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Research Article

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Synthesis of some new cyclohexene carboxylic acid derivatives as potent anti-tumor agents

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ABSTRACT

*Cyclohexenone derivative **(2)** prepared by reaction of butanoic acid **(1)** with ethyl acetoacetate reacts with aromatic aldehydes, hydrazine hydrate, thiosemicarbazide to give arylidene, indazole and thiotriazole derivatives. The behavior of the obtained thiotriazole towards hydrazine hydrate, aromatic amines, sodium nitrite, acetylacetone, anthranilic acid, thiourea, benzalacetophenone has been studied. The structure of all synthesized compounds were confirmed by micro analytical and spectral data. The antitumor activity of some of the synthesized compounds were tested .*

Keywords: cyclohexenone, indazole and thiotriazole derivatives, antitumor activity.

INTRODUCTION

In the last several decades, cyclohexenone and indole derivatives have received considerable attention due to their wide range of applications. Cyclohexenone derivatives exhibit antifungal**[1]**'fluorescent**[2]**and anticancer**[3]**activity.

On the other hand, indole derivatives exhibit antioxidant[4],anti-microbial**[5,6],** antimalarial**[7]**anti-proliferative **[8]**anti-tumor **[9]**and also used as antivascular agents**[10]**

Encouraged by these reports, the present investigation deals with synthesizing a new series of cyclohexenone containing the 2-phenyl indolemoiety hoping to improve the antitumor activity of the new compounds.

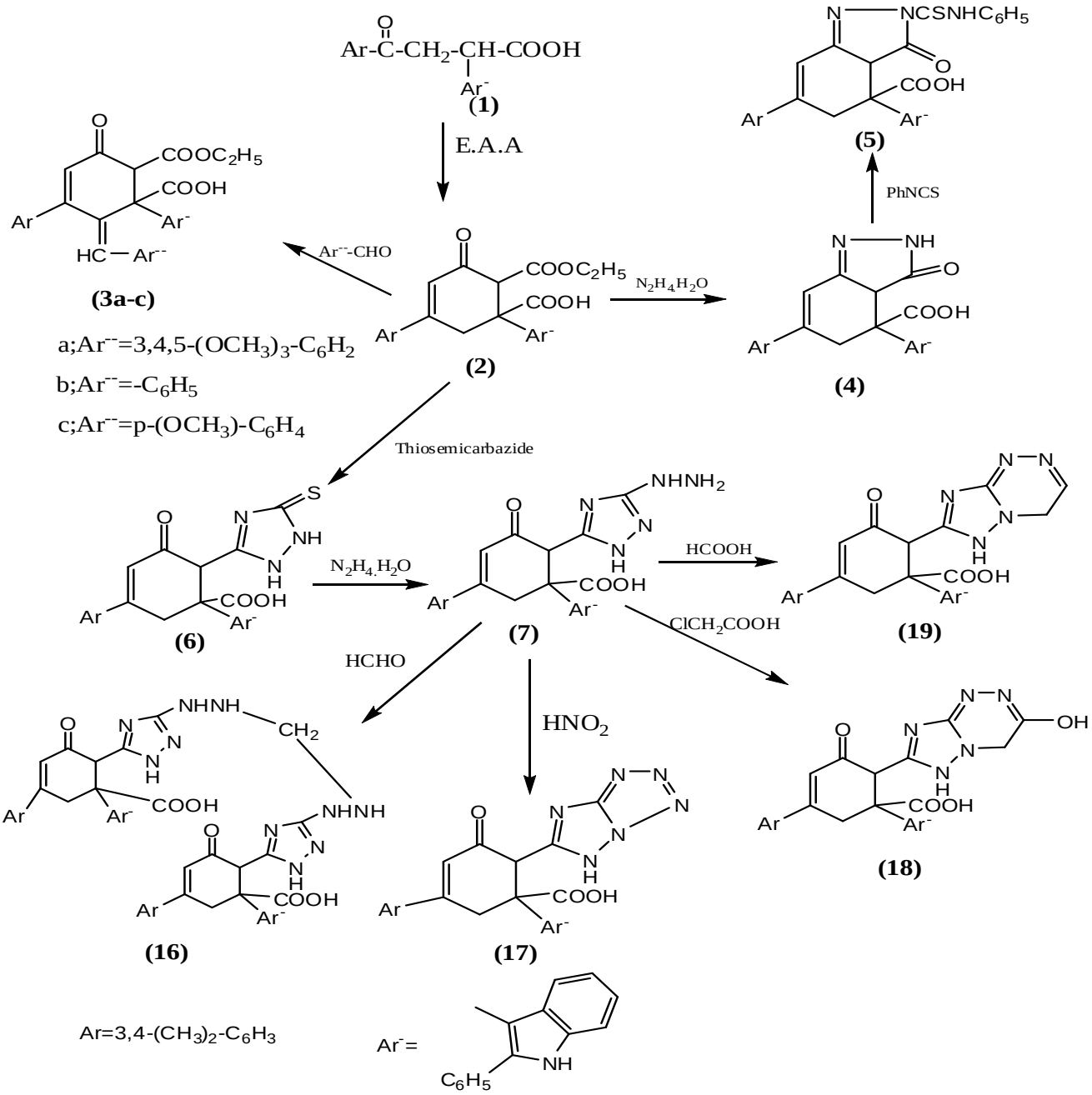
EXEPERIMENTAL SECTION

All melting points are uncorrected .IR spectra (KBr) were recorded with a Perkin Elmer Spectrum RXIFT-IR system .^{1}HNMR were measured with a Varian Gemini 200 MHZ instrument using TMS as internal standard and mass spectra were measured with a Shimadzu GC-MS-QP 100 EX mass spectrometer.

