Biochemical Study to Thiosemicarbazone Derivatives

Ibrahim El-Sayed Mohy El-Deen[1] ، Lamia Abd E l- Latif Ali Barakat[2] ، Sozan Ahmed Ibrahim El-Deek[3]
[1]Professor of Organic Chemistry، Faculty of Science Port said University.[2]Assistant professor of Biochemistry ، Faculty of Science Port said University.، [1] ieldeen@yahoo.com2 lamiaabarakat@yahoo.com.3sozan.gazy@gmail.com

ABSTRACT

There have been tremendous development in the chemotherapy of cancer and researches are still developing new and more effective drugs to combat this disease. Thiosemicarbazides and thiosemicarbazone derivatives are important and useful compounds with diverse pharmacological properties.In the present study, we evaluated the in vitro cytotoxic activity of new thiosemicarbaz -ones derivatives[1-[(6-acetyl pyridine-2-YL ethylidene) amino]-thiourea(3) and 2,4- di -acetyl pyridine- bis- 3- thiosemicarbazone(4) against MCF-7 (human breast cancer), HePG2 (Hepatocellular carcinoma), HCT116 (human colon cancer), PC3 (human pro -state cancer). In order to find new drugs with anticancer activities prepared compounds were evaluated for their in vitro and in vivo anticancer activities.

Thiosemicarbazones exhibited a significant anticancer activity towards MCF-7, HEPG2, HCT116 and PC3 cancer cell lines. Also, in vivo study of, [1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3) and 2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)] compounds revealed a significant anticancer activity towards Ehrlich ascites carcinoma (EAC) cells by reduction of its volume to 65.5 % and 73.3% ($p<0.001$), in the treated groups; respectively. And significantly decrease in the cell count by 65.9% and 78.9%, in treated groups ($p<0.001$); resp -ectively, compared to the positive control group. It turned out that they reduced cell viability of cancer cells in a time and concentration dependent manner in vitro and in vivo studies.

This review discusses current advances of an emerging 'new wave' of thiosemicarbazide/thiosemicarbazone as potent anticancer agents and toxicity caused by them.

Keywords: Anticancer, Antineoplastic, Antitumor,Thiourea, Thiosemicarbazione,

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1. INTRODUCTION

Thiosemicarbazones present a wide range of applications that stretch from their use in analytical chemistry, through pharmacology to nuclear medicine[1–2]. The presence of amide, imine and thione groups makes them potential polydentate ligands[3] and it is not surprising that numerous thiosemicarbazone complexes have been prepared and characterized [4]. In addition, in the last few years there has been a growing attention towards thiosemicarbazones related to their range of biological properties.specifically as antifungal, antiviral, antibacterial and anticancer agents [5-6].

Cancer is an important area of interest in the life sciences as it has been a major killer disease throughout human history. It is not one disease, but a large group of diseases characterized by uncontrolled growth and spread of abnormal cells. Heterocyclic molecules are well known to play a critical role in health care and pharmaceutical drug design. Currently a number of heterocyclic compounds are available commercially as anticancer drugs and great efforts have been put to the identification of novel anticancer targets for novel anticancer drug discovery[7] .

For the survival of any organism, there should be a delicate balance between cell growth and death. This balance can get disturbed in a number of ways, which may lead to abnormal growth of tissue [8] leading to a lethal tumor or cancer [9]. According to WHO, cancer is a leading cause of death worldwide. Antineoplastic or anticancer drugs prevent or inhibit the maturation and proliferation of neoplasms. They travel the body and destroy cancer cells. Many of the side effects associated with antineoplastic agents occur because treatment destroys the body's normal cells in addition to cancerous cells [10].

Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors, which play a direct and/or indirect role in the induction and deterioration of cancers. Emergence of resistance to anticancer drugs poses a major clinical challenge in successful treatment of cancer since some tumor cells develop a particular phenotype, called multidrug resistance (MDR), which makes these cells resistant to other classes of anti -cancer agents to which the tumor cells have not been treated previously. MDR cell lines have been shown to display a complex spectrum of biochemical and cytogenetic changes such as the over-expression of p-glycoprotein, increased levels of glutathione related enzymes, down regulation of mono-oxyg -enases, and altered expression of protein kinase C [11].

