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Synthesis of some new compounds containing the pyrazolyl moiety

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ABSTRACT

*The one pot reaction of pyrazolone **3** with malononitrile , ethylacetoacetate in presence of aromatic aldehyde , ammonium acetate and/or piperidine gave compounds **4-6** .Reaction of compound **4** with ammonium thiocaynate , formamide / formic acid and acetic anhydride gave compounds **7-9**. Reaction of compound **5** with acetic anhydride followed by hydrazine hydrate gave compounds **10** and 11 respectively . Reaction of pyrazolone **3** with glucose , phenyl isothiocaynate ,nitrous acid /aniline , aromatic aldehyde have also been taken onto consideration to give compounds **12,13,15** and the α ,β –unsaturated ketone **16** . Compound **16** was used as key intermediate for preparation of various heterocyclic compounds via its reaction with pyrazolone **3** ,hydrazine hydrate , hydroxylamine hydrochloride , urea , thiourea , ethylcyanoacetate / sodium ethoxide , malononitrile / ammonium acetate or sodium ethoxide and also with ethyl acetoacetate / sodium ethoxide . The newly synthesized compounds were characterized by IR, ^{1}HNMR ,mass spectral data. The antitumor and biological activity has also been taken in consideration.*

Keywords: Pyrazolone ,Pyrazolopyrimidines,Pyrazolopyrazole, Pyranopyrazole , Biological activity , , Antitumor activity.

INTRODUCTION

Pyrazolone is one of the important heterocyclic compounds having five membered ring lactam and an additionalketo (C=O) group . It occurs in many drugs and synthetic products. The compounds having pyrazolone moiety are found to be remarkable anti tubercular, anti-inflammatory, antibacterial, and antitumor activity[1-8].as potential therapeutics for immune thrombocytopenias[9] bacteriostatic[10-15]anticancerous[16], antioxidant [17-20] and fungicidal [21,22.]

The present investigation deals with synthesis of some new pyrazolone derivatives to study their behavior towards some nucleophilic as well as electrophilic reagents in the hope of obtaining new derivatives of biological interest and potential target compounds. The new synthesized pyrazolone were tested for their cytotoxic activity and their structure activity relationship were examined

MATERIALS AND METHODS

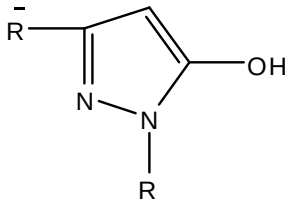
General

All melting points were measured on a GallenKamp melting point apparatus and are uncorrected. IR spectra (KBr) were recorded with a Perkin Elmer Spectrum RXIFT-IR system. ^{1}HNMR(DMSO-D6) were measured with a Varian Gemini 200 MHz instrument using TMS as internal standard and DMSO-d6 as solvent and mass spectra were measured with a Shimadzu GC–MS–QP 100 EX mass spectrometer.

RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in Scheme **1** and **2**

The starting material pyrazolone**3** was prepared using ethyl acetoacetate **1** as β-keto ester and 2,4- dinitro phenyl hydrazine **2**. Compounds unsubstituted at pyrazole C-4 , three isomers are possible assigned as 1H-pyrazol – 5-ol (**A**) , 2,4-dihydro -3H-pyrazol-3-one(**B**) and 1,2 –dihydro -3H-pyrazol-3-one(**C**).



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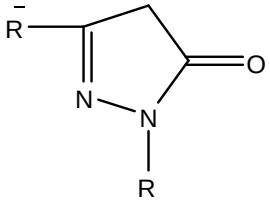

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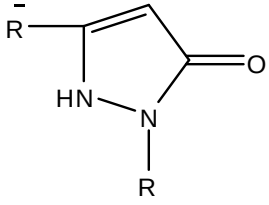


C

The IR spectrum of **3** displayed band at 1658 cm^{-1} due to C=O absorption at position 5 and was devoid for OH and NH which indicated that pyrazolone**3** exists in the keto form.

The bifunctional compound **3** was used as key intermediate for the preparation of several new heterocyclic compounds via its multicomponent reaction with p- hydroxybenzaldehyde and active methylene compounds namely malononitrile and / or ethyl acetoacetate in the presence of ammonium acetate and / or piperidine to give the aminocarbonitrile**4** and **5** and the pyrazolopyridine **6**.

It has beenreported thataminocarbonitrile reacted with ammonium thiocyanate in boiling acetic acid to give (thioxopyrimidinyl) thiourea derivative , while in our investigation we isolated the amino (iminomethyl) thiocyanatsmethane thioimidate**7** through two consecutive addition of two molecules of ammonium thiocyanate to compound **4**.

Treatment of compound 4 with formamide in the presence of formic acid and DMF afforded the open structure pyrazolo(3,4-b) pyridinylformamide**8** .

Acetylation of compounds**4** and **5** with acetic anhydride afforded the acetylated compounds pyrazolo (3,4-b-pyridn-6-yl) acetamide **9** and dihydropyrano (2,3-c) pyrazol-6-yl) acetamide**10** , respectively . The structure of these compounds were confirmed by spectroscopic data and also the reaction of compound **10** with hydrazine hydrate to give the bis compound **11**. The hydrazine hydrate is an ambiendt nucleophile therefore one molecule of it attacks two molecules of compound 10 followed by hydrogenation to afford the bis compound **11**.

Condensation of pyrazolone 3 with glucose in ethanol / DMF / drops of AcOH mixture gave the pentahydroxyhexylidenepyrazolone derivative 12 .

In the presence of piperidine ,pyrazolone 3 reacted with phenyl isothiocyanate through its active methylene group to give the thioamide derivative 13.

Compound 13 is a versatile multifunctional reagent which reacted with hydrazine hydrate to affordpyrazolo (3,4-c) pyrazolethione 14 .

Reaction of pyrazolone 3 with nitrous acid and aniline gave the red dye 4-(2-phenylhydrazono)-1H-pyrazolone derivative 15 .

Knoevenagel condensation reaction of the pyrazolone 3 with 2,4-dimethoxybenzaldehyde in the presence of piperidine gave the pyrazolone derivative 16 .

Pyrazolone 16 reacted with pyrazolone 3 in the presence of sodium ethoxide to give thehydroxypyrazolylpyrazolol17.

Pyrazolone 16 can be used as key intermediate for the preparation of several new heterocyclic compounds, via its reaction with different nitrogen nucleophiles such as hydrazine hydrate , hydroxyl amine hydrochloride , urea and thiourea (Scheme 2,3)

Reaction of compound 16 with hydrazine hydrate gavepyrazolo (3,4-c) pyrazole 18 via β-attack on C=C moiety in compound 16 followed by 1,5- dipolar cyclization , while hydroxylamine hydrochloride gave pyrazolo(3,4-c) isoxazole 19.

Reaction of 16 with urea afforded the pyrazolo(3,4-c) pyrimidinone 20 via β-attack on the C=C moiety in compound 16 , followed by 1,6- intramolecular dipolar cyclization.

However , reaction of thioureawith compound 16 gave the pyrazolo (4,3-e)(1,3) thiazinylthiourea 21 via β-attack on the C=C moiety in compound 16 , followed by 1,6- intramolecular dipolar cyclization through the sulfur atom , then the loss of ammonia through the reaction with another molecule of thiourea.

Reaction of ethyl cyanoacetate in the presence of sodium ethoxide gave the pyrano (2,3-c)pyrazolecarbonitrile 22.

