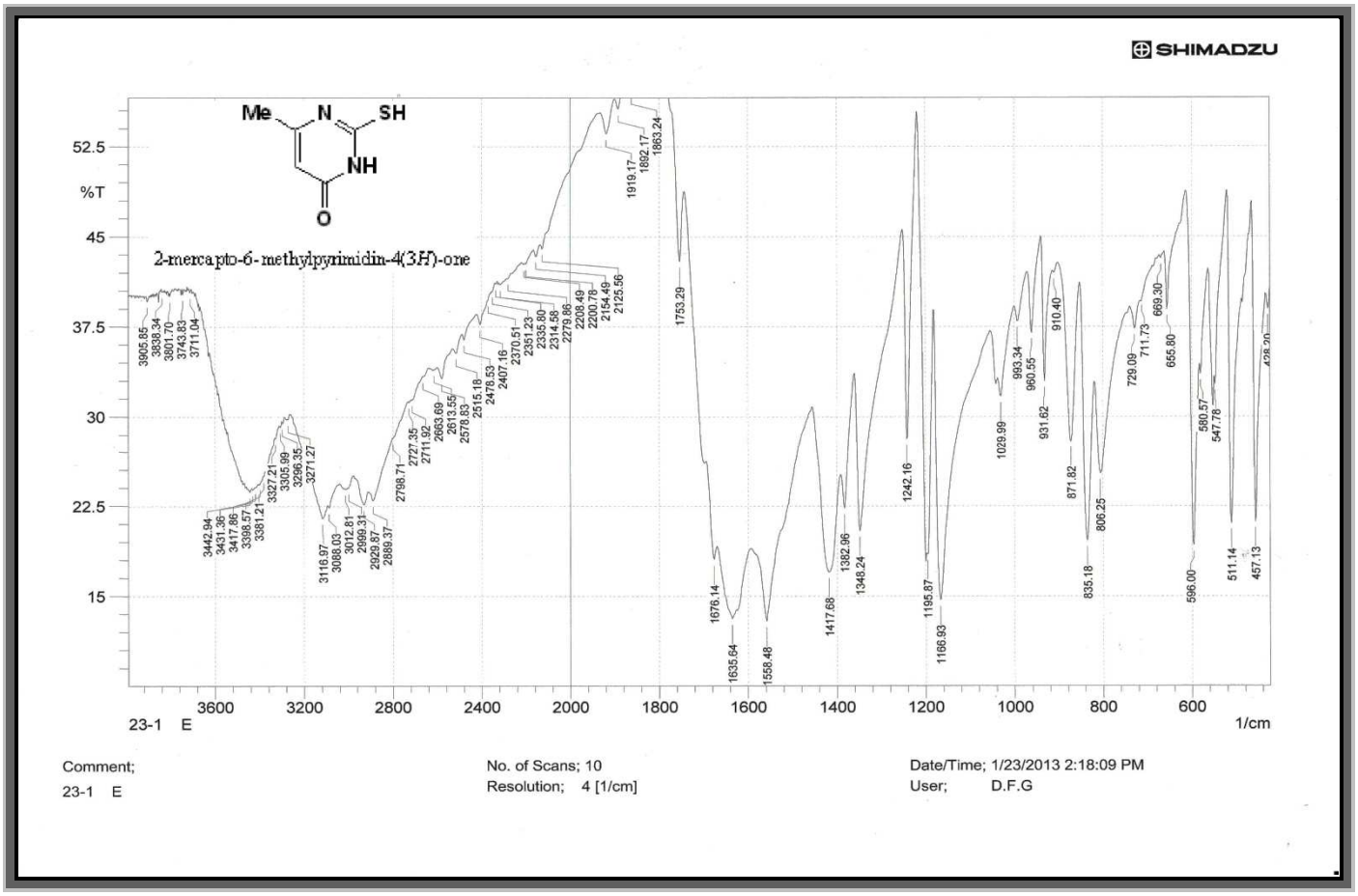


Fig. (3-8): FT-IR spectrum of 2-mercapto-6-methylpyrimidin-4(3H)-one

No.	ν N-H cm^{-1}	ν C-H cm^{-1}	ν S-H cm^{-1}	ν C=O cm^{-1}	ν C=N cm^{-1}	ν C-S cm^{-1}
5	3398.5	3012.8 arm 2889.3 alph	2578.8-2335.8	1635.64	1558.4	596.0

3.4 *Synthesis of 2-hydrazinyl-6-methylpyrimidin-4(3H)-one[4]:*

It was prepared from the reaction of 2-mercapto-6-methylpyrimidin-4(3H)-one with hydrazine hydrate (99%), the reaction takes place by nucleophile removal of (-SH) group by amino in hydrazine [(57)], the following mechanism was suggested. Figure (3-9)

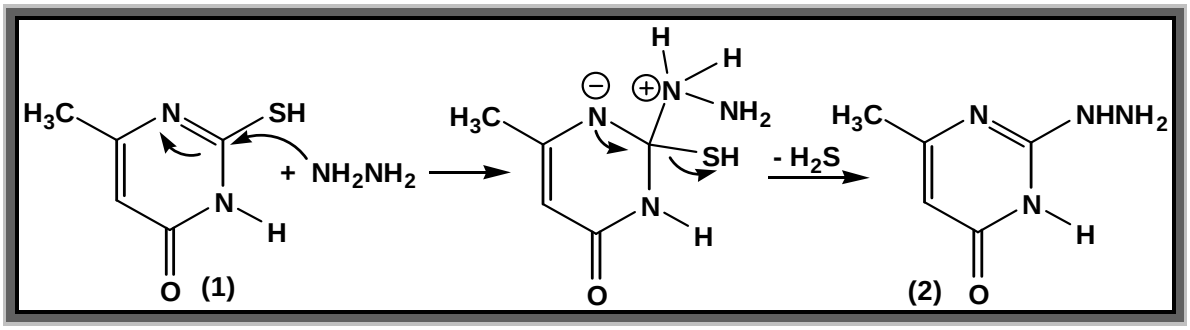


Figure (3-9) mechanism of the preparation 2-hydrazinyl-6-methylpyrimidin-4(3H)-one.

F.T.IR spectral data for 2-hydrazinyl-6-methylpyrimidin-4(3H)-one. the absorption bands at 3300-3209.3cm^{-1} could be attributed to (NH, NH_2) stretching vibration of hydrazine group, The disappearance of bands at 2578.8-2335.8cm^{-1} attributed to (SH) stretching frequency are good evidence for the structure given to (2-hydrazinyl-6-methylpyrimidin-4(3H)-one).

