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SYNTHESIS, MOLECULAR DOCKING, ANTICANCER ACTIVITY OF SOME NEW HETEROCYCLIC COMPOUNDS CONTAINING THE PYRAZOLYL MOIETY

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ABSTRACT

A new series of pyrazolyl derivatives are reported herein. The one pot reaction of pyrazolone **3** with malononitrile, ethyl acetoacetate 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 aromatic aldehyde have also been taken onto consideration to give the α,β –unsaturated ketone **12**. Compound **12** was used as key intermediate for preparation of various heterocyclic compounds via its reaction with pyrazolone **3**, hydrazine hydrate, hydroxylamine hydrochloride, urea, thiourea, ethyl cyanoacetate / 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, molecular docking biological activity has also been taken in consideration.

KEYWORDS: Pyrazolone, Pyrazolopyrimidines, Pyrazolopyrazole, Pyranopyrazole, Biological activity, Molecular docking, Antitum activity.

1. INTRODUCTION

Pyrazolone is one of the important heterocyclic compounds having five membered ring lactam and an additional keto (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

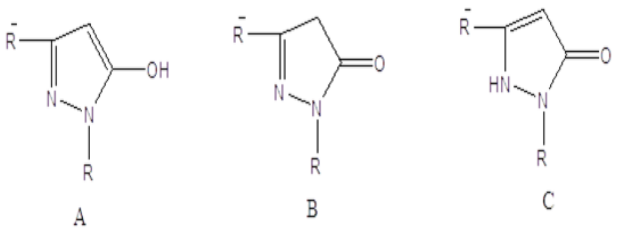
Experimental

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**).



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 3,4 dimethoxybenzaldehyde 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 been reported that aminocarbonitrile reacted with ammonium thiocyanate in boiling acetic acid to give (thioxopyrimidinyl) thiourea derivative, while in our investigation we isolated the amino (iminomethyl) thiocyanats methane 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) pyridinyl formamide **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**.

Knoevenagel condensation reaction of the pyrazolone 3 with p-hydroxybenzaldehyde in the presence of piperidine gave the pyrazolone derivative **12**.

Pyrazolone **12** reacted with pyrazolone **3** in the presence of sodium ethoxide to give the hydroxy pyrazolyl pyrazolol **13**.

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 **12** with hydrazine hydrate gave pyrazolo (3,4-c) pyrazole **14**via β-attack on C=C moiety in compound **12** followed by 1,5- dipolar cyclization, while hydroxylamine hydrochloride gave pyrazolo(3,4-c) isoxazole **15**.

Reaction of **12** with urea afforded the pyrazolo(3,4-c) pyrimidinone **16**via β-attack on the C=C moiety in compound **12**, followed by 1,6- intramolecular dipolar cyclization.

However, reaction of thiourea with compound 16 gave the pyrazolo (4,3-e)(1,3) thiazinyl thiourea **17** via β-attack on the C=C moiety in compound **12**, 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)pyrazole carbonitrile **18**.

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 **12** with ethyl acetoacetate in the presence of ammonium acetate gave the pyrazolo(3,4-b) pyridinyl ethanone 25 via the addition of the nucleophile ethyl acetoacetate on the β-carbon of the electrophile**12** in a conjugate addition reaction in the presence of ammonium acetate followed by cyclization and dehydrogenation.

Synthesis and discussion

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

A mixture of ethylacetoacetate **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 acetic acid. yield 85% and M.P 168˚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-4-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-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 (0.03 mol) and 3,4-dimethoxybenzaldehyde (0.01 mol) was fused 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 70% and M.P 257˚C.

Analysis of **4** $C_{22}H_{17}N_7O_6$ (475) (%) calcd: C, 55.58; H, 3.60; N, 20.62;3. Found:C, 55.40; H, 3.70; N, 22.90.

IR spectrum of **4** showed absorption bands at 1616 cm^{-1}, 23233 cm^{-1},32207 cm^{-1}, 31134,31126 cm^{-1} attributable to √ C=N, √ CN and √ OH and √NH_2. The mass spectrum showed the molecular ion peak at m/z 475 (M,7.03%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.89 (s,1H,OH), 8.91-7.21 (m, 7H,Ar-H), 4.86(s,2H,NH_2),2.54(s,6H,2x OCH_3), 1.34(s,3H, CH_3).

Synthesis of 6-amino-4-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-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 3,4-dimethoxybenzaldehyde (0.01 mol) in presence of 1 ml piperdine was fused for 15 hrs at 160^0C. After cooling, the solid product was washed with EtOH, dried and recrystallized from EtOH. yield 78% and M.P 290˚C.

Analysis of **5** $C_{22}H_{18}N_6O_7$ (478) (%) calcd: C, 55.23; H, 3.79; N, 17.57. Found:C , 55.20; H, 3.81; N, 17.58.

IR spectrum of **4** showed absorption bands at 16223 cm^{-1}, 2233 cm^{-1}, 3345,3299 cm^{-1} attributable to √ C=N, √ CN and √NH_2. The mass spectrum showed the molecular ion peak at m/z 478 (8.1%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 10.89 (s,1H,OH), 7.18-8.01 (m, 7H,Ar-H), 4.52(s,2H,NH_2), 2.54(s,6H,2x OCH_3), 1.39(s,3H, CH_3).

Synthesis of 4-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-3,6-dimethyl-1H-pyrazolo[3,4-b]pyridine (6)

A mixture of pyrazolone **3** (0.01 mol), ethyl acetoacetate (0.01 mol), ammonium acetate (0.03 mol) and 3,4-dimethoxybenzaldehyde (0.01 mol) was fused for 15 hrs at 170^0C. After cooling, the solid product was washed with water, filtered off and recrystallized from EtOH. yield64% and M.P 279˚C.

Analysis of **5** $C_{22}H_{19}N_5O_6$ (449) (%) calcd: C, 58.80; H, 4.26; N, 15.58. Found:C , 58.40; H, 4.60; N, 15.64.

IR spectrum of **6** showed absorption bands at 1606 cm^{-1}, 3314cm^{-1} attributable to √ C=N, √ OH. The mass spectrum showed the molecular ion peak at m/z 447 (16.62%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 6.96-7.91 (m, 8H,Ar-H), 4.31(s,2H,NH_2), 2.44(s,6H,2x OCH_3), 1.19(s,6H,2x CH_3).

Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-(3,4-dimethoxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridin-5-yl)(iminomethyl)thiocyanatomethane thioimidate (7)

A mixture of **4** (0.01 mol) and ammonium thiocyanate (0.03 mol) 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. yield 78% and M.P 270˚C.

Analysis of **7** $C_{24}H_{19}N_9O_6S_2$ (593) (%) calcdC, 48.56; H, 3.23; N, 21.24; S, 10.80. Found: C, 48.50; H, 3.25; N, 21.26; S, 10.82.

IR spectrum of **7** showed absorption bands at 1606 cm^{-1}, 2209 cm^{-1}, 3269cm^{-1}, 3354 cm^{-1}attributable to √ C=N, √ CN, √ OH, √ NH. The mass spectrum showed the molecular ion peak at m/z 593 (7.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 6.96-8.01 (m, 7H,Ar-H),5.41(s,2H,2xNH),4.61(s,2H,NH_2),3.04(s,6H,2x OCH_3), 1.49(s,3H, CH_3).