Synthesis of 3-(3,4-dimethyl phenyl)-6-(ethoxy carbonyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl) cyclohex-3-enecarboxylic acid (2), (E)-3-(3,4-dimethyl phenyl)-6-(ethoxy carbonyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)-2-(3,4,5-trimethoxy benzylidene)cyclohex-3-enecarboxylic acid(3a),(E)-2-benzylidene-3-(3,4-dimethylphenyl)-6-(ethoxy carbonyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid(3b), (E)-3-(3, 4-dimethyl phenyl)-6-(ethoxy carbonyl)-2-(4-methoxy benzylidene)-5-oxo-1-(2-phenyl-1H-indol-3-yl) cyclohex-3-ene carboxylic acid(3c)

A solution of 1 (0.01 mol) in ethanol (20 ml),sodium ethoxide(0.5 sodium metal in10 ml ethanol)and ethylacetoacetate , 3,4,5tri methoxybenzaldehyde, benzaldehyde and/ or anisaldehyde was refluxed for 6h.The solid separated on cooling was crystallized from ethanol to give 2 and3a-c respectively(2 m.p.193^{0}C,3a 215^0 C,3b 212^0 C,3c 219^{0}C).Anal.calcd. for $C_{32}H_{29}NO_5$ C, 75.72; H, 5.76; N, 2.76; Found C, 75.70; H, 5.78; N, 2.72; calcd. for $C_{42}H_{39}NO_8$ C, 73.56; H, 5.73; N, 2.04,Found C, 73.58; H, 5.72; N, 2.03;, for$C_{39}H_{33}NO_5$ C, 78.64; H, 5.58; N,

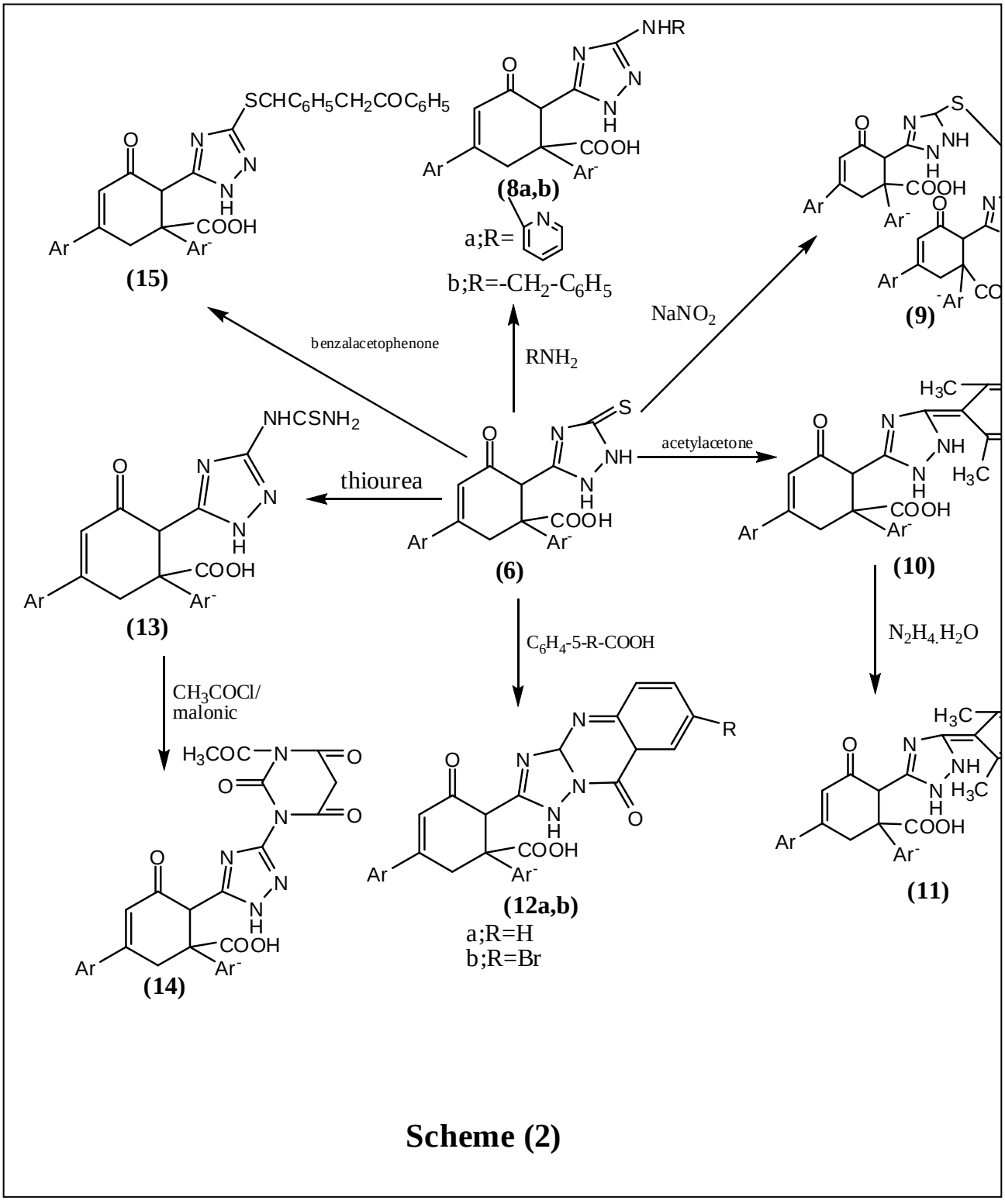
2.35,Found C, 78.64; H, 5.58; N, 2.35; for $C_{40}H_{35}NO_6$ C, 76.78; H, 5.64; N, 2.24; Found C, 76.73; H, 5.66; N, 2.25 %



Scheme(1)

Synthesis of 6-(3,4-dimethyl phenyl)-3-oxo-4-(2-phenyl-1H-indol-3-yl)-3,3a,4,5-tetrahydro-2H-indazole-4-carboxylic acid (4),3-(3,4-dimethylphenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)-6-(5-thioxo-2,5-dihydro-1H-1,2,4-triazol-3-yl)cyclohex-3-enecarboxylic acid (6)

A solution of 2 (0.01 mol) in ethanol (20 ml),and hydrazine hydrate or thiosemicarbazide, was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (4 m.p.230^{0}C,6 m.p.235^{0}C). Anal .calcd.for $C_{30}H_{25}N_3O_3$ C, 75.77; H, 5.30; N, 8.84. FoundC,75.72 ;H,5.35;N,8.83, for$C_{31}H_{26}N_4O_3S$, C, 69.64; H, 4.90; N, 10.48;; S, 6.00 FoundC,69.60;H ,4.95 ;N,10.51; S,5.98 %



Scheme (2)

Synthesis of 6-(3,4-dimethyl phenyl)-3-oxo-4-(2-phenyl-1H-indol-3-yl)-2-(phenylcarbamothioyl)-3,3a,4,5-tetrahydro-2H-indazole-4-carboxylic acid (5)

A solution of 4 (0.01 mol) in ethanol (20 ml),and phenyl isothiocyanate(0.01 mol) was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (5 m.p 259^{0}C). Anal.calcd. For $C_{37}H_{30}N_4O_3S$ C, 72.77; H, 4.95; N, 9.17; S, 5.25 .Found C, 72.73; H, 4.97; N, 9.18; S, 5.26 %

Synthesis of 3-(3,4-dimethyl phenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)-6-(3-(pyridin-2-ylamino)-1H-1,2,4-triazol-5-yl)cyclohex-3-enecarboxylic acid (8a),6-(3-(benzylamino)-1H-1,2,4-triazol-5-yl)-3-(3,4-dimethyl phenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid (8b), 3-(3,4-dimethylphenyl)-6-(5-(2,4-dioxopentan-3-ylidene)-2,5-dihydro-1H-1,2,4-triazol-3-yl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-ene-carboxylic acid (10),3-(3,4-dimethylphenyl)-6-(3-hydrazinyl-1H-1,2,4-triazol-5-yl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid (7).