Biological properties of thiosemicarbazones have been studied since 1956 when Brockman *etaI* reported the antitumour properties of thiosemicarbazones derived from 2-formyl -pyridine. The nature of the substituent attached at 4-N influences the biological activity, while the acid character of the 3NH allows the ligand to be anionic and conjugation to be extended to include the thiosemicarbazones moiety. It has been proposed that this conjugated system enhances the antitumor activity.

Thiosemicarbazones display a broad spectrum of pharmacological properties, including antitumor,antifungal, antibacterial, antiviral and antimalarial activities [12]. Much effort has been devoted to structural variations of the thiosemicarbazones for achieving the ultimate goal of medicinal applications [13–15]. The antitumor activity of such thio- compounds was revealed in their ability to inhibit ribonucleotide reductase (RR), a necessary enzyme for DNA synthesis. The thiosemicarbazone side chain located at a position α to the heterocyclic nitrogen, through a conjugated N-N-S tridentate ligand system, is essential for anticancer activity [15].

Thiosemicarbazones derivatives possess many promising biological activities, such as herbicidal, antimicrobial, antioxidant, antiviral, anti-HIV and antitumor activity[14], and chemotherapeutic agents which exhibit inhibitory activeities against most of the cancers through inhibition of a crucial enzyme obligatory for DNA biosynthesis and cell division, *viz*. ribonucleotide diphosphate reductase (RDR) [16]. Some thiosemicarbazones even increase their anti-tumour activity by their ability to form chelates with specific metal ions [17]. Heterocyclic thiosemicarbazone showed higher activity compared with aromatic thiosemicarbazones[18].

As a result, Thiosemicarbazones derivatives have been the subject of extensive investiga -tions. In the present study for new anticancer agents, we have evaluated the antitumor properties of recently developed synthetic Thiourea derivatives among which two compounds revealed important activity: 1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3) and 2,4- diacetyl pyridine- bis- 3-thiosemicarbazone(4) against breast, liver, colon, prostate cancer cell lines (in vitro study) and against EAC cells (in vivo study).

2-Materials and methods

2. 1.Chemistry

Thiosemicarbazone (thioureas) Replacement of oxygen atom in urea by sulphur atom produces thiourea which has been successfullly used in many infectious diseases. Thiourea can be readily synthesized by different synthetic routes among which condensation of primary and secondary amine with isothiocyanate, thiophosgene or its derivatives constitutes the most widely accepted general methods [19]. Recently Maddani and Prabhu disclosed a concise synthesis of substituted thiourea derivatives in aqueous medium via reaction between the amines and carbon disulphide in the presence of sodium hydroxide [20]. Thioureas displaying biological activities possess specific binding sites known as hydrogen binding area, complementary area and auxiliary binding area. Edrah with the help of X-ray crystallographic data suggested that the distal aryl/heterocyclic ring present in complementary area, occupying different positions depending on bond angles and the atomic distances, affects the potency of a drug [21].

2.2- Experimental

Melting points were determined on MEL- TEMP II melting point apparatus and uncorrected .Infrared spectra were recorded in afrlein-Elmer 1420 spectrometer and aBiorad FTS 7(KBr).NNR spectra were recorded on ageneral electrons QE 300 instrument and chemical shifts were given with respect TMS. Masss pectra were recorded on Gc/Ms with CI[chemical ionization] and a Hewlett – packard MS Engine Thermospray and ionization by electron impact at 70ev. The accelerating voltage was 6 kv,the temperature of the ion source was ≈200$^{°C}$ and the emission current ═ 100mA, microanalyses were conducted using an elemental analyzer 1106.

Syntheses of 1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)

1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3) was prepared according to the procedure reported literature.