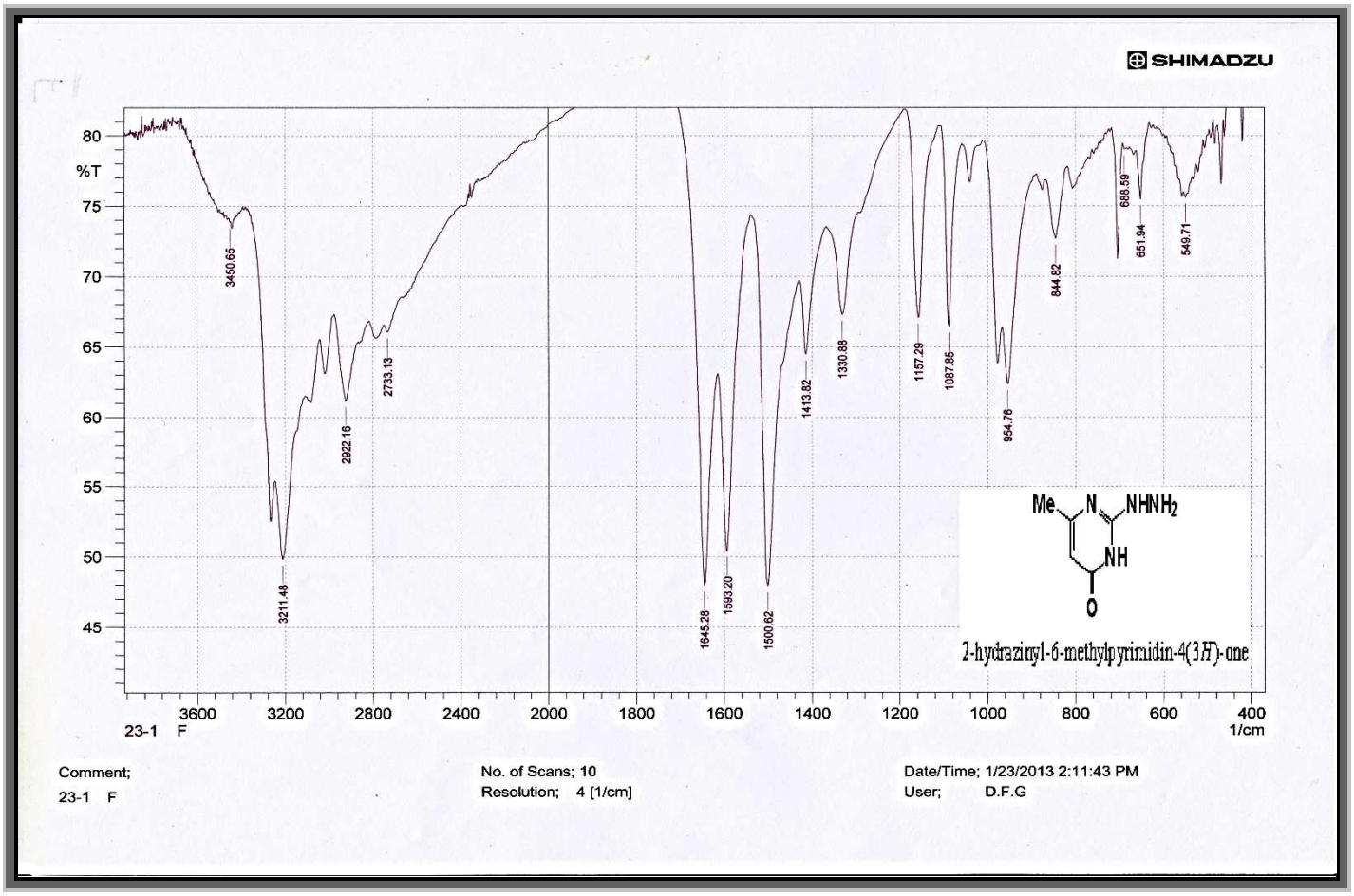


Fig. (3-10): FT-IR spectrum of 2-hydrazinyl-6-methylpyrimidin-4(3H)-one

No.	ν N-H cm^{-1}	ν C-H cm^{-1}	ν C=O cm^{-1}	ν C=N cm^{-1}	ν C-N cm^{-1}
6	3300-3209.3	2922.0(alph) 3016.5(arm)	1645.2	1593.2	1157.2

3.5*Synthesis of 2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidin-4(3H)-one[5]:*

Compounds of Schiff base were prepared, by nucleophilic addition of (-NH) group with aldehyde carbonyl group to form N-substituted hemiaminals that losses water molecule to yield stable compounds shown below in the following mechanism [58]. Figure (3-11)

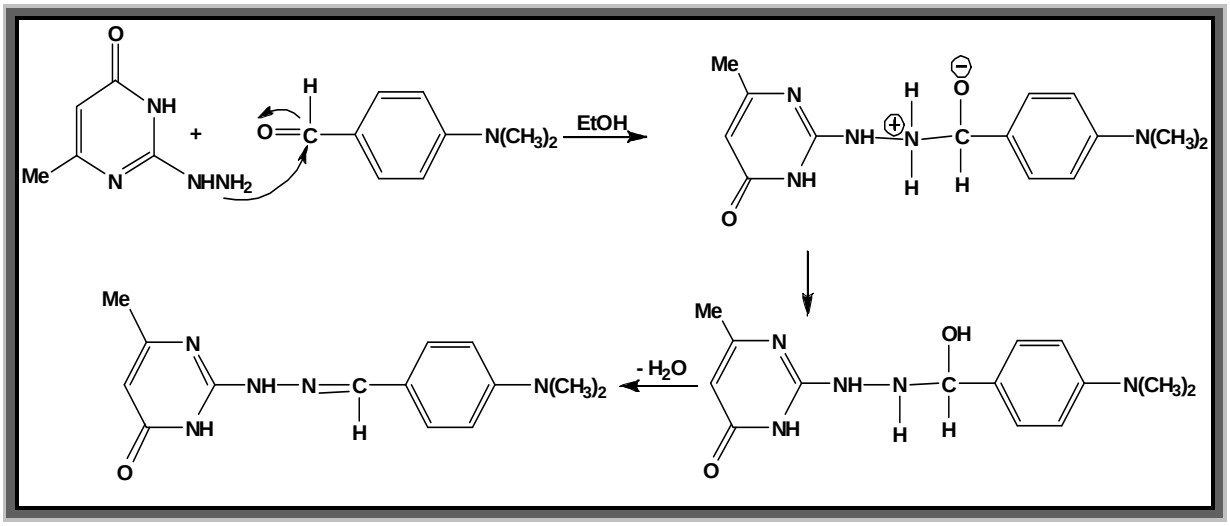


Figure (3-11) mechanism of the preparation

2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidin-4(3H)-one

F.T.IR spectrum of the synthesized of 2-(2-(4-(dimethylamino) benzylidene)hydrazinyl)-6- methylpyrimidin-4(3H)-one, (C=N) which is characteristic to Schiff bases showed peak absorption band at ~ 1600 cm^{-1} which could be attributed to (C=N) stretching vibration, and showed absorption band at 3211.26cm^{-1} which could be attributed to (N-H) of pyrimidine ring and of hydrazine group.

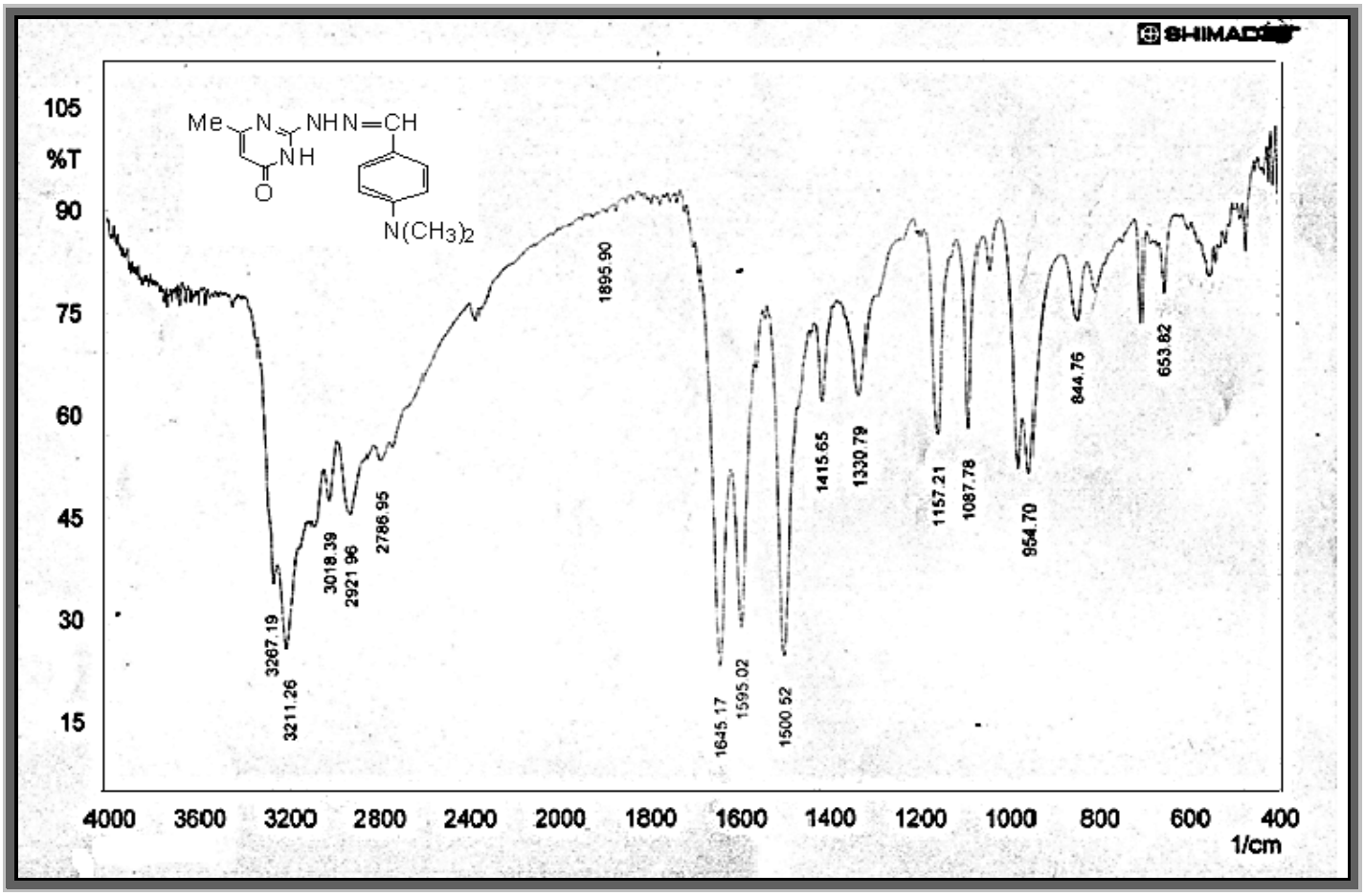
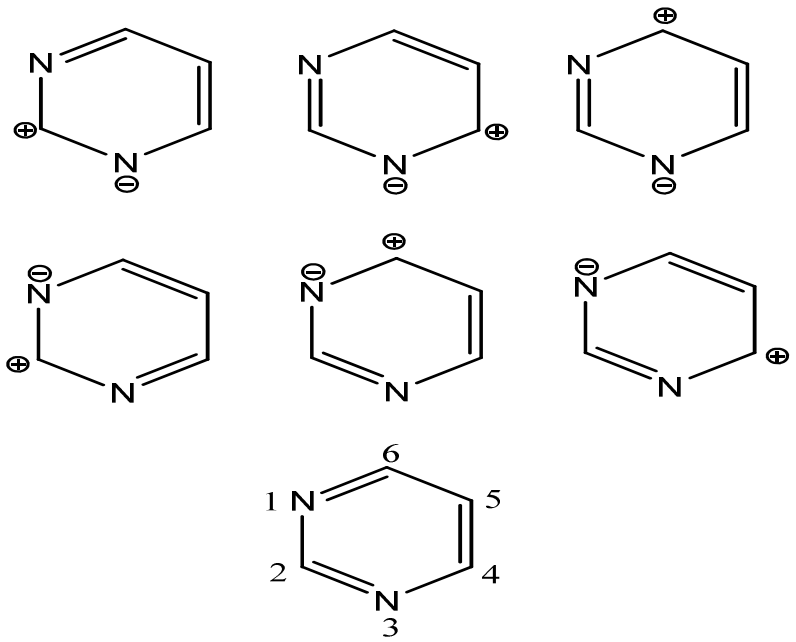


