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Synthesis, Cytotoxicity and Molecular Docking of Some Schiff Bases Derived Quinazolinone Bearing Pyrazoline

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OBJECTIVE of our work based on the synthesis of the quinazolinone derivative, 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-qunazolinones, and evaluated for cell line. quinazolinone derivatives based on 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]-4(3*H*)-qunazolinones were designed, synthesized and evaluated for their in vitro cytotoxic activity against the hepatic carcinoma cell line (HepG2), the results revealed that the tested compounds process inhibitory effects on the growth of HepG2 liver cancer cells. 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]-4(3*H*)-quinazolinone showed the highest inhibition activity against HepG2 cell line (IC_{50} equals 67.8 µg/mL) among the tested compounds. The molecular modelling studies were performed to explore the detailed binding affinity towards the human liver carcinoma HepG2. The obtained results proved that the most active 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]-4(3*H*)-quinazolinone could be useful as a template for future design, adaptation and investigation to construct more active qunazolinone analogs. Moreover, compounds were screened for antibacterial activity and none of them showed noteworthy activity.

Keywords : Quinazolinone, HepG2, Schiff Base, Molecular Docking, Pyrazoline, Azomethines.

Introduction

3*H*)-Quinazolinone heterocycles have attracted)4 much attention of chemists and pharmacologists because of their broad spectrum biological activities. Many derivatives based on the quinazolinone system exhibited diverse range of pharmacological activities including antifungal, antibacterial, [1-3] anti-inflammatory, [4,5] anticonvulsant [6-8] and antitumor [9-11] .activities

Moreover, the quinazolinone skeleton is a building block for many naturally occurring alkaloids [12,13] displaying a wide range of biological activities.

Pyrazoline moiety exhibited a wide application as anti-tumor agents against different human carcinoma cells lines [14-16] and has been reported as cyclooxygenase-2, lipoxygenase, human monoamine oxidase and tyrosinase inhibitors [17-19].

Azomethine derivatives are important class of compounds that occupy a prime position in medicinal and pharmaceutical chemistry for their diverse biological activities. [20-22]

Based on these observations, the synthesis of some azomethines derived from 3-amino-4(3*H*)-quinazolinones bearing pyrazoline moiety could afford an interesting compounds exhibiting high level of biological activity.

Experimental

Material and Physical measurements

All melting points reported are uncorrected and determined by the open capillary tube method on a Buuchi 510 melting point apparatus, Made in UK, Serial no: SG94/06/370, cat no: MPD350. BM2.5. ^{1}H NMR spectra were measured on Bruker (300 MHz) and TMS was used as internal standard at Micro-analytical Laboratory, Cairo University, Cairo, Egypt. IR spectra were recorded on a Perking Elmer 1430 ratio recording infrared spectrophotometer with CDS data station

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using KBr Wafer technique at lab of Ain Shams University, Cairo, Egypt. Mass spectra were measured on a GC-MSQP 1000EX Schimadzu at laboratory of Chemical War, Egypt. The starting material, 2-[3-(4-methoxy-3-methylphenyl)-3-oxoprop-1-enyl]-*4H*-3,1-benzoxazin-4-one **(1)** was prepared according to the described method. [23]

Cytotoxic activity test (*In vitro* bioassay on human tumour cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt.

Synthesis of 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-quinazolinone **(2)**

To a solution of 2-[3-(4-methoxy-3-methylphenyl)-3-oxoprop-1-enyl]-*4H*-3,1-benzoxazin-4-one **(1)** (0.8 gm, 0.0025 mol) in butanol (30 mL), hydrazine hydrate (0.075 mol) was added and the reaction mixture was heated under reflux for 3 hrs. The solid product obtained after cooling, was filtered off, dried and crystallized from butanol to give compound **(2)** as white crystals: Yield: 91%; M.p. 222-224°C; $C_{19}H_{19}N_5O_2$ (349.39): Anal. Found: C, 66.01; H, 5.52; N, 20.01 Calc.: C, 65.32; H, 5.48; N, 20.04. IR (cm^{-1}): $\nu_{C=O}$ 1693, ν_{NH} 3141, ν_{NH2} 3293, 3332; ^{1}H NMR (δ, ppm in DMSO-d6): 6.94-8.15 (m, 8H); 5.64 (s, 2H); 5.26-5.32 (dd, *J* = 11.4, 6.1 Hz, 1H); 3.80 (s, 3H); 3.29-3.37 and 3.71-3.8 (2dd, *J* = 16.9, 6.2 Hz, 2H); 2.16 (s, 3H).

Synthesis of 3-(3-phenylthioureido)-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-qunazolinone **(3)**

A mixture of 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-*1H*-pyrazol-5-yl]-4(*3H*)-qunazolinone **(2)** (1.05 gm, 0.003 mol) and phenyl isothiocyanate (0.003 mol) in butanol (30 mL) was heated under reflux for 10 hrs. After cooling, the solid that separated out was filtered off, dried and crystallized from butanol to give **(3)** as white crystals: Yield: 65%; M.p. 225-226°C; $C_{26}H_{24}N_6O_2S$ (484.57): Anal. Found: C, 64.7; H, 4.81; N, 17.41 Calc.: C, 64.44; H, 4.99; N, 17.34. IR (cm^{-1}): $\nu_{C=S}$ 1133, $\nu_{C=O}$ 1677, ν_{NH} 3184, 3320; ^{1}H NMR (δ, ppm in DMSO-d6): 10.09 (s, H); 7.02-8.15 (m, 13H); 6.62-6.65 (m, 1H); 5.72 (s, 1H); 3.86-3.92 (m, 1H); 3.84 (s, 3H), 3.51-3.58 (m, 1H); 2.20 (s, 3H).

Synthesis of 3-arylidenamino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-quinazolinone **(4a-c)**

A mixture of 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-*1H*-pyrazol-5-yl]-4(*3H*)-quinazolinone **(2)** (1.05 gm, 0.003 mol) and the appropriate aldehydes (0.003 mol) namely benzaldehyde, 4-anisaldehyde and/ or 4-chlorobenzaldehyd in ethanol (50 mL) was heated under reflux for 3 hrs. The solid product obtained after cooling, was filtered off, dried and crystallized from the suitable solvent to give **(4a-c)**.