A solution of 6 (0.01 mol) in ethanol (20 ml),and(0.01 mol) 2-amino pyridine,benzylamine,acetyl acetone or hydrazine hydrate(0.01 mol) was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (8 m.p 270^0C,9m.p. 289^0C,11 m.p.275^0C,13 m.p.250^0C,7 m.p.177^0C),). Anal.calcd. for $C_{36}H_{30}N_6O_3$C, 72.71; H, 5.08; N, 14.13; Found C, 72.73; H, 5.06; N, 14.10;.for $C_{38}H_{33}N_5O_3$ C, 75.10; H, 5.47; N, 11.52; found C, 75.08; H, 5.49; N, 11.55;. for $C_{36}H_{32}N_4O_5$C, 71.98; H, 5.37; N, 9.33;; Found C, 72.00; H, 5.36; N, 9.34; for $C_{31}H_{28}N_6O_3$ C, 69.91; H, 5.30; N, 15.78; O, 9.01,FoundC, 69.89; H, 5.31; N, 15.80%

Synthesis of 6-(5-(3,5-dimethyl-4H-pyrazol-4-ylidene)-2,5-dihydro-1H-1,2,4-triazol-3-yl)-3-(3,4-dimethyl phenyl) -5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid (11)

A solution of 10 (0.01 mol) in ethanol (20 ml),and hydrazine hydrate(0.01 mol) was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (12 m.p 290^0C). Analysis for $C_{36}H_{32}N_6O_3$(%) calcd C, 72.47; H, 5.41; N, 14.08;,found C, 72.69; H, 5.20; N, 14.09%

Synthesis of 3-(3,4-dimethyl phenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)-6-(3-thioureido-1H-1,2,4-triazol-5-yl)-cyclohex -3-enecarboxylic acid (13)

A solution of 6 (0.01 mol) in DMF (30 ml),andthiourea(0.01 mol) was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (14 m.p.215^0C). Analy.calcd. for$C_{32}H_{28}N_6O_3SC$, 66.65; H, 4.89; N, 14.57; S, 5.56.Found C, 66.60; H, 4.92; N, 14.60; S, 5.56 %

Synthesis of 6-(3-(3-acetyl-2,4,6-trioxotetrahydro pyrimidin-1(2H)-yl)-1H-1,2,4-triazol-5-yl)-3-(3,4-dimethyl phenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid (14)

A solution of 13 (0.01 mol) , acetyl chloride (3 ml),andmalonic acid(0.01 mol) was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (15 m.p 275). Analy.calcd. for$C_{37}H_{30}N_6O_7$ C, 66.26; H, 4.51; N, 12.53;Found C, 66.20; H, 4.55; N, 12.49 %

Synthesis of3-(3,4-dimethylphenyl)-5-oxo-6-(9-oxo-1,3a,8a,9-tetrahydro-[1,2,4]triazolo[5,1-b]quinazolin-2-yl)-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid(12a),6-(6-bromo-9-oxo-1,3a,8a,9-tetrahydro-[1,2,4] triazolo[5,1-b] quinazolin-2-yl)-3-(3,4-dimethyl phenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-ene -carboxylic acid(12b) and 3-(3,4-dimethylphenyl)-5-oxo-6-(3-(3-oxo-1,3-diphenylpropylthio)-1H-1,2,4-triazol-5-yl)-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid (15)

A solution of 6 (0.01 mol) in butanol or ethanol (20 ml),andanthranilic acid,5-bromo anthranilic acid or benzalacetophenone(0.01 mol) was refluxed for 6-7h.The solid separated on cooling was crystallized from ethanol (13a m.p 250^0C ,13b m.p.275^0C,16 m.p.289^0C). Analy.calcd. for$C_{38}H_{31}N_5O_4$ C, 73.41; H, 5.03; N, 11.27;,Found C, 73.45; H, 5.01; N, 11.25; for$C_{38}H_{30}BrN_5O_4$ C, 65.15; H, 4.32; Br, 11.41; N, 10.00;,Found C, 65.10; H, 4.35; Br, 11.42; N, 10.03;. for $C_{46}H_{38}N_4O_4S$ C, 74.37; H, 5.16; N, 7.54; S, 4.32, Found C, 74.35; H, 5.56; N, 7.50; S, 4.35%

Synthesis of 6,6'-(3,3'-(2,2'-methylene bis(hydrazine-2,1-diyl))bis(1H-1,2,4-triazole-5,3-diyl)) bis(3-(3,4-dimethyl phenyl) -5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid) (16)

A solution of 7 (0.01 mol) , formaldehyde(0.01 mol) and HCl (3 ml)were added .The mixture was refluxed for 3 h.The solid separated on cooling was crystallized from ethanol (17 m.p 311^0C). Analy.calcd. for$C_{63}H_{56}N_{12}O_6$ C, 70.24; H, 5.24; N, 15.60,Found C, 70.20; H, 5.30; N, 15.53%

Synthesis of 6,6'-(3,3'-disulfanediyl bis(1H-1,2,4-triazole-5,3-diyl)) bis(3-(3,4-dimethylphenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid) (9),6-(5H-[1,2,4] triazolo[1,5-d] tetrazol-6-yl)-3-(3,4-dimethyl phenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid (17)

To a solution of 6,7(0.01 mol) in ethanol(20 ml),sodium nitrite(0.01 mol)and acetic acid(3 ml)were added .The mixture was stirred at room temperature for 4 h. The solid separated was crystallized from ethanol (10 m.p320^0C). Analy.calcd. for$C_{62}H_{50}N_8O_6S_2$ C, 69.77; H, 4.72; N, 10.50; S, 6.01,FoundC, 69.78; H, 4.71; N, 10.50; S, 5.97, for $C_{31}H_{25}N_7O_3$. C, 68.50; H, 4.64; N, 18.04;,Found C, 68.51; H, 4.65; N, 18.05%

Synthesis of3-(3,4-dimethylphenyl)-6-(3-hydroxy-4,6-dihydro-[1,2,4]triazolo[5,1-c][1,2,4]triazin-7-yl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid(18),6-(4,6-dihydro-[1,2,4]triazolo[5,1-c][1,2,4]triazin-7-yl)-3-(3,4-dimethylphenyl)-5-oxo-1-(2-phenyl-1H-indol-3-yl)cyclohex-3-enecarboxylic acid(19)

A solution of 7 (0.01 mol) in xylene or ethanol (20 ml),andchloroacetic acid or formic acid(0.01 mol) was refluxed for 6h.The solid separated on cooling was crystallized from ethanol (19 m.p 224^0C ,20 m.p.211^0C). Analy.calcd for $C_{33}H_{28}N_6O_4$ C, 69.22; H, 4.93; N, 14.68, Found. C, 69.20; H, 4.95; N, 14.66; for$C_{33}H_{28}N_6O_3$ C, 71.21; H, 5.07; N, 15.10;Found C, 71.20; H, 5.09; N, 15.11%.