Fig. (3-12):FT-IRspectrumof2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)

6-methylpyrimidin-4(3H)-one

No.	ν N-H cm^{-1}	ν C-H cm^{-1}	ν C=O cm^{-1}	ν C=N cm^{-1}	ν C-N cm^{-1}
7	3211.26	3018.39arm 2921.96alph	1645.17	1600	1157.2

Pyrimidine could be represented as a resonance hybrid of the four pairs of equivalent canonical structure [59].



The electronic effect of nitrogen atoms of the m-diazine ring system reinforce each other, resulting in a marked electron deficiency at the positions 2, 4 and 6; there is a loss of electrons at 5-position also, through induction. Consequently, nucleophilic substitution would be expected to occur at the 2,4 and 6-positions. It has been shown that pyrimidines substituted with a mercapto group at 2,4and 6-position exist in aqueous solution predominantly as the thiopyrimidines. The mercaptopyrimidines adopt the tautomeric keto structure as long as the aromaticity of the ring can be maintained by a suitable electron distribution, mercapto groups at positions 2, 4, or 6 of a pyrimidine can be replaced by amino groups on treatment with ammonia[60].The 5-position of the pyrimidine ring is more aromatic in character. Various electrophilic substitutions at this position have been reported[61]. However, in order that weakly reactive electrophilic agents should attack the pyrimidine nucleus, such electronegative

substituents as the potentially tautomeric hydroxy, mercapto and amino group are required in two or three of the 2,4 and 6-positions.

3.6Ultraviolet spectrum for prepared compounds:

Electronic Spectral studies show that pyrimidine spectrum is similar to spectrum of benzene because of the electronic symmetry of the ring in both of them, but the distinguishing differences between their spectra were clearly shown by increasing absorbance intensitis of pyrimidine ring as compared with intensity of benzene ring. The existence of electronic pair on the nitrogen atom in pyrimidine ring leads to excite one of the electronic pair and obtained electronic transition $n \longrightarrow \pi^*$ **(62)** state.

The conjugated ring system of the purines and pyrimidines in nucleic acid result in marked absorption in the ultraviolet region of the spectrum, with absorption maxima near 260-280 nm **(63)**

Ultra violet spectrums for the prepared compounds in absolute DMSO are showed in figures (3-13) to (3-17) and table (3-1).

Table (3-1):*Absorption ultra violet bands for the prepared compounds*

Compounds	$\lambda max\,(nm)$
5,6-diaminouracil-2,4-diol hydrochloride.[1]	253nm
5,6-diamino2-mercaptopyrimidine-4-ol hydrochloride.[2]	271nm

2-mercapto-6-methylpyrimidin-4 (3H)-one. [3]	292nm, 271nm
2-hydrazinyl-6-methylpyrimidin-4(3H)-one. [4]	404nm, 251nm
2-(2-(4-(dimethylamino) benzylidene)hydrazinyl)-6-methyl pyrimidin-4(3H)-one. [5]	264nm, 355nm

Ultra violet spectrum for (5,6-diaminopyrimidine-2,4- diol hydrochloride) appeared one band, at λ max=253nm due to electronic transition state represents (π-π*), they have a bond system that absorb ultraviolet in nucleic acids and their derivatives, and that can be explained due to carbonyl group presence which do as electron-withdrawing group causing an inductive effect on pyrimidine ring in (5,6-diaminopyrimidine-2,4-diol hydrochloride), hence decrease the transition intensity.

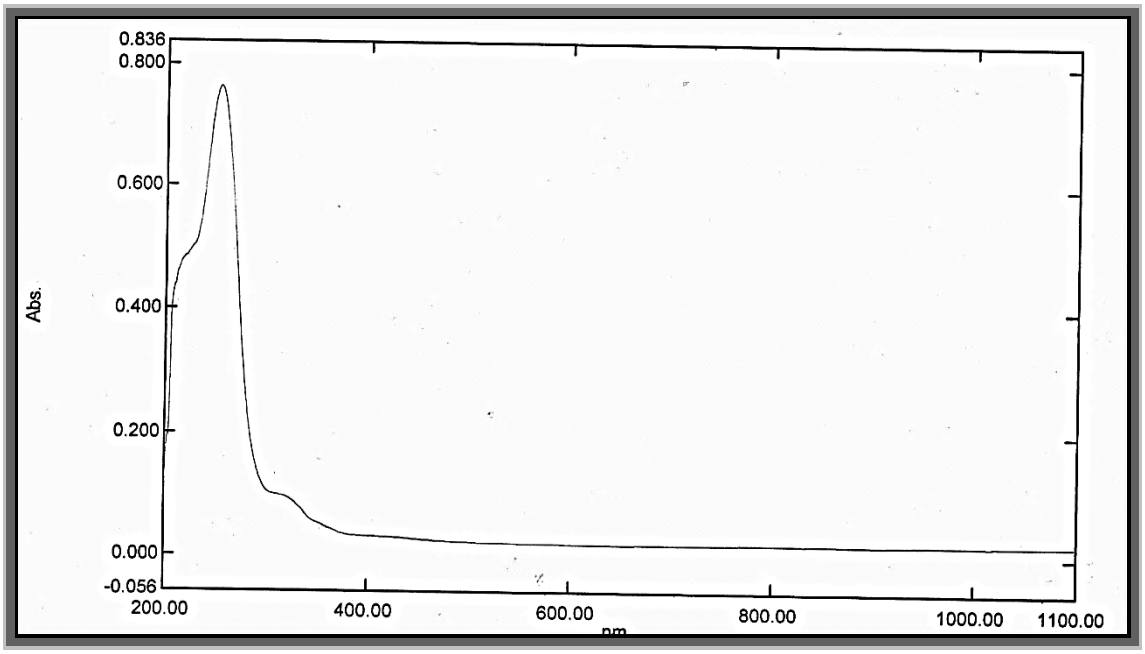


Fig. (3-13): UV.Spectrum of 5,6-diaminouracil-2,4-diol hydrochloride

Ultra violet spectrum for (5,6-diamino-2-mercaptopyrimidine-4-ol hydrochloride) appeared one band, at λ max = 271nm due to electronic transition (π-π*) state.