2.4.a *3-(benzylidenamino)-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-quinazolinone* **(4a):** From butanol; White crystals; Yield: 75%; M.p. 183-184°C; $C_{26}H_{23}N_5O_2$ (437.50): Anal. Found: C, 71.5; H, 5.28; N, 16.2 Calc.: for C, 71.38; H, 5.30; N, 16.01. IR (cm^{-1}): ν_{CO} 1674, ν_{NH} 3278; ^{1}H NMR (δ, ppm in DMSO-d6): 6.93-8.15 (m, 13H); 5.5-5.52 (d, *J* = 6.0 Hz, 1H); 5.1-5.15 (dd, *J* = 11.2, 2.5 Hz, 1H); 3.97-4.04 (dd, *J* = 17.0, 2.6 Hz 1H); 3.80 (s, 3H); 3.49-3.58 (dd, *J* = 17.0, 11.2 Hz 1H); 2.14 (s, 3H); MS: m/z: 437 (43.5%) (M^+).

2.4.b *3-(4-methoxybenzylidenamino)-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-quinazolinone* **(4b):** From butanol; White crystals; Yield: 69%; M.p. 195-196°C; $C_{27}H_{25}N_5O_3$ (467.52): Anal. Found: C, 68.99; H, 5.41; N, 14.87 Calc.: C, 69.36; H, 5.39; N, 14.98. IR (cm^1): ν_{CO} 1683, ν_{NH} 3237; ^{1}H NMR (δ, ppm in DMSO-d6): 6.94-8.14 (m, 12H); 5.45-5.47 (d, *J* = 5.5 Hz, 1H); 5.08-5.13 (dd, *J* = 2.4, 2.3 Hz, 1H); 3.94-4.0 (dd, *J* = 16.7, 2.1 Hz, 1H); 3.76 and 3.8 (2s, 6H); 3.48-3.58 (dd, *J* = 16.7, 2.1 Hz, 1H); 2.15 (s, 3H); MS: m/z: 467.5 (31.1%) (M^+).

2.4.c *3-(4-chlorobenzylidenamino)-2-(3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4(3H)-quinazolinone* **(4c):** From butanol; White crystals; Yield: 87%; M.p. 217-219°C; $C_{26}H_{22}ClN_5O_2$ (471.94): Anal. Found: C, 66.21; H, 4.81; N, 14.75 Calc.: C, 66.17; H, 4.70; N, 14.84. IR (cm^{-1}): $\nu_{C=O}$ 1682, ν_{NH} 3226; ^{1}H NMR (δ, ppm in DMSO-d6): 6.94-8.14 (12H, m); 5.49- 5.51 (d, *J* = 0.6 Hz,

1H); 5.10-5.14 (dd, *J* = 11.2, 2.5 Hz, 1H); 3.98-4.04 (dd, *J* = 17.0, 2.6 Hz, 1H); 3.81 (s, 3H); (m, 1H), 2.11 (s, 3H).

Synthesis of 3-(cyclohexylidenamino)-2-(3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4(3H)-quinazolinone **(5)**

A mixture of 3-aminoquinazolinone **(2)** (1.05 gm, 0.003 mol) and cyclohexanone (0.45 gm 0.003 mol) in ethanol (50 mL) was heated under reflux for 6 hrs. The solid product obtained after concentration and cooling, was filtered off, dried and crystallized from the petroleum ether to give **(5)** as white crystals; Yield: 81%; M.p. 213-215°C; $C_{25}H_{27}N_5O_2$ (427.51): Anal. Found: C, 70.79; H, 5.47; N, 16.28 Calc.: C, 70.24; H, 5.89; N, 16.38. IR (cm^{-1}): $\nu_{C=O}$ 1701, ν_{NH} 3210; ^{1}H NMR (δ, ppm in DMSO-d6): 6.96-8.19 (m, 9H); 3.81 (s, 3H); 2.14 (s, 3H); 1.65-2.11 (m, 10H); MS: m/z: 427.5 (64.6%) (M^+).

Synthesis of 2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-3-(2-oxoindolin-3-ylidenamino)-4(3H)-quinazolinone **(6)**

A mixture of 3-aminoquinazolinone **(2)** (1.05 gm, 0.003 mol) and isatin (0.003 mol) in butanol (40 mL) was heated under reflux for 6 hrs. The solid product obtained after cooling, was filtered off, dried and crystallized from butanol to give **(6)** as yellow crystals; Yield: 92%; M.p. 268-270°C; $C_{27}H_{22}N_6O_3$ (478.50): Anal. Found: C, 67.81; H, 4.67; N, 17.7 Calc.: C, 67.77; H, 4.63; N, 17.56. IR (cm^{-1}): $\nu_{C=O}$ 1655, 1712, ν_{2NH} 3216 and 3283; ^{1}H NMR (δ, ppm in DMSO-d6): 10.84 (s, H); 6.91-8.13 (m, 12H); 5.33-5.36 (dd, *J* = 10.7, 2.1 Hz, 1H); 3.79-3.84 (dd, *J* = 17.1, 2.2 Hz, 1H); 3.78 (s, 3H); 3.59-3.66 (dd, *J* = 17.0, 10.8 Hz, 1H); 2.14 (s, 3H).

3,3'-(1E,1'E)-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(azan-1-yl-1-ylidene)bis(2-(3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinazolin-4(3H)-one) **(7)**

A mixture of 3-aminoquinazolinone **(2)** (1.05 gm, 0.003 mol) and terephthalaldehyde (0.0015 mol) in ethanol (50 mL) was refluxed for 6 hrs. The solid product obtained was filtered off, dried and crystallized from butanol to give compound **(7)** as white crystals, decomposed at 295°C; $C_{46}H_{40}N_{10}O_4$ (796.87): Anal. Found: C, 68.1; H, 4.25; N, 17.12 Calc.: C, 69.33; H, 5.06; N, 17.58. IR (cm^{-1}): $\nu_{C=O}$ 1663, 1692, ν_{2NH} 3219, 3268; ^{1}H NMR (δ, ppm in DMSO-d6): 6.89-8.13 (m, 22H); 5.11-5.14 and 5.31-5.33 (2dd, *J* = 11.6, 8.6 Hz, 2H); 3.62-4.02 (m, 2H); 3.75-3.78 (2s, 6H); 3.41-3.55 (m, 2H); 2.10-2.13 (2s, 6H).

