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Synthesis, Anticancer Activity and Docking Study of Quinoline, Diazepine, Pyrimidine And Pyridine Derivatives

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Abstract: *New classes of quinoline derivatives **2a, b, 7a, b**, diazepine derivatives **3a, b, 8a, b**, pyrimidine derivatives **4a, b, 5a, b; 6** had been synthesized, via reactions between visnagine carbaldehyde **1** and different reagents. The structures of the products were elucidated by spectra and elemental analyses. In the present work, the derivative products have been tested, along with reference compounds, for their cytotoxic potential against two tumor cell lines. The anticancer activity results indicated that the products **3b, 5b** and **7b** showed growth inhibition activity against HEPG2 cell line and products **4b, 6**, and **8a** showed growth inhibition activity against MCF-7. Moreover, we attempted an in-silico approach to gain insights into their binding modes against cyclin-dependent protein kinase 2 (CDK-2) which is involved in cell cycle, and receptor protein B-cell lymphoma 2 (BCL-2) which is involved in cell apoptosis. These targets were selected based on their key roles in cancer progression via the regulation of the cell cycle and DNA replication. Molecular-docking analyses revealed that compound **4b** was the best docked ligand against both targets, as it displayed the lowest binding energy, and critical hydrogen bonds and hydrophobic interactions with the targets.*

Key word*: anticancer activity, cytotoxicity, diazepine, human cancer cell lines, molecular docking, quinoline, Pyrimidine.*

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I. Introduction

Today, like never before, researchers are completing a lot of research to encourage, counteract or beat malignancy. In spite of the fact that there are numerous anticancer medications right now utilized, numerous new synthetic classes of anticancer specialists influencing an assortment of organic targets have risen and are under clinical preliminaries to deliver novel compelling and less dangerous medications.

N-heterocyclic derivatives occur in natural products, dyes and organic compounds,. In the past, there is an interest in the development and pharmacology of heteroaromatic organic compounds [1,2]. Also, recently the researches on multi-component reactions are an important in organic chemistry due to their higher efficiency, atom economy and have synthesis of heterocyclic, as a majority of drug [3]. Multi-component reactions have become increasingly to guarantee molecular diversity and complexity [4]. As part of our research program is to widen an organic reaction improvement methodology. One of the strategies to achieve this goal is the development of total synthesis [5].

Herein, we report the synthesis, characterization and anticancer activity of the new derivatives. The anticancer activity of each compound in the study has been determined using the MTT colorimetric assay [6, 7] in two cancer cell lines hepatic cell line (HEPG2) and breast cell line (MCF-7). The anticancer activity of the synthesized products was tested along with reference compounds, 5-Flurouracil (5-FU) and Doxorubicin (DOX).

Molecular docking provides a rapid way to evaluate likely binders from large chemical libraries with minimal costs, and it is being widely used as a vital component of the drug discovery process [8], therefore, we employed a molecular docking study to determine the possible mechanism of action of the tested compounds against two proteins -cyclin-dependent protein kinase 2 (CDK-2), and receptor protein B-cell lymphoma 2 (BCL-2) - which are implicated significantly in cancer progression.

II. Material And Methods

Experimental Chemistry:

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. The melting points were measured in degrees centigrade and determined using BucHI 510 apparatus. Elemental analyses were carried out in the micro analytical unit of the National Research Centre. IR spectra were recorded on a Mattson-5000 FTIR spectrometer using KBr Wafer technique.^{1}H-NMR, ^{13}C NMR spectra were determined on a Varian-Gemini-300 MHz and JeolEx-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift δ= 0 ppm). Mass Spectra were determined on Finnegan MatSSQ 7000 mode: EI, 70Ev (Thermo Inst. Sys. Inc., USA). The purity of the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates. TLC Silica gel 60 F254 25 Aluminum sheets 20 x 20 cm.

General Procedure for the Preparation of Compounds 2a, b:

A mixture of visnagine carbaldehyde **1** (1.0 mol), malononitrile (1.0 mol), Anisidine/ or p-bromoaniline (1.0 mol) in ethanol in presence of TEA was refluxed for 7 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was washed with ethyl acetate (10 mL) and filtered. The filtrate was evaporated, and the crude product was recrystallized from ethanol (5 mL).