Sulforhodamine-B(SRB)assay of cytotoxic activity:

MCF7 (breast carcinoma cell line), HEPG2 (hepatocellular carcinoma cell line), HCT116(colon carcinoma cell line) were obtained frozen in liquid nitrogen (-180°C) from the American type culture collection. The tumor cell lines were maintained in the National cancer Institute, Cairo, Egypt, by serial sub-culturing . Potential cytotoxicity of **2,3a,6,7,8a,b,10,11,13,14** and **17**were tested using the method of Skehan et al.

Principle :

The sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a bright pink aminoxanthrene dye with two sulfonic group . It is a protein stain that binds to the amino groups of intracellular proteins under mildy acidic conditions to provide a sensitive index of cellular protein content .

Procedure

1-Cells were used when 90% confluence was reached in T25 flasks . Adherent cell lines were harvested with 0.025 % trypsin. Viability was determined by trypan blue exclusion using the inverted microscope (Olympus 1x70, Tokyo, Japan) .

2-Cells were seeded in 96- well microtiter plates at a concentration of $5x10^4$ - 10^5 cell / well in a fresh medium and left to attach to the plates for 24 hrs.

3-After 24 hrs., cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200µl volume / well using fresh medium and incubation was continued for 24, 48 and 72 hrs . Control cells were treated with vehicle alone. For each drug concentration, 4 wells were used .

4-Following 24, 48 and 72 hrs. treatment, the cells were fixed with 50 µl cold 50 % trichloroacetic acid for 1 hr. at 4ºC .

5-Wells were washed 5 times with distilled water and stained for 30 min. at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid .

6-The wells were then washed 4 times with 1 % acetic acid .

7-The plates were air-dried and the dye was solubilized with 100µl / well or 10 mMtris base (Ph 10.5) for 5m in on a shaker (orbital shaker 0520, Boeco, Germany) at 1600 rpm.

8- The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter tech ∑ 960, U.S.A.). The mean background absorbance was automatically substracted and mean values of each drug concentration was calculated. The relation between survival fraction and compound concentration was plotted to get the survival curve of each tumor cell lines (Fig.1), The IC50 values (the concentrations of thymoquinonerequired to produce 50% inhibition of cell growth (Fig. 2).

RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in Schemes(**1,2)**

Treatment of 4-(3,4-dimethylphenyl)-4-oxo-2-(2-phenyl-1H-indol-3-yl) butanoic acid**[11](1)** with ethylacetocetate in ethanol and sodium ethoxide yielded cyclohexenonederivative**(2).**Its IR spectrum showed υC=O at 1672, υC=C at 1601,and NH/OH at3443..Its mass spectrum showed the parent ion peak at m/z 507(36.04%).The^1 HNMR (DMSO-d_6)spectrum exhibited signals at (ppm): 12.34 (s,1H,OH), 11.49(s,1H,NH), 6.887.86(m,13H,ArH), 2.50(q,2H,$\underline{CH_2}CH_3$),2.51(t,3H,$CH_2\underline{CH_3}$),2.48(s,6H,2 XCH_3),2.49(s,2H,CH_2),3.45(s,1H,CH).

Condensation of **(2)**with 3,4,5-trimethoxybenzaldehyde, benzaldehyde and anisaldeyde give(**3a-c)**. Its IR spectra showed υC=O at 1686, 1635, 1698, υC=C at 1588, 1598, 1587and NH/OH at 3442,34411,3269.the mass spectrum of 3a showed the parent ion peak at m/z 685(24.2%).The^1 HNMR of **3a** (DMSO-d_6)spectrum exhibited signals at(ppm):12.44(s, 1H, OH), 11.34(s, 1H, NH), 6.88-7.86(m, 16H, ArH), 3.17(q, 2H, $\underline{CH_2}CH_3$), 2.52(t, 3H, $CH_2\underline{CH_3}$), 4.49(s, 1H, CH), 3.85(s, 9H, 3$XOCH_3$), 2.49(s, 6H, 2 XCH_3).

It was stated that indazole derivatives showed antiamoebic**[12]**,anti –inflammatory**[13]**antimicrobial**[14]**activities .This prompted the author to synthesize new indazole derivatives through the reaction of **(2)**with hydrazine hydrate in bioling ethanol to give the indazole derivative**(4).** Its IR spectrum showed υC=O at 1686, υC=N at 1602 ,and

NH/OH at 3448, Its mass spectrum showed the parent ion peak at m/z 475(1.02%).The ^{1}HNMR(DMSO-d_6)spectrum exhibited signals at(ppm): 12.48 (s,1H,OH), 11.46 (s,1H,NH), 11.33 (s,1H,NH), 6.887.86 (m,13H,ArH), 2.49 (s,6H,2XCH$_3$) 2.51(s,2H,CH$_2$),3.48(s,1H,CH).

The nucleophilic addition of**(4)** to phenylisothiocyanate gave the adduct **(5)** . Its IR spectrum showed υC=O at 1663, υC=N at 1602, υC=S at 1446 ,and NH/OH at 3442,.Its mass spectrum showed the parent ion peak at m/z610(0.76%).The ^{1}HNMR (DMSO-d_6) spectrum exhibited signals at(ppm): 12.37(s,1H,OH), 11.89(s,2H,2xNH), 4.23(s,1H,NH),6.88-8.09(m,18H,ArH), 2.30(s,6H,2XCH$_3$) 2.47(s,2H,CH$_2$).

The present investigation also deals with triazolethione**(6)** derivative through the reaction of **(2)**with thiosemicarbazide in boiling ethanol in presence of sodium ethoxide. Its IR spectrum showed υC=O at 1672, υC=N at 1602, andC=S at1450 and NH/OH at 3426. Its mass spectrum showed the parent ion peak at m/z 534(30.05%). The ^{1}HNMR (DMSOd$_6$) spectrum exhibited signals at (ppm):12.39(s, 1H, OH), 11.77(s, 1H, NH), 2.23(s, 2H, 2xNH), 6.50-8.18(m, 13H, ArH), 2.32(s, 6H, 2XCH$_3$), 2.46(s, 2H, CH$_2$), 3.48(s, 1H, CH).