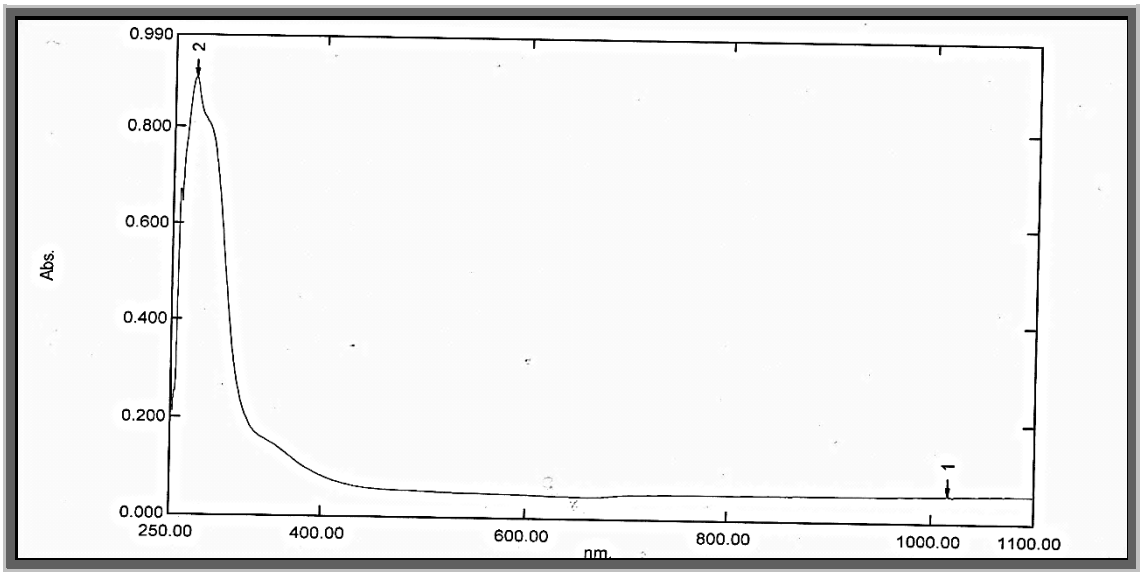


Fig. (3-14): UV.Spectrum of 5,6-diamino2-mercaptopyrimidine-4-ol hydrochloride

Ultra violet spectrum for (2-mercapto-6-methylpyrimidin-4(3H)-one) shows two bands, a band at λ max =271nm is due to pyrimidine or may be electronic transition represents (π-π*), they have a bond system that absorb ultraviolet in pyrimidine and their derivatives, the second band at λ max = (292nm) is due to (C=S and C=O) or may be an electronic transition representing (n-π*) state.

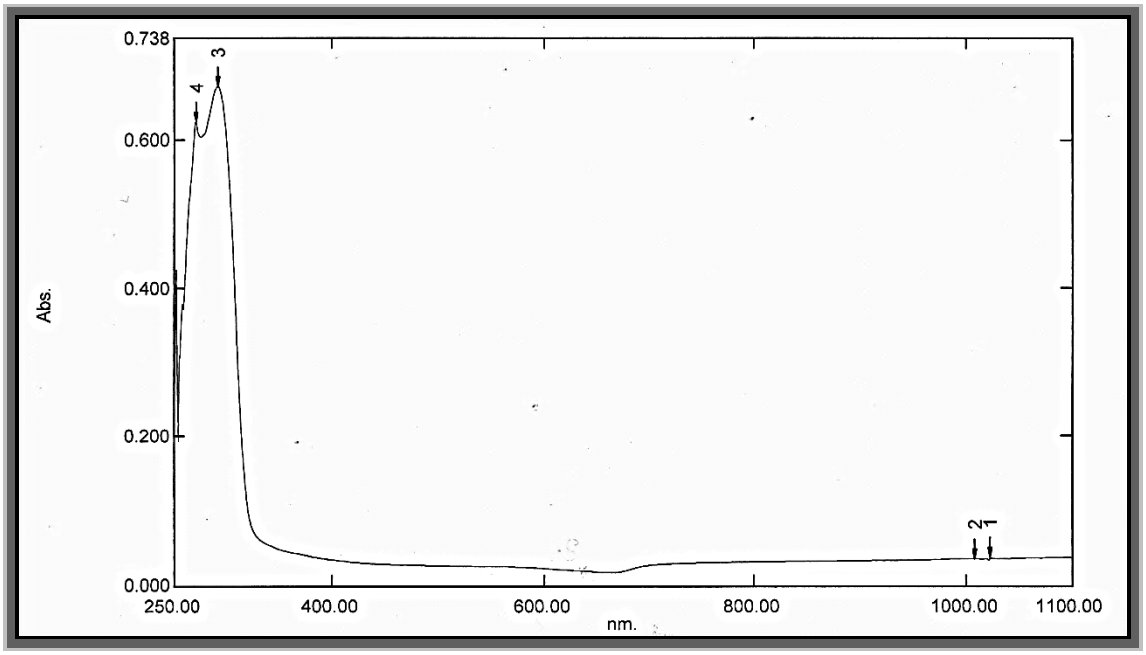


Fig. (3-15): UV.Spectrum of 2-mercapto-6-methylpyrimidin-4(3H) one

Ultra violet spectrum for (2-hydrazinyl-6-methylpyrimidin-4(3H)-one) appeared two bands, a band at λ max =251nm belongs to pyrimidine, they have a bond system that absorb ultraviolet in pyrimidine and their derivatives, the other band at λ max = (404nm) belongs to(C=N (strong intensity) and C=O) or may be an electronic transition representing (n-π*).

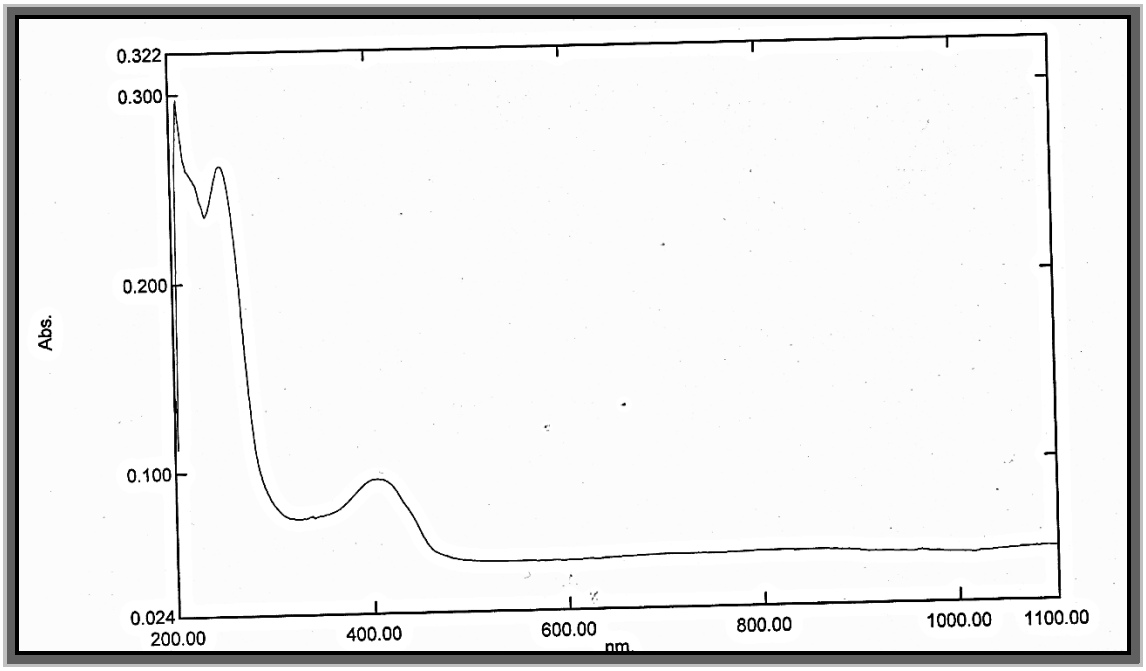


Fig. (3-16): UV.Spectrum of 2-hydrazinyl-6-methylpyrimidin-4(3H)one

Ultra violet spectrum for (2-(2-(4-(dimethylamino)benzylidene) hydrazinyl)-6- methylpyrimidin-4(3H)-one) appeared two bands, first at λ max =264nm belongs to pyrimidine, they have a bond system that absorb ultraviolet in pyrimidine and their derivatives, second band at λ max = (355nm) belongs to(C=N (strong intensity) and C=O) or may be an electronic transition representing (n-π*).

