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RESEARCH ARTICLE

SYNTHESIS OF NOVEL [1,2,4]TRIAZOLO[3,4-*B*][1,3,4]THIADIAZOLE-6(5H)-THIONE, 5,8-DIHYDRO-[1,2,4] TRIAZOLO[4,3-*B*][1,2,4,5]TETRAZINE AND 5,10-DIHYDRO - [1,2,4]TRIAZOLO[4,3-*B*][1,2,4] BENZOTRIAZINE DERIVATIVES AND STUDY THEIR BIOLOGICAL ACTIVITY.

A.Y.Hasssan[1], Mona.S.Kadh[2], N.M.Saleh[2] and E.S.Abou-Amra[3].

1. Prof. of Organic Chemistry, Faculty of Science (Girls), Al-Azhar University.
2. Assist. Prof. of Organic Chemistry, Faculty of Science (Girls) Al-Azhar University.
3. Demonstrator at Organic Chemistry Department, Faculty of Science (Girls) Al-Azhar University.

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Abstract

Nitrogen-containing heterocycles are of particular interest and significant importance for the discovery of potent bioactive agents in pharmaceutical industry. The present study reports the synthesis of new heterocycles including 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole derivatives (**2**, **6**, $\mathbf{8_{a,c}}$,$\mathbf{9_{a,b}}$,$\mathbf{10_a}$, and $\mathbf{11_{a,b}}$) by cyclocondensation reaction of $\mathbf{1_{a-c}}$with CS_2, ethylcyanoacetate, cyclohexanone, ethyl chloroformate, trichloroacetic acid and oxalic acid scheme (1,2).[1,2,4]triazolo[4,3-*b*][1,2,4, 5]tetrazine derivative**13** was prepared by the reaction of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol $\mathbf{1_b}$ with hydrazine hydrate followed by cyclocondensation with *para*-chlorobenzaldehyde scheme (3).[1,2,4]triazolo[4,3-*b*][1,2,4] benzotriazine derivative **14** which prepared by cyclocondensation of $\mathbf{1_b}$ with *ortho*-aminothiophenol scheme (3).The structures of newly synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral data. Some of These compounds (**7,** $\mathbf{8_c}$, and $\mathbf{9_a}$) were exhibited significant growth inhibition against (HepG2) cancer cell line when screened for their in vitro cytotoxic activity against human hepatocellular liver carcinoma (HepG2). The results of such studies are discussed in this paper.

Introduction:-

The design, synthesis and production of molecules having value as human therapeutic agents remain one of the main objectives of organic and medicinal chemistry. The chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. For example, a large number of 1,2,4-triazoles have been incorporated into a wide variety of therapeutically interesting drug candidates possessing antimicrobial [1-5], anti-inflammatory [6], analgesic [7] and anticancer activities [8,9]. Among these heterocycles, the mercapto and thione substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives [10,11]. In addition to these important biological applications, mercapto 1,2,4-triazoles are also of great utility

Corresponding Author:-A.Y.Hasssan
Address:-Prof. of Organic Chemistry, Faculty of Science (Girls), Al-Azhar University.

inpreparativeorganic chemistry as useful intermediates for the preparation of some triazolothiadiazoles, triazolotetrazine, triazolotriazine, triazolothiadiazines and triazolothia-diazepins. The amino and mercapto groups are ready made nucleophilic centers for the synthesis of condensed heterocyclic rings [12]. Meanwhile, the triazolothiadiazoles and triazolothiadiazines exhibiting broad spectrum biological profile have matured into indispensable heterocyclic scaffolds [13,14]. New analogs of triazolothiadiazoles, triazolo- thiadiazines and triazolothiadiazepins bearing different substituents on the 3 and 6 positions were found to possess significant anticancer activity.

For this reason, much attention has been directed in our laboratory for synthesis of new compounds containing [1,2,4]triazole nucleus fused with a different substituted [1, 3, 4] thiadiazole, [1,2,4,5]tetrazine and [1,2,4] triazine moieties, in order to study their anticancer activity.

The present study describes the synthesis and characterization of novel triazolothia- diazoles, triazolotetrazine and triazolotriazine derivatives and evaluation of their anticancer activities.

Materials and Methods:-

Melting points of all the compounds are determind in open capillary method and are uncorrected. IR spectra are recorded in KBr pellets on Schimadzu FT-IR Affinity-1 spectrometer.^{1}H NMR spectra are recorded in DMSO-d_6 solvent on Bruker High Performance Digital FT-NMR Spectrometer Avancelll 400 MHz using TMS as internal stander and Mass spectra were done in the regional center for mycology and biotechnology, at Al-Azhar University.

Preparation of 3-(Trifluoromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-6-(5H)-thione (2):-

Carbon disulfide (3.3 cm^3, 10 mmol) was added to a mixture of compound $\mathbf{1_a}$ (1.84 g, 10 mmol) in ethanolic KOH solution (0.56 g, 10 mmol of KOH in 30cm^{3}EtOH). The reaction mixture was heated under reflux for 6h, the solvent was removed under reduced pressure, and then the residue was poured in to an ice-water mixture with stirring and acidified with 10% HCl. The separated solid was filtered off and washed by ethanol, methanol, acetone, DMF, dioxan and benzene.

Compound **2**: white powder, (226.2); yield 1.65g (73%); m.p.: >360^{o}C. IR(KBr,cm^{-1}): 3324(NH); 3110(SH);and 1624(C=N). ^{1}H NMR(DMSO-d$_6$,δppm): 8.20 (s,1H, NH, D_2O exchangeable).

6-(Ethylthio)-3-(trifluoromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (3):-

The thiol derivative **2** (2.26 g, 10mmol)was dissolved in an aqueous solution of KOH (0.53 g, 10mmol in 20 cm^3 H_2O). Ethyl iodide (1.56 g, 10mmol) was added with continuous shaking for a few minutes until the product separated. The excess of ethyl iodide was removed by heating on a water bath and the crude ethyl thio ether was separated by filtration.

Compound **3**: White crystals, (254.26); yield 1.96g (77%); m.p.: 270-272°C .IR (KBr, cm^{-1}):2926(CH-aliphatic); and 1630,1624(C=N).

6-Hydrazinyl-3-(trifluoromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole (4):-

A solution of compound **3** (2.54 g, 10mmol) in 10 cm^{3}EtOH and 0.5 g $N_2H_4.H_2O$ (10mmol) was heated under reflux for 6 h. The solvent was removed under reduced pressure and the precipitated solid was filtered off, washed with EtOH, and recrystallized from ethanol.