The resulting thione**(6)**has been used as starting material for the preparation of a series of new compounds .Reaction of (6) with hydrazine hydrate in boiling ethanol give the hydrazide derivative **(7)**. Its IR spectrum was devoid ofC=S and showed υC=O at 1672, υC=N at 1602, NH/OH at 3443. Its mass spectrum showed the parent ion peak at m/z532(8.81%). The ^{1}HNMR (DMSO-d_6) spectrum exhibited signals at(ppm): 12.43 (s,1H,OH), 11.50(s, 2H, 2xNH), 2.07(s, 1H, NH), 2.15(s, 2H, NH$_2$), 6.88-7.86(m, 13H, ArH), 2.33(s, 6H, 2XCH$_3$), 2.54(s, 2H, CH$_2$), 3.18(s, 1H, CH).

Treatment of **(6)**with 2-amino pyridine and/ or benzylamine in ethanol give the amino derivative of triazole**(8a,b).** Their IR spectra was devoid of C=S and showed υC=O at 1674, 1739, υC=N at 1602, 1595, υ NH/OH at 3443, 3313.The mass spectrum of **8a** showed molecular ion peak at m/z 596(5.12%) (M+2), while for **8b** showed the parent ion peak at m/z 607(13.25%). The ^{1}HNMR(DMSO-d_6) spectrum of 8a exhibited signals at(ppm): 12.39(s, 1H, OH), 11.51(s, 2H, 2xNH), 4.46(s, 1H, NH), 6.86-8.61(m,18H, ArH), 2.26(s, 6H, 2XCH$_3$), 2.46(s, 2H, CH$_2$), while for **8b** exhibited signals at(ppm): 12.54 (s, 1H, OH), 11.54(s, 2H, 2xNH), 3.78(s, 1H, NHCH$_2$), 6.86-7.86(m, 18H, ArH), 2.34(s, 6H, 2XCH$_3$), 2.10(s, 2H, CH$_2$), 3.47(s, 1H, CH), 3.82(s, 2H, NHCH$_2$).

On the other hand, compound**(6)** was oxidized to the disulfane derivative**(9)** upon treatment with sodium nitrite/acetic acid mixture. Its IR spectrum was devoidof υC=S, showed υC=O at 1639, υC=N at 1569, υ NH/OH at 3440, υS-S at 1384, and its mass spectrum showed the parent ion peak at m/z 1066(1.04%). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at (ppm): 12.34(s, 2H, 2xOH), 11.25(s, 4H, 4xNH), 3.30(s, 2H, 2xCH), 6.86-8.30(m, 26H, ArH), 2.26 (s,12H, 4xCH$_3$), 2.50(s, 4H, 2xCH$_2$).

It was stated that pyrazole derivatives showed anticancer**[15],** anti-inflammatory**[16],** antioxidant**[17],** antimicrobial**[18],** molluscicidal**[19]**, anti-angiogenic**[20]**, antitumor**[21]**activities. This prompted the author to synthesize new pyrazole derivative, through the reaction of **(6)** with acetylacetone followed by cyclization with the binucleophile hydrazine hydrate to give the pyrazole derivative **(11).** The IR spectrum of **(10)** showed υC=O at 1673, υC=N at 1612,υ NH/OH at 3436. Its mass spectrum showed an ion peak at m/z 598(1.14%). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at(ppm):12.29(s, 1H, OH), 11.36(s, 1H, NH), 6.86-7.88(m, 13H, ArH), 2.05(s, 2H, 2XNH), 2.38(s, 6H, 2XCOCH$_3$), 2.10(s, 2H, CH$_2$), 2.25(s, 6H, 2XCH$_3$), 3.48(s, 1H, CH). The IR spectrum of **(11)** showed υC=O at 1662, υC=N at 1608, υ NH/OH at 3436. Its mass spectrum showed the parent ion peak at m/z 596(10.01%). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signal at(ppm): 12.32(s, 1H, OH), 11.51(s, 1H, NH), 6.85-7.82(m, 13H, ArH), 2.28(s, 6H, 2XCH$_3$), 3.35(s, 1H, CH), 2.46(s, 6H, 2XNCH$_3$), 2.46(s, 2H, 2xNH).

Reaction of**(6)** with anthranilic acid and 5-bromo-anthranilic acid gave the quinazolinone derivatives **(12a,b)** . The IR spectrum of **(12a)** showed υC=O at 1666, υC=N at 1608, υ NH/OH at 3440, while for**(12b)**υC=O at 1666, υC=N at 1608, υ NH/OH at 3444 and υC-Br at 670. The mass spectrum of **(12a)** showed an ion peak at m/z 624(9.69%) (M+3). Its ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at(ppm): 12.32(s, 1H, OH), 11.48(s, 2H, 2xNH), 7.02-8.02(m, 17H, ArH), 3.36(s, 1H, CH), 2.23(s, 6H, 2XCH$_3$), 3.48(s, 1H, CH), 3.32(s, 1H, NCHN), 3.18(s, 1H, CH$_2$).

Treatment of **(6)**withthiourea in DMF give the thiourea derivative **(13).** Its IR spectrum showed υC=O at 1666, υC=N at 1608, NH/OH at 3437 and C=S at 1446. Its mass spectrum showed an ion peak at m/z 578(12.14%) (M+2). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at(ppm): 12.24(s, 1H, OH), 11.42(s, 3H, 3xNH), 11.31(s, 2H, NH$_2$), 3.31(s, 1H, CH), 6.86-7.84 (m, 13H, ArH), 2.28(s, 6H, 2XCH$_3$), 2.51(s,2H,CH$_2$).

The thioxodihydropyrimidiedione**(14)** can be prepared through the one-pot reaction of **(13),** malonic acid and acetyl chloride. Its IR spectrum was devoid C=S, showed υC=O at 1666, υC=N at 1600, NH/OH at 3410. Its mass spectrum showed an ion peak at m/z 670(100%). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at(ppm):12.36(s, 1H, OH), 11.50(s, 2H, 2xNH), 3.70(s, 1H, CH), 6.86-7.88(m, 13H, ArH), 2.22(s, 6H, 2XCH_3), 3.45(s, 2H, CH_2), 2.43(s, 3H, $COCH_3$), 3.17(s, 2H, CH_2).