Synthesis of N-(5-cyano-4-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-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 onto water. The solid product that separated was filtered off, washed well with water, dried and recrystallized from EtOH. yield 69% and M.P 278˚C.

Analysis of **8** $C_{23}H_{17}N_7O_7$ (503) (%) calcd: C, 54.87; H, 3.40; N, 19.48.Found: C, 54.88; H, 3.35; N, 19.52.

IR spectrum of **8** showed absorption bands at 1615 cm^{-1}, 1668 cm^{-1}, 2208 cm^{-1}, 3269cm^{-1},3331cm^{-1}attributable to √ C=N, √ C=O, √ CN, √ OH, √ NH. The mass spectrum showed the molecular ion peak at m/z 503 (6.52%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.78(s,1H,NH),10.99(s,1H,OH), 7.16-8.82 (m, 8H,Ar-H), 2.54(s,6H,2x OCH_3), 1.34(s,3H, CH_3).

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

Amixture of 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** 82% and M.P 291˚C. Yield of **10** 70% and M.P 297˚C.

Analysis of **9** $C_{24}H_{19}N_7O_7$ (517) (%) calcd: C, 55.71; H, 3.70; N, 18.95;;.Found: C, 55.85; H, 3.60; N, 18.91;while for **10** $C_{24}H_{19}N_6O_8$ (518) (%) calcd: C, 55.49; H, 3.69; N, 16.18;;. Found: C, 55.40; H, 3.74; N, 16.19.

IR spectrum of **9** showed absorption bands at 1601 cm^{-1}, 1665 cm^{-1}, 2206 cm^{-1}, 3325cm^{-1}attributable to √ C=N, √ C=O, √ CN, √ OH/ √ NH. while for **10** showed absorption bands 1599 cm^{-1}, 1675 cm^{-1}, 2206 cm^{-1}, 3316 cm^{-1}attributable to √ C=N, √ C=O, √ CN, √ OH/ √ NH. The mass spectrum of **9** showed the molecular ion peak at m/z 517 (18.55%) and for **10** is 477(2.88%). The ^{1}H-NMR(DMSO-d6) of **9** showed signal bands δppm at 11.75(s,1H,NH),10.89(s,1H,OH), 6.89-8.01 (m, 7H,Ar-H), 2.85(s,6H,2x OCH_3), 1.78(s,6H,2x CH_3), while **10** showed signal bands δppm at 11.24(s,1H,NH),10.73(s,1H,OH), 7.13-8.15 (m, 8H,Ar-H), 2.94(s,6H,2x OCH_3), 1.81(s,6H,2x CH_3).

Synthesis of 6,6- -(1,1-hydrazine-1,2-diyl)bis(ethane-1,1-diyl)bis(azanedinyl)bis (1-(2,4-dinitrophenyl)-4-(3,4-dimethoxyphenyl)-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 onto ice/HCl(1:3). The solid product that separated was filtered off, washed well with water, dried and recrystallized from EtOH. yield 72% and M.P 276˚C.

Analysis of **8** $C_{48}H_{44}N_{14}O_{14}$ (1040) (%) calcd; C, 55.38; H, 4.26; N, 18.84; O,. Found: C, 55.48; H, 4.22; N, 18.78.

IR spectrum of**11** showed absorption bands at 1609 cm^{-1}, 2209 cm^{-1}, 3296cm^{-1}, 3325cm^{-1}attributable to √ C=N, √ CN, √ OH, √ NH. The mass spectrum showed the molecular ion peak at m/z 1040 (3.14%).

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

Amixture of **3** (0.01 mol), 4-hydroxybenzaldehyde(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. yield 78% and M.P 190˚C.

Analysis of **12** $C_{17}H_{12}N_4O_6$ (368) (%) calcd: C, 55.44; H, 3.28; N, 15.21;.Found C, 55.30; H, 3.38; N, 15.25.

IR spectrum of **12** showed absorption bands at 1603 cm^{-1}, 1668 cm^{-1}, attributable to √ C=N, √ C=O. The mass spectrum showed the molecular ion peak at m/z 368 (15.19%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at10.83(s,1H, OH), 7.12-8.01 (m, 7H,Ar-H), 1.59(s,3H, CH_3).

Synthesis of 4,4'-((4-hydroxyphenyl)methylene)bis(1-(2,4-dinitrophenyl)-3-methyl-1H-pyrazol-5-ol) (13)

Compound **12** (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 100^0C. The reaction mixture was poured onto ice/HCl. The solid product that separated was filtered off, washed well with water, dried and recrystallized from EtOH. yield 80% and M.P 197˚C.

Analysis of **13** $C_{27}H_{20}N_8O_{11}$ (632) (%) calcd: C, 51.27; H, 3.19; N, 17.72;.Found C, 51.20; H, 3.21; N, 17.77.

IR spectrum of **13** 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 of 4-(6-(2,4-dinitrophenyl)-4-methyl-2,3,3a,6-tetrahydropyrazolo[3,4-c]pyrazol-3-yl)phenol (14)

Amixture of **12** (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. yield 74% and M.P 250˚C.

Analysis of **14** $C_{17}H_{14}N_6O_5$ (382)(%) calcd: C, 53.40; H, 3.69; N, 21.98. Found: C, 53.55; H, 3.59; N, 21.93.

IR spectrum of **14** showed absorption bands at 1591 cm^{-1}, 3316 cm^{-1}, attributable to √ C=N, √ NH. The mass spectrum showed the molecular ion peak at m/z 382 (14.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.24(s,1H,NH), 10.84(s,1H,OH), 7.01-7.91 (m, 8H,Ar-H), 1.89(s,3H, CH_3).

Synthesis of 4-(6-(2,4-dinitrophenyl)-4-methyl-3a,6-dihydro-3H-pyrazolo[3,4-c]isoxazol-3-yl)phenol (15)

Amixture of **12** (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. yield 64% and M.P 223˚C.

Analysis of **15** $C_{17}H_{15}N_5O_6$ (383)(%) calcd: C, 53.27; H, 3.42; N, 18.27;. Found C, 53.20; H, 3.46; N, 18.30.

IR spectrum of **15** showed absorption bands at 1604 cm^{-1}, attributable to √ C=N,. The mass spectrum showed the molecular ion peak at m/z 383 (10.02%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.29(s,1H,NH), 10.42(s,1H,OH), 6.91-8.01 (m, 7H,Ar-H),3.05(s,1H,CH), 2.01(s,3H, CH_3).

Synthesis of 1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(3H)-one (16)

Amixture of **12**(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. yield 70% and M.P 204˚C.

Analysis of **16** $C_{18}H_{14}N_6O_6$ (410)(%) calcd: C, 52.69; H, 3.44; N, 20.48. Found C, 52.90; H, 3.33; N, 20.38.

IR spectrum of **16** showed absorption bands at1606 cm^{-1}, 1681 cm^{-1},3310 cm^{-1} attributable to √ C=N, √ C=O, √ NH.