Compound **4**: fine white crystals, (224.17); yield 1.78g (79%); m.p.: 80-82^{o}C. IR (KBr, cm^{-1}): 3244, 3142 ($NHNH_2$); 1640 (C=N-thiadiazole);and 1624 (C=N).^{1}H NMR (DMSO-d$_6$, δppm): 3.38 (s, 2H, NH_2, D_2O exchangeable);and 8.05 (br.s, 1H, NH, D_2O exchangeable).

6-(Trifluoromethyl)bis([1,2,4]triazolo)[3,4-*b*:4',3'-*d*][1,3,4]thiadiazole-3-(2H)-thione(5):-

A mixture of an alcoholic KOH solution (0.56 g, 10 mmol in 7 cm^3 EtOH) and 3cm^3 H_2O was added to a solution of compound **4** (2.24g, 10mmol) in 25 cm^{3}EtOH with stirring. CS_2(0.76 g, 10mmol) was added dropwise with continuous stirring and the reaction mixture was refluxed until the H_2S ceased for 20 h. The reaction mixture was concentrated, cooled to room temperature and poured in to 100 cm^3 of an ice-water mixture, then acidified with conc. HCl. The precipitate was filtered off and crystallized from ethanol.

Compound **5**: yellow powder, (266.23); yield 2.00g (75%); m.p.: 158-160^{o}C. IR (KBr, cm-^{1}): 3199 (NH); and 1624, 1619 (C=N). ^{1}H NMR (DMSO-d_6,δppm): 8.29 (s, 1H, NH, D_2O exchangeable).

Ethyl2-(3-(trifluoromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)acetate(6):-
An equimolar mixture of compound **1$_a$** (1.84 g, 10 mmol) and ethyl cyano acetate (1.13 g, 10 mmol) in (10 ml) of ortho phosphoric acid was heated under reflux 3h at 120^{o}C. The mixture was cooled, dilute with 100 ml of water and made alkaline by adding sodium hydrogen carbonate. The solid obtained by scratching was collected by filtration, dried and recrystallized by ethanol.

Compound **6**: Fine white crystals,(280.23); yield 1.89g (67%); m.p.:60^{o}C. IR (KBr, cm^{-1}):2970-2909(CH-aliphatic); 1717(C=O); 1640(C=N-thiadiazole);and1624(C=N-triazole);^{1}H NMR (DMSO,δppm):1.46(t, 3H, CH_3); 3.35(q, 2H, OCH_2);and 4.80(s, 2H, CH_2). MS: m/z **(%):** 280($M^{+\cdot}$, 1.22), 207(37.25), 140(3.96), 81(86.01), 57(100), and 55(40.34).

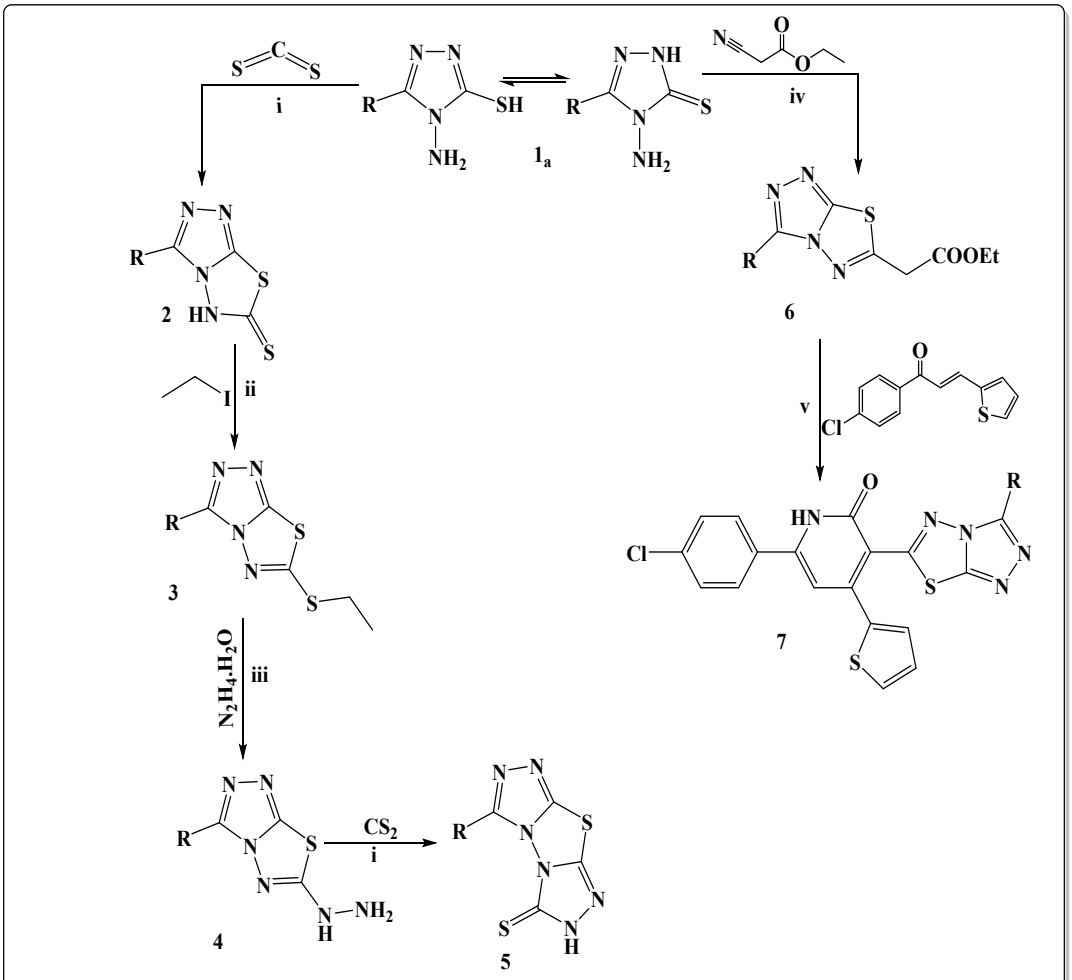
6-(4-chlorophenyl)-4-(thiophen-3-yl)-3-[3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-6-yl]pyridin-2-(1H)-one(7):-
A mixture of compound **6** (2.82g, 10mmol), chalcone (2.08g, 10mmol) and (80mmol)of ammonium acetate in (20ml) of ethanol was heated under reflux 12h. A solid product separated and was filtered off, washed with water, ethanol and crystallized from diethyl ether.