Interestingly , the product of the reaction of malononitrile with compound 16 depends on the reaction conditions , in the presence of ammonium acetate and / or sodium ethoxide compounds 23 and 24 were obtained , respectively .

Similarly ,reaction of compound 16 with ethyl acetoacetate in the presence of ammonium acetate gave thepyrazolo(3,4-b) pyridinylethanone 25 via the addition of the nucleophile ethyl acetoacetate on the β- carbon of the electrophile 16 in a conjugate addition reaction in the presence of ammonium acetate followed by cyclization and dehydrogenation

DISCUSSION

Synthesis of 1-(2,4-dinitrophenyl)-3-methyl-1H-pyrazol-5(4H)-one (3)
A mixture ofethylacetoacetate**1** (0.01 mol) and 2,4-dinitrophenylhydrazine **2** (0.01 mol) in acetic acid (50ml)was refluxed for4 hrs and left to cool. The separated solid was filtered off, washed with ethanol , dried and recrystallized from aceticacid.yield 85% and M.P168 ˚C.

Analysis of **3**$C_{10}H_8N_4O_5$(264) (%) calcdC: 45.46; H, 3.05; N, 21.21.Found:C , 45.40; H, 3.09; N, 21.22.

The IR spectrum of **3**showed absorption bands at 1658cm^{-1} and 1608 cm^{-1} attributable to √ C=O and √ C=N.

Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (4)
A mixture of pyrazolone**3**(0.01 mol) , malononitrile(0.01 mol), ammonium acetate (0.03mol) and p-hydroxybenzaldehyde(0.01 mol)wasfused for 15 hrs at 140-170 ^{0}C, the reaction mixture was poured onto water ,to give the solid product 4 ,which was washed with water dried and recrystallized from EtOH .yield 72% and M.P250 ˚C.

Analysis of **4**$C_{20}H_{13}N_7O_5$(431) (%) calcd: C, 55.69; H, 3.04; N, 22.73..Found:C , 55.40; H, 3.29; N, 22.82.

IR spectrum of **4**showed absorption bands at 1623 cm^{-1}, 2236 cm^{-1},3107 cm^{-1}, 3334,3226 cm^{-1} attributable to √ C=N , √ CN and √ OH and √NH_2 . The mass spectrum showed the molecular ion peak at m/z 432 (M+1,0.03%). The ^{1}H-

NMR(DMSO-d6) showed signal bands δppm at 9.89 (s,1H,OH), 6.91-7.91 (m, 7H,Ar-H), 4.84(s,2H,NH_2), 2.34(s,3H, CH_3).

Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5)
A mixture of pyrazolone**3**(0.01 mol) , malononitrile(0.01 mol), and p-hydroxybenzaldehyde(0.01 mol) in presence of 1 ml piperdinewasfused for 15 hrs at 160 ^{0}C. After cooling , the solid product was washed withEtOH , dried and recrystallized from EtOH .yield 68% and M.P280 ˚C.

Analysis of **5**$C_{20}H_{14}N_6O_6$(434) (%) calcd: C, 55.30; H, 3.25; N, 19.35..Found:C , 55.40; H, 3.20; N, 19.30.

IR spectrum of **4**showed absorption bands at 1613 cm^{-1}, 2233 cm^{-1}, 3345,3289 cm^{-1} attributable to √ C=N , √ CN and √NH_2 . The mass spectrum showed the molecular ion peak at m/z 434 (0.1%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 10.29 (s,1H,OH), 6.78-8.01 (m, 7H,Ar-H), 4.22(s,2H,NH_2), 1.39(s,3H, CH_3).

Synthesis of 4-(1-(2,4-dinitrophenyl)-3,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)phenol(6)
A mixture of pyrazolone**3**(0.01 mol) , ethylacetoacetate (0.01 mol), ammonium acetate (0.03mol) and p-hydroxybenzaldehyde(0.01 mol)wasfused for 15 hrs at 170 ^{0}C. After cooling , the solid product was washed withwater , filtered off and recrystallized from EtOH . yield74% andM.P278 ˚C.

Analysis of **5**$C_{20}H_{15}N_5O_5$(405) (%) calcd: C, 59.26; H, 3.73; N, 17.28..Found:C , 59.40; H, 3.69; N, 17.18.

IR spectrum of **6**showed absorption bands at 1618 cm^{-1}, 3356cm^{-1} attributable to √ C=N , √ OH . The mass spectrum showed the molecular ion peak at m/z 407 (26.62%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 6.96-8.11 (m, 8H,Ar-H), 4.01(s,2H,NH_2), 2.19(s,6H,2X CH_3).

Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridin-5-yl)(iminomethyl)thiocyanatomethane thioimidate(7)
A mixture of **4** (0.01 mol) and ammonium thiocyanate (0.03mol) in acetic acid (10 ml) was refluxed for 10 hrs. The reaction mixture was poured onto water ,filtered off , washed with water , dried and recrystallized from EtOH.yield68% and M.P266 ˚C.

Analysis of **7**$C_{22}H_{15}N_9O_5S_2$ (549) (%) calcd:C, 48.08; H, 2.75; N, 22.94; S, 11.67. Found: C, 48.01; H, 2.77; N, 22.96; S, 11.70.

IR spectrum of **7**showed absorption bands at 1616 cm^{-1}, 2206 cm^{-1},3069cm^{-1},3354cm^{-1}attributable to √ C=N ,√ CN, √ OH,√ NH. The mass spectrum showed the molecular ion peak at m/z 547 (3.32%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 6.76-8.91 (m, 7H,Ar-H),5.11(s,2H,2XNH),4.51(s,2H,NH_2), 3.19(s,3H, CH_3).

Synthesis of N-(5-cyano-1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridin-6-yl)formamide (8)
Amixture of **4** (0.01 mol) , formic acid(5 ml), formamide(10 ml) and dimethylformamide (5 ml) was refluxed for 2 hrs ,the reaction mixture was poured ontowater .The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield79% and M.P273 ˚C.

Analysis of**8** $C_{21}H_{13}N_7O_6$(459) (%) calcd: C, 54.91; H, 2.85; N, 21.34 .Found: C, 54.88; H, 2.83; N, 21.37.

IR spectrum of **8**showed absorption bands at 1612 cm^{-1}, 1657 cm^{-1}, 2206 cm^{-1},3069cm^{-1},3461cm^{-1}attributable to √ C=N ,√ C=O,√ CN, √ OH,√ NH. The mass spectrum showed the molecular ion peak at m/z 459 (1.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.45(s,1H,NH) ,10.91(s,1H,OH), 7.06-8.51 (m, 8H,Ar-H),), 2.34(s,3H, CH_3).

Synthesis of N-(5-cyano-1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridin-6-yl) acetamide (9), N-(5-cyano-1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c] pyrazol-6-yl)acetamide(10)

Amixtureof compound**4 and/or 5** (0.01 mol) , and acetic anhydride(20 ml was refluxed for 5 hrs ,the reaction mixture was poured onto water .The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.Yield of **9**72% and M.P286˚C.Yield of **10**75% and M.P298˚C.

Analysis of**9** $C_{22}H_{15}N_7O_6$(473) (%) calcd: C, 55.82; H, 3.19; N, 20.71;.Found: C, 55.85; H, 3.17; N, 20.68;while for **10**$C_{22}H_{16}N_6O_7$(476) (%) calcd: C, 55.47; H, 3.39; N, 17.64;.Found: C, 55.40; H, 3.42; N, 17.70.