2,4- Diacetyl pyridine (0.01 mole) was dissolved in absolute ethanol (40 ml) and mixed in a round bottomed flask with 30 ml of absolute ethanolic solution containing thiosemicarbazide (0.01 ml). The reaction mixture was heated under refux for 3 hours and then allowed to cool to room temperature and kept for 34 hours . The solid obtained was filtered off and washed with cold ethanol and then recrystalized from ethanol to give 3 (scheme 1) as pale yellow crystals, yield 71% , m.p. 250$^{°C}$,

IR (KBr) : 3456, 3178(NH_2),3255(NH), 1705-1632(C=N), 1603,1589(C=C),1401(C=S)cm^{-1}.

^{1}H-NMR (DEMSO-d_6). S 2.40 (S, 3H, CH_3), 2.63 (S, 3H, CH_3), 7.60 (S, 2H, NH_2), 7.91- 8.81 (m, 5H,H-pyridine), 10.30- 10.32 (S, 1H, NH) ppm.

^{13}C-NMR (DMSO – d6): δ194.56 (C=S), 179.67 (C=O), 153.95, 137.97,136.99, 124.96, 121,42(C- pyridine), 148.45(C=N), 25.81, 12.3(4CH_3) ppm.

MS (m/z; k): 237(M^{+1}, 9.20), 236(M^{+} , 67.31), 221(20.03), 144(10.31), 180(19.36), 176 (77.61), 162(13.20), 148(100),130(97.30),116(58.20), 106(78.30),105(75.10),104(27.30), 93(24.10),90(18.20),79(27.30),78(68.30),77(64.20),76(22.50),75(17 .20),60(88.30),52(18.30), 51(34.20).Anol . Calcd for $C_{10}H_{12}N_4S_2$:C, 50.84; H, 5.08, N, 23.73. Found : C, 50.67 , H, 4.47, N, 23.57.

Syntheses of 2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)

2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)) was prepared according to the procedure reported literature.

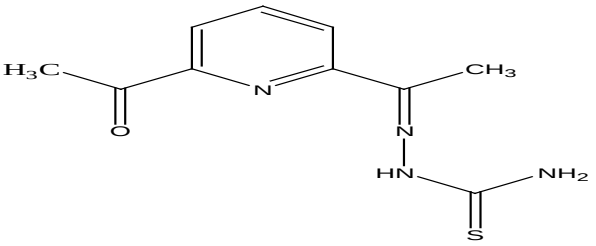
A mixture of 2,4- diacetyl pyridine (0.01 mole) and thiosemicarbazide(0.02 mole) in absolute ethanol (70 ml) in a round bottomed flask . The reaction mixture was heated under reflux for 4 hours, and then allowed to cool to room temperature and kept for 24 . The solid formed was filterated off, washed with cold ethanol,dried and reccrystalized from acetic acid to give 4 as yellow crystals, yield 67%, m.p.

IR (KBr) : 3428, 3161(NH_2), 3251(NH), 1650-1635(br-C=N), 1602,1566(C=C),
1442(C=S)cm^{-1}.

^{1}H-NMR (DEMSO-d_6). S 2.44 (S, 6H, 2*CH_3), 775 (t, 1H, py -H, J=8 H_2), 8.06 (br-s, 4H, 2 * NH_2), 8.59 (d, 2H, py – H, J=8H_2), 10.30- 10.32 (br-S, 2H, 2*NH) ppm.

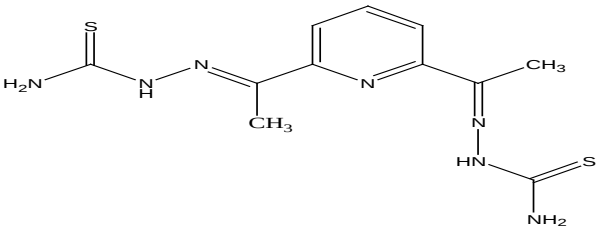
^{13}C-NMR (DMSO – d6): δ179.54 (2*C=S), 159.00 (C=N of pyridine ring), 148.53, 146.89 (2*C+N of ethylidene amino), 138.95, 137.10, 125.03, 121.31 (C – pyridine), 12.50 (2*CH_3) ppm.