Compound **7**: Yellow powder,(479.88); yield 2.38g (49%);m.p.: 92-94^{o}C.IR (KBr, cm^{-1}):3446(OH tautomer); 3292(NH); 3105(CH-aromatic);1680(C=O);1651(C=N-thiadiazole);and 1624(C=N).^{1}H NMR(DMSO, δppm): 7.18 (s, 1H, CH-pyridinone); 7.61-8.12(m, 10H, Ar-H);and 13.12(NH).MS: m/z (%):480($M^{+\cdot}$+1, 0.89), 152(72.93), 136(100), 85(70.02), and 75(78.76).

3'-(trifluoromethyl)-5'H-spiro[cyclohexane-1,6'-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadi-azole](8$_a$) and 3',3'''-(phenylmethylene)bis(sulfanediyl)bis(methylene)bis(5'H-spiro [cyclohexane-1,6'-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole])(8$_c$):-
An equimolar mixture of compound **1$_{a,c}$** (1.84g, 4.12g, 10 mmol) and cyclohexanone (0.98 g, 10 mmol) in glacial acetic acid (20 ml), few drops of Conc. $H_2SO_4$was refluxed for 16 h. The reaction mixture was cooled to room temperature, poured on to crushed ice and allowed to stand overnight. The separated solid was filtered off, washed thoroughly with water, dried, and crystallized by proper solvent.

Compound **8$_a$**: Black powder, crystallized by benzene,(264.07); yield 1.82g (69%); m.p.:196-198^{o}C. IR (KBr, cm^{-1}): 3124(NH); 2931,2858(CH-aliphatic);and 1624(C=N).

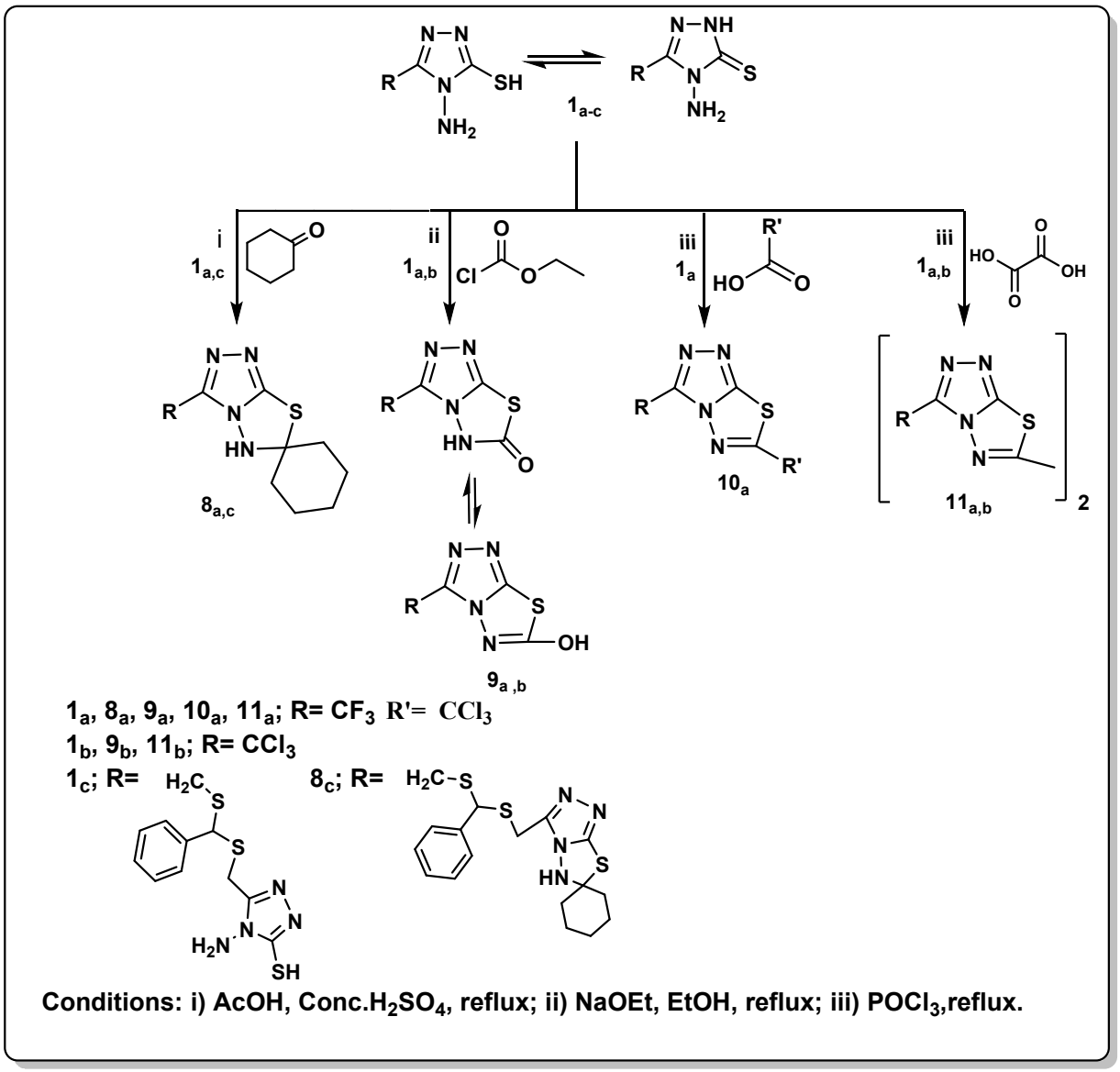
Compound **8$_c$**: Coffee powder, washed by ethanol, methanol, acetone, ethyl acetate and benzene,(572.83); yield 2.61g (46%); m.p.: > 360^{o}C. IR (KBr, cm^{-1}): 3197, 3180 (2NH); 3088 (CH-aromatic); 2931, 2856 (CH-aliphatic); 1624 (C=N); and 1600 (C=C). ^{1}H NMR (DMSO-d_6,δppm):1.23(t, 8H, $4CH_2$); 1.76-1.99 (m, 8H, $4CH_2$); 3.70 (s, 4H, 2(–S–CH_2))**;** 4.89 (s, 1H, -S- CH); 7.09-8.25 (m, 7H,Ar-H);and 11.26, 11.29 (2s, 2H, 2(NH) D_2O exchangeable).