The nucleophilic addition of**(6)** to benzalacetophenone gave the adduct **(15).** Its IR spectrum showed υC=O at 1658, υC=N at 1600, υC-S at 1446 and NH/OH at 3436. Its mass spectrum showed an ion peak at m/z 740(43.86%). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at(ppm):12.32(s, 1H, OH), 11.41(s, 2H, 2xNH), 3.41(s, 1H, CH), 7.12-8.16(m, 23H, ArH), 2.30(s, 6H, 2XCH_3), 3.47(s, 1H, SCH), 3.30(s, 2H, $COCH_2$), 2.49(s, 2H, CH_2).

On the other hand compound **(7)** can be used as a key intermediate for the preparation of series of new compound thus,**(7)** reacts with formaldehyde / HCl to form the dimer compound**(16).** Its IR spectrum showed υC=O at 1670, υC=N at 1608, NH/OH at 3408. Its mass spectrum showed an ion peak at m/z 1076(1.05%). The ^{1}HNMR(DMSO-d_6) spectrum exhibited signals at(ppm): 12.26(s, 2H, 2xOH), 11.42(s, 4H, 4xNH), 6.84-7.88 (m, 26H, ArH), 2.51(s, 2H, 2XNCHN), 2.23(s, 12H, 4XCH_3), 3.81(s, 2H, 2xCH), 4.60(s, 4H, 4XNH), 3.14(s, 2H, 2xCH)2. 48(s, 2H, 2xCH).

Treatment of **(7)**with sodium nitrite /acetic acid mixture give the tetrazole derivative **(17).** Its IR spectrum showed υC=O at 1727, υC=N at 1589, NH/OH at 3405and N=N at 1176. Its mass spectrum showed the parent ion peak at m/z 543(1.12%). The ^{1}HNMR (DMSO-d_6) spectrum exhibited signals at(ppm): 12.45(s, 1H, OH), 11.56(s, 1H, NH), 3.31(s, 1H, CH), 6.88-7.87(m, 13H, ArH), 2.28(s, 6H, 2XCH_3), 2.51(s, 2H, CH_2), 3.51(s, 1H, CH).

On the other hand **(7)** react with chloroacetic acid in boiling xylene to form the triazolotriazinecompound**(18).** Its IR spectrum showed υC=O at 1670, υC=N at 1600, NH/OH at 3428. Its mass spectrum showed an ion peak at m/z 570(17.72%). The ^{1}HNMR (DMSO-d_6) spectrum exhibited signals at(ppm): 12.29(s, 2H, 2xOH), 11.65(s, 2H, 2xNH), 3.80(s, 2H, 2xCH), 6.88-7.87(m,13H, ArH), 2.28(s, 6H, 2XCH_3), 3.11(s, H, CH), 2.51(s, 2H, CH_2).

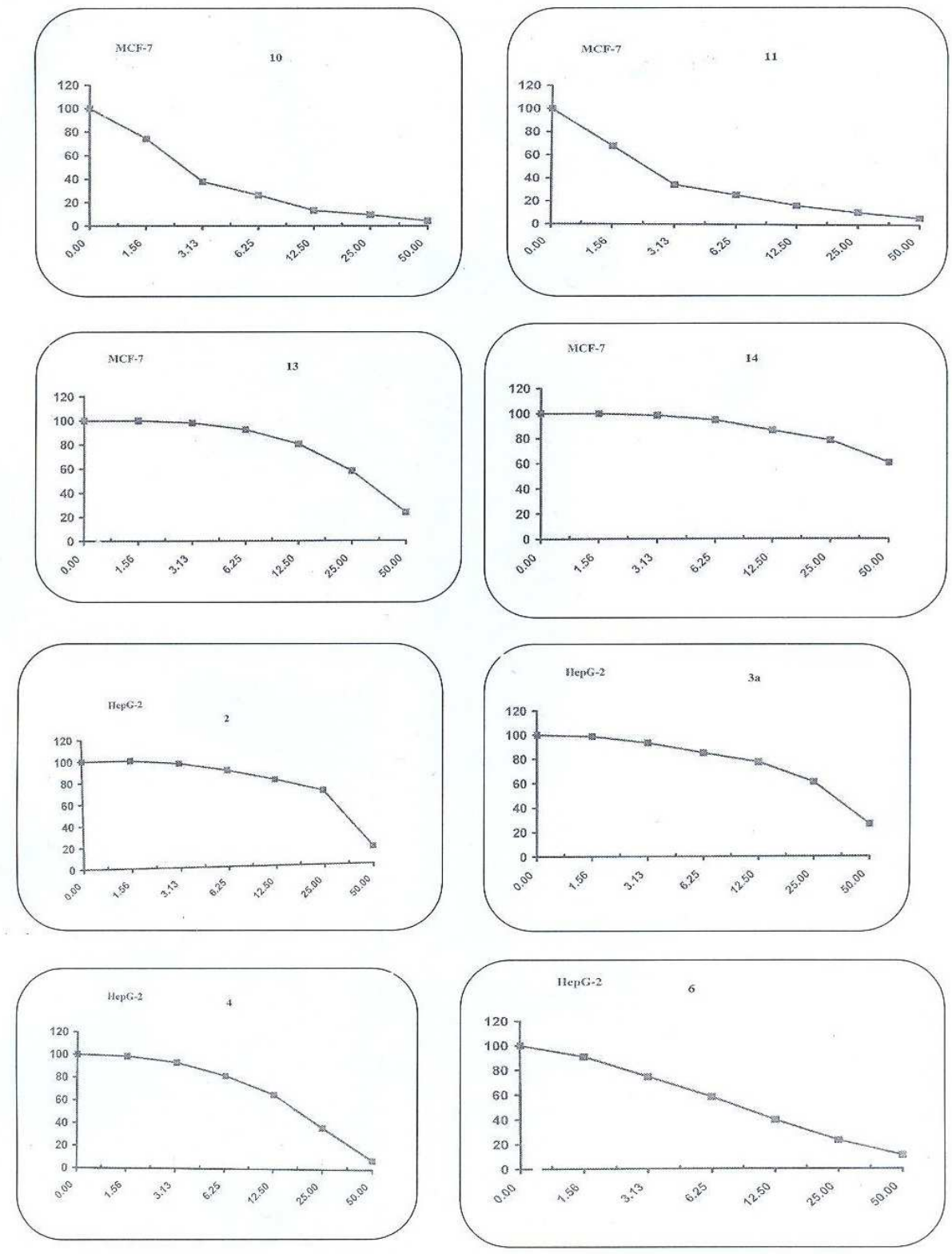
Reaction of **(7)** with formic acid in boiling ethanol form the triazolotriazine derivative**(19).** Its IR spectrum showed υC=O at 1662, υC=N at 1608, NH/OH at 3401. Its mass spectrum showed the parent ion peak at m/z 556(15.21%). The ^{1}HNMR (DMSO-d_6) spectrum exhibited signals at(ppm): 12.38(s, 1H, OH), 11.85(s, 2H, 2xNH), 3.80(s, 1H, CH), 6.88-7.87(m, 14H, ArH), 2.25(s, 6H, 2XCH_3), 3.11(s, 1H, CH), 2.51(s, 2H, NCH_2), 2.30(s, 2H, CH_2).