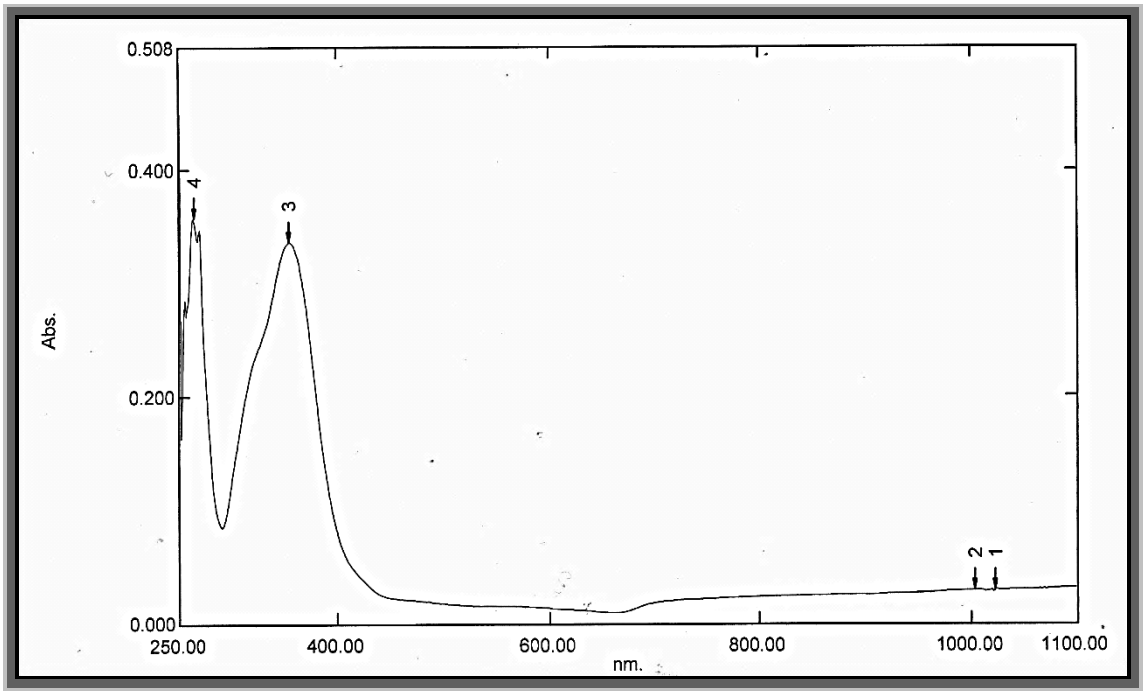


Fig. (3-17): UV.Spectrum of 2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-6-methylpyrimidin-4(3H)-one

3.7*Results of the Biological Study:*

Biological significance of pyrimidine derivatives is due to structural as they DNA & RNA and these derivatives have the ability of interactions with rapid generation processes of cancer cells and causes of disease. Structural modifications of nitrogen base producing isomers with the following effects:

a. Inhibition of some enzymes which are essential in production of nucleic acids in the cell.
b. Combination of nucleic acids leading to stop their production in the cell.

Preparation of highly selective nitrogen base isomers which serve the mentioned mechanisms finally give extensive biological activities that work as anticancer [(64)].

3.7.1 ***Cytotoxic effect of synthesized compounds on HepG2 cell line.***

Tumor cell line (HepG2) was used in this study for one time of exposure (48 hours). Cell line was subjected to six concentrations of synthesized compounds (600, 300, 150, 75 and 37.5µg/ml). Using Neutral Red assay, the optical density measured at transmitting wave length 492nm .The Neutral Red assay is a cell survival/viability assay based on the ability of viable cells to incorporate and bind Neutral Red dye. The Neutral Red uptake assay provides one of the most used cytotoxicity tests with many biomedical and environmental applications. Therefore it was selected for determination of synthesized compounds cytotoxicity [(65)]. Following exposure to synthesized compounds, cells are incubated in the presence of Neutral Red dye. The dye readily penetrates cell membranes and accumulates intracellular in lysosomes. As Neutral Red is a vital stain, it was used for staining living cells. Changes of the cells brought about by the action of the synthesized compounds causes a decreased in the uptake and binding of Neutral Red. After washing cells with PBS and treating with destaining solution to release any excess of dye taken up, the cells damage level was evaluated by measuring the optical density of treated cell solution and comparing it to untreated negative control samples. Microtiter plate reader equipped with 492nm filter. Results obtained may be discussed as follows:

After cancer cell line (HepG2) was treated with synthesized compounds, results showed significant cytotoxic effect started at the certain concentration and continued to the higher concentrations reaching the last concentration when compared with the control, as showed in the (table 3-2).

Table (3-2): Cytotoxicity Effect of Synthesized Compounds (at Different Conc.)on HepG2 Tumor Cell Line after, Incubation for 48 Hours Measured at 492nm.

Compounds	Concentration µg/ml	Absorbance of compounds	Inhibition Rate% ±SE	Viable Cell%	$IC_{50\%}$
5,6-diaminouracil-2,4-diol hydrochloride [1]	37.5	1	9.00±0.57 (e)	90.90	432.6141
	75	0.968	12.00±0.00 (d)	88.00	
	150	0.788	28.30±0.34 (c)	71.63	
	300	0.599	45.50±0.28 (b)	54.45	
	600	0.425	61.3±0.17 (a)	38.63	
5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride [2]	37.5	0.978	11.00±0.57 (c)	88.90	447.9962
	75	0.967	12.00±1.15 (c)	87.90	
	150	0.967	12.00±0.00 (c)	87.90	
	300	0.703	36.00±0.57 (b)	63.90	
	600	0.368	66.5±0.28 (a)	33.45	
2-mercapto-6-methyl pyrimidine-4(3H)-one [3]	37.5	1.099	0.09±0.00 (d)	99.09	531.6179
	75	1.097	0.27±0.00 (d)	99.09	
	150	1.056	4.00±0.57 (c)	96.00	
	300	1.037	5.7±0.11 (b)	93.63	
	600	0.371	66.2±0.11 (a)	33.72	
2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one [5]	37.5	0.997	9.30±0.17 (c)	90.63	451.5608
	75	1.075	2.20±0.20 (e)	97.27	
	150	1.03	6.30±0.00 (d)	93.63	
	300	0.765	30.40±0.40 (b)	69.54	
	600	0.422	61.60±0.11 (a)	38.36	

**($P<0.0001$), different letters(a,b,c,d, and e)= significant differences between mean, Abs. of negative control = 1.1

3.7.1.1*Cytotoxic effect of 5,6-diaminouracil-2,4-diol hydrochloride[1]:*

Results indicated in table (3-2) showed that 5,6-diaminouracil-2,4-diol hydrochloride has significant cytotoxic effect on HepG2 cell line ($P<0.0001$) started at the lower concentration and continued to the higher concentrations reaching the last concentration, growth inhibition of HepG2 cell line was increase gradually with the increase of 5,6-diaminouracil-2,4-diol hydrochloride concentration treated with, when compared with the negative control (the same cell line without any treatment). Maximum inhibitory effect of 5,6-diaminouracil-2,4-diol hydrochloride was reached 61.3% growth when the cell culture of HepG2 was treated with 600µg/ml of 5,6-diaminouracil-2,4diol hydrochloride, then the inhibitory effect was decreased to 9% after treatment with 37.5µg/ml of 5,6-diaminouracil-2,4-diol hydrochloride. As shown in figure (3-18)