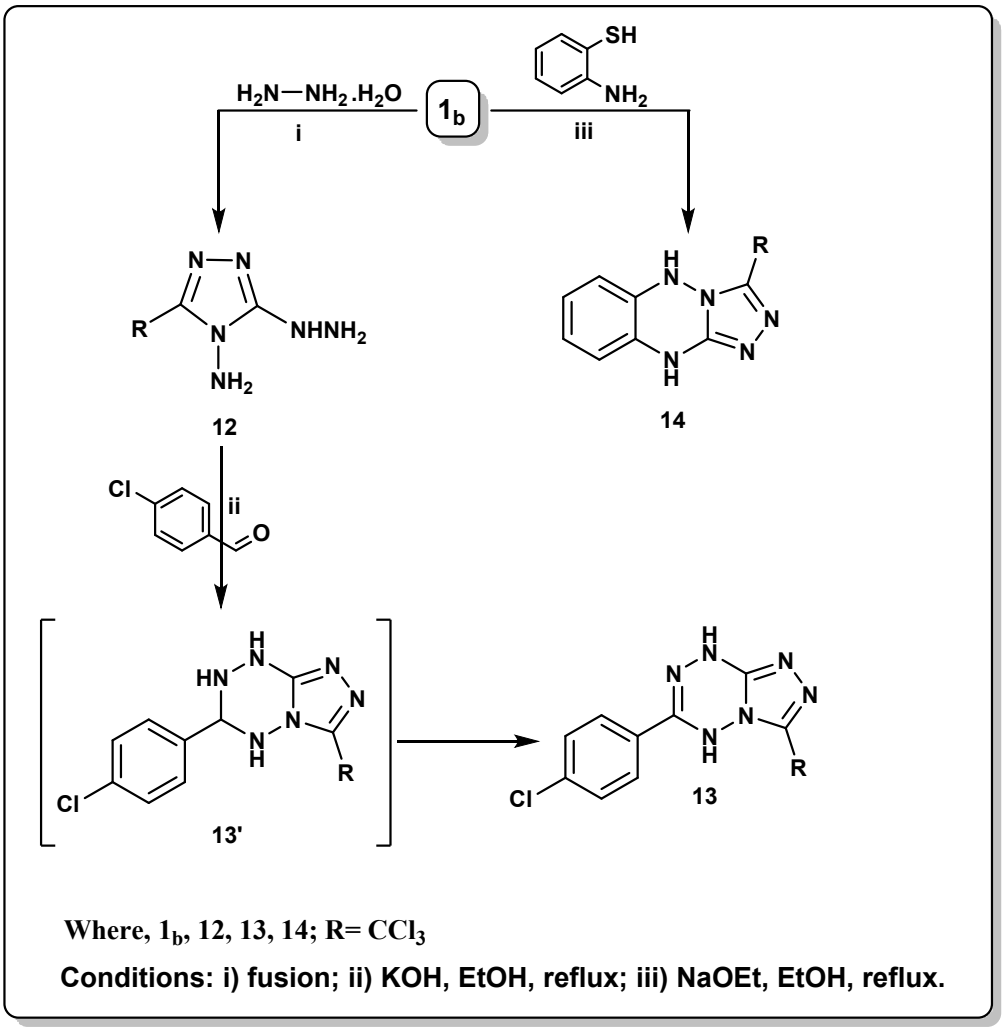
Where: 1_a, 2, 3, 4, 5, 6, 7; R= CF_3

Conditions: i) KOH, EtOH, reflux; ii) aq. KOH, stirring ; iii) EtOH, reflux; iv) ortho phosphoric acid, reflux; v) CH_3COONH_4, EtOH, reflux .

Scheme (1)


1a-c
i
1a,c
ii
1a,b
iii
1a
iii
1a,b
8a,c
9a ,b
10a
11a,b
2
1a, 8a, 9a, 10a, 11a; R= CF3 R'= CCl3
1b, 9b, 11b; R= CCl3
1c; R=
8c; R=
Conditions: i) AcOH, Conc.H2SO4, reflux; ii) NaOEt, EtOH, reflux; iii) POCl3,reflux.

Scheme (2)



Scheme (3)

3-(Trifluoromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-(5H)-one(9_a)and3-(trichloromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-(5H)-one(9_b):-

An equimolar mixture of compound $\mathbf{1_{a,b}}$ (1.84g, 2.33g, 10 mmol) and ethyl chloroformate (1.08 g, 10 mmol) in ethanolic sodium ethoxide solution [(prepared by dissolving sodium metal (0.24 g, 10 mmol) in absolute ethanol (30 ml)] was heated under reflux 20 h. The reaction mixture was cooled, poured on to crushed ice, the solid obtained was filtered off and washed by ethanol, hexane, DMF, and benzene.

Compound $\mathbf{9_a}$: White crystals, (210.1); yield 1.20g (57%); m.p.: >360^{o}C. IR(KBr, cm^{-1}): 3468 (OH, NH tautomer); 1692 (C=O); 1630 (C=N-thiadiazole); and 1624 (C=N).^{1}H NMR (DMSO-d_6, δppm): 8.20 (s, 1H, NH, D_2O exchangeable). MS: m/z (%):210($M^{+\cdot}$, 15.60), 139(100), and 81(78.05).

Compound $\mathbf{9_b}$: Brown powder,(259.5); yield 1.26g (49%);m.p.: 254-256^{o}C. IR (KBr, cm^{-1}): 3438 (OH); 1640 (C=N, thiadiazole ring); and 1624 (C=N).MS: m/z (%): 259($M^{+\cdot}$, 2.71); 84(84.83); 60(78.55); 55(80.06); and 43(100).

6-(Trichloromethyl)-3-(trifluoromethyl)-[1,2,4]triazole[3,4-*b*][1,3,4]thiadiazole(10_a), 3,3'-bis(trifluoromethyl)-6,6'-bi[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole(11_a) and3,3'-bis(trichloromethyl)-6,6'-bi[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole(11_b):-

An equimolar mixture of compound $\mathbf{1_{a,b}}$ (1.84g, 2.33g, 10 mmol) and acid derivatives (oxalic acid ortrichloro acetic acid) (0.90g, 1.63g, 10 mmol) in presence of phosphorous oxychloride (10 ml) was refluxed at (150-180)^{o}C for 6h.The reaction mixture was allowed to cool and then poured on to crushed ice while scratching. The resulting solid was filtered off and crystallized by proper solvent.

Compound **10_a**: Shine white crystals, recrystallized by ethanol,(311.5); yield1.96g (63%);m.p.: 136-138^oC. IR(KBr,cm^{-1}): 1651 (C=N-thiadiazole);and 1624 (C=N). MS: m/z (%):311($M^{+\cdot}$,4.87), and 70 (100).

Compound **11_a**: Off white powder, crystallized by dioxane,(386.26); yield2.11 g (54%); m.p.:264-266^oC. IR(KBr,cm^{-1}):1651 (C=N-thiadiazole);and 1624 (C=N). MS: m/z(%): 387($M^{+\cdot}$+1, 0.82), 386($M^{+\cdot}$,6.22),and 69 (100).