IR spectrum of **9**showed absorption bands at 1613 cm^{-1}, 1675 cm^{-1}, 2206 cm^{-1}, 3355 cm^{-1}attributable to √ C=N ,√ C=O,√ CN, √ OH/√ NH .while for **10**showed absorption bands 1595 cm^{-1}, 1666 cm^{-1}, 2206 cm^{-1}, 3356 cm^{-1}attributable to √ C=N ,√ C=O,√ CN, √ OH/√ NH. The mass spectrum of **9** showed the molecular ion peak at m/z 473 (10.55%) and for **10** is 477(2.88%). The ^{1}H-NMR(DMSO-d6) of **9** showed signal bands δppm at 11.65(s,1H,NH) ,10.89(s,1H,OH), 7.01-8.01 (m, 7H,Ar-H),), 2.28(s,6H,2x CH_3), while **10**showed signal bands δppm at 11.74(s,1H,NH) ,10.95(s,1H,OH), 7.03-8.41 (m, 8H,Ar-H),), 1.98(s,6H,2x CH_3)

Synthesis of 6,6˙-(1,1-hydrazine-1,2-diyl)bis(ethane-1,1-diyl)bis(azanediyl)bis (1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5- carbonitrile(11)

Amixture of **10** (0.01 mol) , hydrazine hydrate(0.01 mol)in ethanol (50 ml) was refluxed for 2 hrs ,the reaction mixture was poured ontoice/HCl(1:3) .The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield72% and M.P 212 ˚C.

Analysis of**8** $C_{44}H_{36}N_{14}O_{12}$(951) (%) calcd: C, 55.46; H, 3.78; N, 20.59 .Found: C, 55.48; H, 3.77; N, 20.58.

IR spectrum of**11**showed absorption bands at 1599 cm^{-1}, 2216 cm^{-1},3226cm^{-1},3425cm^{-1}attributable to √ C=N ,√ CN, √ OH,√ NH. The mass spectrum showed the molecular ion peak at m/z 950 (2.04%).

Synthesis of 1-(2,4-dinitrophenyl)-3-methyl-4-(2,3,4,5,6-pentahydroxyhexylidene)-1H-pyrazol-5(4H)-one (12)

A mixture of **3** (0.01 mol) , glucose (0.01 mol)in water (1 ml),ethanol(50 ml), and dimethylformamide(5 ml) was heated on water bath for 2 hrs.The solid product that separated was filtered off , washed well with water(3x10 ml) , dried and recrystallized from EtOH.yield69% and M.P 232 ˚C.

Analysis of**12** $C_{16}H_{18}N_4O_{10}$(426) (%) calcd: C, 45.08; H, 4.26; N, 13.14.Found: C, 45.01; H, 4.30; N, 13.17.

IR spectrum of **12**showed absorption bands at1613cm^{-1}, 1698 cm^{-1}, 3341cm^{-1}attributable to √ C=N ,√ C=O√ OH. The mass spectrum showed the molecular ion peak at m/z 426 (55.55%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.45(s,1H,NH) ,10.98(s,5H,5xOH), 7.36-8.11 (m, 4H,Ar-H),), 3.34(s,4H, 4xCHOH),3.21(s,2H,CH_2OH),1,99(s,3H,CH_3).

Synthesis of 1-(2,4-dinitrophenyl)-3-methyl-5-oxo-N-phenyl-4,5-dihydro-1H-pyrazole-4-carbothioamide (13)

A mixture of **3** (0.01 mol) , and phenylisothiocyanate(0.01 mol) in dry acetone (50 ml) in presence of piperidine (0.5 ml) was heated on water bath for 4 hrs .The reaction mixture was poured ontoice/HCl.The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield81% and M.P 289 ˚C.

Analysis of**13** $C_{17}H_{13}N_5O_5S$(399) (%) calcd: C, 51.12; H, 3.28; N, 17.54; S, 8.03.Found: C, 51.09; H, 3.30; N, 17.55; S, 8.03.

IR spectrum of **13**showed absorption bands at 1613 cm^{-1}, 1674 cm^{-1}, 3169cm^{-1},attributable to √ C=N ,√ C=O,√ OH,. The mass spectrum showed the molecular ion peak at m/z 400 (0.09%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 7.12-8.01 (m, 8H,Ar-H),4.45(s,1H,NH),2.55(s,1H.CH) ,1.34(s,3H, CH_3).

Synthesis of 6-(2,4-dinitrophenyl)-4-methyl-2-phenyl-1,6-dihydropyrazolo[3,4-c]pyrazole-3(6H)-thione (14)

Amixture of **13** (0.01 mol) , and hydrazine hydrate(0.01 mol) in ethanol (50 ml)was refluxed for 5 hrs .The reaction mixture was poured ontoice/HCl.The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield58% and M.P330 ˚C.

Analysis of**14** $C_{17}H_{12}N_6O_4S$(396) (%) calcd: C, 51.51; H, 3.05; N, 21.20; S, 8.09.Found C, 51.45; H, 3.07; N, 21.22; S, 8.11.

IR spectrum of **14**showed absorption bands at 1436 cm^{-1}, 1613 cm^{-1}, 3226cm^{-1},attributable to √ C=S ,√ C=N,√ NH,. The mass spectrum showed the molecular ion peak at m/z 398 (4.09%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 6.92-8.11 (m, 8H,Ar-H),4.23(s,1H,NH),1.83(s,3H, CH_3).

Synthesis of 1-(2,4-dinitrophenyl)-3-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (15)
Aniline(0.01 mol) was dissolved in con. HCl (5ml) and the solution was then cooled to 0-5^{0}C .Solution of sodium nitrite (0.01 mol) in water (3 ml) was then added to this solution dropwise with vigorous stirring during about 1 hr ,while cooling at0-5^{0}C . The clear diazonium salt solution was then added dropwise to a well cooled(0-5^{0}C) and stirred solution of pyrazolone**3** (0.01 mol) in sodium acetate (1 gm,dissolved in 5 ml of 25 % aqueows ethanol). Stirring was continued for 4 hrs atto 0-5^{0}C. The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield71% and M.P221 ˚C.

Analysis of**15** $C_{16}H_{12}N_6O_5$(368) (%) calcd: C, 52.18; H, 3.28; N, 22.82;.FoundC, 52.22; H, 3.26; N, 22.80.

IR spectrum of **15**showed absorption bands at 1608 cm^{-1}, 1663 cm^{-1}, 3226cm^{-1},attributable to √ C=N ,√ C=O,√ NH,. The mass spectrum showed the molecular ion peak at m/z 370 (11.01%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.23(s,1H,NH),6.82-8.51 (m, 8H,Ar-H),2.13(s,3H, CH_3).

Synthesis of4-(2,4-dimethoxybenzylidene)-1-(2,4-dinitrophenyl)-3-methyl-1H-pyrazol-5(4H)-one (16)
Amixture of **3** (0.01 mol) , 2,4- dimethoxybenzaldehyde(0.01 mol) in ethanol (50 ml) in presence of piperidine (1 ml) was refluxed for 5 hrs .The solid product that separated was filtered off , washed well with ethanol , dried and recrystallized from EtOH.yield74% and M.P197 ˚C.

Analysis of**16** $C_{19}H_{16}N_4O_7$(412) (%) calcd: C, 55.34; H, 3.91; N, 13.59.FoundC, 55.30; H, 3.93; N, 13.61.

IR spectrum of **16**showed absorption bands at 1598 cm^{-1}, 1673 cm^{-1}, ,attributable to √ C=N ,√ C=O .The mass spectrum showed the molecular ion peak at m/z 414 (14.19%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 7.12-8.31 (m, 7H,Ar-H),3.23(s,6H,2x OCH_3),1.99(s,3H, CH_3).