Anol . Caclo for $C_{11}H_{15}N_6S_2$:C, 44.74, H, 5.08, N, 28.47. Found :C, 44.47 , H, 4.47, N, 28.28.



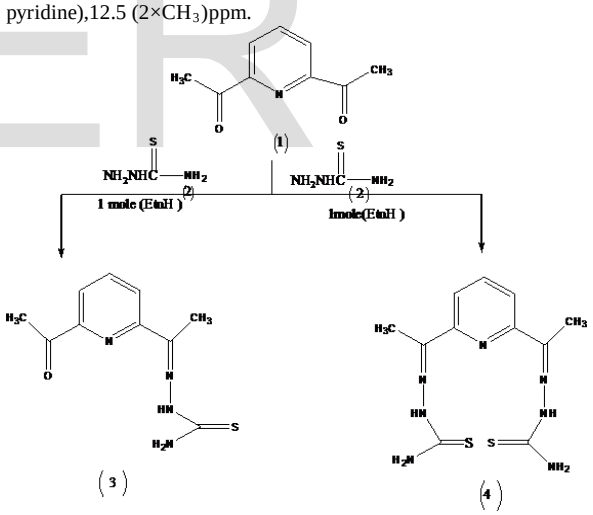
1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)

C═S≈194,56 ,C═O≈179.69 ,C═N(pyridine)≈153.45 , C═N≈(148.45)
137.97, 136.49, 129.96; 121.42 (C—pyridine) 25.81, 12.38(2 × CH_3)



2,4- diacetyl pyridine – bis(thiosemicarbazone)
(pyridine – 2,4 di(ethylidene amino)- 1,-1 bis - thiourea)

(H-NMR(Dms)) 2.44(S,6H,2×CH_3), 7.75- 8.69 (m,3H, H pyridine and 4H,2×NH_2), 10.30- 10.32(br.s, 2H, 2×NH)ppm.^{13}C-NMR(Dmso-d_6): 179.54(2×C═S), 154(C═N of pyridine), 148.53(2×CH_3N), 137.10, 138.95- 125.03, 121.3(C— of pyridine),12.5 (2×CH_3)ppm.



Scheme 1

1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3) 、 2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)

In vitro study:

Cytotoxicity: cytotoxic activity of compounds [**1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)** & **2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)**] was performed on a panel of human tumor cell lines (MCF-7(human breast cancer), HePG2 (Hepatocellular carcinoma),HCT116 (human colon cancer), PC3 (human prostate cancer) at differ -ent concentrations. The cytotoxicity was carried out

In vivo study:

Toxicity studies: Approximate LD50 of [**1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)** and **2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)**] in mice were determined according to the method Meier and Theakston(22)

.**Dose response curve:** Dose response curve of 3a and 3b in mice

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was determined according to the method Crump et al., (23).

Experimental design: 30 female Swiss albino mice were divided into 3 groups each one contains of 10 mice: Group I"served as positive control; i.p. injected with 2.5x106 of Ehrlich ascites carcinoma "EAC" cells.
Group II "[**1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)**therapeuticgroup, injected i.p. with 10 mg/kg one day after EAC injection and repeated doses of [**1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)** day after day;
Group III "**2,4- diacetyl pyridine- bis- 3- thiosemicarbazone(4)**] therapeutic group",
injected i.p. with 7.5mg/kg one day after EAC injection and repeated doses of **2,4- diacetyl pyridine- bis- 3-thiosemicarbazone(4)**] injected day after day.
After the end of the experiment, EAC cells were collected from mice, and viability study was assayed.
Cell Viability and Counting of EAC cells: the counting and viability of EAC cells was determined by the Trypan Blue Exclusion Method (24), where the total and viable cells (nonstained) were counted at magnifiation × 40; as the number of cells/ml was determined in the studied groups.
analysis
Statistical analysis was performed using SPSS software II version 14 (14). The effect of each parameter was assessed using the one way analysis of variance. Individual differences between groups were examined using Dunnett's test and those at p <0.05 was considered statistically signifiant.