Compound **11_b**: Black powder, washed by ethanol, methanol, acetone, DMF, hexane and benzene,(484.99); yield 2.41 g (49%);m.p.:>360^oC.IR (KBr,cm^{-1}):1651 (C=N-thiadiazole);and 1624 (C=N). MS: m/z(%): 485($M^{+\cdot}$,2.06), and 40 (100).

3-Hydrazino-5-trichloromethyl-[1,2,4]triazol-4-yl amine(12):-
An equimolar mixture of compound **1_b** (2.33g, 10 mmol) and N_2H_4 99% (0.32 g, 0.31 ml, 10 mmol) was heated under reflux for 30 h. The reaction mixture was cooled, triturate with ethanol, concentrated and the solid obtained was recrystallized from ethanol.

Compound 1**2**: off white crystals, (231.47); yield 1.56g (67%); m.p.: 66-68^oC.IR (KBr, cm^{-1}): 3375, 3240 ($2NH_2$, NH); and 1624 (C=N).

6-(4-chloro-phenyl)-3-trichloromethyl-5,8-dihydro-[1,2,4]triazolo[4,3-*b*][1,2,4,5] tetr-azine (13):-
An equimolar mixture of compound 1**2**(2.31g, 10 mmol) and parachlorobenzaldehyde (1.4 g, 10 mmol) in alcoholic KOH [(0.56 g, 10 mmol) KOH in 30 ml ethanol] was heated under reflux for 6 h. The reaction mixture was allowed to cool and then poured on to crushed ice with scratching. The resulting solid was filtered off and recrystallized from acetone.

Compound **13**: shiny yellow crystals, (352.01); yield 2.08g (59%); m.p.: 188-190^oC. IR (KBr, cm^{-1}): 3402, 3365(2NH); 3047(CH-aromatic);and 1624(C=N).^{1}H NMR (DMSO-d_6, $^\delta$ppm): 7.59 (d, 2H, AA'XX' (P-Cl phenyl) J=7.2 Hz); 7.90 (d, 2H, AA'XX' (P-Cl phenyl) J=7.2 Hz); and 8.72 (s, 2H, 2NH, D_2O exchangeable). MS: m/z(%): 352($M^{+\cdot}$, 8.37); 256(65.14); 98(100); and 76(23.80).

3-(Trichloromethyl)-5,10 –dihydro[1,2,4]triazolo [4,3-*b*][1,2,4] benzotriazine (14):-
An equimolar mixture of compound **1_b** (2.33 g, 10 mmol) and ortho amino thiophenol (1.25 g, 10 mmol) in ethanolic sodium ethoxide solution [(prepared by dissolving sodium metal (0.24 g, 10 mmol) in absolute ethanol (30 ml)] was heated under reflux 20 h. The reaction mixture was cooled, poured on to crushed ice, the solid obtained was filtered off and washed by dioxan, THF, acetone, CCl_4 and petroleum ether 60-80.

Compound **14**: coffee powder, (290.54); yield 1.42g (49%); m.p.: 236-238^oC. IR (KBr, cm^{-1}):3417,3331(2NH); 3050(CH-aromatic);and1627(C=N).^{1}H NMR(DMSO-d_6,δppm): 5.58 (br.s,NH, D_2O exchangeable); and 6.41-7.42(m,4H,Ar-H).

Results and Discussion:-

In this article, we have synthesized compounds [**2**, **3**, **4**, **5**, **6**, **7**,**10_a**, **$8_{a,c}$**, (**9**, **$11)_{a,b}$**,**12**, **13**and**14**].The structures of the synthesized compounds have been supported by IR, ^{1}H NMR, and mass spectral studies.

In the present investigation, 4-amino- 5-trifluoromethyl-4H-1,2,4-triazole-3-thiol **1_a**was refluxed with CS_2 and ethanolic KOH for 6 h to afford the target compound; 3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-6 (5H)-thione**2**.The **IR** spectrum of **2**lacked the absorption band due to NH_2 group and it showed the absorption band at 3324 cm^{-1} corresponding to NH function of thiadiazole .The **^{1}H NMR** spectrum of **2** showed a deuterium oxide exchangeable singlet at δ 8.20 ppm due to NH proton.

The target compound; 6-(Ethylthio)-3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazole **3** was prepared through shaking 3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-6-(5H)-thione **2** in an aqueous solution of KOH with ethyl iodide for a few minutes until the product separated.The**IR** spectrum of**3**lacked the absorption band due to NH group but it showed an absorption band at 2926 cm^{-1} corresponding to CH-aliphatic.

6-(Hydrazino)-3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3, 4]thiadiazole**4** was obtained through refluxing of 6-(Ethylthio)-3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3, 4]thiadiazole**3** with excess hydrazine hydrate in absolute

ethanol for 6 h. The **IR** spectrum of **4** exhibited absorption bands at (3244, 3142) cm^{-1} due to NH, NH_2 functions. It also revealed an additional band at 1640 cm^{-1} corresponding to C=N of the thiadiazolering.The **^{1}H NMR** spectrum of **4** showed two deuterium oxide exchangeable singlets at δ(3.38 , 8.05) ppm attributed to NH_2 and NH protons; respectively.

In our present work , aqueous ethanolic solution of 6-(Hydrazino)-3-(trifluoromethyl) [1,2,4]triazolo[3,4-*b*][1,3, 4]thiadiazole**4** was stirring with CS_2 in the presence of ethanolic KOH solution, then the reaction mixture was refluxed 20 h until elimination of H_2S to afford 6-(trifluoromethyl)bis[1,2, 4]triazolo[3,4-*b*:4′,3′-*d*][1,3, 4]thiadiazole-3-(2H)-thione**5**. The **IR** spectrum of **5** lacked the absorption band due to NH_2 group and it showed an absorption band due to NH group of the new triazole ring at 3199cm^{-1}. In addition to it lacked the absorption band due to C=N of the thiadiazole ring and it showed an absorption band at 1619 cm^{-1} corresponding to C=N function group of the new triazole ring. The **^{1}H NMR** spectrum of **5** showed a deuterium oxide exchangeable singlet at δ 8.29 ppm due to NH proton.

Ethyl-2-(3-(trifluoromethyl)[1,2, 4]triazolo[3,4-*b*][1,3, 4]thiadiazol-6-yl)acetate **6** was prepared by refluxing 4-amino-5-trifluor-methyl-4H-1,2,4-triazole-3-thiol $\mathbf{1_a}$ with ethyl cyanoacetate in the presence of ortho phosphoric acid at 120^{o}C for 3 hours. Compound **6** was formed via nucleophilic addition of 1,2,4-triazole amino thiol derivative $\mathbf{1_a}$ at the cyano group of ethyl cyanoacetate, followed by cyclization with elimination of ammonia.The**IR** spectrum of **6** contained an absorption band at 1717 cm^{-1}, typical of ester carbonyl. Its **^{1}H NMR** spectrum showed a methylene proton singlet at δ 4.80ppm and signals from the ethyl protons at δ 1.46 (t) and δ 3.35ppm (q).The **electron impact mass** spectrum of compound **6** showed a peak at m/z 280 (1.22%) corresponding to $M^{+\cdot}$. While its base peak appeared at m/z 57 (100%).