The mass spectrum showed the molecular ion peak at m/z 410 (10.31%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.24(s,1H,NH), 10.54(s,1H,OH) 7.01-8.31 (m, 7H,Ar-H),3.15(s,1H,CH), 2.09(s,3H, CH_3).

Synthesis of 1-(1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-1,6-dihydropyrazolo[4,3-e][1,3]thiazin-6-yl)thiourea (17)

Amixture of **12** (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. yield 73% and M.P 234˚C.

Analysis of **17** $C_{19}H_{15}N_6O_5S_2$ (485)(%) calcd: C, 47.00; H, 3.11; N, 20.20; S, 13.21.Found C, 47.08; H, 3.09; N, 20.18; S, 13.19.

IR spectrum of **17** showed absorption bands at 1420 cm^{-1},1605 cm^{-1}, 3120-3101 cm^{-1},3319 cm^{-1} attributable to √ C=S, √ C=N, √ NH_2, √ NH. The mass spectrum showed the molecular ion peak at m/z 485(8.12%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 11.28(s,1H,NH), 10.98(s,1H,OH),6.91-8.01 (m, 7H,Ar-H),3.15(s,1H,CH), 1.49(s,3H, CH_3).

Synthesis of 1-(2,4-dinitrophenyl)-4-(4-hydroxyphenyl)-3-methyl-6-oxo-1,6-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (18)

Amixture of **12** (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. yield 77% and M.P 250˚C.

Analysis of **18** $C_{20}H_{11}N_5O_7$ (433)(%) calcd: C, 55.43; H, 2.56; N, 16.16;.Found C, 55.30; H, 2.66; N, 16.20.

IR spectrum of**18** showed absorption bands at 1607 cm^{-1},1679 cm^{-1} , 2220 cm^{-1} attributable to √ C=N , √ C=O, √ CN . The mass spectrum showed the molecular ion peak at m/z 433 (11.12%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at 10.88(s,1H,OH), 7.09-8.14 (m, 8H,Ar-H) , 1.97(s,3H, CH_3).

Synthesis of 2-[5-amino-4-(4-hydroxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl-4-9-dihydro-1H-pyrazolo[4',3':5,6]pyrido(2,3-d)pyrimidin-7-yl]acetonitril(19)

Amixture of **12** (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. yield 73% and M.P 267˚C.

Analysis of **19** $C_{23}H_{17}N_9O_5$ (499)(%) calcd: C, 55.31; H, 3.43; N, 25.24.Found C, 55.20; H, 3.50; N, 25.28.

IR spectrum of **19** showed absorption bands at 1601 cm^{-1}, 2223 cm^{-1} ,3374 attributable to √ C=N, √ CN, √ NH. The mass spectrum showed the molecular ion peak at m/z 499 (14.12%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at,11.60(s,1H,NH), 10.90(s,1H,OH),7.01-8.01 (m, 7H,Ar-H),4.53(s,2H,NH_2), 2.01(s,3H, CH_3).

Synthesis of 2-[5-amino-4-((4-hydroxyphenyl)-1-(2,4-dinitrophenyl)-3-methyl1,4-dihydropyrazolo[4',3':5,6]pyrano [2,3-d]pyrimidin-7-yl]acetonitrile (20)

Amixture of **12** (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. yield 69% and M.P 240˚C.

Analysis of **20** $C_{23}H_{18}N_8O_6$(500)(%) calcd: C, 55.20; H, 3.22; N, 22.39.Found C, 55.18; H, 3.21; N, 22.38.

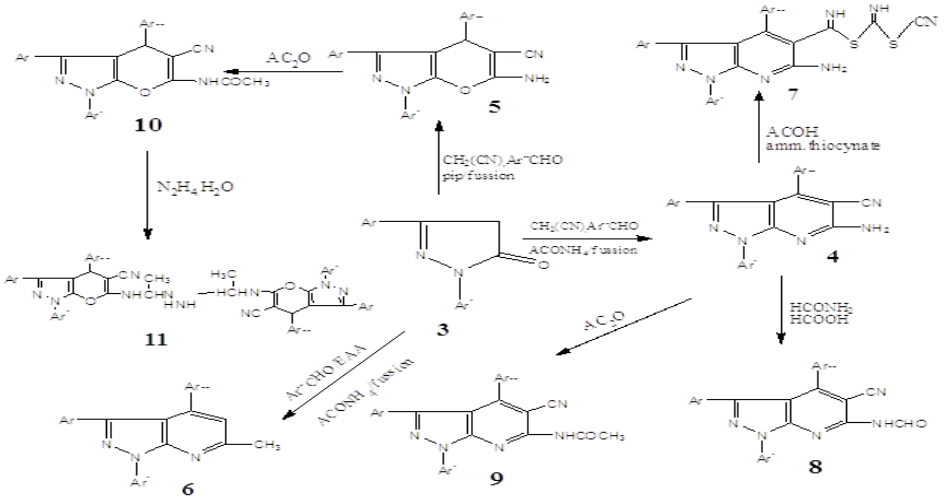
IR spectrum of **20** showed absorption bands at 1598 cm^{-1}, 2234 cm^{-1} ,3398attributable to √ C=N, √ CN, √ NH. The mass spectrum showed the molecular ion peak at m/z 500 (14.12%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at,11.42(s,1H,NH), 10.92(s,1H,OH),6.69-8.01 (m, 7H,Ar-H),4.23(s,2H,NH_2), 2.11(s,3H, CH_3).

Synthesis of 4-(4-hydroxyphenyl)-1-(2,4-dinitrophenyl)-6-hydroxy-3-methyl-1,3a,4,7a-tetrahydropyrano[2,3-c]pyrazol-5-yl)ethanone(21)

Amixture of **12** (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. yield 70% and M.P223˚C.

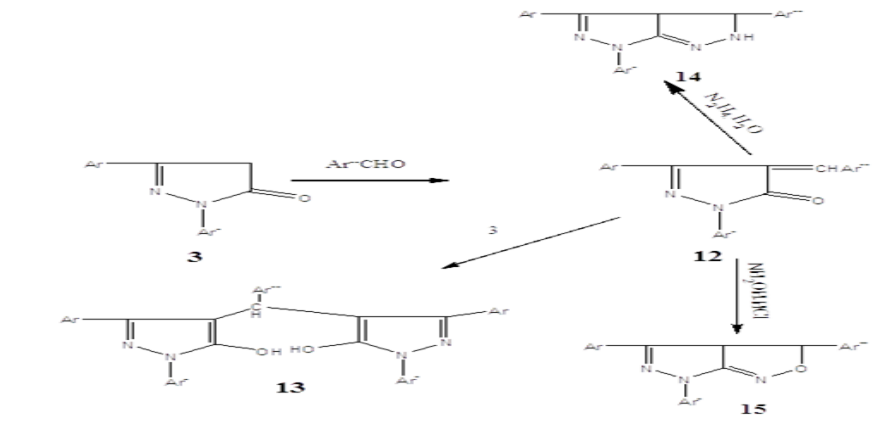
Analysis of **21** $C_{21}H_{16}N_4O_8$(452)(%) calcd: C, 55.76; H, 3.56; N, 12.39;.Found C, 55.50; H, 3.70; N, 12.51.