3. Results:

Cytotoxicity: The in vitro cytotoxic activities of compounds ([**1-[(6-acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)** and **2,4-diacetyl pyridine- bis- 3- thiosemicarbazone(4)**]) were showed in table (1) and figures (1-4). Minimum Inhibitory concentrations of synthesized compound ([**1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)** were found to be 50 µg/ml against MCF-7, HePG2, HCT16, and PC3 cell lines; respectively. While, Minimum Inhibitory concentrations of synthesized compound **2,4- diacetyl pyridine- bis- 3-thiosemicarbazone (4)**were found to be 50 µg/ml against HePG2, HCT16, and PC3 cell lines ,and 25mg/ml against MCF-7

Table 1: Minimum inhibitory concentration of compounds (1)and (2) against MCF-7, HEPG2, HCT116, and PC3 cell line.

	MCF7	HEP2	HCT16	P3
Compd(1)	22.2	25	25	43.1
Compd(2)	25	25	41.9	31.1

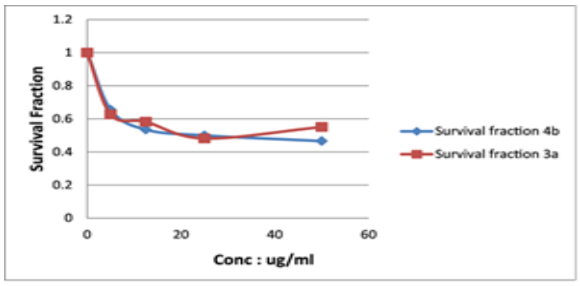


Figure (1): Minimum inhibitory concentration of compounds 3a and 3b against MCF-7 cell line.

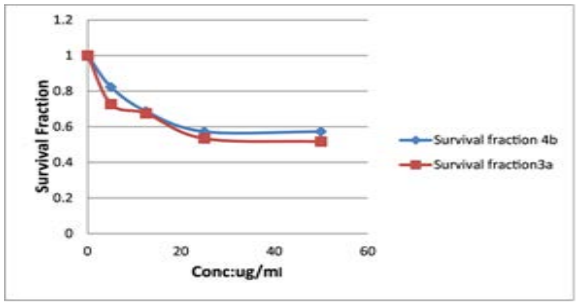


Figure (2): Minimum inhibitory concentration of compounds 3a and 3b against HePG2 cell line.

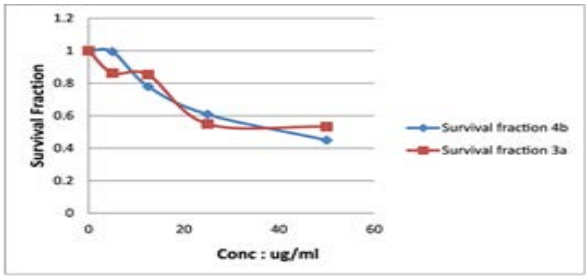


Figure (3): Minimum Inhibitory Concentration of compounds 3a and 3b against HCT16 cell line.

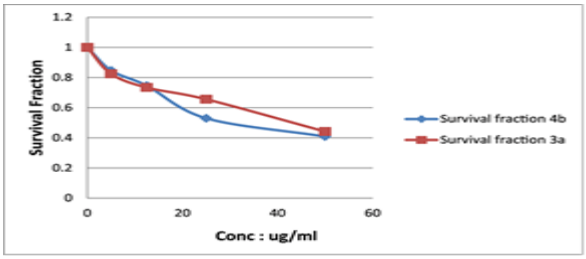


Figure (4): Minimum Inhibitory Concentration of Compounds 3a and 3b against PC3 cell line.

Determination of median lethal dose (LD50) of two compounds: our results revealed that, doses to 50 mg /kg ([**1-[(6- acetyl pyridine-2-YL ethylidene) amino]-thiourea(3)** and up to 400 mg /kg were considered safe for compound 2,4**- diacetyl pyridine- bis- 3- thiosemicarbazone (4)]**; where no mortality were observed.
Dose-response curve: the most effective doses were found to be 10 mg /kg and 7.5 mg /kg for compounds compound 1 and 2; respectively, Fig. (5).