Molecule **6** possess a chemically active side chain which makes it capable of undergoing further transformations, so acetate **6**was treated with (E)-1-(4-chlorophenyl)-3-(thiophen-2-yl)prop-2-en-1-one in presence of 8 equiv. of ammonium acetate in ethanol to obtain 6-(4-chlorophenyl)-4-(thiophen-2-yl)-3-(3-(trifluoromethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)pyridin-2(1H)-one **7** .The **IR** spectrum of **7** exhibited broad absorption band at 3446 cm^{-1} due to the tautomeric OH function .In addition to an absorption band corresponding to NH moiety of the pyridinone ring at 3292 cm^{-1}. It also showed an absorption band at 1680 cm^{-1} corresponding to amidic C=O function. The **^{1}H NMR** spectrum of **7** contained a singlet at δ13.12 ppm and δ 7.18ppm attributed to NH and CH pyridinone protons; respectively. Other protons appeared at their expected chemical shifts.The**electron impact mass** spectrum of compound **7** showed a peak at m/z 480 (0.89%) corresponding to $M^{+\cdot}$. While its base peak appeared at m/z 136 (100%).

The interaction of 4-amino-3-mercapto-1,2,4-triazole derivatives$\mathbf{1_{a,c}}$ with cyclo-hexanone in boiling acetic acid, containing a few drops of conc. H_2SO_4, for 16h afforded the spiro[cyclohexane-1,6'-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole] derivatives $\mathbf{8_{a,c}}$ .The **IR** spectra of $\mathbf{8_{a,c}}$ lacked the absorption bands due to NH_2 function and showed an absorption band at (3124-3180)cm^{-1} due to NH function of thiadiazine. In addition to showed absorption bands at (2931, 2858, 2856) cm^{-1}due to CH-aliphatic.other functions appeared at their expected frequencies.The**^{1}H NMR** spectrum of $\mathbf{8_c}$ revealed two deuterium oxide exchangeable singlets at δ11.26 ppm and δ11.29 ppm attributed to 2NH protons. In addition to the spectrum showed a triplet signal at δ 1.23ppm integrated for 8H attributed to $4CH_2$ and a multiplet at δ (1.76-1.99) ppm integrated for 8H due to $4CH_2$ of bis(spiro[cyclohexane-1,6'-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole) protons. Other protons appeared at their expected chemical shifts.

4-amino-4H-1,2,4-triazolo-3-thiol derivatives $\mathbf{1_{a,b}}$ was refluxed with ethyl chloroformate in ethanol containing sodium ethoxide for 20 h to yield the target compound; 3-substituted[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-(5H)-one $\mathbf{9_{a,b}}$ by elimination of HCl and EtOH molecules **.**The **IR** spectrum of $\mathbf{9_a}$ revealed a broad absorption band corresponding to tautomeric OH and NH functions at 3468 cm^{-1} and additional band due to amidic C=O group at 1692 cm^{-1}. However, the **IR** spectrum of compound $\mathbf{9_b}$ showed absorption band at 3438 cm^{-1} due to OH function group. The **^{1}H NMR** spectrum of $\mathbf{9_a}$ revealed a deuterium oxide exchangeable singlet at δ 8.20 ppm attributed to thiadiazole NH proton.The**electron impact mass** spectra of compounds $\mathbf{9_{a,b}}$ revealed the molecular ion peaks at m/z 210 ($M^{+\cdot}$, 15.60%) and 259($M^{+\cdot}$, 2.71%); respectively. In addition to showed the base peaks at m/z 139 (100%) and 43(100%) ; respectively.

The target compounds; 6-(trichloromethyl)-3-(trifluoromethyl)[1,2,4]triazole[3,4-*b*][1,3,4]thiadiazole**10$_a$** and substituted [1,2, 4]triazole[3,4-*b*][1,3, 4]thiadiazole**11$_{a,b}$** were obtained through a one-pot reaction by condensation 4-amino-4H-1,2,4-triazole-3-thiol derivatives **1$_a$** and **1$_{a,b}$** with tri chloroacetic acid and oxalic acid in the presence of phosphorus oxy chloride ; respectively, followed by an intramolecular ring closure to form the five-membered ring .The structure of the synthesized compounds was firmly established on the basis of their **IR** analysis. Compound **10$_a$** and**11$_{a,b}$** showed absences of absorption bands respect to NH and NH_2 functions, indicates the formation cyclic. In addition to reveled absorption bands at 1651cm^{-1} corresponding to C=N of thiadiazolering.The**electron impact mass** spectrum of compound **10$_a$** revealed the molecular ion peak at m/z 311 ($M^{+\cdot}$, 4.87%) and the base peak at m/z 70 (100%) ,while the **electron impact mass** spectrum of compound **11$_a$** revealed its molecular ion peak at m/z 386($M^{+\cdot}$, 6.22) The spectrum of **11$_a$**displayed its base peak at m/z 69(100%) .

Fusion of 1,2,4 triazole amino thiol derivative **1$_b$** with hydrazine hydrate led to the formation of 3-hydrazinyl-4H-1,2,4-triazol-4-amine derivative 1**2**because of the expulsion of H_2S. Interaction of**12**with p-chlorobenzaldehyde using KOH and ethanol as a media of the reaction caused cyclization to give 6-(4-chlorophenyl)-5,6,7,8-tetrahydro[1,2, 4]triazolo[4,3-*b*][1,2,4, 5]tetrazinederivative 1**3'** which undergo auto oxidation to prevent formation of aldehyde result in the formation of 6-(4-chlorophenyl)-5,8-dihydro[1,2,4]triazolo[4,3-*b*][1,2,4, 5]tetrazine derivative**13** .The **IR** spectrum of **12**lacked the absorption band due to SH thiol and it showed the absorption bands at 3375cm^{-1} and3240cm^{-1} corresponding to NH_2 and NH functions. Also **IR** spectrum of 1**3** showed absorption bands at (3402, 3365)cm^{-1} according to 2NH functions , absorption band at 3047 cm^{-1} for CH-aromatic and at 1624cm^{-1}due to (C=N) .The **^{1}H NMR** spectrum of 1**3** showed a deuterium oxide exchangeable singlet at δ 8.72 ppm due to 2NH protons. Other protons appeared at their expected chemical shifts.The**electron impact mass** spectrum of compound 1**3** showed a peak at m/z 352 (8.37%) corresponding to $M^{+\cdot}$.While its base peak appeared at m/z 98 (100%).