Synthesis of4,4'-((2,4-dimethoxyphenyl)methylene)bis(1-(2,4-dinitrophenyl)-3-methyl-1H-pyrazol-5-ol) (17)
Compound **16** (0.01 mol) was added at room temperature with stirring to a mixture of pyrazolone**3** (0.01 mol) ,and sodium ethoxide (0.5 gm sodium in 10 ml ethanol) ,then the mixture was stirred for another 1 hr at 100^{0}C. .The reaction mixture was poured ontoice/HCl.The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield74% and M.P190 ˚C.

Analysis of**17** $C_{29}H_{24}N_8O_{12}$(676) (%) calcd: C, 51.48; H, 3.58; N, 16.56;.Found C, 51.52; H, 3.56; N, 16.54.

IR spectrum of **17**showed absorption bands at 1625 cm^{-1}, 3340cm^{-1},attributable to √ C=N,√ OH,. The mass spectrum showed the molecular ion peak at m/z 676 (11.21%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.23(s,2H,2xOH),6.87-8.02 (m, 10H,Ar-H),3.41(s,6H,2x OCH_3), ,2.13(s,6H,2X CH_3).

Synthesis of4-(2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c] pyrazole(18)
Amixture of **16** (0.01 mol) , hydrazine hydrate(0.01 mol) in ethanol (20 ml) was refluxed for 5 hrs .The solid product that separated after cooling was filtered off , washed well with water , dried and recrystallized from EtOH.yield84% and M.P270 ˚C.

Analysis of **18** $C_{19}H_{18}N_6O_6$ (426)(%) calcd: C, 53.52; H, 4.26; N, 19.71. Found: C, 53.55; H, 4.25; N, 19.69.

IR spectrum of **18**showed absorption bands at 1599 cm^{-1}, 3226 cm^{-1}, ,attributable to √ C=N ,√ NH .The mass spectrum showed the molecular ion peak at m/z 425 (10.12%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.34(s,1H,NH) , 7.01-8.11 (m, 8H,Ar-H),3.41(s,6H,2x OCH_3),2.01(s,3H, CH_3).

Synthesis of 3-(2,4-dimethoxyphenyl)-6-(2,4-dinitrophenyl)-4-methyl-3a,6-dihydro-3H-pyrazolo[3,4-c]isoxazole(19)

Amixture of **16** (0.01 mol) , hydroxylamine hydrochloride(0.01 mol) in pyridine (10 ml) was refluxed for 6 hrs .The solid product that separated after cooling was filtered off , washed well with water , dried and recrystallized from EtOH.yield59% and M.P230 ˚C.

Analysis of **19**$C_{19}H_{17}N_5O_7$ (427)(%) calcd: C, 53.40; H, 4.01; N, 16.39. FoundC, 53.45; H, 3.98; N, 16.36.

IR spectrum of **19**showed absorption bands at 1614 cm^{-1}, attributable to √ C=N ,.The mass spectrum showed the molecular ion peak at m/z 425 (13.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.34(s,1H,NH) , 7.21-8.01 (m, 7H,Ar-H),3.65(s,1H,CH)3.21(s,6H,2x OCH_3),2.21(s,3H, CH_3).

Synthesis of 4-(2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl-4,5-dihydro-1H-pyrazolo[3,4-d] pyrimidin -6(3aH)-one (20)

Amixture of **16** (0.01 mol) , and urea (0.01 mol) in glacial acetic acid (20 ml) was refluxed for 5hrs .The solid product that separated after cooling was filtered off , washed well with water , dried and recrystallized from EtOH.yield77% and M.P214 ˚C.

Analysis of **20**$C_{20}H_{18}N_6O_7$ (454)(%) calcd: C, 52.86; H, 3.99; N, 18.50. FoundC, 52.90; H, 3.97; N, 18.548.

IR spectrum of **20**showed absorption bands at1600cm^{-1},1711 cm^{-1},3363 cm^{-1} attributable to √ C=N ,√ C=O ,√ NH.The mass spectrum showed the molecular ion peak at m/z 454 (1.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.04(s,1H,NH) , 7.01-8.31 (m, 7H,Ar-H),3.45(s,1H,CH)3.01(s,6H,2x OCH_3),2.09(s,3H, CH_3).

Synthesis of1-(4-(2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl-1,4-dihydropyrazolo[4,3-e][1,3]thiazin -6-yl)thiourea (21)

Amixture of **16** (0.01 mol) , and thiourea(0.01 mol) in glacial acetic acid (20 ml) was refluxed for 5hrs .The solid product that separated after cooling was filtered off , washed well with water , dried and recrystallized from EtOH.yield61% and M.P241 ˚C.

Analysis of **21**$C_{20}H_{18}N_6O_6$ (529)(%) calcd: C, 47.63; H, 3.62; N, 18.52; S, 12.11.FoundC, 47.58; H, 3.64; N, 18.53; S, 6.83.S, 12.12.

IR spectrum of **21**showed absorption bands at 1421cm^{-1},1615cm^{-1}, 3110-3001 cm^{-1} ,3279 cm^{-1} attributable to √ C=S ,√ C=N ,√ NH_2,√ NH.The mass spectrum showed the molecular ion peak at m/z 454 (1.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.18(s,1H,NH) , 6.91-8.01 (m, 7H,Ar-H),3.15(s,1H,CH),2.99 (s,6H,2x OCH_3),1.49(s,3H, CH_3).

Synthesisof 4-(2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl-6-oxo-1,6-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (22)

Amixture of **16** (0.01 mol) , and ethylcyanoacetate(0.01 mol) in sodium ethoxide (0.5 gm in20 ml) was refluxed for 5hrs .The solid product that separated after cooling and pouring into ice was filtered off , washed well with water , dried and recrystallized from EtOH.yield57% and M.P256 ˚C.

Analysis of **22**$C_{22}H_{15}N_5O_8$ (477)(%) calcd: C, 55.35; H, 3.17; N, 14.67.FoundC, 55.30; H, 3.20; N, 14.69.