Synthesis of 3-(N,N-diacetylamino)-2-[1-acetyl-3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-quinazolinone **(8).**

A solution of 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-*1H*-pyrazol-5-yl]-4(*3H*)-quinazolinone **(2)** (1 gm) in acetic anhydride (10 mL) was refluxed for 4 hrs, the solid product obtained, after evaporation of most of the solvent and cooling, was filtered off, washed with ethanol and crystallized from benzene to give **(8)** as white crystals; Yield: 89%; M.p. 243-246°C; $C_{25}H_{25}N_5O_5$ (475.50): Anal. Found: C, 63.3; H, 5.21; N, 14.82 Calc.: C, 63.15; H, 5.30; N, 14.73. IR (cm^{-1}): $\nu_{C=O}$ 1650, $\nu_{3C=O}$ 1701, 1718, and 1754; ^{1}H NMR (δ, ppm in DMSO-d6): 6.99-8.18 (m, 7H); 5.64-5.70 (q, 1H); 3.83 (s, 3H); 3.56-3.66 (dd, *J* = 12.0, 12.0 Hz, 1H); 3.01-3.03 and 3.07-3.09 (dd, *J* = 5.7, 6.0 Hz, 1H); 2.32, 2.38 and 2.48 (3s, 9H); 2.19 (s, 3H).

Synthesis of 3-amino-2-[1-benzoyl-3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-4(3H)-quinazolinone **(9)**

To a solution of 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-*1H*-pyrazol-5-yl]-4(*3H*)-quinazolinone **(2)** (1.05 gm, 0.003 mol) in dry benzene (50 mL); freshly distilled benzoyl chloride (0.45 mL, 0.003 mol) was added and the reaction mixture was heated under reflux for 6 hrs. The solid that separated after concentration and cooling was filtered off, dried and crystallized from benzene to give **(9)** as white crystals; Yield: 56%; M.p. 228-230°C; $C_{26}H_{23}N_5O_3$ (453.49): Anal. Found: C, 68.76; H, 5.09; N, 15.53 Calc.: C, 68.86; H, 5.11; N, 15.44. IR (cm^{-1}): $\nu_{C=O}$ 1677, ν_{NH2} 3227, 3317; ^{1}H NMR (δ, ppm in DMSO-d6): 6.98-8.14 (m, 13H); 6.24-6.28 (dd, *J* = 12.0, 4.6 Hz, 1H); 5.76 (s, 2H); 3.81-3.88 (dd, *J* = 18.1, 12.2 Hz, 1H); 3.8 (s, 3H); 3.52-3.57 (dd, *J* = 18.0, 4.7 Hz, 1H); 2.13 (s, 3H).

Synthesis of 2-[2-(3-(4-methoxy-3- methylphenyl) -1H-pyrazol-5-yl)-4-oxoquinazolin-3(4H)-carbamoyl]benzoic acid **(10)**

A mixture of 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-*1H*-pyrazol-5-yl]-4(*3H*)-quinazolinone **(2)** (1.05 gm, 0.003 mol) and appropriate anhydride namely, maleic anhydride and/ or phthalic anhydride (0.003 mol) in butanol (50 mL) was heated under reflux for 6

hrs. The solid product obtained after cooling, was filtered off, dried and crystallized from butanol to give **(10)** white crystals; Yield: 72%; M.p. 269°C; $C_{27}H_{21}N_5O_5$ (495.49): Anal. Found: C, 65.7; H, 4.19; N, 14.15 Calc. C, 65.45; H, 4.27; N, 14.13. IR (cm^{-1}): ν_{CO} 1661, ν_{NH} 3275, ν_{OH} 3445; ^1H NMR (δ, ppm in DMSO-d6): 13.67 (br, H); 6.92-8.33 (m, 14H); 3.87 (s, 3H); 2.22 (s, 3H).

Biological Activity

The antibacterial activities of the synthesized compounds were screened *in vitro* against strains of Gram-positive bacteria [*Bacillus subtilis*, and *Flavo*] and Gram-negative bacteria [P*seudomonas aeruginosa* and *Entero*]. The well technique of the agar diffusion method was used to determine the approximate activity. [24] The compounds were tested at 1mg/ mL concentration and the activity was determined by measuring the diameter of the inhibition zones in millimeters.

The data showed that the newly synthesized compounds have slightly or no activity against the mentioned organisms, Except compound (10) which exhibited moderate inhibitory activities at 250 µg/ mL.

Cytotoxic effect on human cell line (HePG2)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan. [25]

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in EMEM medium for HepG2, 1% antibiotic-antimycotic mixture (10,000U/ mL Potassium Penicillin, 10,000 µg/ mL Streptomycin Sulphate and 25 µg/ mL Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/ well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 hrs under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/mL). After 48 hrs of incubation, medium was aspirated, 40 µl MTT salt (2.5 µg/ mL) were added to each well and incubated for further four hours at 37 ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% Sodium dodecyl sulphate (SDS) in deionised water was added to each well and incubated overnight at 37 ºC. A positive control which composed of 100 µg/ mL was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions Table 1. [26, 27]

The absorbance was then measured using a micro plate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated

TABLE 1. Positive control Adrinamycin (Doxorubicin) [Mw= 579.99] .

	IC_{50} µg/mL	IC_{50} µM
HepG2	21.6	37.8
HCT116	37.6	65.1
A549	28.3	48.8
MCF7	26.1	45.02
PC3	23.8	41.1

according to the formula:

((Reading of extract / Reading of negative control) -1) x 100. A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

Molecular modelling and docking studies

All molecular modelling studies were performed on a Hewlett- Packard Pentium Dual-Core T4300 2.10 GHz running Windows 7 Ultimate using molecular operating environment (MOE) 2008.10 molecular modelling software. [28] The 3D structure of the HepG2 was downloaded in PDB format from the Protein Data Bank (PDB) web site and used in the docking studies. [29] The docking studies were performed after deleting the co-crystallized inhibitors from the active site. The target compounds were then docked within the active site of the crystallized structures using the MOE dock tool in MOE, performed with the default values. The active site was defined by all the amino acid residues involved in the interaction with the co-crystallized inhibitors.