8-amino-6,9-dihydro-5-methoxy-9-(4-methoxyphenyl)-2-methyl-4-oxo-*4H*-pyrano*[3,2-g]* quinoline-7-carbonitrile 2a:

Brown color, 33%=1.3gm yield, m.p. 215-216^{o}C. Mol. Formula $C_{22}H_{19}N_3O_4$. Mol. Wt.: 389.4. Elemental analysis, calc.: C, 67.86; H, 4.92; N, 10.79, found: C, 66.22; H, 4.23; N, 11.00. IR(KBr)(cm^{-1}): 3457 (NH_2); 2186 (CN); 1650 (CO); ^{1}HNMR (DMSO-d6) ppm:7.31, 6.60 (dd, 4H, aromatic ring), 6.50 (s, 1H, CH-3 pyranone ring), 5.91 (s, 1H, CH-10), 3.32 (s, 2H.CH_2-6), 3.89, 3.95 (ss, 6H, 2OCH_3), 2.21 (s, 2H, NH_2 exchangeable with D_2O) and 1.6 (s, 3H, CH_3). Exact Mass: 389.14 m/e: 389 (10%), 390 (88%), 391.14 (67%),

8-amino-9-(4-bromophenyl)-6, 9-dihydro-5-methoxy-2-methyl-4-oxo-*4H*-pyrano *[3, 2-g]* quinoline -7-carbonitrile 2b:

Brown color,23%=1.0gm yield, m.p. 213-215^{o}C. Mol. Formula $C_{21}H_{16}BrN_3O_3$. Mol. Wt.: 438.27. Elemental analysis, calc.: C, 57.55; H, 3.68; Br, 18.23; N, 9.59, found: C, 56.32; H, 3.23; Br, 17.00; N, 9.22. IR (KBr) (cm^{-1}): 3433 (NH_2); 2123 (CN); 1660 (CO). ^{1}HNMR (DMSO-d6) ppm: 7.40, 6.89 (dd, 4H, aromatic ring), 6.60 (s, 1H, CH-3 pyranone ring), 5.93 (s, 1H, CH-10), 3.33 (s, 2H.CH_2-6), 3.88 (s, 3H, OCH_3), 2.24 (s, 2H, NH_2 exchangeable with D_2O) and 1.6 (s, 3H, CH_3).^{13}C NMR (100 MHz, DMSO- d6) ppm: 174.9(CO), 167.1, 164.3, 159.3, 154.3, 150.5, 130.5, 117.8, 116.1, 115.9, 112.7, 110.9, 109.4, 101.2, 98.40, 65.05, 56.04(OCH_3), 25.8(CH_3), 18.2(CH_2). Exact Mass: 438.04, m/e: 438, (33%), 439 (9%), 440 (67%).

General Procedure for the Preparation of Compounds 3a, b:

A mixture of visnagine carbaldehyde **1** (1.0 mol), malononitrile (1.0 mol), hydrazine/ or phenyl hydrazine (1.0 mol) in ethanol in presence of TEA, was refluxing for 5 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was washed with ethanol (10 mL) and filtered. The filtrate was evaporated, and the crude product was recrystallized from ethanol (5 mL).

3-amino-1, 7-dihydro-6-methoxy-9-methyl-7-oxochromeno [7, 6-c] [1, 2] diazepine-4-carbonitrile 3a:

Deep brown color; 34% =1.0gm yield, m.p. 194-195^{o}C. Mol. Formula $C_{15}H_{13}N_4O_3$, Mol. Wt.: 297.29 Elemental analysis, calc.: C, 60.60; H, 4.41; N, 18.85. IR (KBr) (cm^{-1}): 3433 (NH_2); 2123 (CN); 1660 (CO).^{1}HNMR (DMSO-d6) ppm:7.0(s, H, NH exchangeable with D_2O), 6.5 (s, 1H, CH-3 pyranone ring), 5.9 (s, 1H, CH-11), 5.5 (s, 2H.CH-6), 3.9 (s, 3H, OCH_3), 2.2 (s, 2H, NH_2 exchangeable with D_2O) and 1.7 (s, 3H, CH_3). Exact Mass: 297.29, m/e: 297.10 (11.0%), 298.10 (17.8%), 299.11 (1.3%),

3-amino-1, 7-dihydro-6-methoxy-9-methyl-7-oxo-1-phenylchromeno [7, 6-c] [1, 2] diazepine-4-carbonitrile 3b:

Deep brown color, 27%=1gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{21}H_{16}N_4O_3$. Mol. Wt.: 372.38. Elemental analysis, calc.: C, 67.73; H, 4.33; N, 15.05. IR (KBr) (cm^{-1}): 3457 (NH_2); 2186 (CN); 1650 (CO), ^{1}HNMR (DMSO-d6) ppm: 7.3- 6.4(m, 5H, aromatic ring), 6.2 (s, 1H, CH-3 pyranone ring), 5.9 (s, 1H, CH-11), 5.1 (s, H.CH-6), 3.7(s, 3H, OCH_3), 2.0 (s, 2H, NH_2 exchangeable with D_2O), 1.8 (s, 3H, CH_3). Exact Mass: 372.12, m/e: 372.12 (100.0%), 373.13 (23.0%), 374.13 (3.1%), 373.12 (1.5%),

General Procedure for the Preparation of Compounds 4a, b:

A mixture of compound 1 (10 mmol), acetic acid (10 ml) in isopropanol (10 ml) was heated under reflux for 12 h; the reaction mixture was allowed to cool to room temperature. The formed solid was collected by filtration, washed by ethanol (20 ml), dried, and crystallized from ethanol.

5-((7-(4-methoxyphenylamino)-5-methoxy-2-methyl-4-oxo-*4H*-chromen-6-yl)methylene)-2-methylpyrimidin-4(5H)-one 4a:

Brown color, 27%=1.16gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{24}H_{21}N_3O_5$, Mol. Wt.: 431.44. Elemental analysis, calc.: C, 66.81; H, 4.91; N, 9.74. IR (KBr) (cm^{-1}): 3327 (NH); 1720, 1662 (2CO),^{1}HNMR (DMSO-d6) ppm: 7.93, 7.61 (ss, 2H, CH=C-CH=N), 7.32, 6.64 (dd, 4H, aromatic ring); 6.38 (s, 1H, CH-3 pyranone ring),

6.13 (s, 1H, CH-8), 4.18(s, 1H, NH exchangeable with D_2O), 3.96 , 4,00 (ss, 6H, $2OCH_3$), 1.8, 1.1 (ss, 6H, $2CH_3$). ^{13}C NMR (100 MHz, DMSO- d6) ppm: 190.2, 182.1due to (2CO), 164.1, 163.2, 163.1, 162.6 , 160.1, 156.1, 151.1, 133.3, 121.2, 115.3, 115,2, 152.1, 110.3, 106.3, 101.0, 100.2 , 57.5, 56.1 due to ($2OCH_3$) and 18.7, 20.9 due to (2 CH_3). Exact Mass: 431.15, m/e: 431.00 (22.0%), 432.11 (17.1%), 433.11 (9%).

5-((7-(4-bromophenylamino)-5-methoxy-2-methyl-4-oxo-*4H*-chromen-6-yl)methylene)-2-methylpyrimidin-4(5H)-one 4b:

Brown color, 40%=1.9gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{23}H_{18}BrN_3O_4$, Mol. Wt.: 480.31, Elemental analysis, calc.: C, 57.51; H, 3.78; Br, 16.64; N, 8.75. IR (KBr) (cm^{-1}): 3242 (NH); 1723, 1669 (2CO), ^{1}HNMR (DMSO-d6) ppm: 7.81, 7.60 (ss, 2H.CH=C-CH=N), 7.53, 6.82 (dd, 4H, aromatic ring); 6.4 (s, 1H, CH-3 pyranone ring), 6.0 (s, 1H, CH-8),4.12(s, 1H, NH exchangeable with D_2O), 3.9 6 (s, 3H, OCH_3), 2.21, 1.80 (ss, 6H, $2CH_3$). Exact Mass: 479.05, m/e: 481.00 (44.0%), 482.05 (24.3%), 483.05 (9%),

General Procedure for the Preparation of Compounds 5a, b, 6:

A mixture of visnagine carbaldehyde **1** (1.0 mol), and ethyl acetoacetate/or acetyl acetone/ or hexanone (1.0 mmol) was added to a solution of EtOH (15 ml) and/ TEA. The mixture was refluxing for 4-5 h until the completion of reaction was confirmed by TLC. The precipitated compounds were obtained after removal of the solvent. Obtained solids were washed with water and diethyl ether. The crude products were then dissolved in acetone or methanol. The solution was concentrated in vacuum; the resulting oil was dissolved in minimal volume of aqueous ethanol and allowed to crystallize. The pure compounds were obtained.