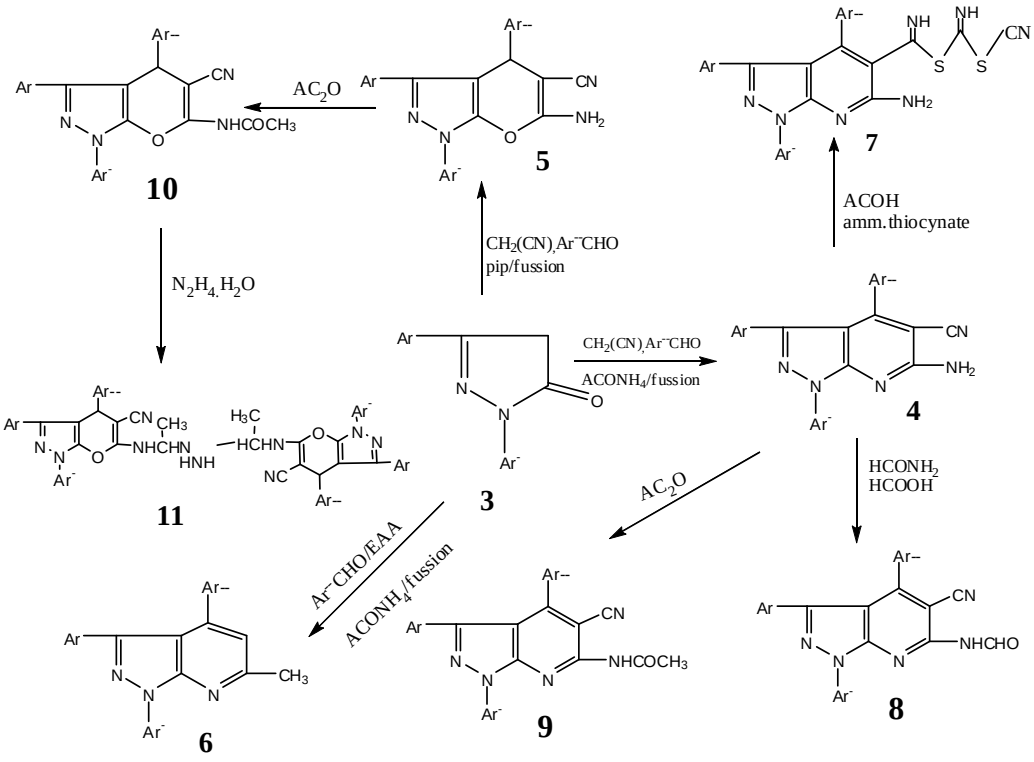
IR spectrum of **22**showed absorption bands at 1617cm^{-1},1709cm^{-1}, 2220 cm^{-1} attributable to √ C=N ,√ C=O,√ CN. The mass spectrum showed the molecular ion peak at m/z 479 (5.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 6.89-8.14 (m, 8H,Ar-H),3.09 (s,6H,2x OCH_3),1.87(s,3H, CH_3).

Synthesisof 2-[5-amino-4-(2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl-4-9-dihydro-1H-pyrazolo[4',3':5,6]pyrido(2,3-d)pyrimidin-7-yl]acetonitril(23)

Amixture of **16** (0.01 mol) ,malononitrile(0.01 mol) and ammonium acetate(2 gm) was fused for 5 hrs at 170^{0}C. After cooling the solid washed well with water ,filtered off , dried and recrystallized from EtOH.yield63% and M.P270 ˚C.

Analysis of **23**$C_{25}H_{21}N_9O_6$ (543)(%) calcd: C, 55.25; H, 3.89; N, 23.19.FoundC, 55.20; H, 3.91; N, 23.22.

IR spectrum of **23**showed absorption bands at 1598cm^{-1}, 2234 cm^{-1} ,3398attributable to √ C=N ,,√ CN,√ NH. The mass spectrum showed the molecular ion peak at m/z 545 (4.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at ,11.42(s,1H,NH),6.69-8.01 (m, 7H,Ar-H),4.23(s,2H,NH_2),3.43 (s,6H,2x OCH_3),2.11(s,3H, CH_3).



Ar=CH_3 **Ar$^-$=$_2$,4-$(NO_2)_2C_6H_3$** **Ar^{--}=4-$(OH)C_6H_4$**

Scheme 1

Synthesis of 2-[5-amino-4-((2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl1,4-dihydropyrazolo[4$^-$,3$^-$:5,6]pyrano [2,3-d]pyrimidin-7-yl]acetonitrile (24)

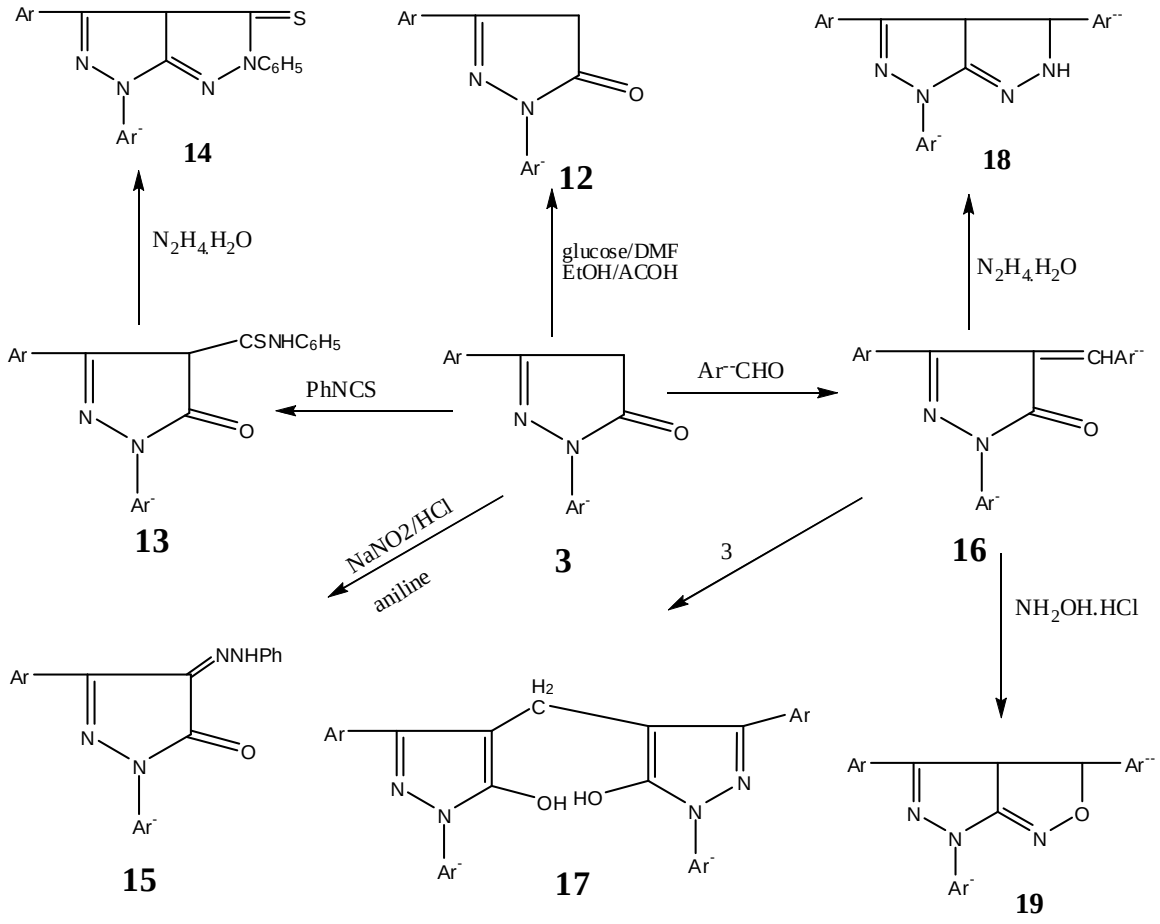
Amixture of **16** (0.01 mol) ,malononitrile(0.01 mol) and sodium ethoxide(0.5 gm in 20 ml) was refluxed for 6 hrs. The mixture was poured onto ice /HCl. The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield54% and M.P232 ˚C.

Analysis of **24**$C_{25}H_{20}N_8O_7$(544)(%) calcd: C, 55.15; H, 3.70; N, 20.58.FoundC, 55.10; H, 3.72; N, 20.61.
IR spectrum of **24**showed absorption bands at 1598cm^{-1}, 2234 cm^{-1} ,3398attributable to √ C=N ,,√ CN,√ NH. The mass spectrum showed the molecular ion peak at m/z 545 (4.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at ,11.42(s,1H,NH),6.69-8.01 (m, 7H,Ar-H),4.23(s,2H,NH_2),3.43 (s,6H,2x OCH_3),2.11(s,3H, CH_3).