Results and Discussion

Chemistry

The starting material 3-amino-4(3*H*)-qunazolinone **(2)** was synthesized from the reaction of 2-[3-(4-methoxy-3-methylphenyl)-3-oxoprop-1-enyl]-4*H*-3,1-benzoxazin-4-one **(1)** with excess hydrazine hydrate.

The presence of the pyrazoline unit in **(2)** was clearly established by ^{1}H NMR. The ^{1}H NMR spectrum (DMSO-d6) of **(2)** revealed the presence of two signals at 3.33 ppm as doublet of doublet (J = 14.6, 9.1 Hz) and 3.73 ppm doublets of doublets (J = 16.9, 6.2 Hz) for the two magnetically nonequivalent protons at the position-4 of the pyrazoline ring Ha and Hb. The Hc proton at C-5 also appeared as a doublet of doublets in the region of 5.29 ppm (J_1 = 11.4, 6.1) due to vicinal coupling with two nonmagnetically equivalent geminal protons of the C-4 carbon. The part of the ^{1}H NMR spectrum showing these peaks is shown in the following Figure 1.

When the 3-aminoquinazolinone **(2)** was allowed to react with phenylisothicyanate in boiling ethanol, it afforded the corresponding phenyl thiourea derivative **(3)**.

The azomethine derivatives **(4a-c)** and **(5)** have been synthesized by refluxing the 3-aminoquinazolinone **(2)** with aldehydes namely benzaldehyde, 4-chlorobenzaldehyde, 4-anisaldehyde and/ or cyclohexanone, respectively in ethanol.

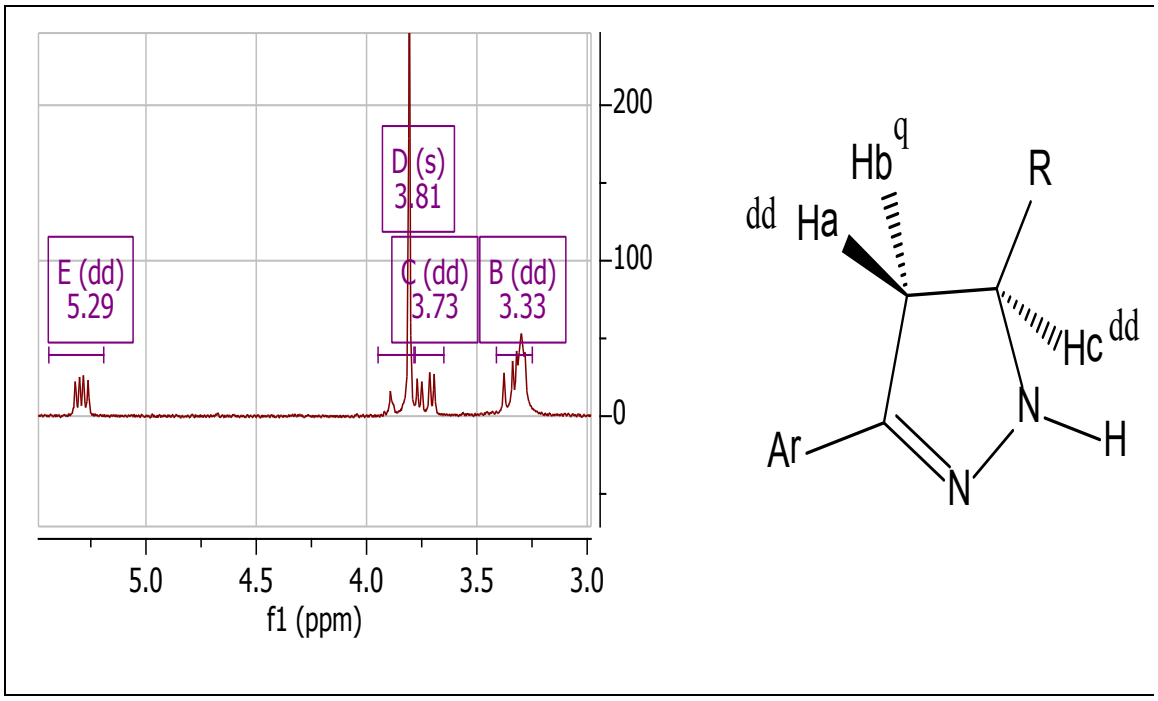
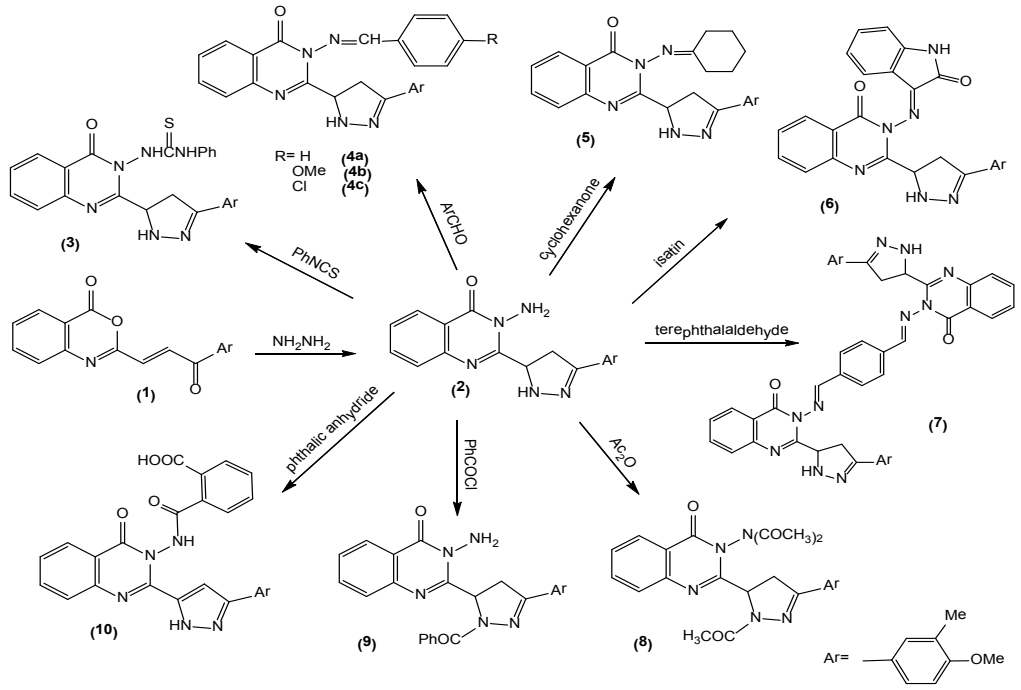


Fig. 1. The ^{1}H NMR spectrum of 3-amino-4(3H)-qunazolinone (2).