Ethyl 5-cyano-4-(7-hydroxy-5-methoxy-2-methyl-4-oxo-*4H*-chromen-6-yl)-2-methyl pyridine-3-carboxylate 5a:

Yellow color, 27%= 1.1gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{21}H_{18}N_2O_6$, Mol. Wt.: 394.38, Elemental analysis, calc.: C, 63.96; H, 4.60; N, 7.10. IR (KBr) (cm^{-1}): 3437(OH), 2216(CN), 1657, 1626 (2CO), ^{1}HNMR (DMSO-d6) ppm: 8.78 (s, 1H, CH=N), 6.56 (s, 1H, CH-3 pyranone ring),), 6.16 (s, 1H, CH-8 benzopyranone ring), 5.29 (s, 1H, OH exchangeable with D_2O), 4.12 (m, 2 H, CH_2), 3.67 (s, 3H, OCH_3), 2.18 (s, 3H, CH_3 in o-pyridine ring), 1.98 (s, 3H, CH_3) and 1.32 (t, 3H. CH_3). Exact Mass: 394.12, m/e: 394.11 (24.6%), 395.10 (55.1%), 396.00 (11%),

5-acetyl-4-(7-hydroxy-5-methoxy-2-methyl-4-oxo-*4H*-chromen-6-yl)-6-methyl pyridine -3-carbonitrile 5b:

Brown color, 33%=1.5gm yield, m.p. 243-244^{o}C. Mol. Formula $C_{20}H_{16}N_2O_5$, Mol. Wt.: 364.35, Elemental analysis, calc.: C, 65.93; H, 4.43; N, 7.69. IR (KBr) (cm^{-1}): 3437(OH), 2216(CN), 1657, 1626 (2CO), ^{1}HNMR (DMSO-d6) ppm: 8.78 (s, 1H, CH=N), 6.56 (s, 1H, CH-3 pyranone ring), 6.21 (s, 1H, CH-8 benzopyranone ring), 5.22 (s, 1H, OH exchangeable with D_2O), 3.97 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3), 2.38 (s, 3H, CH_3 in o-pyridine ring) and 1.60 (s, 3H. CH_3). Exact Mass: 364.11, m/e: 364.00 (10.0%), 365.12 (44.0%), 366.01 (65%). ^{13}C NMR (100 MHz, DMSO6): d 199.3, 180.0(2CO), 169.7, 165.2, 162.7, 160.9, 160.2, 152.4, 131.1, 115.9, 110.3, 108.8, 107.3, 100.3, 98.0, 59.9(OCH_3) and 39.5, 20.5, 18.8($3CH_3$).

5, 6, 7, 8 -tetrahydro-4-(7-hydroxy-5-methoxy-2-methyl-4-oxo-*4H*-chromen-6-yl) quinoline-3-carbonitrile 6:

Deep brown, 32%=1.2gm yield, m.p. 225-226^{o}C. Mol. Formula $C_{21}H_{18}N_2O_4$. Mol. Wt.: 362.38. Elemental analysis, calc.: C, 69.60; H, 5.01; N, 7.73, IR (KBr) (cm^{-1}): 3429(OH), 2298(CN), 1647, (CO),^{1}HNMR (DMSO-d6): ppm: 9.03 (s, 1H, CH=N), 6.95 (1H, s, CH-3 pyranone ring), 5.43 (s,1H, OH exchangeable with D_2O), 3.68 (s, 3H, OCH_3), 2.03 (s, H, CH-8), 1.75-1.27 (m, 8H, $4CH_2$). Exact Mass: 362.13, m/e: 362.01 (11.1%), 363.00 (43.1%), 364.22 (10 %),

General Procedure for the Preparation of Compounds 7a, b:

Refluxing a mixture of visnagine carbaldehyde **1** (1.0 mol), ethyl acetoacetate and anisidine/ or p- bromoaniline (1.0 mol) in ethanol as solvent and TEA as catalyst for 6 hrs, until the completion of reaction was confirmed by (TLC). The precipitated was obtained after filtration. The residue was recrystallized with ethanol/acetone.