Cytotoxicity against different human cancer cell lines in vitroFor evaluation of anti-tumor cytotoxicity of compounds **2,3a,6,7,8a,b,10,11,13,14** and **17**, three different human cancer cell lines were used : MCF7 (breast carcinoma cell line), HEPG2 (hepatocellular carcinoma cell line), HCT116(colon carcinoma cell line). Cytotoxicity and IC50 values of the tested compounds are shown in Fig. I and II. The survival fraction was gradually decreased as the concentration of the tested compounds was increased (Table 1).

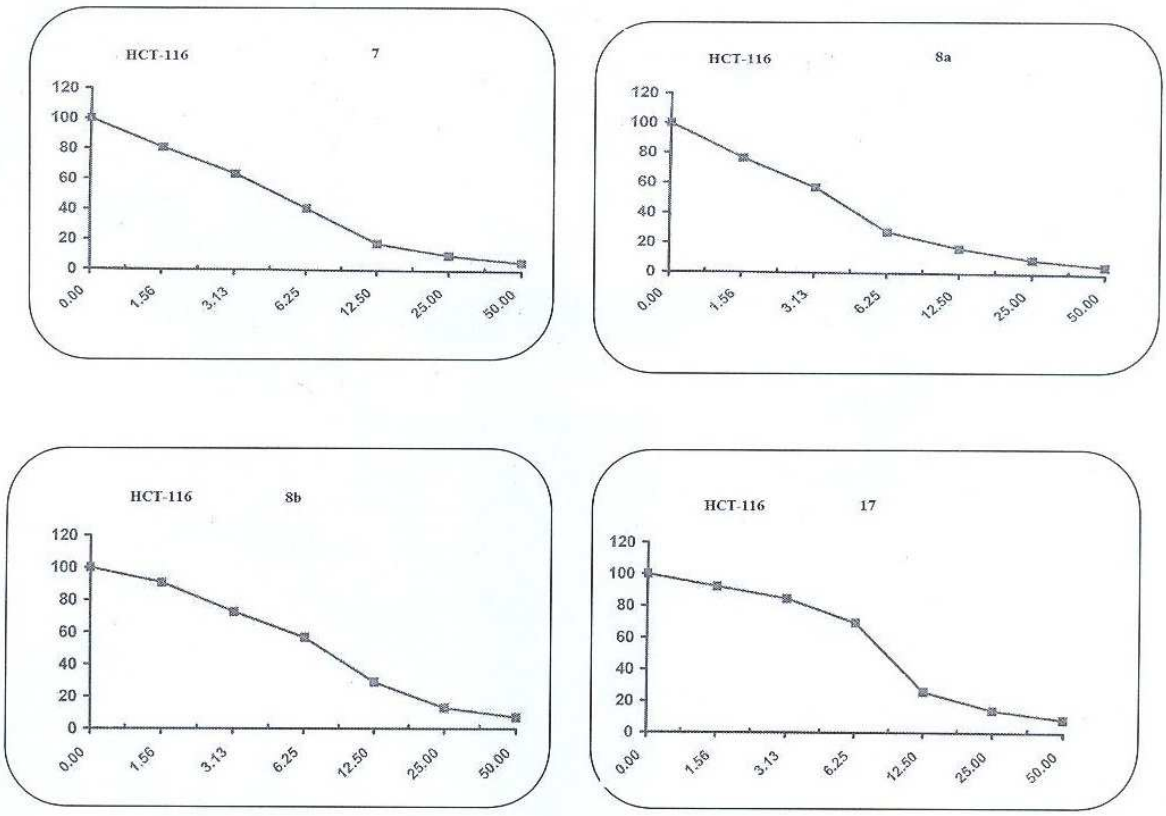
From figure II, it has been shown that **6,7,8a,b,9,10,11,17** are the compounds of lowest IC50 which means that they are the most effective cytotoxic drugs, accordingly compounds 10 and **11** can beused as very potent cytotoxic drug for breast carcinoma cell, **6** for liver carcinoma cell and 7,8a,b,17 as colon carcinoma cell cytotoxic drug, while **4** as moderate cytotoxic drug for liver carcinoma cell respectively, while the remaining compounds are very weak cytotoxic drug .

Table(1):Effect of some new prepared compounds on different types of tumor cells as cytotoxic drug

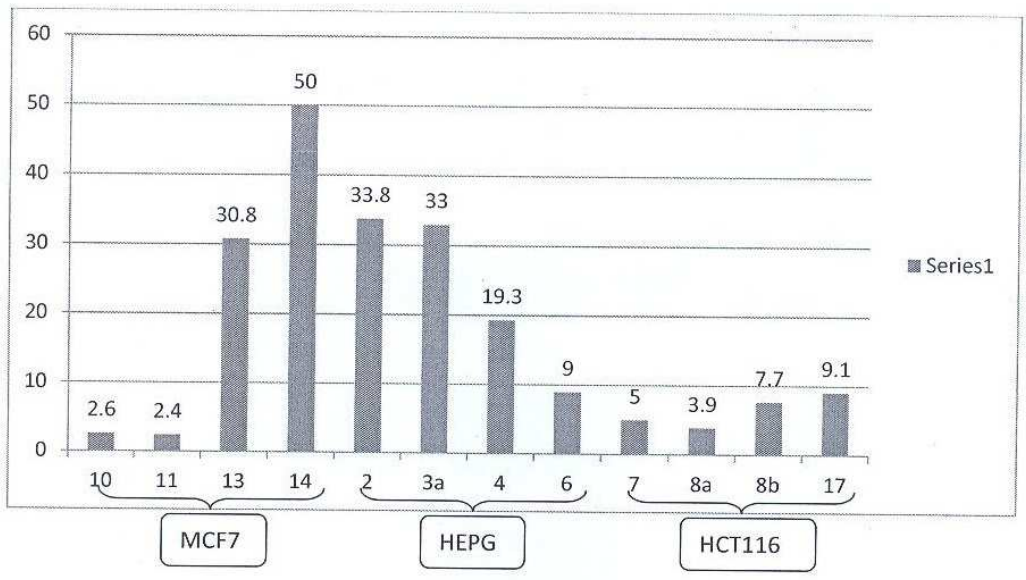
Conc.µg/ml	MCF7				HEPG2				HCT116			
	10	11	13	14	2	3a	4	6	7	8a	8b	17
0	100	100	100	100	100	100	100	100	100	100	100	100
1.56	74.23	67.91	100	100	100	98.65	98.57	90.82	80.98	76.72	90.64	92.23
3.125	37.86	34.63	98.16	98.48	96.42	93.17	93.42	74.56	63.77	57.18	72.58	84.71
6.25	26.41	25.94	92.54	94.77	89.14	84.86	82.14	58.25	41.12	28.02	56.79	69.68
12.5	13.37	16.86	80.43	86.35	79.68	77.33	65.93	39.67	17.93	17.25	28.89	26.14
25	9.62	10.86	58.04	78.42	68.47	61.03	36.84	23.18	10.26	9.72	12.94	14.27
50	4.35	6.14	23.67	60.56	16.19	26.46	8.15	10.86	5.88	5.43	7.18	8.36