Synthesis of 4-(2,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-6-hydroxy-3-methyl-1,3a,4,7a-tetrahydro pyrano [2,3-c]pyrazol-5-yl)ethanone(25)

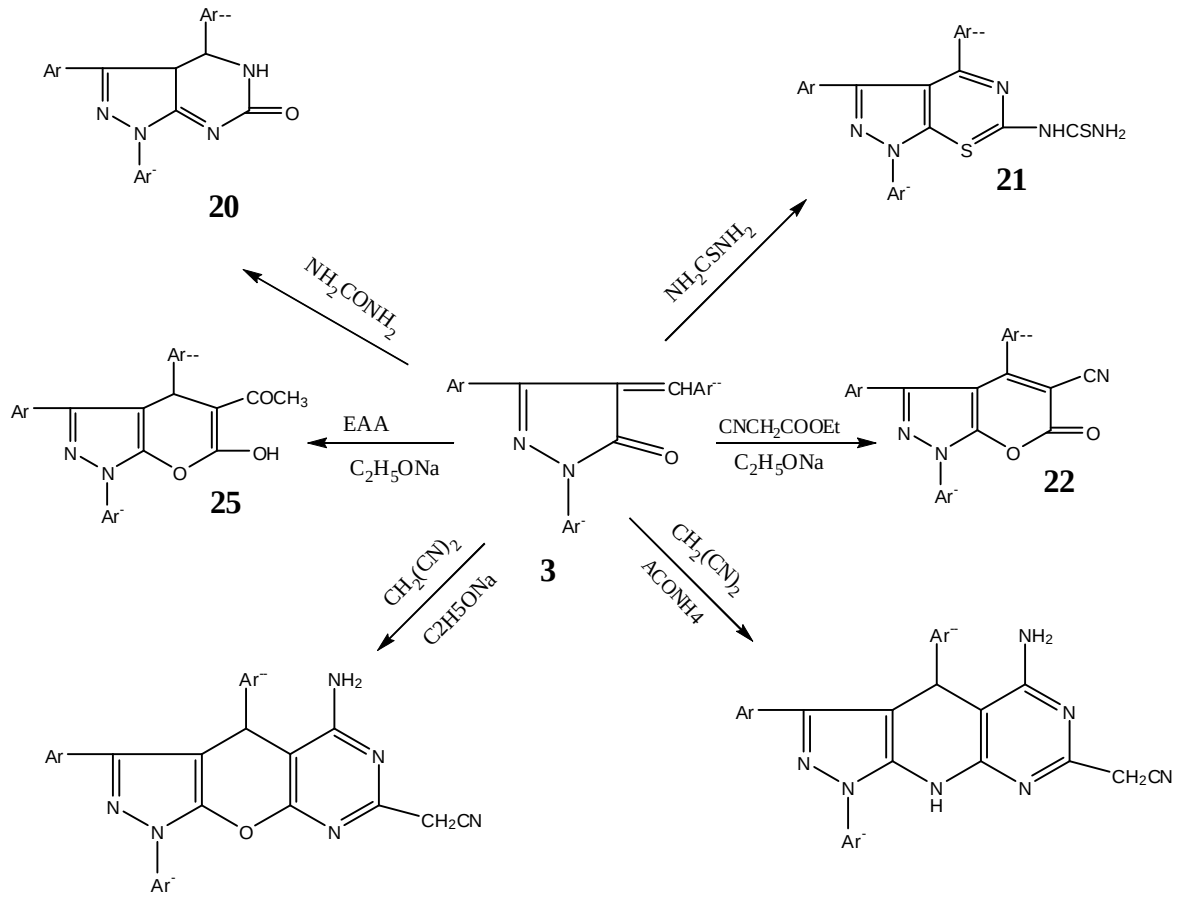
Amixture of **16** (0.01 mol) ,ethylacetoacetate(0.01 mol) and sodium ethoxide(0.5 gm in 20 ml) was refluxed for 6 hrs. The mixture was poured onto ice /HCl. The solid product that separated was filtered off , washed well with water , dried and recrystallized from EtOH.yield73% and M.P214˚C.

Analysis of **25**$C_{23}H_{22}N_4O_9$(496)(%) calcd: C, 55.65; H, 4.06; N, 11.29.FoundC, 55.50; H, 4.16; N, 11.33.
IR spectrum of **25**showed absorption bands at 1613cm^{-1}, 1675 cm^{-1} ,3355attributable to √ C=N ,,√ C=O,√ OH. The mass spectrum showed the molecular ion peak at m/z 498 (7.03%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at ,10.42(s,1H,OH),6.99-8.06 (m, 7H,Ar-H),3.13 (s,6H,2x OCH_3),2.35(s,3H,$COCH_3$),1.91(s,3H, CH_3).



Ar=CH_3 **Ar$^-$=$_2$,4-$(NO_2)_2C_6H_3$** **Ar^{--}= 2,4-$(OCH_3)_2C_6H_3$**

Scheme 2



Scheme 3

4. Cytotoxic assay

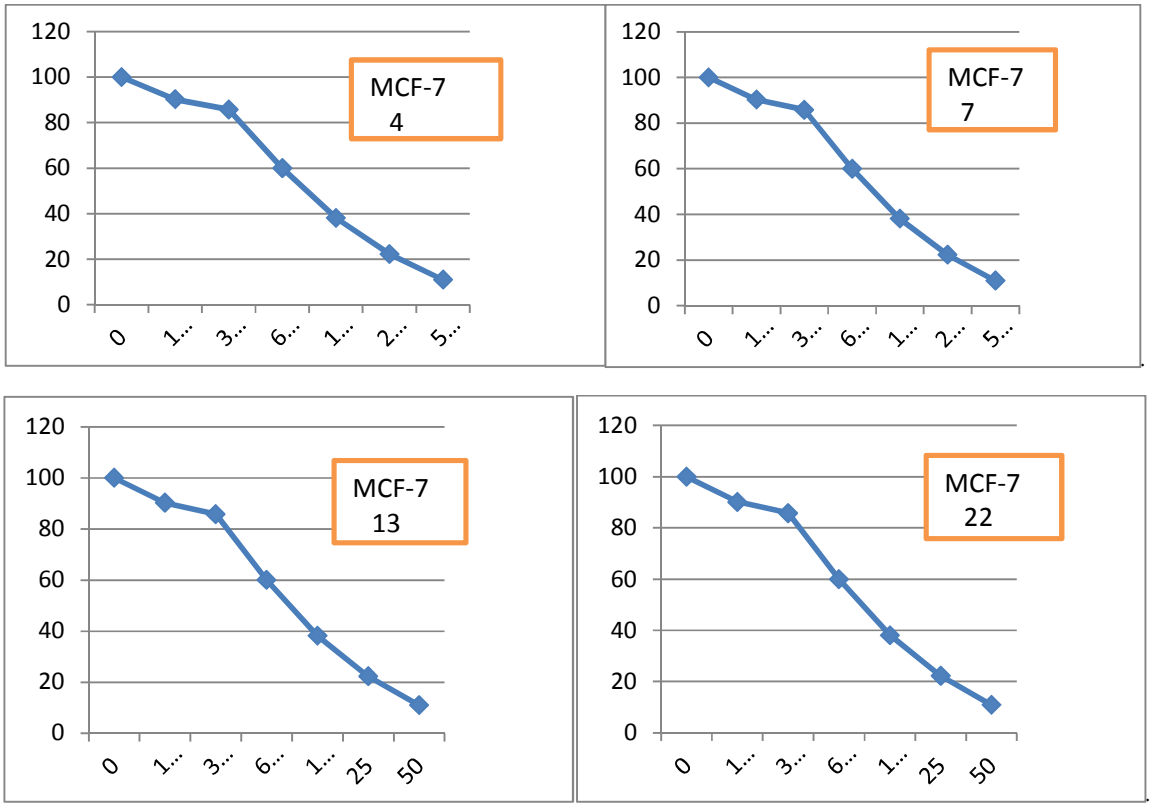
Cytotoxic assay was performed using the modified method as previously described (Tengchaisir.T,1998). Cancer cells were grown in Ham´s/F12 medium containing 2 m Ml-glutamine supplemented with 100 U/ml penicillin, streptomycin and 10% FBS.