Ethyl 6, 9-dihydro-5-methoxy-9-(4-methoxyphenyl)-2, 8-dimethyl-4-oxo-*4H*-pyrano [3, 2-g] quinoline-7-carboxylate 7a:

Brown color, 17%=0.7gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{25}H_{25}NO_6$, Mol. Wt.: 435.47. Elemental analysis, calc.: C, 68.95; H, 5.79; N, 3.22, IR (KBr) (cm^{-1}):1754, 1657 (2CO) ^{1}HNMR (DMSO-d6) ppm: 6.50-6.30 (dd, 4H, arom.), 6.35 (1H, s, CH-3 pyranone ring), 5.63 (s, H, CH-10), 4.32 (m, 2H, CH_2). 3.88. 3, 98 (s s, 6H, $2OCH_3$), 3.0 (s, 2H, CH_2), 1.97 (t, 3H, CH_3) and 1.21, (s, 3H, CH_3), Exact Mass: 435.17, m/e: 435.00 (23.1%), 436.12 (12.1%), 437.11 (41.1%),

Ethyl9-(4-bromophenyl)-6, 9-dihydro-5-methoxy-2, 8-dimethyl-4-oxo-*4H*-pyrano [3, 2-g] quinoline-7-carboxylate 7b:

Pale yellow color, 17%=0.8gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{24}H_{22}BrNO_5$, Mol. Wt.: 484.34. Elemental analysis, calc.: C, 59.52; H, 4.58; Br, 16.50; N, 2.89, IR (KBr) (cm^{-1}):1754, 1678 (2CO). ^{1}H NMR (DMSO-d6) ppm: 6.57-6.32 (dd, 4H, arom.), 6.22 (1H, s, CH-3 pyranone ring), 5.68 (s, H, CH-10), 4.14 (m, 2H, CH_2), 3.98(s, 3H, OCH_3), 3.1(s, 2H, CH_2), 1.87 (t, 2H, CH_3) and 1.77 (s, 3H, CH_3). Exact Mass: 483.07, m/e: 485.01 (40.4%), 483.12 (54.3%), 484.00 (26.3%).

General Procedure for the Preparation of Compounds 8a, b:
A mixture of visnagine carbaldehyde **1** (1.0 mol), ethyl acetoacetate and hydrazine hydrate/ or phenyl hydrazine (1.0 mol) was refluxed in ethanol as solvent and TEA as catalyst for 5 hrs. until the completion of reaction was confirmed by (TLC). The precipitate was obtained after filtration and was recrystallized with DMF.

Ethyl 1, 7-dihydro-6-methoxy-3, 9-dimethyl-7-oxochromeno *[7, 6-c]* [1, 2] diazepine-4-carboxylate 8a:
Yellow color, 17%=0.6gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{18}H_{18}N_2O_5$, Mol. Wt.: 342.35. Elemental analysis, calc.: C, 63.15; H, 5.30; N, 8.18. IR (KBr) (cm^{-1}): 1778, 1618 (2CO). ^{1}H NMR (DMSO-d6) ppm: 7.7 (s, 1H, CH=C), 7.1 (s, H, NH exchangeable with D_2O), 6.35 (1H, s, CH-3 pyranone ring), 5.63 (s, H, CH-11), 4.32 (m, 2H, CH_2), 3.78 (s, 3H, OCH_3), 1.89 (s, 3H, CH_3), 1.37 (t, 3H, CH_3) and 1.09 (s, 3H, CH_3). Exact Mass: 342.12, m/e: 342.12 (22.0%), 343.00 (29.9%), 344.11(39.1%),

Ethyl 1, 7-dihydro-6-methoxy-3, 9-dimethyl-7-oxo-1-phenylchromeno *[7, 6-c]* [1, 2] diazepine -4-carboxylate 8b:
Yellow color, 16%=0.7gm yield, m.p. 205-206^{o}C. Mol. Formula $C_{24}H_{22}N_2O_5$, Mol. Wt.: 418.44. Elemental analysis, calc.: C, 68.89; H, 5.30; N, 6.69. IR (KBr) (cm^{-1}): 1770, 1620 (2CO). ^{1}H NMR (DMSO-d6) ppm: 7.9 (s, 1H, CH=C), 7.00-6.44 (m, 5H, arom.), 6.32 (1H, s, CH-3 pyranone ring), 5.61 (s, H, CH-11), 4.12 (m, 2H, CH_2), 3.88 (s, 3H, OCH_3), 1.77 (s, 3H, CH_3), 1.32 (t, 3H, CH_3),0.9 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO6): d 182.0, 164.8(2CO), 164.1, 161.2, 158.1, 155.6, 155.0, 148.0, 129.3, 123.9, 118.9, 110.3, 105.2, 102.1, 100.2, 95.1, 61.3(OCH_2), 56.1(OCH_3) and 21.0, 17.3, 14.0($3CH_3$) ppm. Exact Mass: 418.15, m/e: 418.00 (11.0%), 419.01 (36.3%), 420.02 (24.1%).