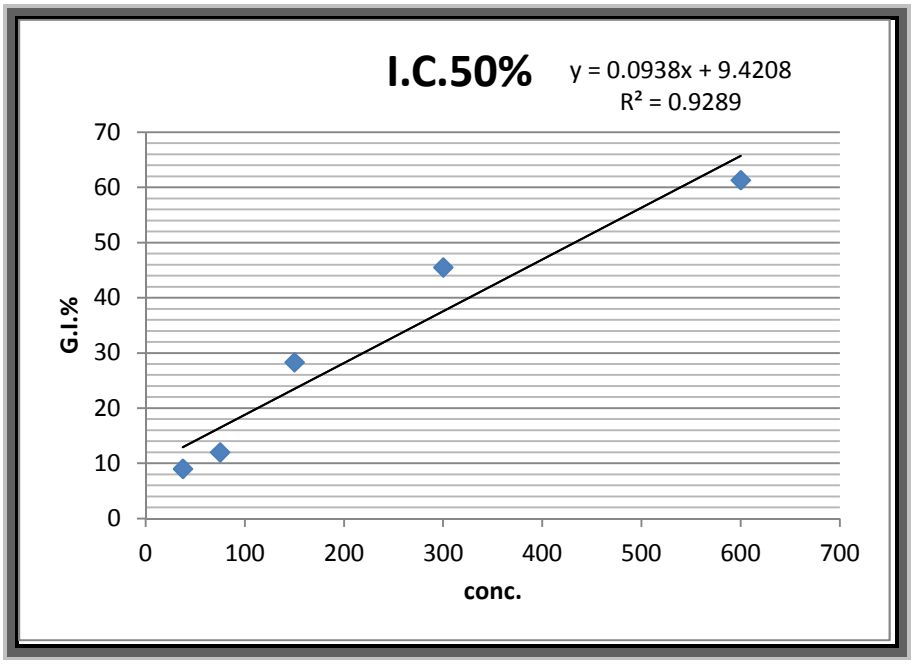


Fig. (3-18): Linear regression between growth inhibition and concentration of 5,6-diaminouraci-2,4-diol hydrochloride

3.7.1.2*Cytotoxic effect of 5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride[2]:*

After HepG2 cell line was treated with (5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride)and results indicated in table (3-2) showed that 5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride had significant cytotoxicity effect(P<0.0001)on HepG2 cell line in concentration range between 600 µg/ml to 300 µg/ml with inhibition rate 66.5% and 36% respectively. And there is no significant difference between the concentrations (37.3, 75 and 150 µg/ml), and confirms this conclusion relationship moral negative regression, as shown in the equation of a straight line to HepG2 cell line. As shown in figure (3-19).

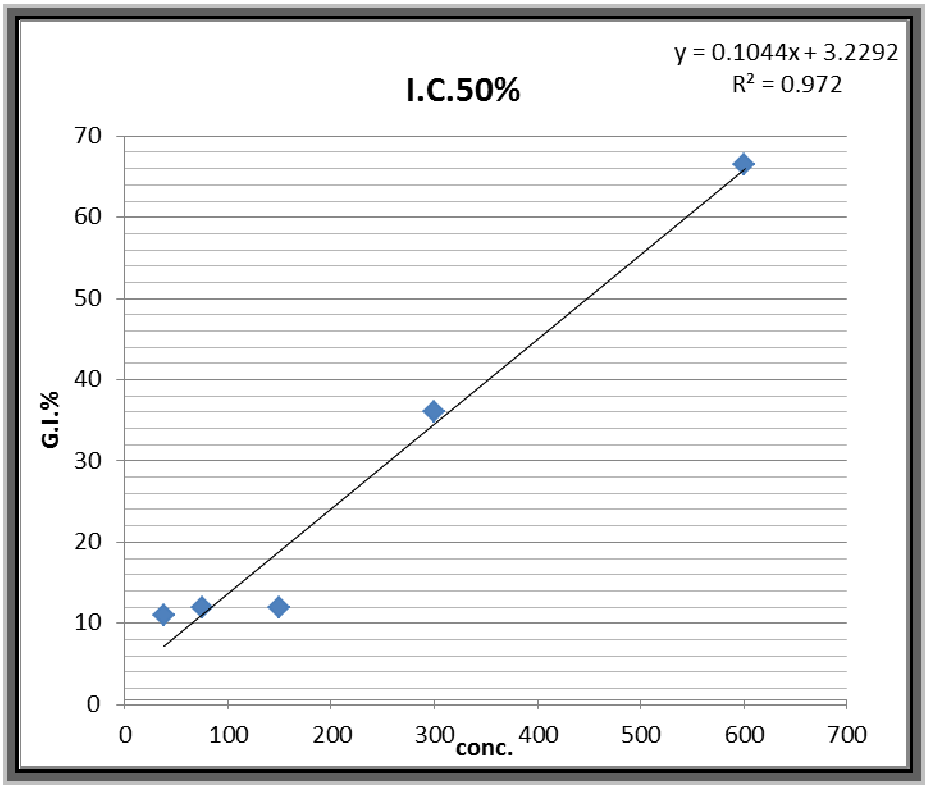


Fig. (3-19): Linear regression between growth inhibition and concentration of 5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride

3.7.1.3*Cytotoxic effect of 2-mercapto-6-methylpyrimidin-4(3H)-one[3]:*

Results indicated in table (3-2) showed that low toxic effect at concentrations (37.3, 75, 150 and 300 µg/ml), and there was no significant difference between these concentrations, but there were significant difference between these concentrations and the control treatment. This result confirms the negative moral regression relationship. As shown in Figure (3-20)

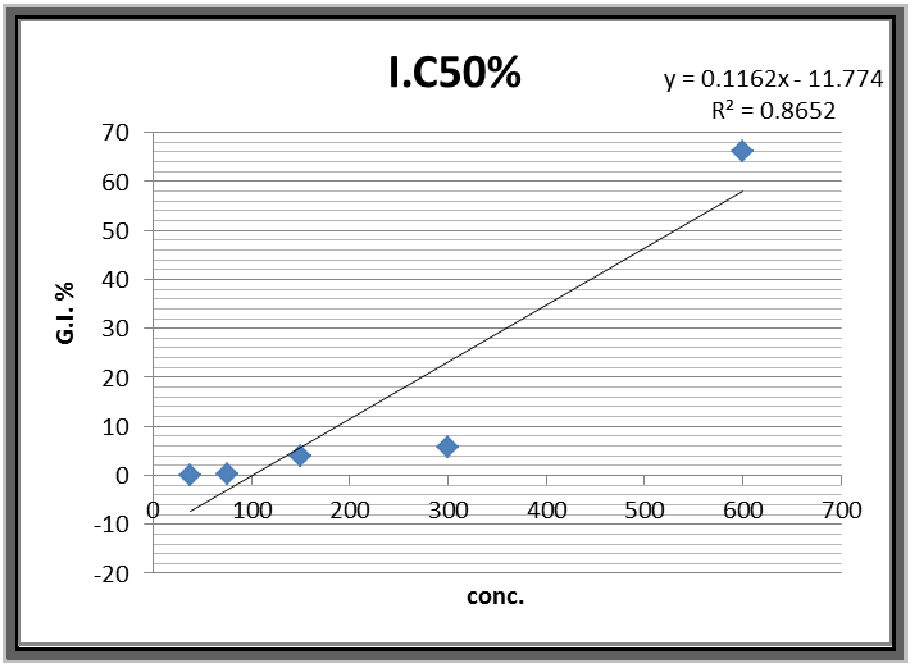


Fig. (3-20): Linear regression between growth inhibition and concentration of 2-mercapto-6-methyl pyrimidine

3.7.1.4 Cytotoxic effect *of2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one[5]:*

Results indicated in table (3-2) showed that the inhibitory effect of 2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one on growth of HepG2 cell line at the concentrations of 37.5 , 75 and150 µg/ml with low growth inhibition percentage **9.3**% , **2.2** % and **6.3** % , respectively. With the presence of significant differences between

concentrations, as well as between concentrations and the control treatment. Figure (3-21) showed the relationship of moral negative regression and equation of a straight line of HepG2 cell line.