Expulsion of H_2S gas can be observed in the reaction of 4-amino-4H-1,2, 4triazole-3-thiol derivative **1$_b$** with *ortho*-amino thiophenol which led to the production of 5,10-dihydro [1,2, 4]triazolo[4,3-*b*][1,2, 4]benzotriazine derivative **14**. The **IR** spectrum of **14** reveled absorption bands at 3417cm^{-1} and 3331 cm^{-1} corresponding to 2NH function groups of triazine, absorption band at 3050 according to CH-aromatic and at 1627 for (C=N).**^{1}H NMR** spectrum of **14** showed a deuterium oxide exchangeable broad singlet at δ 5.58ppm due to 2NH protons and at 6.41-7.42 due to (m,4H,Ar-H).

Biological Activity:-

Anticancer screening studies:-

Some of the compounds were screened for their in vitro cytotoxic activity against human hepatocellular liver carcinoma (HepG2) in the regional center for mycology and biotechnology, at Al-Azhar University. Doxorubicin was used as the reference drug in this study. It is well documented that doxorubicin induces its antitumor activity through several mechanisms including inhibition of topoisomerase II, DNA intercalation, generation of reactive oxygen species and DNA single and double strand breaks.

Cytotoxicity evaluation using viability assay:-

For cytotoxicity assay, the cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well micro titer plate incubated for 24h at 37^{o}C in a humidified incubator with 5% CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of tested sample in fresh maintenance medium and incubated at 37^{o}C. A control of untreated cells was made in the absence of tested sample. A positive control containing Doxorubicin drug was also tested as reference drug for comparison. Six wells were used for each concentration of the test sample. Every 24h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet (Mosmann, 1983; Gangadevi and Muthumary, 2007) followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader (SunRise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation.The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots.

The six dose growth inhibition percent and the IC_{50} values of the tested compounds **6,7, 8_c,9_a, 10_a,11_a, 13** and**14**against liver HepG2 are represented in tables (**1**).

Table 1:- Six dose growth inhibition percent and IC_{50} values of the tested compounds against HepG2 cell line.

Sample concentration (µg/ml) Compound No.	Growth inhibition %						IC_{50} (µg/ml)
	50	25	12.5	6.25	3.125	1.56	
6	27.94	14.68	5.17	1.72	0	0	**>50**
7	93.65	87.42	76.74	68.56	54.37	36.16	**2.75**
8_c	88.36	79.53	70.68	38.22	19.39	9.15	**8.52**
9_a	91.69	80.11	71.28	60.35	32.57	21.81	**5.09**
10_a	40.52	23.69	10.53	3.98	0.84	0	**>50**
11_a	54.37	29.58	13.79	6.46	1.24	0	**45.6**
13	65.68	52.17	23.79	9.36	1.82	0	**24**
14	21.94	10.75	3.53	0.88	0	0	**>50**
Doxorubicin	**89.05**	**85.71**	**83.10**	**78.68**	**69.68**	**51.75**	**4.65**