Determination of Anticancer Activities

Cell Culture

For anticancer activity screening of the tested compounds, liver HEPG2 and breast MCF-7, cell lines as well as the normal cell line (human normal melanocyte, HFB4) were obtained from National Cancer Institute, Cairo University. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of culture medium.

In vitro Cell Proliferation and Cell Viability Assay (Trypan Blue Exclusion Assay)

Trypan blue exclusion assay was performed as previously described [9]. This assay is used to assess the effect of newly synthesized products on viability of HEPG2 and MCF7 cells. Approximately 0.75×10^5 cells/ml was seeded in a six well tissue culture plate and different concentrations of compounds were added after 24 h. For the determination of growth rate, smaller aliquots were collected in a 0.5 ml tubes, trypan blue (0.4%) was added to the cell suspension, and the number of cells viable cells (unlabeled), number of non-viable cells (blue), and the number of damaged cells (slightly blue) was determined using a haemocytometer. Viability is just the ratio of live cells divided by total number of cells. The media was not changed during the induction period. Each experiment was repeated a minimum of three times and the results are presented as graphs.

MTT Assay

The synthesized products were applied on the two cell lines, hepatic cell line(HEPG2) and breast carcinoma cell line (MCF-7) in comparison to the known anticancer drugs: 5-FU and DOX. Cell survival was further assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction assay which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet form azan product that can be detected spectro photometrically. Exponentially growing cells (HEPG2 and MCF-7) were plated in triplicate in 96-well sterilized plates at a density of 1×10^4 cells/well. After 24 h, cells were treated with escalating doses of the compounds under investigation and incubated in 5% CO_2 atmosphere with high humidity. After 48 and 72 h of compound exposure, the cells were incubated with MTT (0.5 mg/ml) for another 4 h at 37°C. The blue MTT formazan precipitate was then, solubilized in detergent (50% final concentration of N, N-dimethylformamide and 10% of sodium dodecyl sulphate) and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multi-well ELISA plate reader. The mean absorbance of medium control was the blank and was subtracted. IC_{50} values (concentration of compound causing 50% inhibition of cell growth) were estimated after 72 h exposure of compound. The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control. The 5-fluorouracil and doxorubicin anticancer drugs were used as positive control, and cells without samples were used as negative control. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines with the specified compound. 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 products.

Molecular docking study

The structures of all tested compounds were modeled using the Chemsketch software (http://www.acdlabs.com/resources/ freeware/). The structures were optimized and energy minimized using the VEGAZZ software [10]. The optimized compounds were used to perform molecular docking. The three-dimensional structures of the two molecular targets (receptors) were obtained from Protein Data Bank (PDB) (www.rcsb.org): CDK-2 (PDB:1di8, https://www.rcsb.org/pdb/explore/explore.do?structureId=1di8), and BCL-2 (PDB:2o2f, https://www.rcsb.org/pdb/explore/explore.do?structureId=2o2f). The steps for receptor preparation included the removal of heteroatoms (solvent and ions), the addition of polar hydrogen, and the assignment of charges. The active sites were defined using grid boxes of appropriate sizes around the bound cocrystal ligands as is shown in **Table 1**. These compounds were docked into the active site of the CDK2, and BCL-2 to study their interaction in silico and to correlate their anti-cancer activity. The docking study was performed using Auto dock vina [11], and Chimera for visualization [12].