Scheme 1. Synthesis and reactivity of 3-amino-4(3H)-qunazolinone.

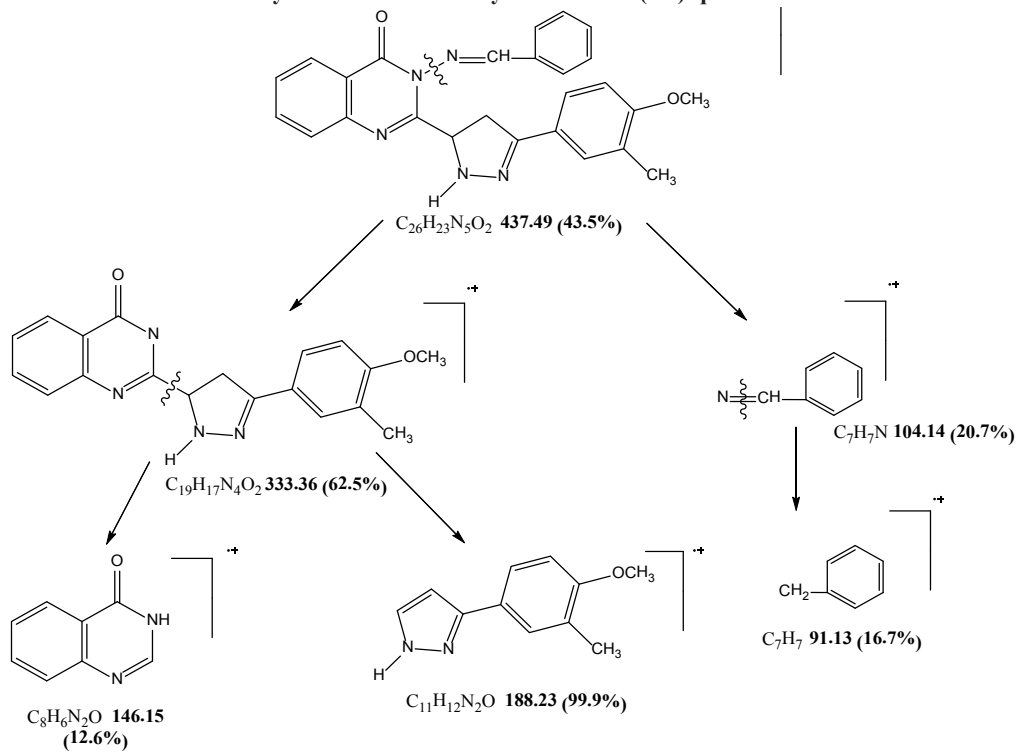


Fig. 2. Suggested Fragmentation Pattern of Compound (4a).

The mass spectra of compound **(4a)** showed molecular ion peak at m/z 437 (43.5%) corresponding to the molecular formula $C_{26}H_{23}N_5O_2$ (437.49). The molecular ion underwent fragmentation as shown in the following pattern Figure 2.

Similarly, the treatment of 3-aminoquinazolinone **(2)** with isatin and/ or terephthalaldehyde in boiling ethanol, it afforded the corresponding azomethines **(6)** and **(7)**, respectively.

The acetylation of 3-aminoquinaolinone **(2)** with acetic anhydride led to the formation of the

corresponding triacetyl derivative **(8)**. The ^{1}H NMR spectrum of **(8)** also showed the appearance of new three singlets in the aliphatic region at 2.32, 2.38, 2.63 ppm characteristic for the three acetyl groups.

However, the acylation of 3-aminoquinazolinone **(2)** with benzoyl chloride afforded the corresponding 3-amino-2-[1-benzoylpyrazolin-5-yl]-4(3*H*)-quinazolinone derivative **(9)**.

When the 3-aminoquinazolinone **(2)** was treated with phthalic anhydride in refluxing benzene, the corresponding carbamoylbenzoic acid derivative **(10)** was obtained.

All the new compounds were tested for their antibacterial activity *in vitro* against strains of Gram-positive bacteria [*Bacillus subtilis*, and *Flavo*] and Gram-negative bacteria [P*seudomonas aeruginosa* and *Entero*] by measuring the inhibition zone produced by each compound using the agar plate diffusion method. However, none of the compounds herein showed any noteworthy activity.

Molecular modelling and energy minimization

The force field and wave function methods were used here in this work as shown in Table 1and in Figures 3 and 4. The semi-empirical calculation AM1 Hamiltonian was used as apart from mopac program under molecular operating environment (MOE) 2008.10. [22] The most stable spatial structure of the compound 2 (Scheme 1) was evaluated and illustrated in Fig. 3 and Table 1(as representative example).