Dose mg/kg	5	10	15	20
Count of EAC (3a)	20(×10^6)	16(×10^6)	22(×10^6)	27.6(×10^6)
Count of EAC(4b)	18(×10^6)	18(×10^6)	27.4(×10^6)	32.4(×10^6)

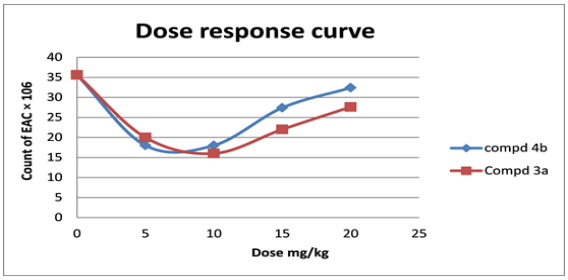


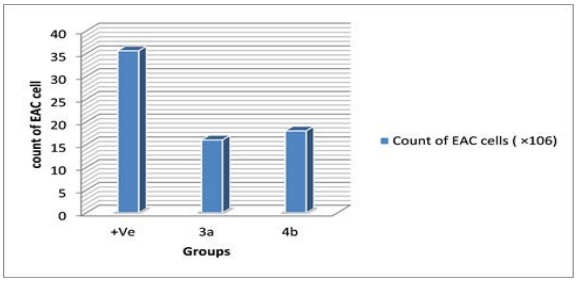
Fig. (5) Dose response curve for compound 3a & 3b

Volume and Counting of EAC cells: Table (2) summarizes the effect of compounds 3a and 3b on EAC cells volume and count. The mean volume of EAC in the positive control group was found to be 2.5 ±0.5 (ml). This value was significantly decreased by 55.5%, and by 73.3%, (p<0.001) for compounds 3a & 4b treated groups; respectively, Fig (6 a). Also, the mean count of EAC cells in the positive control group was found to be 35.6(×10^6), which significantly decreased by 65.9% and 78.9%, (p<0.001) for compounds 3a & 4b treated groups; respectively, compared to the positive control group, Fig (6 b).

Table (2): Effect of compounds 3a & 3b on the volume and count of EAC in the studied groups:

Parameter	Positive control	Cpd 3a treated group	Cpd 4b treated group
Volume of	4.5 ± 0.5	2.5± 0.56	1.7± 0.54

Ascitis Fluid (ml)			
% change	-------	55.5%	73.3%
Count of EAC cells (×106)	35.6(×10^6)	16(×10^6)	18(×10^6)
% change	----------	78.9%	65.9%



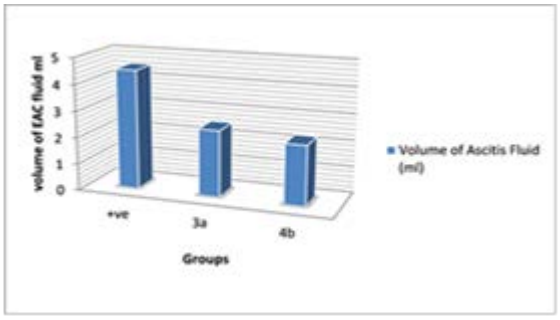


Figure (6): (a) Effect of compound 3a and 3b on EAC volume and (b) Effect of compound 3a and 3b on EAC count.