IR spectrum of **21** showed absorption bands at 1598 cm^{-1}, 1689 cm^{-1} ,3310attributable to √ C=N, √ C=O, √ OH . The mass spectrum showed the molecular ion peak at m/z 452 (12.73%). The ^{1}H-NMR(DMSO-d6) showed signal bands δppm at,10.72(s,2H,2xOH),6.99-7.96 (m, 7H,Ar-H), 2.41(s,3H,$COCH_3$), 1.81(s,3H, CH_3).



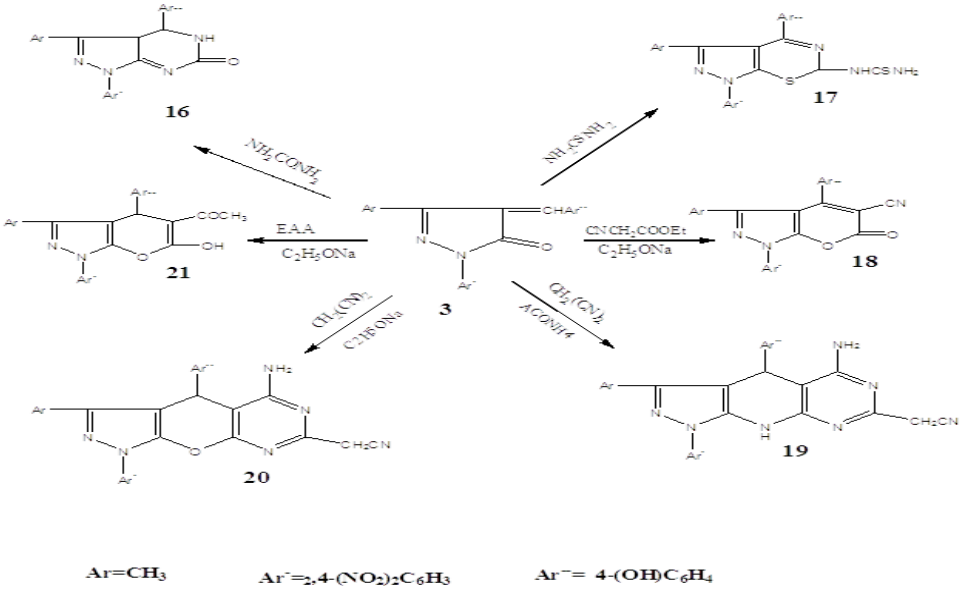
Ar=CH_3 Ar^{-}=2,4$(NO_2)_2C_6H_3$ Ar^{--}=3,4-$(OCH_3)C_6H_4$

Scheme 1



Ar=CH_3 Ar^{-}=2,4$(NO_2)_2C_6H_3$ Ar^{--}=4-$(OH)C_6H_4$

Scheme 2



Scheme 3

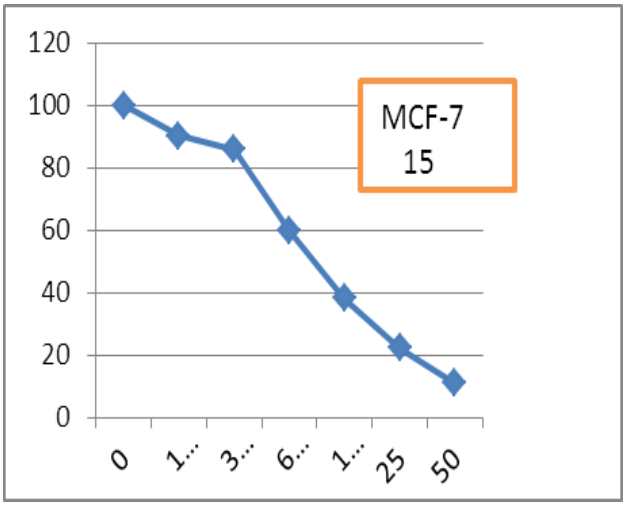
4. Cytotoxic assay

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 37ºC. 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 values were determined as the drug and sample concentrations at 50% inhibition of cell growth (Fig.1)