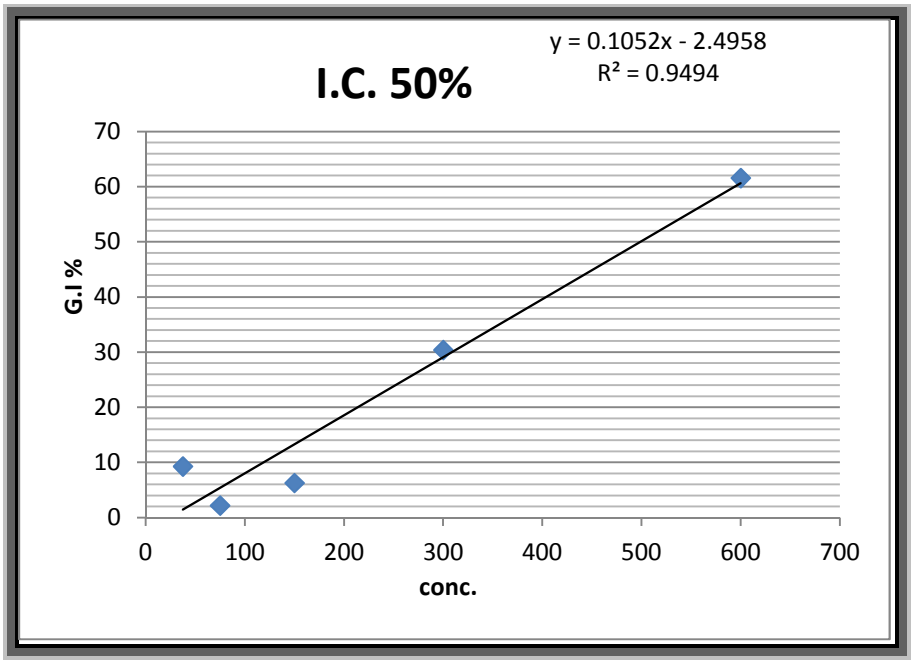


Fig. (3-21): Linear regression between growth inhibition and concentration of 2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one

3.7.2 *Cytotoxic Effect of synthesis compounds on MCF7 cell line, in vitro study,((MTT assay)):*

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cytotoxicity assay is based on the ability of a mitochondrial dehydrogenase enzymes from viable cells to cleave the tetrazolium rings
of the pale yellow MTT and form dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of surviving cells is directly proportional to the level of the formazan created.

After cancer cell line (MCF7) was treated with the synthesized compounds, results showed significant cytotoxic effect started at the certain concentration and continued to the higher concentrations reaching the last concentration when compared with the control.as showed in the (table 3-3).

Table (3-3):Cytotoxicity Effect of Synthesized Compounds (at Different Conc.)on MCF7 Tumor Cell Line after, Incubation for 24 Hours Measured at 570nm.

compounds	Concentration µg/ml	Absorbance of compounds	Viable cell% ±SE	Growth Inhibition%	$IC_{50\%}$
5,6-diaminouracil-2,4-diol hydrochloride.[1]	3.125	0.646	96.12±0.22 (a)	3.86	21.9
	6.25	0.540	80.35±0.30 (b)	19.64	
	12.5	0.463	68.89±0.34 (c)	31.10	
	25	0.318	47.31±0.22 (d)	52.67	
	50	0.190	28.32±0.26 (e)	71.72	
	100	0.084	12.54±0.30 (f)	87.50	
5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride.[2]	3.125	0.645	95.97±0.30 (a)	4.01	23.2
	6.25	0.558	83.03±0.30 (b)	16.96	
	12.5	0.467	69.53±0.34 (c)	30.5	
	25	0.335	49.84±0.30 (d)	50.14	
	50	0.202	30.10±0.52 (e)	69.94	
	100	0.090	13.38±0.39 (f)	86.60	
2-mercapto-6-methyl pyrimidine-4(3H)-one.[3]	3.125	0.661	98.36±0.17 (a)	1.63	22.6
	6.25	0.559	83.18±0.17 (b)	16.81	
	12.5	0.478	71.17±0.21 (c)	28.86	
	25	0.342	50.98±0.48 (d)	49.10	
	50	0.175	26.13±0.26 (e)	73.95	
	100	0.074	11.00±0.25 (f)	88.98	
2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one.[5]	3.125	0.653	97.16±0.17 (a)	2.82	20.9
	6.25	0.566	84.27±0.17 (b)	15.77	
	12.5	0.447	66.56±0.21 (c)	33.48	
	25	0.320	47.71±0.48 (d)	52.38	
	50	0.169	25.14±0.26 (e)	74.85	
	100	0.055	8.27±0.25 (f)	91.81	

**($P<0.0001$), different letters(a,b,c,d,e and f)= significant differences between mean, Abs. of negative control = 0.672

The results showed in table (3-3) that all synthesized compound have effective toxicity on cancerous trend line. The concentration 100µg/ml is the most toxic and most daunting of this line cancerous compared with treatment control, as its effect was highly significant compared with the rest of concentrations, and that cell division of MCF7 cell line was decreased gradually with the increase of concentrations treated with when compared with the control, and the results showed that the 2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one(e) is the most toxic as cell divisionof MCF7 cell line with 8.27 at 100µg/ml.These results confirmed the relationship negative moral regression that showed the relationship between the concentrations of synthesized compounds and cell division of MCF7. As showed in figures (3-22), (3-23), (3-24) and (3-25).

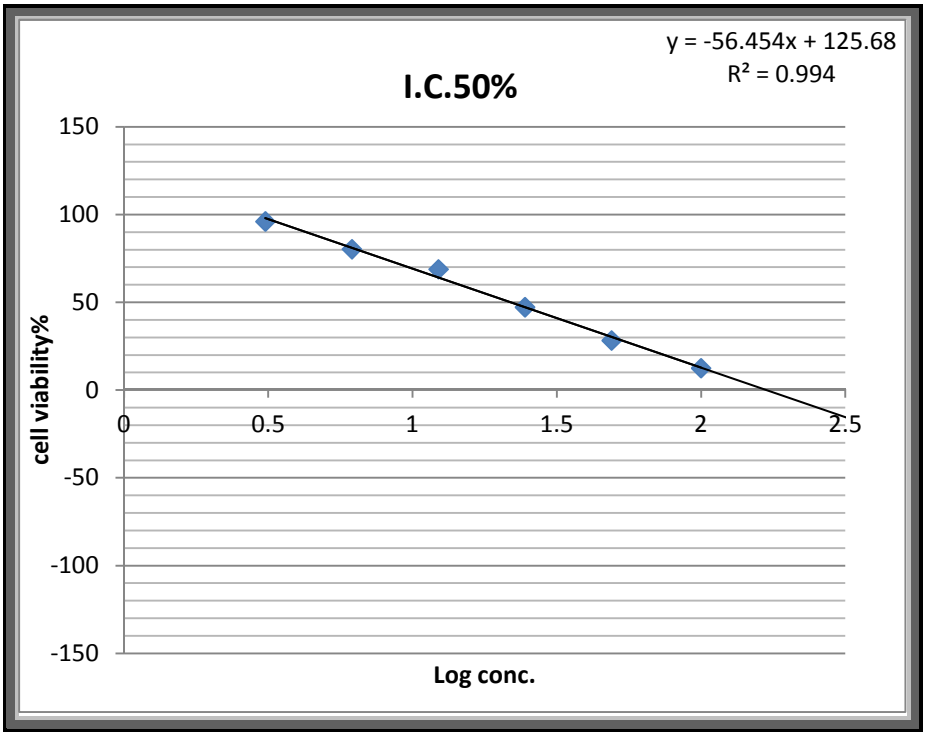


Fig. (3-22): Linear regression between cell viability and concentration of 5,6-diaminouracil-2,4-diol hydrochloride

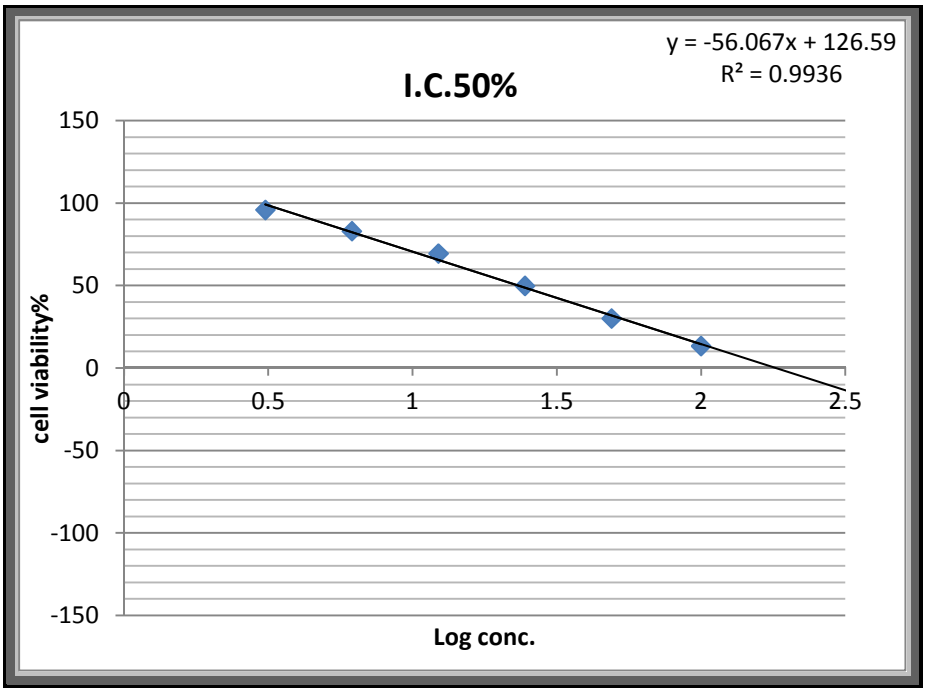


Fig. (3-23): Linear regression between cell viability and concentration of 5,6-diamino-2-mercapto pyrimidine-4-ol hydrochloride