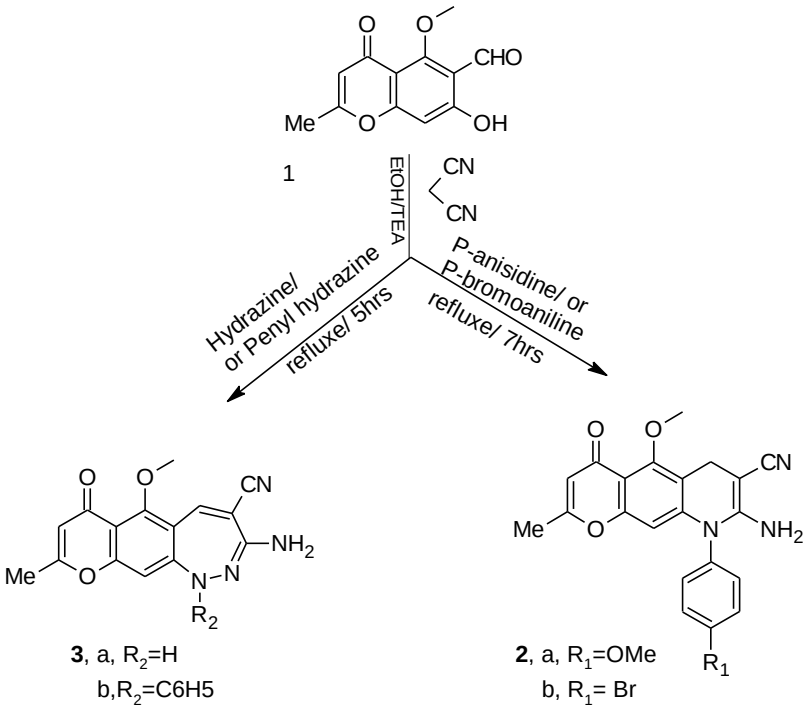
Table 1: Protein targets, cocrystal ligand, and grid box dimensions.

Protein targets	Cocrystal ligand	Grid box Dimensions		
		Center (X, Y, Z)		
1DI8	DTQ (4-[3-Hydroxyanilino]-6,7- Dimethoxyquinazoline)	-7.623	49.881	11.367
2O2F	LI10 4-(4-Benzyl-4-Methoxypiperidin-1-Yl)-N- [(4-{[1,1-Dimethyl-2-(Phenylthio)Ethyl]Amino}-3- Nitrophenyl)Sulfonyl]Benzamide	-0.024	3.142	-0.361

III. Results And Discussion

Chemistry

In one-pot of three-component reaction of carbaldehyde **1** which was utilized as a starting material, anisidine/ or p-bromoaniline and malononitrile in ethanol as solvent using triethylamine as catalyst for preparation of substituted quinoline-7-carbonitrile **2a, b (Figure 1).** IR spectra showed two peaks for NH_2, CN. ^{1}HNMR spectra displaced single signals representing CH_3, NH_2, CH_2 in quinoline ring, OCH_3, CH-10 and CH-3 pyranone ring groups respectively. The aromatic protons were observed at the excepted regions. ^{13}C-NMR showed 19 signals for compound **2b** a characteristic for 174.9(CO), 60.01(OCH_3), 25.8(CH_3) and 19.2(CH_2).
Also, herein, we report a facile one-pot synthesis of diazepine-4-carbonitrile derivatives **3a, b (Figure 1)** *via* three-component coupling of carbaldehyde **1**, malononitrile and hydrazine/ or phenyl hydrazine in ethanol and TEA. The IR spectrum of **3a, b** exhibited two peaks for NH_2, CN.^{1}HNMR spectra displaced single signals representing CH_3, NH_2, OCH_3, CH-6, CH-11 and CH-3 pyranone ring groups respectively. The aromatic protons were observed at the excepted regions.



3, a, R_2=H
b,R_2=C6H5

2, a, R_1=OMe
b, R_1= Br

Fig 1: synthesis of compounds **2a, b and 3a, b**

Multi-component reaction *via* condensation of quinoline -7-carbonitrile derivatives **2a, b** and carboxylic acid was reported as pyrimidinone derivatives **4a,b (Figure2)**. The formation of **4a,b** were thoroughly characterized by spectroscopic techniques. IR spectra showed three singlet peaks for NH, 2CO groups and peak of cyano, nitro groups of **4a,b** were disappeared.[1]HNMR spectra displaced a single signals representing $2CH_3$,$2OCH_3$, NH , CH-8 andCH-3 pyranone ring, CH=C-CH=N groups respectively. The aromatic protons were observed at the excepted regions. Also, in [13]CNMR spectrum showed 22 signals for compound **4a** resonated at 190.2, 182.1due to (2CO) , 57.5, 56.1 due to ($2OCH_3$) and 18.7, 20.9 due to (2 CH_3).