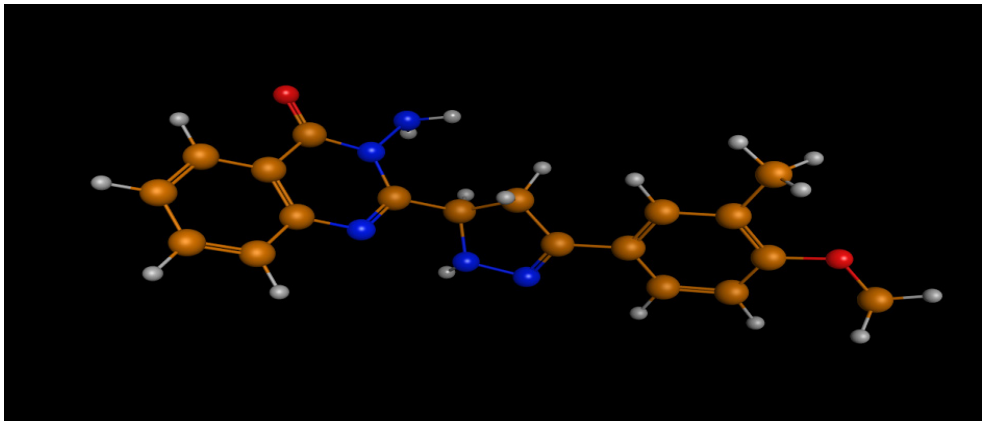
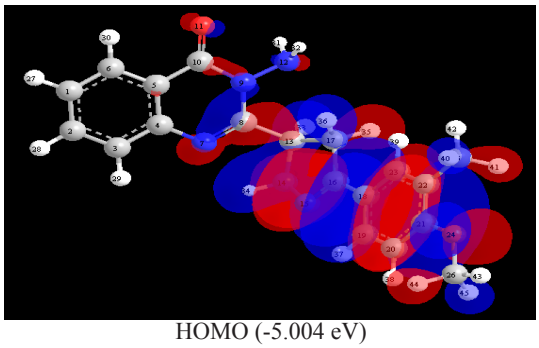
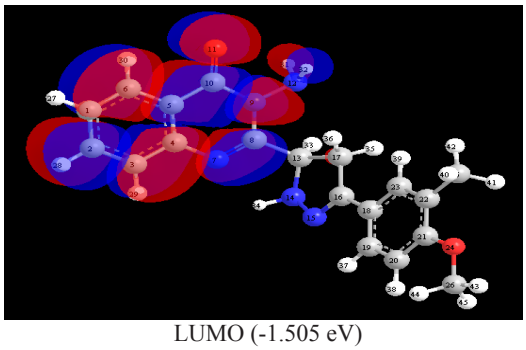


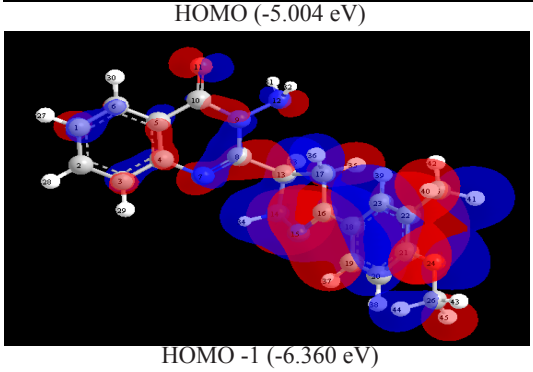
Fig. 3 . Spatial arrangement structure after application of MMFF94 energy minimization function for compound 2



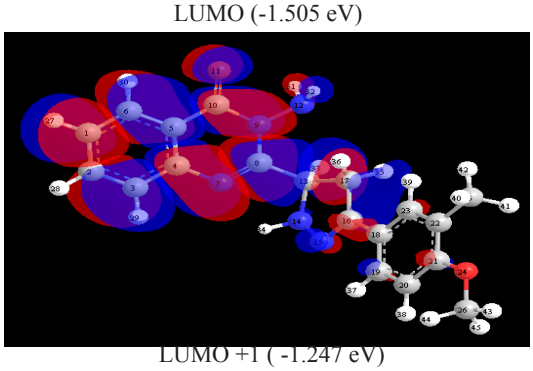
HOMO (-5.004 eV)



LUMO (-1.505 eV)



HOMO -1 (-6.360 eV)



LUMO +1 (-1.247 eV)

Fig. 4. HOMO and LUMO view and their energy for the compound 2.

TABLE 2 . The types and the values of energy evaluated from the molecular modelling calculation for the compound 2

Energy type	Energy value
Stretching	1.4105
Bending	8.2471
Stretching-bending	0.1233
Torsion	-11.6616
Non-1,4VDW	-2.1681
1,4VDW	24.6885
Dipole/Dipole	-9.0035
Total energy	11.6363 kcal/mol

The Van der Waals energy plays the major part in potential energy for the target compound. The HOMO and LUMO levels for the compound **2** (Fig. 4) indicate the higher electronic stability of the target compound which strongly pronounced from the total energy value (11.6363 kcal/ mol) which listed in Table 2.

Human liver carcinoma HepG2

The docking process of the target compounds with the human liver carcinoma HepG2 (PDB code 5EQG) were shown in the Fig.5–8 and listed in Table 3. The variation of the binding sites and energy accompanied with each compounds were cited in 2D and 3D views. The obtained results could be analysed as the following:

[a] The binding energy of the interaction of the target ligands with the receptor was founding in the range -24.847 to -32.308 kcal/ mol, which reveal the similarity of the interaction mechanism for all target compounds.

[b] The obtained binding energy values reveal the promising target compounds which could be used as anticancer drugs.

[c] The obtained results go with some extent with the *in vitro* studies.

[d] The order of the binding energy of the synthesized compounds could be arranged as:

$$4c > 4a > 4b > 2$$

[e] The strategic enhanced activities view for the synthesized compounds were signed by the