Conc.µg/ml	MCF-7		HEPG-2		HCT-116			
	15	20	17	18	4	7	9	12
0	100	100	100	100	100	100	100	100
1.56	71.04	69.01	95.65	97.57	82.32	76.03	90.00	90.23
3.125	31.53	33.98	81.17	91.42	60.07	55.18	71.60	85.81
6.25	19.16	21.43	60.86	80.14	35.10	40.22	59.89	59.98
12.5	12.01	15.87	37.98	60.03	19.90	19.25	26.80	38.14
25	8.11	10.05	22.43	30.14	12.65	10.02	14.94	22.27
50	3.35	5.14	11.06	7.10	5.44	2.03	6.98	10.98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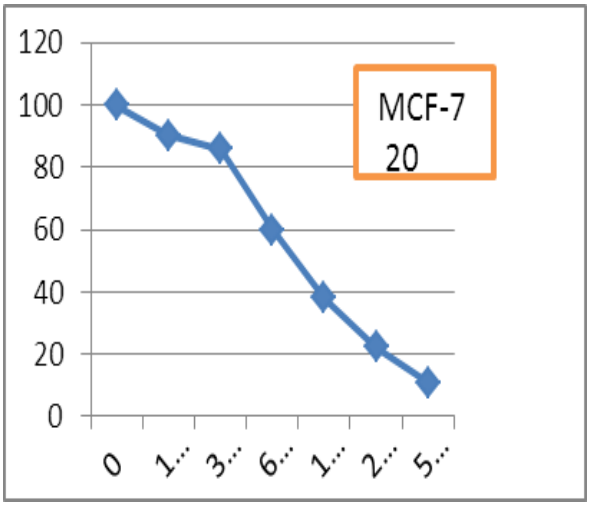
100
80
60
40
20
0
MCF-7
15
0
1...
3...
6...
1...
25
50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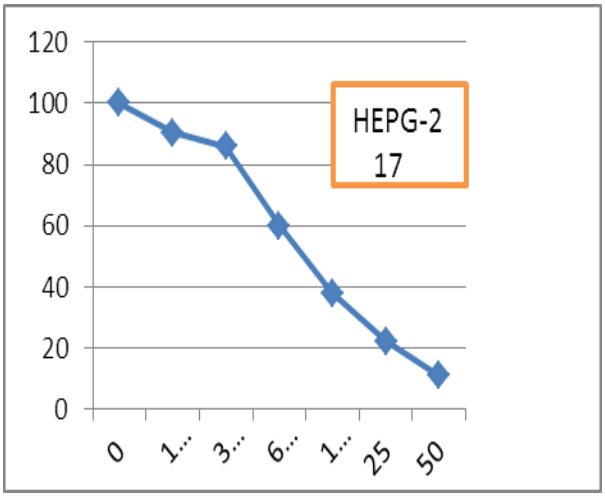
MCF-7
20
0
1...
3...
6...
1...
2...
5...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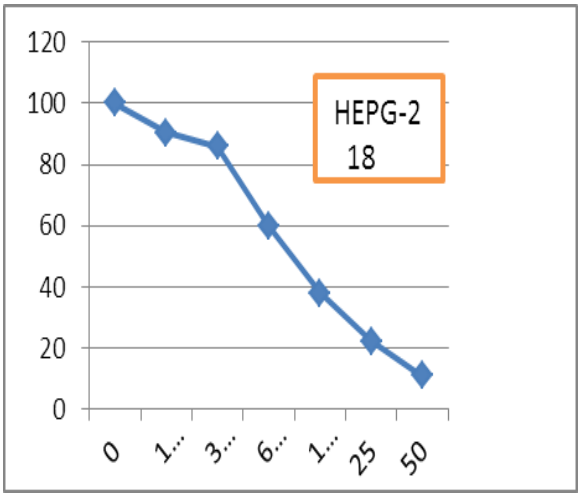
40
20
0
HEPG-2
17
0
1...
3...
6...
1...
25
50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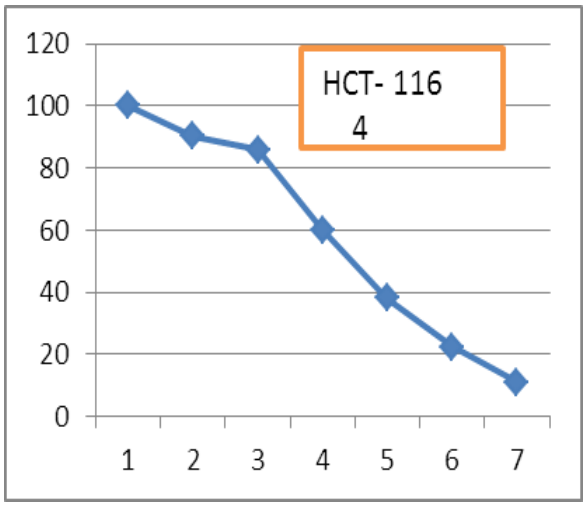
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4
1
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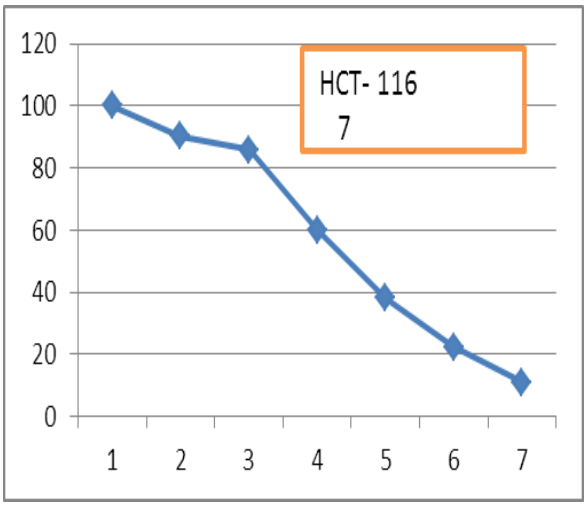
3
4
5
6
7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HCT- 116
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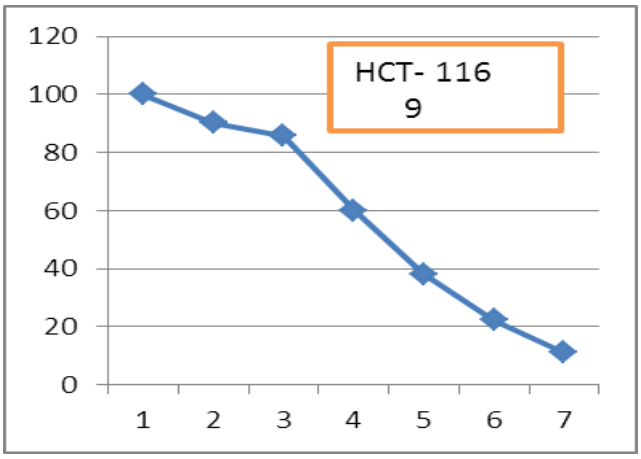


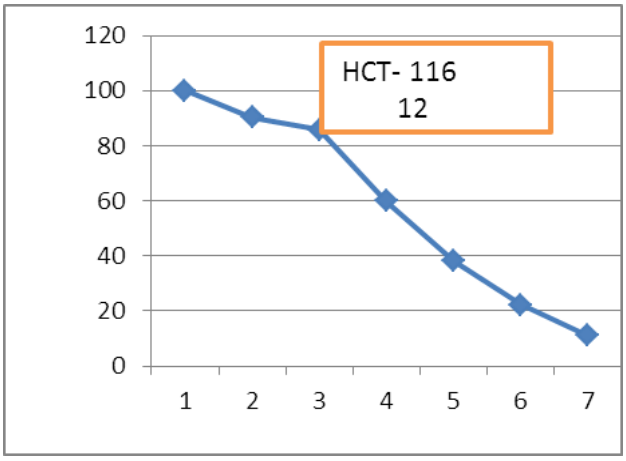


Table (2): Cytotoxic activity of pyrazolyl derivatives (4,7,9,12,15,17,18,20)

Cell lines[a]	IC50 (µg/ml)[b,c]							
	4	**7**	**9**	**12**	**15**	**17**	**18**	**20**
Hep G-2	NT	NT	NT	NT	NT	9	19.3	NT
HCT	7.7	5	3.9	9.1	NT	NT	NT	NT
MCF-7	NT	NT	NT	NT	2.4	NT	NT	2.6

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 (**4,7,9,12,15,17,18,20**) against three cancer lines using a modified method.

The results which were listed in (Table 2) showed that compounds **4,7,9,12,15,17** and **20** have shown the highest activity toward the tested cancer cell lines (Hep G-2)(MCF-7) and (HCT) respectively with IC50 of 7.7, 5, 3.9, 9.1, 9, 2.6 µg/ml and 4.98 µg/ml respectively. Fig **(2).**

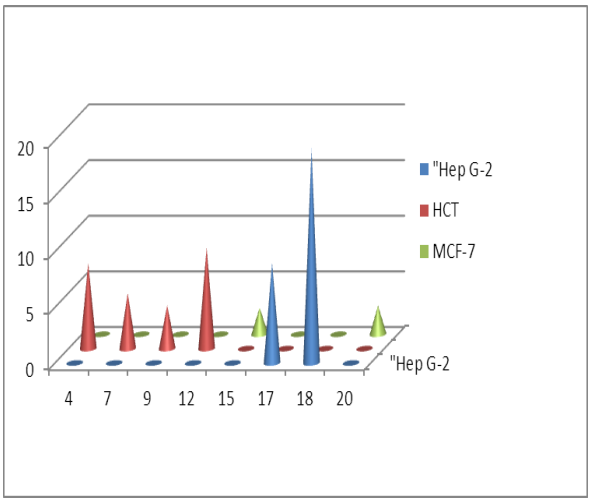


Figure (2): Cytotoxic activity of some pyrazole derivatives

6-Molecular Docking

Docking simulation[23] study was performed in Medicinal Chemistry Department, Faculty of pharmacy, Assiut University using Molecular Operating Environment (MOE®) version 2014.09, Chemical Computing Group Inc., Montreal, Canada. The computational software operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory.