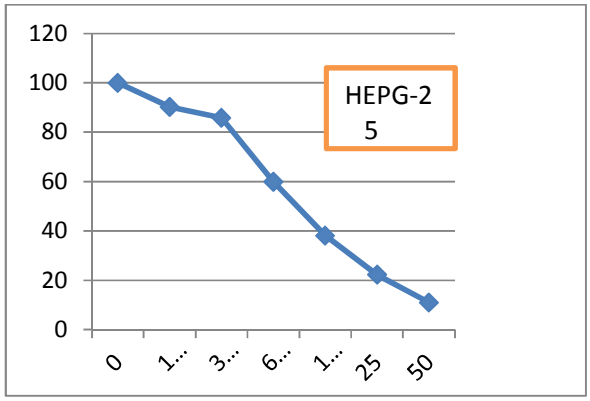
Except Hep G-2 cell was grown in DMEM. Briefly , cell lines(Table 1) suspended in RPMI- 1640 containing 10% FBD were seeded at 1x104 cells(100 µL) per well ina96—well plate , and incubated in humidifiedatmosphere , 95% air 5% CO2 at 37 ºC . After 24h, additional medium (100 µL) containing the tested compound and vehicle was added to a final concentration of 50 µg/ml , 0.2% DMSO , and further incubated for 3 days. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH after which absorbance was measured at 550 nm whereas Hep G2, HCT and MCF-7 cells were stained by MTT.

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

Conc.µg/ml	MCF7				HEPG2				HCT116			
	4	7	13	22	5	9	16	25	2	14	19	23
0	100	100	100	100	100	100	100	100	100	100	100	100
1.56	74.24	69.01	88.98	79.00	87.00	95.65	97.57	89.92	82.32	76.03	90.00	90.23
3.125	31.53	33.98	65.16	72.48	66.42	81.17	91.42	76.56	60.07	55.18	71.60	85.81
6.25	22.16	21.43	48.54	56.77	59.14	60.86	80.14	54.09	35.10	40.22	59.89	59.98
12.5	10.01	15.87	43.67	34.53	49.68	37.98	60.03	22.67	19.90	19.25	26.80	38.14
25	8.11	10.05	33.42	22.65	11.47	22.43	30.14	11.21	12.65	10.02	14.94	22.27
50	3.35	5.14	15.67	2.66	4.19	11.06	7.10	4.98	5.44	2.03	6.98	10.98

IC50 values were determined as the drug and sample concentrations at 50% inhibition ofcell growth (Fig.1)

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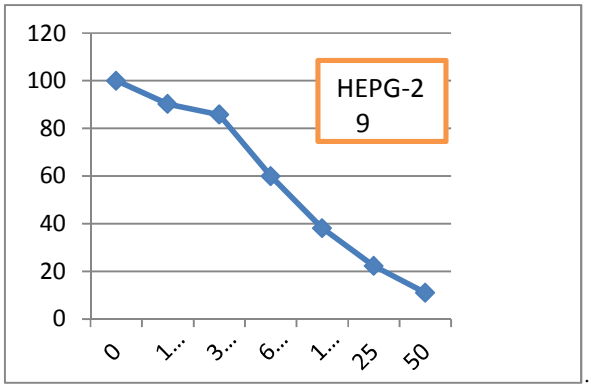
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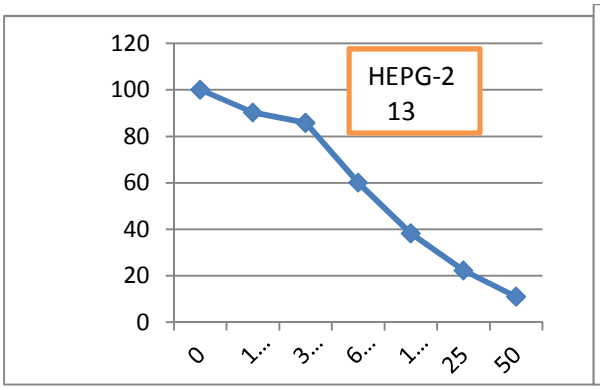
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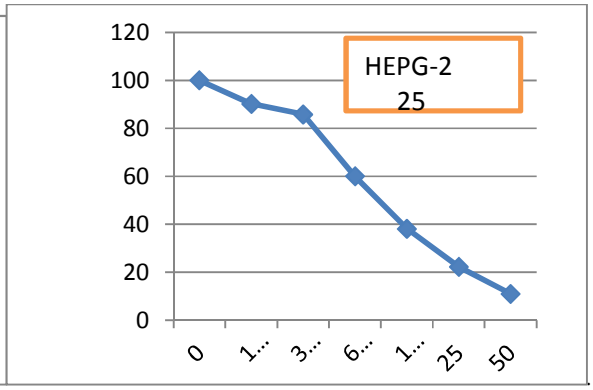
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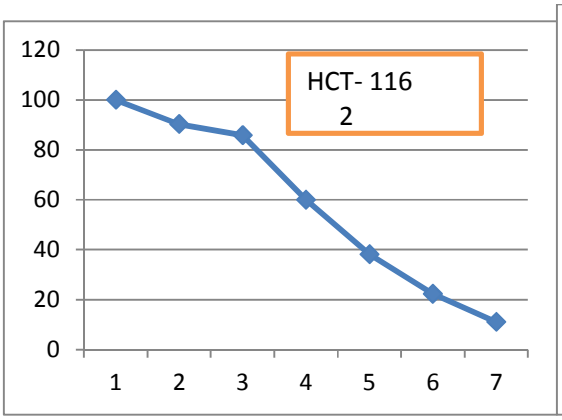
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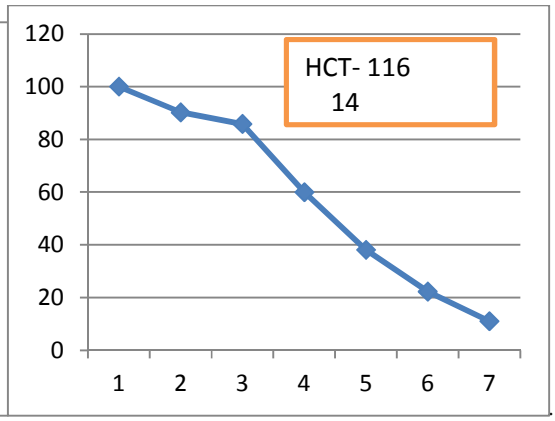
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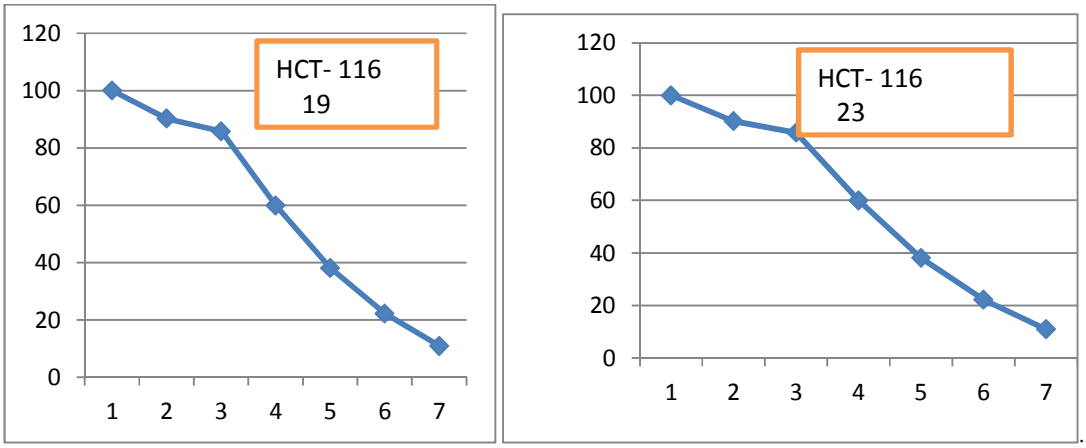