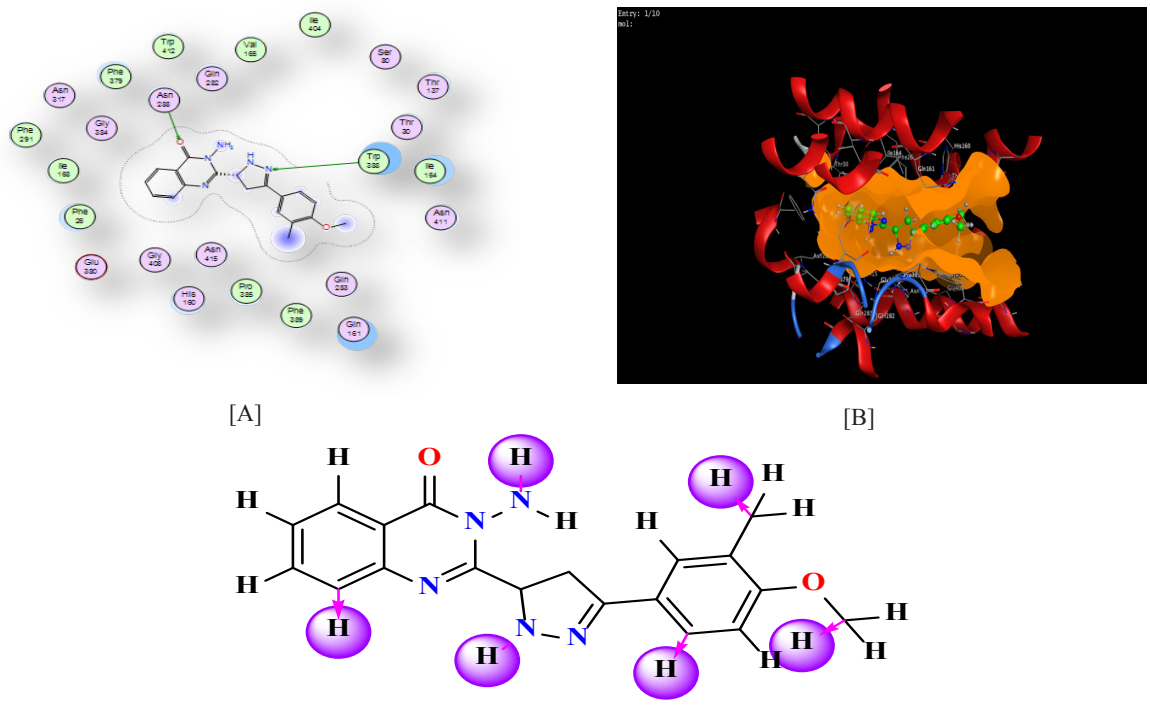
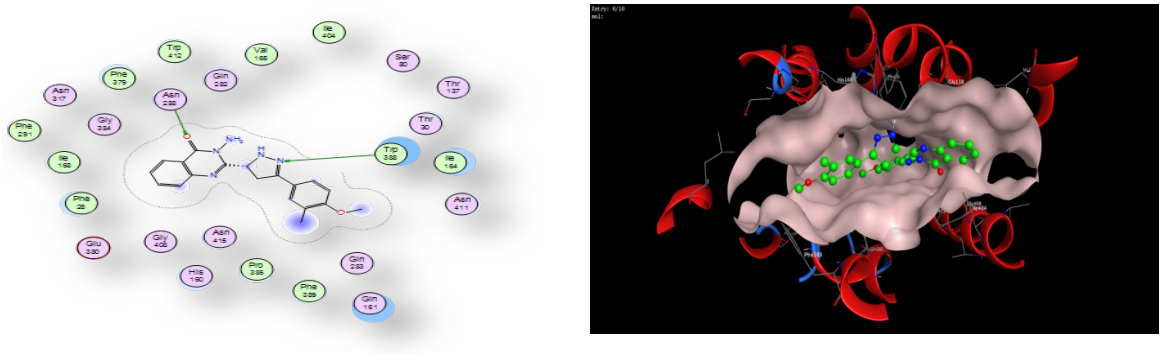
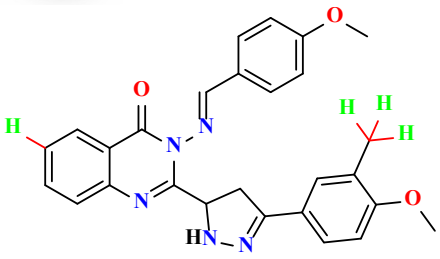


Fig. 5. Docking model of the interaction of compound 2 with HepG2 (PDB code 5EQG) bonding sites: [A] 2D view, [B] 3D view and [C] promising sites.

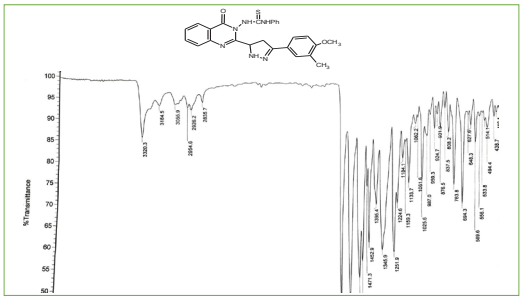


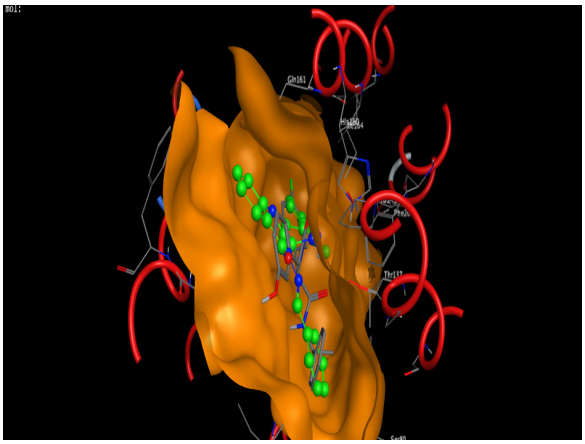
[A] [B]



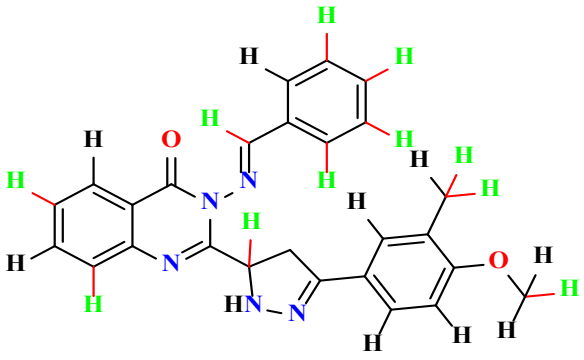
[C]

Fig. 6. Docking model of the interaction of compound (4c) with HepG2 (PDB code 5EQG) bonding sites: [A] 2D view, [B] 3D view and [C] promising sites.