Docking on the active site of cyclin dependent kinase-2 (CDK-2) was performed for synthesized compounds **(4,7,9,15,17,19).** All the minimizations were performed with MOE until a root mean standard deviation (RMSD) gradient of 0.01 kcal/mol and RMS distance of 0.1Å with MFF94X force field and the partial charges were automatically calculated. The X-ray crystallographic structure of CDK-2 with its ligand was obtained from the protein data bank. The enzyme was prepared for docking studies where: (i) Acting on only one chain of amino acids containing one molecule of the inhibitor. (ii) 3D protonation for the amino acid side chain and the ligand. (iii) Deleting all water of crystallization away from the active site. (iv) Isolation of the active site and recognition of the amino acids. (v) Studying the interaction of the ligand with the amino acids of the active site.

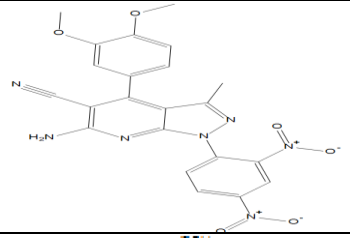
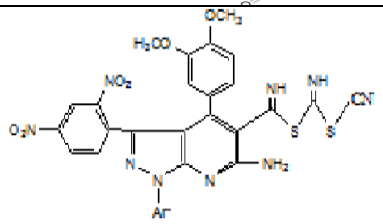
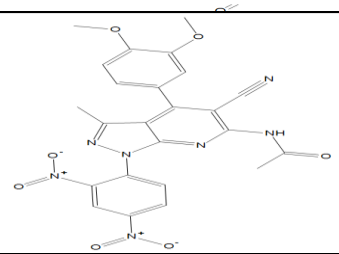
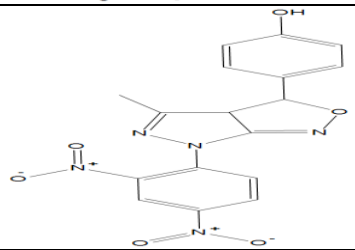
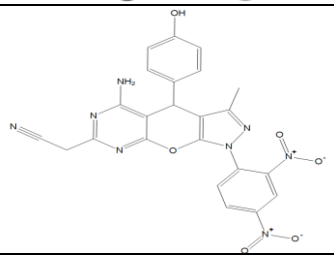
All the above procedures were taken and the 2D interactions of ligand with the amino acids of the active site are shown figure (1).

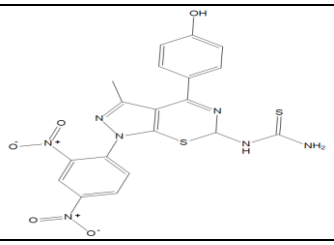
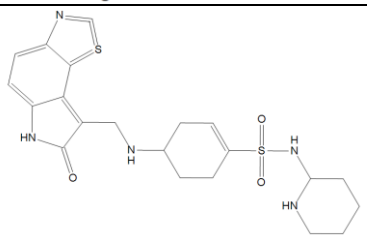
Preparation of the synthesized compounds for docking was achieved via their 3D structure built by MOE. Certain procedures should be taken before docking which includes: (i) 3D protonation of the structures. (ii) Running conformational analysis using systemic search. (iii) Selecting the least energetic conformer. (iv) Applying the same docking protocol used with the ligand. The previous measures were taken and docking for the synthesized compounds was applied. Energy scoring (S) was measured and illustrated in table (3).

Molecular Docking Study

The protein data bank file (1FVV) was selected for testing the molecular docking on the active site of CDK-2. The file contains CDK-2 co-crystallized. Docking protocol was verified by re-docking of the co-crystallized ligand in the vicinity of the active site of the enzyme with energy score (S)= −7.7722 kcal/mol.

Table (3): Energy scoring (S) measured for the tested compounds.

No.	Compound	Score
4		-8.0429
7		-6.7728
9		-7.4467
15		-6.7838
19		-6.8126

17		-7.3589
Co-crystalized ligand (Reference)		-7.7722

From figure (**3**) the ligand interacts with the active site of CDK2 by four interactions: the sulphonate group interacts with Lys 89 with a hydrogen bond of 3.13 A. On the other hand the carbonyl group of pyrolone interacts with Leu 83 with a hydrogen bond of 2.96 A and finally the NH group of pyrolone interacts with Glu 81 with a hydrogen bond of 1.98 A. in order to visualize these interactions in a better manner 3D interactions were illustrated in fig (**4**).

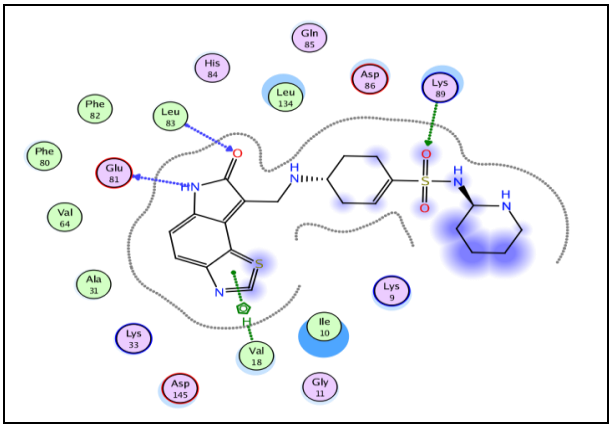


Figure (3) interactions of the ligand on the active site of CDK2

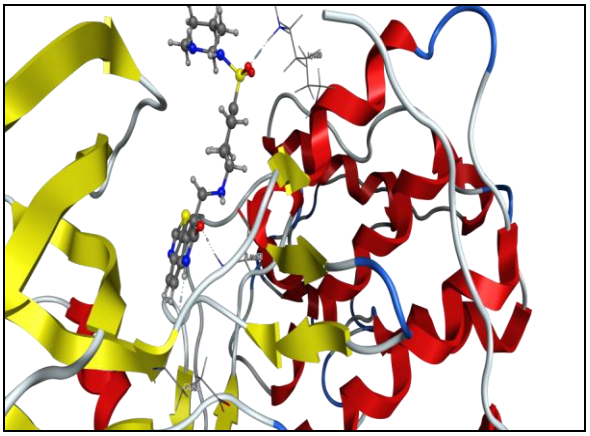


Figure (4) 3D interactions of the ligand on the active site of CDK2

Compound **4** showed the best energy score (S)=−8.0429 kcal/mol and interacted with Leu 298 with a hydrogen bond of 3.75 Å through a sulufur atom of pyrazlyl moiety and interacted also with Gln 131 with a hydrogen bond of 3.23 Å through its O-−N+ of the benzene ring. Another two hydrogen bonds with Lys 20 and Ile 10 were recorded with bond length 3.88 and 4.39 Å, respectively (fig **5,6**).