4. Discussion:

Cancer is a group of diseases of higher multicellular organisms. It is characterized by alterations in the expression of multiple genes, leading to dysregulation of the normal cellular program for cell division and cell differentiation (25). This result is an imbalance of cell replication and cell death that favors growth of a tumor cell population. Heterocyclic moieties can be found in a large number of compounds which display biological activity. The biological activity of the compounds is mainly dependent on their molecular structures (26). Schiff-base compounds have been used as fine chemicals and medical substrates [30]. Azomethine group (-C = N-)-containing compounds, typically known as Schiff's bases, have been synthesized via condensation of primary amines with active carbonyls. It is well established that the biological activity of hydrazone compounds is associated with the presence of the active (-CO-NHN = C-) pharmacophore and these compounds form a significant category of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antitumoral [31,32], antifungal [33-34], antibacterial [35,36], antimicrobial [37]. Schiff base complexes have a broad range of biological properties: antitumor, antiviral, antifungal, antibacterial [10]. They are also used in the treatment for diabetes and AIDS. As biological models, they help in understanding the structure of biomolecules and biological processes occurring in living organisms. They participate, inter alia, in photosynthesis and oxygen transport in organisms. They are involved in the treatment of cancer drug resistance, and often tested as antimalarials. It also could be used for the immobilization of enzymes [39, 40]. Biological activity Schiff bases are characterized by an imine group –N=CH-, which helps to clarify the mechanism of transamination and racemization reaction in biological system [41]. It exhibits antibacterial and antifungal effect in their biological properties [42, 43]. Metal-imine complexes have been widely investigated due to antitumor and herbicidal use. They can work as models for biologically important species [42].

All these findings encouraged us to explore the synthesis of Schiff bases [3a, 4-b] thiosemicarb -azone derivatives and examine their activities as in vitro anti-cancer against some different cell lines such as [MCF-7(human breast cancer), HePG2 (Hepatocellular carcinoma), HCT116 (human colon cancer), PC3 (human prostate cancer)] to assess their cytotoxicity effects. The results indicated that compound 3a,4b have cytotoxicity potency. Compound 3a showed a very potent activity against MCF-7, HePG2 and HCT116 with minimum inhibitory concentration (MIC) [22.2, 25, and 25, µg/ml, respectively] but compound 3a showed low activity against PC3 with minimum inhibitory concentration 43.1 µg/ml . Compound 4b showed a very potent activity against MCF-7, HePG2 and PC3 with minimum inhibitory concentration [25, 25, and 31.1 µg/ml, respectively] , but showed low activity against HCT116 with minimum inhibitory concentration [41.9µg/ml, respectively] for all cell lines compared with doxorubicin as reference drug. Thio - and pyridinethiosemicarbazones are important sulphur and nitrogen bearing organic reagents. The metal chelates of these reagents find a wide range of applications in medicine[38].

In Vivo study; doses until to 50 mg / kg were be safe in compound 3a(IC50═ 50) and up to400 mg/kg were be safe in compound 4b. We found that, 10 mg /kg and 7.5 mg /kg were considered to be the most effective dose of compounds 3a & 3b; respectively. In Vivo antitumor activity results against Ehrlich ascites carcinoma cells for compound 3a, 4b revealed that, the mean volume of EAC in the positive control group was found to be 4.5 ±0.5 (ml) as Amer (44) who reported that, the mean volume of EAC was 5.0±0.5 (ml). This value was significantly de- creased by 55.6% and by 73.3% ($p<0.01$) in compounds 3a & 4b treated groups; respectively, as shown in Fig (6 a). Also, the mean count of EAC cells in the positive control group was found to be 35.6± (×106), which significantly decreased by 78.9%, and 65.9% ($p<0.001$) in comp - ounds 3a & 4b treated groups; respectively, compared to the pos - itive control group, Fig (6 b). This indicates that compound 3 b has in vivo antitumor activity against EAC more than compound 3 a.

5- Conclusion:

The in vitro cytotoxic activity for the compounds 3 a,4 b against the human breast tumor cells (MCF-7), human hepatocellular cancer cells (HePG2), HCT16 (colon cancer), and PC3 (prostate cancer). Compound3a and 4b exhibits minimum inhibitory concentration against all cell lines at higher doses than compound 3a. Also, in vivo effect of the compounds 3a, and 3b exhibited significant anticancer activity towards EAC cells by reduction of their volume and count. On the basis of these results, compound 3a and4b may be considered as attractive leads in the future development of potential anticancer agents.

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REFFERANCE

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