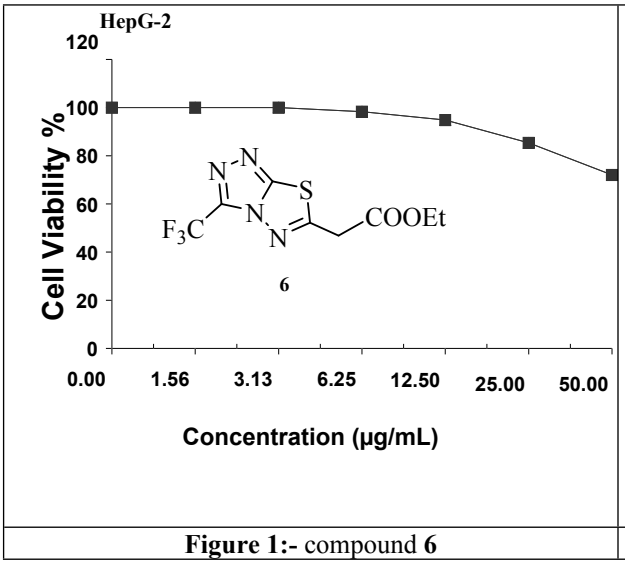


Figure 1:- compound **6**

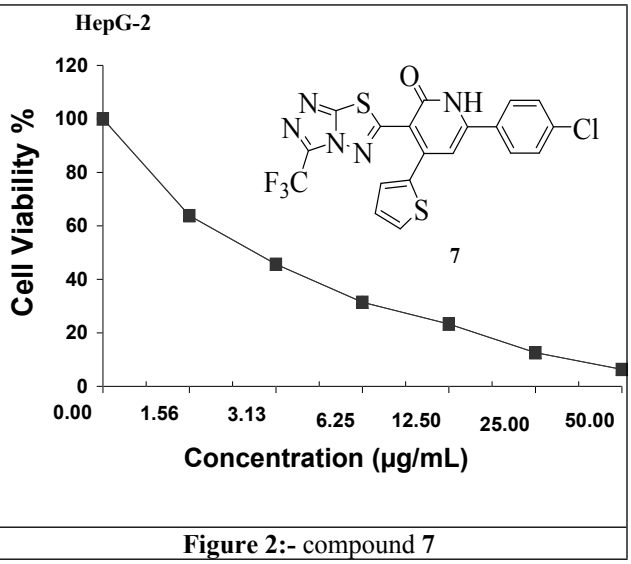


Figure 2:- compound **7**

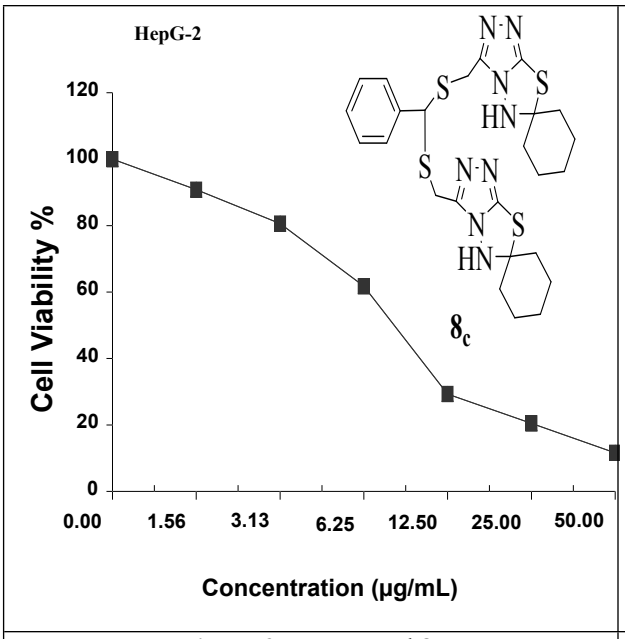


Figure 3:- compound **8_c**

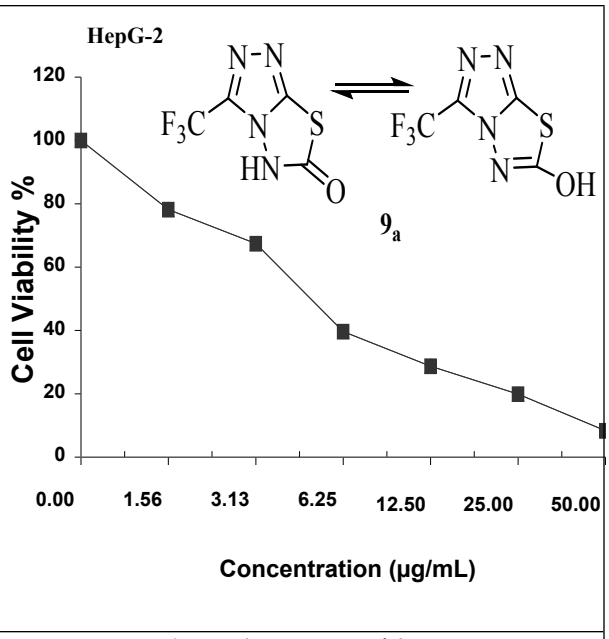


Figure 4:- compound **9_a**

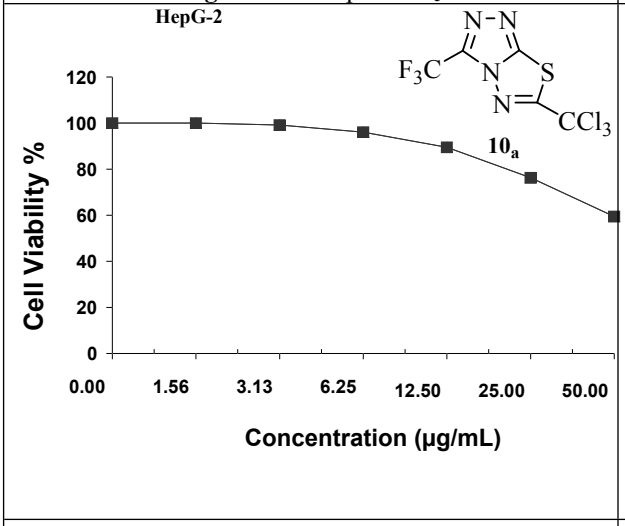


Figure 5:- compound **10_a**

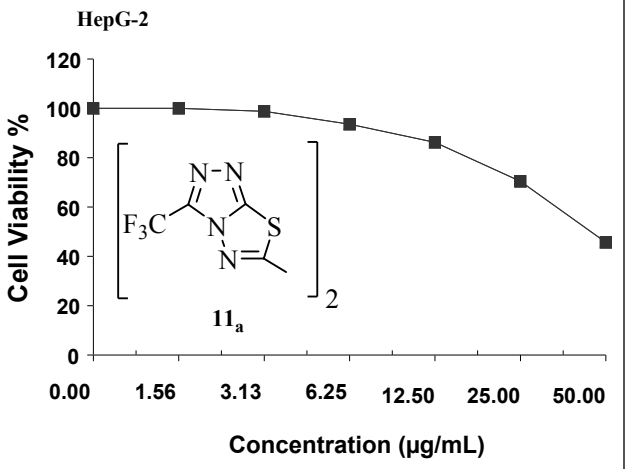


Figure 6:- compound **11_a**

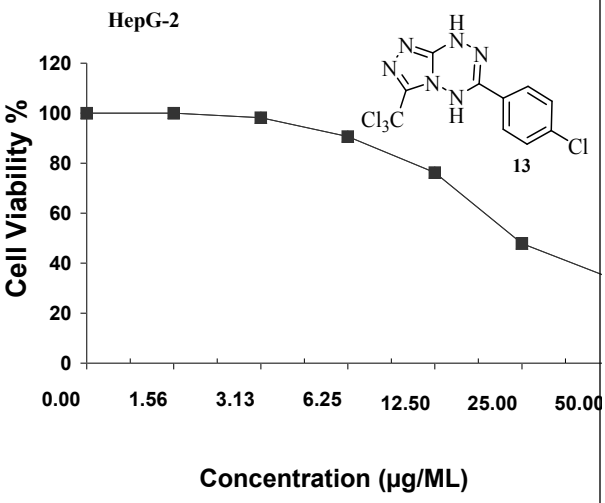


Figure 7:- compound **13**

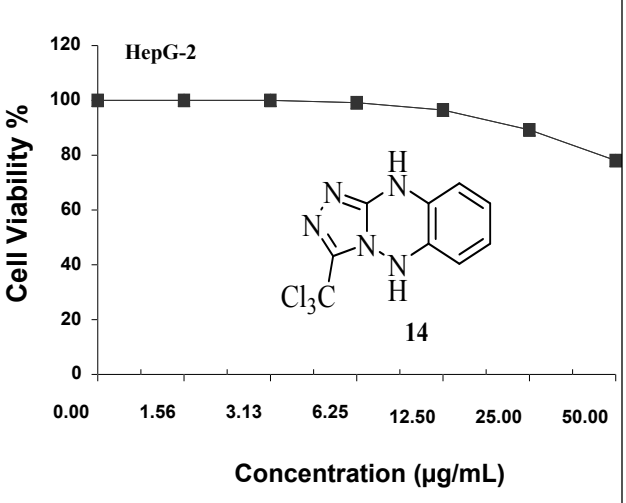


Figure 8:- compound **14**

Conclusion:-

The present research reports the successful synthesis, characterization and anticancer activity of newtriazolothiadiazole, triazolotetrazine and triazolotriazinederivatives.It can be concluded that compounds **7, 8$_c$,** and**9$_a$**showed extremely high activity against HepG2 with IC_{50} values ranging from 02.12-12.00µg/ml from which compound **7**was more potent than the reference drug doxorubicin against HepG2 cell line showing IC_{50} value 02.75 µg/ml (doxorubicin 4.65 µg/ml).

In view of the increasing significance of triazolothiadiazole, triazolotetrazine and triazolotriazine scaffolds, we speculate that the design and exciting developments of novel synthetic methodologies combined with potential biological profile will provide a future insight in this rapidly evolving research area.

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