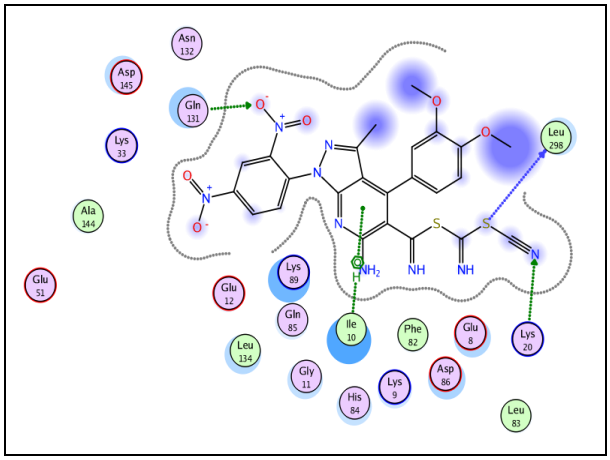


Figure (5) interactions of the compound 4 on the active site of CDK2

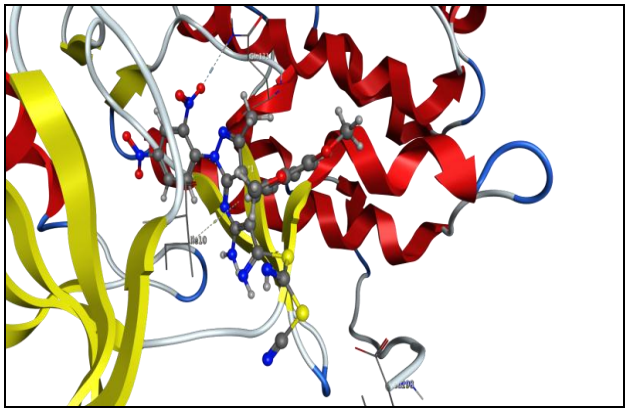


Figure (6) 3D interactions of compound 4 on the active site of CDK2

Compound **7** revealed energy score (S)=−8.0429 kcal/mol and interacted with Asp 86 with one hydrogen bond of 3.40Å through its C=O, in addition to another two hydrogen bonds with Glu 12 and Leu 134 with bond distance 2.99 and 4.28 Å, respectively (fig **7,8**).

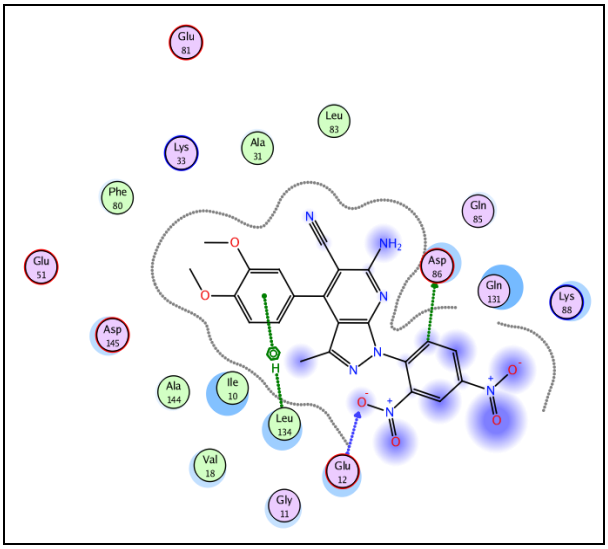


Figure (7) interactions of the compound7 on the active site of CDK2

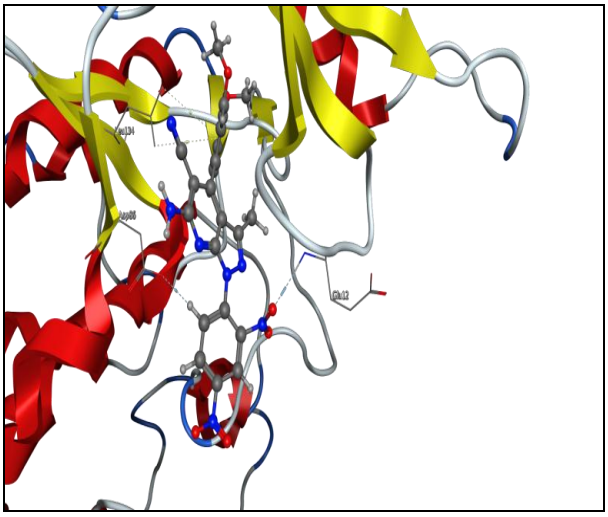


Figure (8) 3D interactions of compound 7 on the active site of CDK2

The energy score for compound **9** was (S)=−7.4467 kcal/mol and interacted with only two amino acids Gln 131 with a hydrogen bond of 2.98 Å and Tyr 15 with a hydrogen bond of 3.49 Å through N=O of the benzene ring of pyrazolyl moiety (fig **9,10**).

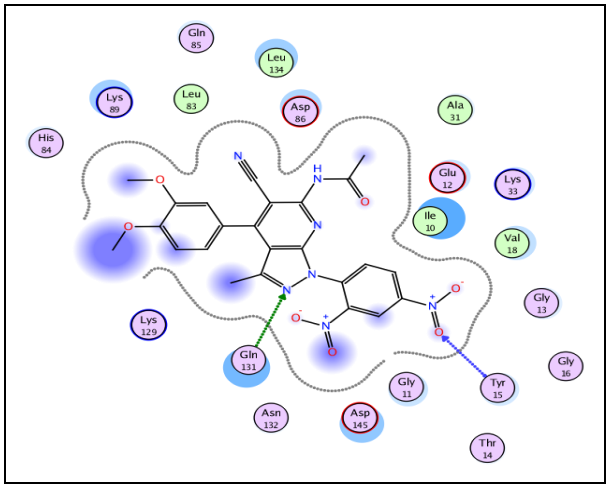


Figure (9) interactions of the compound 9 on the active site of CDK2

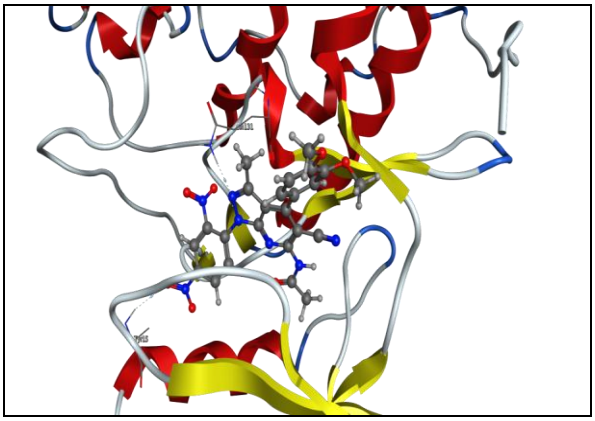


Figure (10) 3D interactions of the compound 9 on the active site of CDK2

Compound **15** revealed energy score (S)=−6.7838 kcal/mol and interacted with Glu 51 with one hydrogen bond of 3.15Å through O=H of the ligand phenyl ring, in addition to hydrogen bonds with Lys 89 with bond distance 3.17 (fig **11,12**).