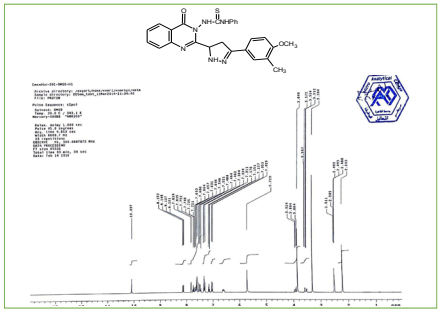


[A] [B]

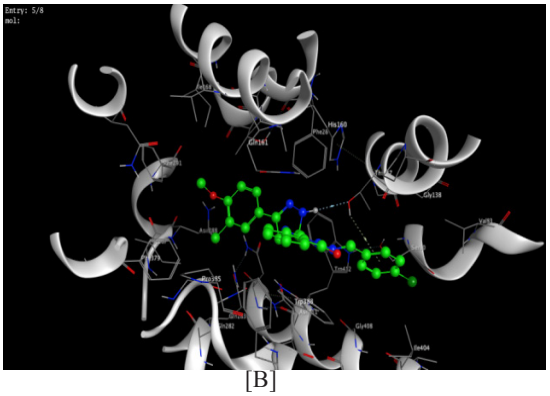


[C]

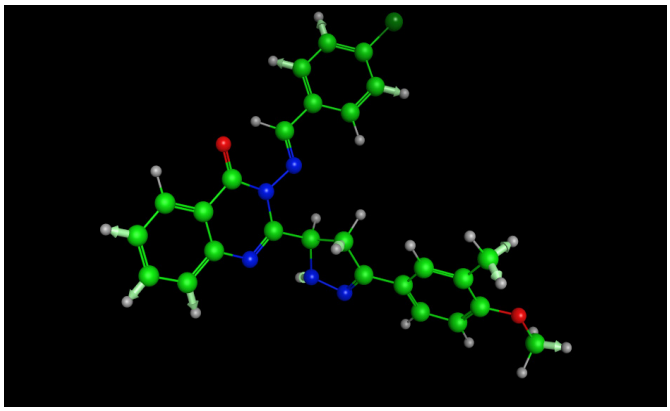
Fig.7. Docking model of the interaction of compound (4a) with HepG2 (PDB code 5EQG) bonding sites: [A] 2D view, [B] 3D view and [C] promising sites.



[A]



[B]



[C]

Fig. 8. Docking model of the interaction of compound (4b) with HepG2 (PDB code 5EQG) bonding sites: [A] 2D view, [B] 3D view and [C] promising sites.

TABLE 3 . The docking data for the HepG2 (code 5EQG) with the target compounds .

Compound	Ligand	Receptor	Type	Distance (Å)	Energy (kcal/mol)	Binding energy (kcal/mol)
2	O11	ND2 ASN288	H-acceptor	3.05	-2.4	-24.847
	N15	NE1 TRP388	H-acceptor	3.16	-1.0	
4c	N19	O THR137	H-donor	3.17	-1.0	-32.308
	C54	6-ring TRP412	H-pi	4.29	-0.6	
4b	N19	O GLY384	H-donor	2.98	-1.6	-31.011
4a	N 19	OG1 THR137	H-donor	3.38	-0.4	-30.943
	Cl56	O SER80 OG1	H-donor	3.43	-0.3	
	6-ring	THR137	pi-H	4.34	-0.6	
	6-ring	5-ring TRP388	pi-pi	3.51	-0.0	

arrows for the compounds 2 and 4b, labelled hydrogen for compounds 4c and 4a.

[f] The new substitutions according the strategic views open new gate for some new derivatives could be has activities exceeding the market drugs.

Cytotoxicity assay in vitro

This method of investigation was carried out to estimate the effect of our novel prepared compounds on human liver carcinoma (HepG2). This cell line was selected to investigate the effect of the new compounds against one of the most common cancer type in Egypt, hepatocellular carcinoma (HCC). [30, 31]

All the target compounds gave the predictable

effects which predicted from the docking studies. The obtained data could be summarized as the following:

[1] The order of the activity of the target compounds, based on the counting of the death cells fraction as: 2 > 4a > 4b > 4c

[2] The order of 4a and 4b compounds go well with the docking studies, meanwhile the reverse order was obtained for the compounds 2 and 4c, which indicate other interaction mechanism could be proceed.

[3] Compound 2 gave IC_{50} = 67.8 µg/mL (194 µM) and IC_{90} = 119.7. The obtained result gave a hope to continuo with these derivatives.

Conclusion

In the present study, we have synthesized some new azomethines using 3-amino-2-[3-(4-methoxy-3-methylphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]-4(3*H*)-qunazolinone **(2)** as starting material. The newly synthesized compounds were screened for different bacterial strains and showed slightly or no activity against the mentioned organisms. The cytotoxic study revealed that the order of 4a and 4b compounds go well with the docking studies, meanwhile the reverse order was obtained for the compounds 2 and 4c. The obtained result showed that compound 2 could be useful as a template for future synthesis to produce more active qunazolinone analogs.

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Supplementary Information

IR, NMR and mass spectra of the synthesized compounds are shown in Figures S1 to S25.

Conflicts of interest

Nothing to declare

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تخليق ودراسة النشاط السام للخلايا والنمذجة الجزيئية لبعض قواعد شيف لمركبات الكينازوليون المحتوي علي البيرازولين.

سحر سعيد أحمد السقا[1]، محمد حلمي عبد الحميد سليمان[1] و إيمان محمد عبد الله الشلقاني[2]

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تم تحضير مشتقات كونازولينون جديدة ودراسة نشاطها السام للخلايا في الجسم (HepG2) وأظهرت النتائج أن المركبات المختبرة تعمل على تثبيط التأثيرات على نمو خلايا سرطان الكبد HepG2. أظهر المركب 2 أعلى نشاط تثبيط (IC50 يساوي 67.8 ميكروجرام / مل) بين المركبات المختبرة. تم إجراء دراسات النمذجة الجزيئية لاستكشاف الارتباط الملزم المفصل تجاه سرطان الكبد البشري HepG2. أثبتت النتائج التي تم الحصول عليها أن المركب الأكثر نشاطا 2 يمكن أن يكون مفيدا كنموذج للتصميم والتكيف والتحقيق المستقبلي لبناء نظائر كونازولينون أكثر نشاطا. علاوة على ذلك ، تم فحص المركبات من أجل نشاط مضاد للبكتيريا ولم يظهر أي منها نشاطًا جديرًا بالملاحظة.