Fig(1); Anti-tumor cytotoxicity of different concentration of new prepared compounds aganist different human cancer cell lines *in vitro*



Fig(1):Continued



Fig(2):0-10 Very potent cytotoxic drug , 10-20 Moderate cytotoxic drug , >20 Very weak cytotoxic drug .

CONCLUSION

A series of 2,3a,6,7,8a,b,10,11,13,14 and 17 compounds have different anti-tumor effects and IC50 values of theme were discussed .Compounds 10 and 11 can be used as very potent cytotoxic drug for breast carcinoma cell, 6 for liver carcinoma cell and 7,8a,b,17as colon carcinoma cell cytotoxic drug, while 4 as moderate cytotoxic drug for liver carcinoma cell respectively, while the remaining compounds are very weak cytotoxic drug .

REFERENCES

[1]J.Y. Li, James K. Harper, David M. Grant, Bob Oka Tombe, Bharat Bashyal, W.M. Hess, *Phytochemistry*.**2001**,56(5), 463–468.
[2]A.Badshah; S.Nawaz,; M.Nazar;S. Shah;A. Hasan.; *J.of Fluorescence*, **2010** ,20(5). 1049-1059.
[3]J.Ruprich;A. Prout,; Dickson, John; Younglove, Brent; Nolan, Lawrence; Baxi, Khyati; LeBlanc, Regan; Forrest, Lori; Hills, Patrice; Holt Jr, Herman; Mackay, Hilary; Brown, Toni; Mooberry, Susan L.; Lee, Moses; *Letters in Drug Design & Discovery* .**2007**.4(2), 144-148
[4]M. S. Esteräo; C. Carvalho; L. M. Ferreira; E. Ternandes; M. M. B. Marques.;. *Tetrahedron Lett*, **2011**, 52(1), 101-107.
[5] G. Sellilto; A. Faruolo; P. de Carprarüs; S. Altamura; G. Poonerra; G. Ciliberto.; *Bioorg Med. Chem*., **2010**, 18(16), 6143-6150.
[6]J. S. Biradar; B. Sasidhar; R. Parvean, *Eur J. Med. Chem*., **2010**., 45(9), 4074-4079
[7]M. Frederich; M. Tits;L. Angenot.;Potential antimalarial activity of indole alkaloids Transactions of the Royal *Society of Tropical Medicine and Hygiene*, **2008**, 102(1), 11-19.
[8] W. Peng, M. Świtalska, Li Wang, Z.-Wu Mei, Y. Edazawa, Cui-Qing Pang, I. El-Tantawy El-Sayed, J. Wietrzyk, T. Inokuchi.; *Eur. J. Med. Chem*., **2012**., 58,441-451.
[9] M. R. P. Queiroz;A. S. Abew;M. S. D. Carvalko;P. M. T. Ferreva;NNazarelk; M S Noxumenls., *Bioorg Med. Chem.*, **2008**, 16(10), 5584-5589.
[10]Z. Riedl;K. Monseurs; G. Kraysovszky;P. Dunkel; B. U. W. Maes;P. Tapolesányi; O. Egyed; S. Boros;P. Malyus; L. Pulers;G. L. F. Lemiére;G. Hajós.; *Tetrahedron*, **2006**, 62(1), 121-129.
[11]N.F.Abd El-Ghaffar,Mona,KhMohamed,MonaS.Kadah,AzzaM.Radwan,GalalH.Sayed and Sara N.Abd El-Al; *J.Chem.Pharma Res*.,**2011**,3(3),248-259.
[12]L.F.Vallejo ,R.Castillo ,L.Yépez-Mulia.; *J Biomol Screen.* **2011**;16(8):862-868.
[13]A.Thangadurai, M.Minu, S.Wakode, S.Agrawal, B.Narasimhan.; *Chem. Research*. **2012**, 21(7), 1509-1523.
[14] H. Al Bay, B.Quaddouri,G.Abdelkarim, R.Touzani,B.Nour-Eddine,M.H.Abdellah, M.Bellaoui and S. El Kadiri .; *Drug Design & Discovery*.**2009**;7(1) 41-45.
[15] P. Bandgar; J. V.Totre; S.S. Gawande; C. N. Khobragade; S.C. Warangkar and P.D. Kadam.; *Bioorg. Med. Chem* (**2010**),18 (16), 6149-6155.
[16]N. J. Thumar and M.P. Pat, *Saudi Pharmaceutical Journal* (**2011**),19(2), 75-83 .
[17] A.A. Fadda; E. Abdel-Latif and Rasha E. El-Mekawy.; *Eur. J. Med. Chem.* (**2009**), 44(3), 1250-1256.
[18] Z. Ratkovic; Z.D. Juranic; T. Stanojkovic, D. Manojlovic; R.D. Vukicevic; N. *Bioorg. Chem*.(**2010**), 38(1), 26-32 .
[19]M.N.El-Shimaa,G. Abdel Hafez;,A.Abou-Rahma; M. Abdel-Aziz; M.F. Radwan and H.H. Farag.; *Bioorg. Med. Chem.*(**2009**) 17(11), 3829-3837 **.**
[20]M.S. Mohamed; R. Kamel and S.S. Fatahala.; *Eur. J. Med. Chem*.(**2010**), 45(7), 2994-3004 **.**
[21]R. Aggarwal; G. Sumran; N. Garg and A. Aggarwal.; *Eur. J. Med. Chem*.(**2011**) 46(7), 3038-3046 **.**