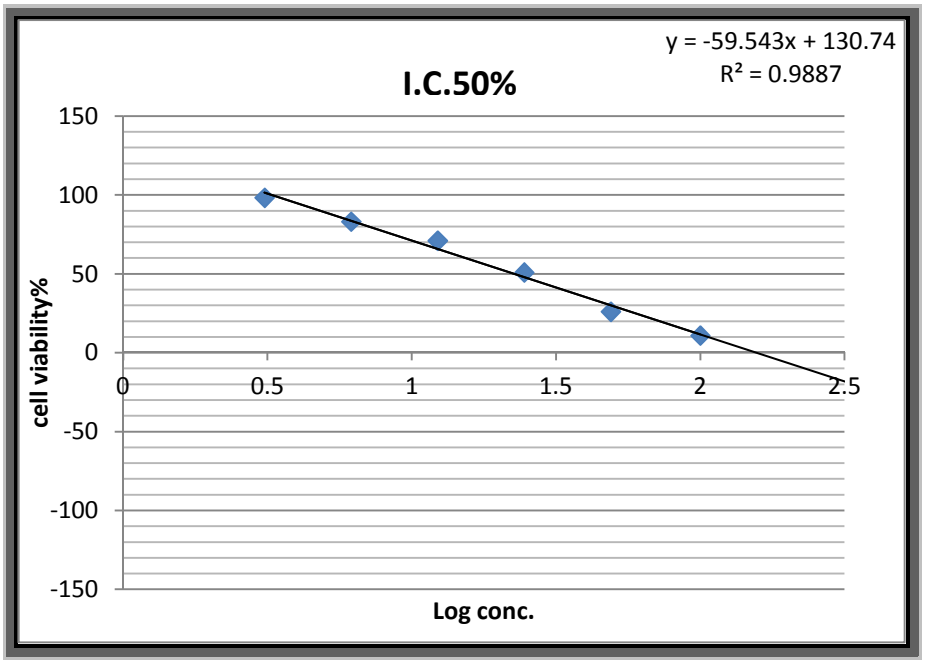


Fig. (3-24): Linear regression between cell viability and concentration of 2-mercapto-6-methyl pyrimidine

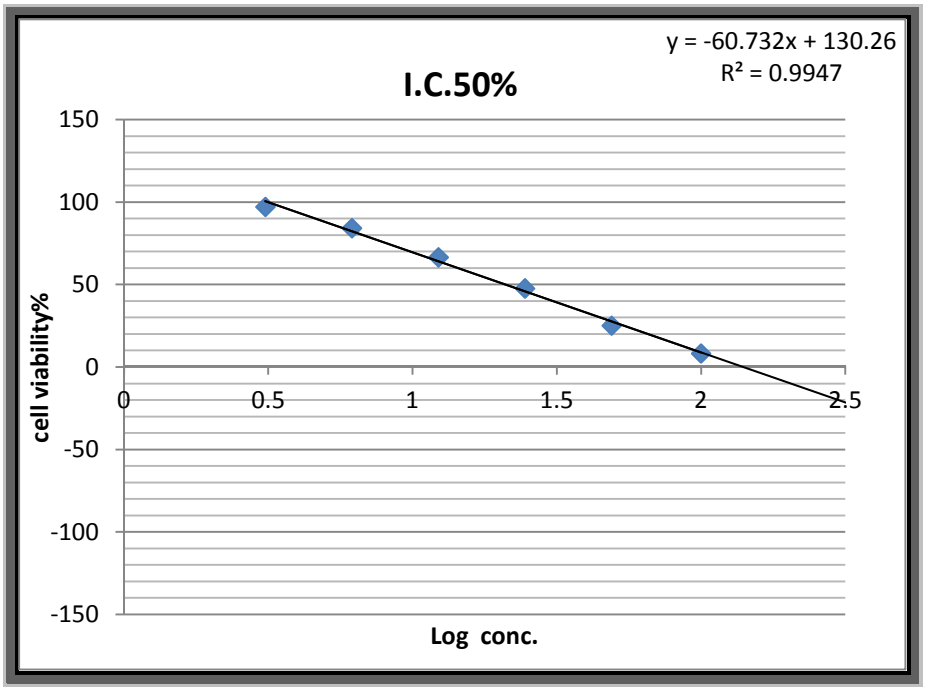


Fig. (3-25): Linear regression between cell viability and concentration of 2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one

We consider that the results from the MTT and NR assays must be analyzed separately because they evaluate two different metabolic functions: the reduction of enzymatic activities in the MTT assay and the membrane integrity in the NR assay.That inhibitory effect and toxic for cancer cells treated with pyrimidine derivatives due to the presence of many active groups in the structural formula of these synthesized compounds, including: the presence of nitrogen heterocyclic rings and the presence of 2-thiouracil moiety which is essential for the inhibition activity [(66)]. Diverse mechanisms of actions were reported to be encountered with the chemotherapeutic bioactivity of pyrimidine derivatives including inhibition of kinases, inhibition of enzymes involved in pyrimidine biosynthesis, incorporation into RNA and DNA which subsequently cause misreading and inhibition of DNA polymerase[(67)].

There are three goals associated with the use of the pyrimidine derivatives:

1. Structural damage of the DNA of the affected cancer cells.

2. Inhibition of the synthesis of new DNA strands which stops DNA replication.

3. Prevents mitosis or the actual splitting of the original cell into two new cells. Stopping mitosis stops cell division (replication) of the tumor cell and may ultimately halt the progression of the cancer.

3.8 *Conclusions and Recommendations:*

3.8.1 *Conclusions:*

From the gained results of the current study, we can conclude the following:

1. Most of the pyrimidine compounds have, as derivatives for the nitrogen bases, a biological activity such as cytotoxic effect, which differ by the difference of their concentrations.
2. Pyrimidines are the important heterocyclic compounds which show promising anticancer activity. The present results suggests that all synthesized compounds (1,2,3 and 5) induced cytotoxicity on HepG2 and MCF7 cell lines. And may indicate that the first compound (5,6-diaminouracil-2,4-diol hydrochloride) is the best compared with other compounds and gives the best value of IC50% (432.61) trend line liver cancer. And the fifth compound ((2-(2-(4-(dimethyl amine) benzylidene) hydrazinyl)-6-methyl pyrimidine-4(3H)-one) is the best direction of a line of breast cancer because it gives the best value of IC50% (20.9).

3.8.2 ***Recommendations:***

1. Synthesis of nucleoside analogues with different types of nitrogen bases such as diaminouracil and thiouracil, and synthesis of pyrimidine derivatives by using different methods of preparation as the second and third methods mentioned during this study.
2. The ability of improving this studies to show its cytotoxicity and mutagenicity carcinogenic and if it succeeds it can be used as gene therapy in the future.

CHAPTER ONE

INTRODUCTION

CHAPTER THREE

RESULTS & DISCUSSION

CHAPTER TWO

EXPERIMENTAL PART

REFERENCES

List of Errors

Error	Correction	Page
which	who	acknowledgement
soil	soul	acknowledgement
Plays	Play	Abstract
kinase	phosphate	List of abbrevations
M.P	m.p.	List of abbrevations
Nitrogen base	Nitrogen bases	1
pinner	Pinner	4
Reaction[9]	Type one[9]	6
et al	*et al*	8,11
Oriotic acid	Orotic acid	8
Sodiumhypochlorate	Sodiumhypochlorite	10
Alejandro *et al*	Alejandro *et al*[10]	11
Gibson *et al*	Gibson *et al*[11]	11
Thio-one	Thio-none	16
Blwomycin	Bleomycin	20
Methods	Method	24
4-N,N-dimethylamion benzyldehyde	4-N,N-dimethylamion benzaldehyde	29
(600,300,150,75and 37.5 µg/ml)	(37.5,75,150,300and 600 µg/ml)	38
Equal number	10000 cells	39
(100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml and 3.125µg/ml)	(3.125µg/ml, 6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml)	40

References:

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