Table (2): Cytotoxic activity of pyrazolyl derivatives (2, 4,5,6,7, 9,13,14,16,19,22,23,25)

Cell lines[a]	IC50 (µg/ml)[b,c]											
	2	**4**	**5**	**7**	**9**	**13**	**14**	**16**	**19**	**22**	**23**	**25**
Hep G-2	NT	NT	4.19	NT	11.06	NT	NT	7.10	NT	NT	NT	4.98
HCT	5.44	NT	NT	NT	NT	NT	2.03	NT	6.98	NT	10.98	NT
MCF-7	NT	3.35	NT	5.14	NT	15.67	NT	NT	NT	2.66	NT	NT

NT: indicates not tested

[a] *Cancer cell lines were hepatocellular carcinoma cell line (Hep G-2); colon carcinoma cell line (HCT); breast carcinoma cell line (MCF-7).*

[b] *When IC50 > 50 µg/ml denotes inactive compound.*

[c] *The assays were performed in triplicate.*

5. Cytotoxic activity:

Cytotoxic activity of pyrazolyl analogs (**2,4,5,7,9,13,14,16,19,22,23,25**) against three cancer lines using a modified method.

The results which were listed in (Table 2) showed that compounds **2,4,5,7,14,22** and **25** have shown the highest activity toward the tested cancer cell lines (Hep G-2)(MCF-7) and (HCT) respectively with IC50 of 5.44,3.35,4.19,5.14,2.66,2.03, µg/ml and 4.98µg/ml respectively.Fig **(2).**

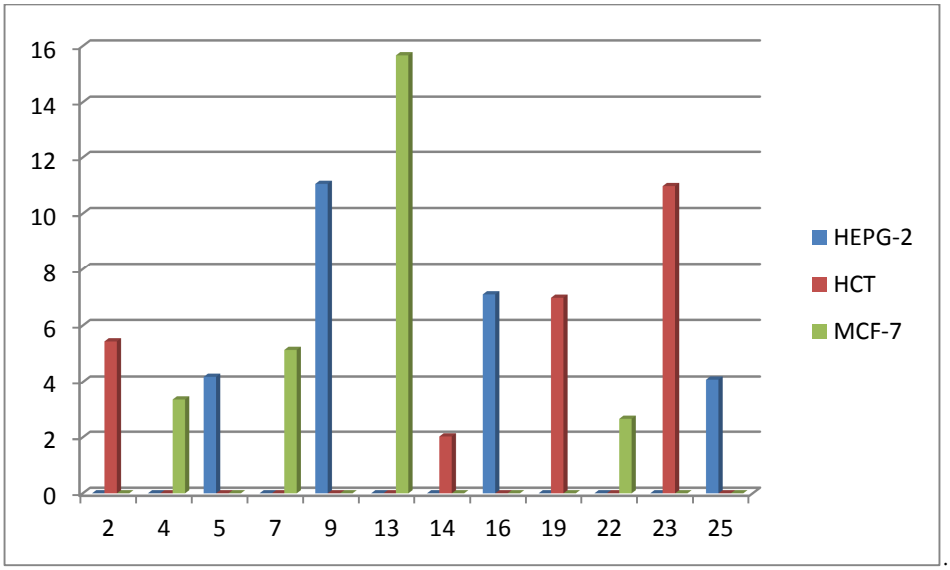

Figure (2): Cytotoxic activity of some pyrazole derivatives

6-Antimicrobial activity

Antimicrobial activity of pyrazolyl analogs (**3,6,15,20**) against three different gram +ve and gram -ve showed significant activity.

Table(3):antimicrobial activity

Samples / *Con.*	*3*			*6*			*15*			*20*		
	1	**5**	**2.5**	**1**	**5**	**2.5**	**1**	**5**	**2.5**	**1**	**5**	**2.5**
Aspergillusfungatus	0	++	+	0	+	0	+++	0	0	0	0	++
Penicilliumitalicum	+++	0	+	0	+	0	0	0	0	++	0	+
Syncephala strum racemosum	0	0	0	+++	0	0	+	0	0	0	0	++
Candida albicans	0	++	0	0	+	0	+++	0	0	0	0	0
Staphylococcus aureus	0	0	+++	+	0	0	0	+	0	0	+	0
Pseudomonas aeruginosa	+++	0	0	0	+	0	0	++	++	0	0	0
Bacillus sbutilis	0	0	0	++	0	+	0	0	0	++	+	0
Escherichia coli	+	++	0	0	+	0	0	0	+	0	0	++

Inhibition values =0.1-0.5 cm beyond control=+; Inhibition values =0.6-1.0cm beyond control=++; Inhibition values =1.1-1.5 cm beyond control=+++;0= not tested

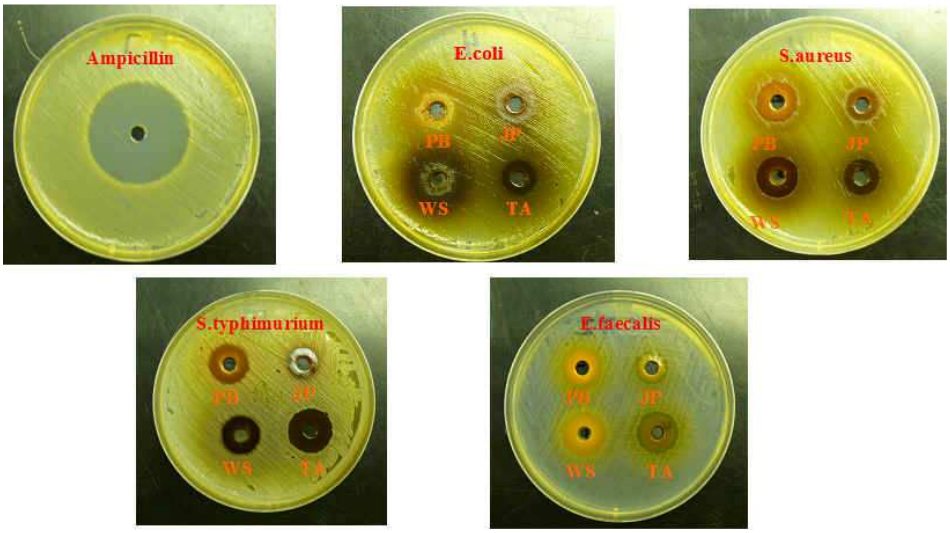

Figure (3): inhibition zone of some pyrazole derivatives

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