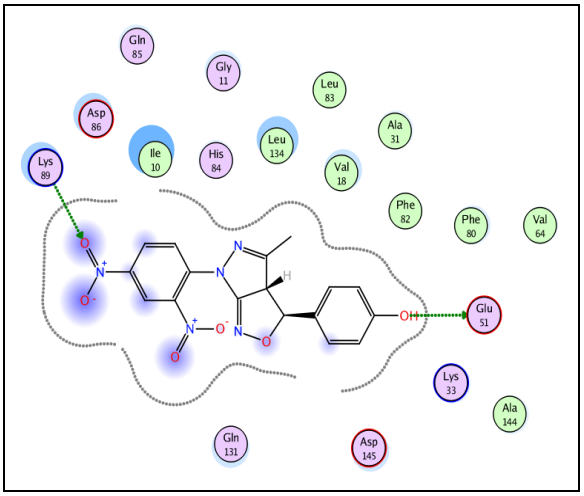


Figure (11) interactions of the compound 15 on the active site of CDK2

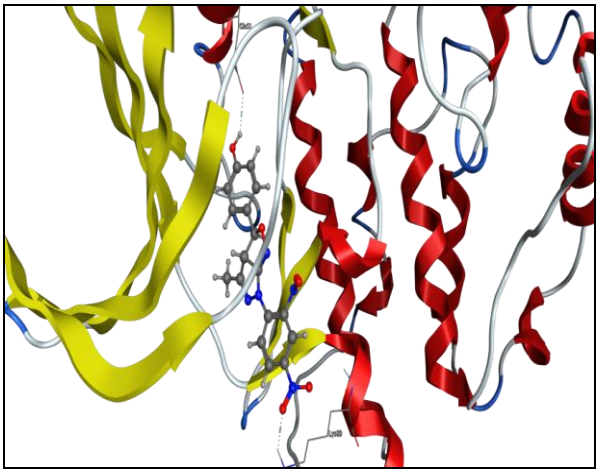


Figure (12) 3D interactions of the compound 15 on the active site of CDK2

The energy score for compound **19** was (S)=−6.8126 kcal/mol and interacted with Asp 145 with one hydrogen bond of 2.79Å through the hydroxyl group of the ligand phenyl ring, Lys 88 and Asp 86 with bond distance 3.36 and 3.07 Å, respectively, in addition to Glu 85 with bond length 4.15 Å (fig **13,14**).

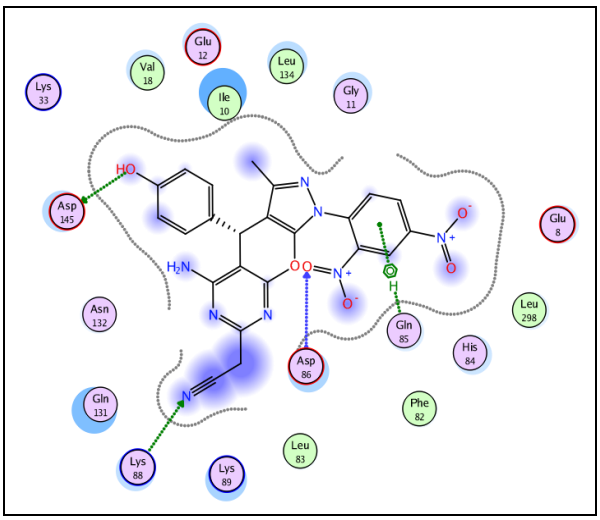


Figure (13) interactions of the compound 19 on the active site of CDK2

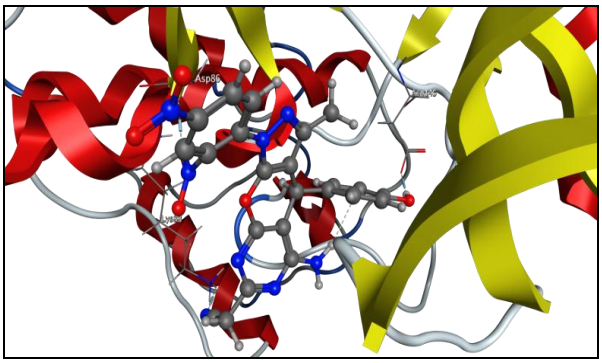


Figure (14) 3D interactions of compound 19on the active site of CDK2

Figures (**15,16**) illustrated the interaction of compound **17** with CDK-2 active site. Compound 6 revealed energy score (S)=−7.3589 kcal/mol and interacted with only one amino acid, Asp 145 with a hydrogen bond of 3.60Å through the sulfur atom of thiazinyl ring.

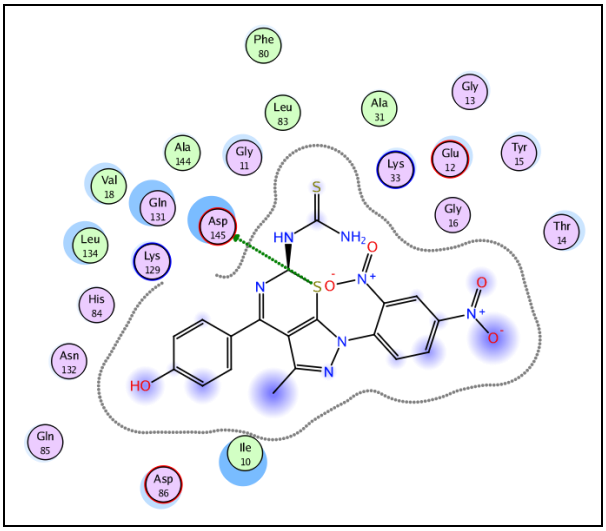


Figure (15) interactions of the compound 17 on the active site of CDK2

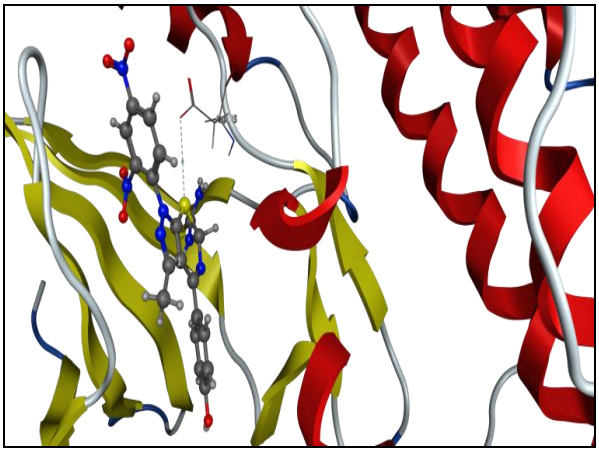


Figure (16) 3D interactions of compound 17 on the active site of CDK2

DISCUSSION

Cyclin dependent kinases (CDKs) are considered a potential target for anti-cancer medication. If it is possible to selectively interrupt the cell cycle regulation in cancer cells by interfering with CDK action, the cell will die. Docking studies were performed in this work to identify the nature and amount of interactions of the synthesized pyrazolyl derivatives with CDK-2 enzyme. All tested compounds possessed an inhibitory activity against CDK-2 since all compounds occupied the pocket of the active site of the enzyme. The higher interaction energy observed for compound 1 rationalizes the sufficient binding into the CDK-2 active site than that of the rest compounds confirming its potent inhibitory activity. Compounds **9** and **17** possess also potent inhibitory activity much higher than **7, 15** and **19** compounds.

REFERENCE

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