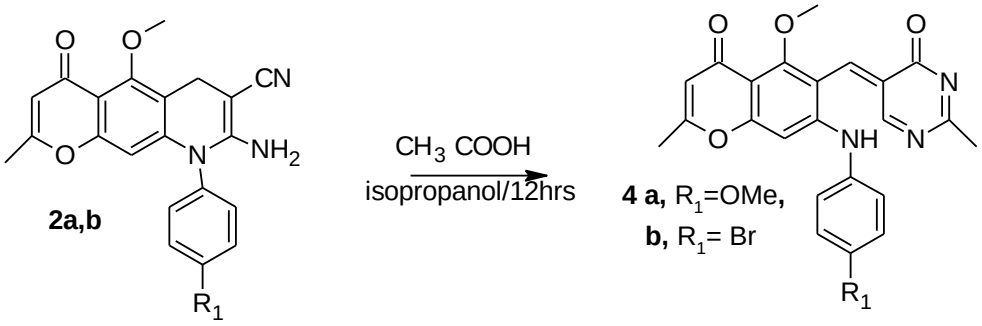


Fig 2: synthesis of compounds **4a,b**

New pyrimidine derivatives **5a, b and 6 (Figure 3)** were synthesized at the refluxing of equivalent amounts of carbaldehyde **1**, malononitrile and ethylacetoacetate/ or cyclohexane.IR spectrum of compounds **5a, b, 6**exhibited disappearing of aldehydic group and signals peak of (CN, OH) were appeared. [1]HNMR of new compounds **5a, b and 6** were in agreement with their molecular structures. All compounds display single signals due to $3CH_3$, OCH_3, OH, CH-8, CH-3, CH=N.[13]C-NMR showed 19 signals for compound **5b** a characteristic for 199.3, 180.0(2CO), 59.9(OCH_3) and 39.5, 20.5, 18.8 ($3CH_3$).

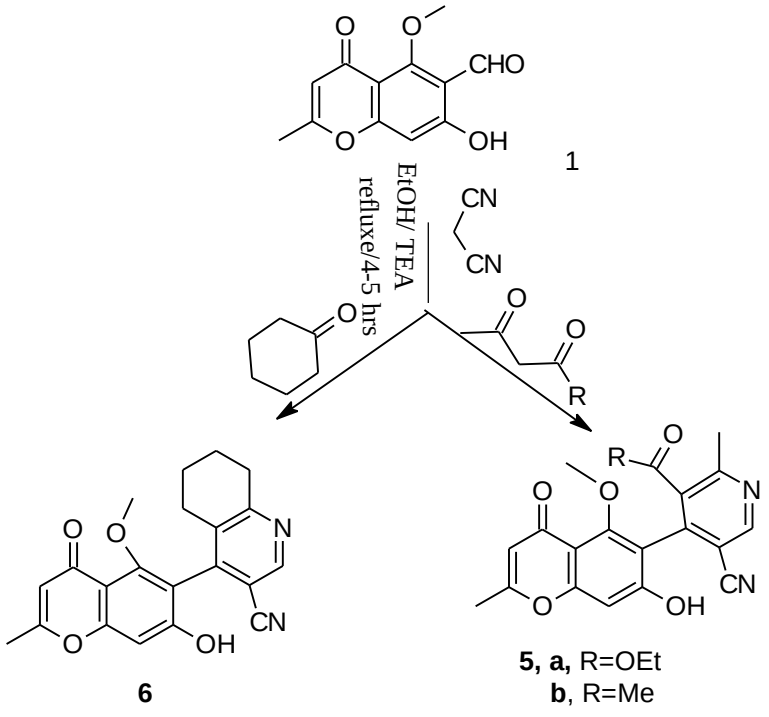


Fig 3: synthesis of compounds **5a, b and 6**

Here, we preparation of quinoline-7-carboxylate **7a, b (Figure 4)**using TEA as catalyst and ethanol as solvent at the reaction of carbaldehyde 1, ethylacetoacetate and anisidine/ or p- bromoaniline via one-pot multi-component reactions (MCRs). This reaction was agreed with [13- 15].Also, three-component coupling of carbaldehyde **1**, malononitrile and, and hydrazine hydrate/ or phenyl hydrazine hydrate have been accomplished in the presence of ethanol /TEA to afford the corresponding diazepine -4 - carboxylate derivatives **8a, b (Figure 3)**.IR spectrum single peaks of aldehyde and hydroxyl of **5a, b and 6** were disappeared and carbonyl group of ester was appeared.[1]HNMR spectra appeared a multiple signal for (CH_2) and triple signals for (CH_3).[13]CNMR

spectrum showed 21 signals for compound **8b** resonated at 182.0, 164.8due to (2CO), 57.5, 56.1 due to (1OCH_{3+} 1 CH_2), 21.0, 17.3, 14.0 due to (3 CH_3).

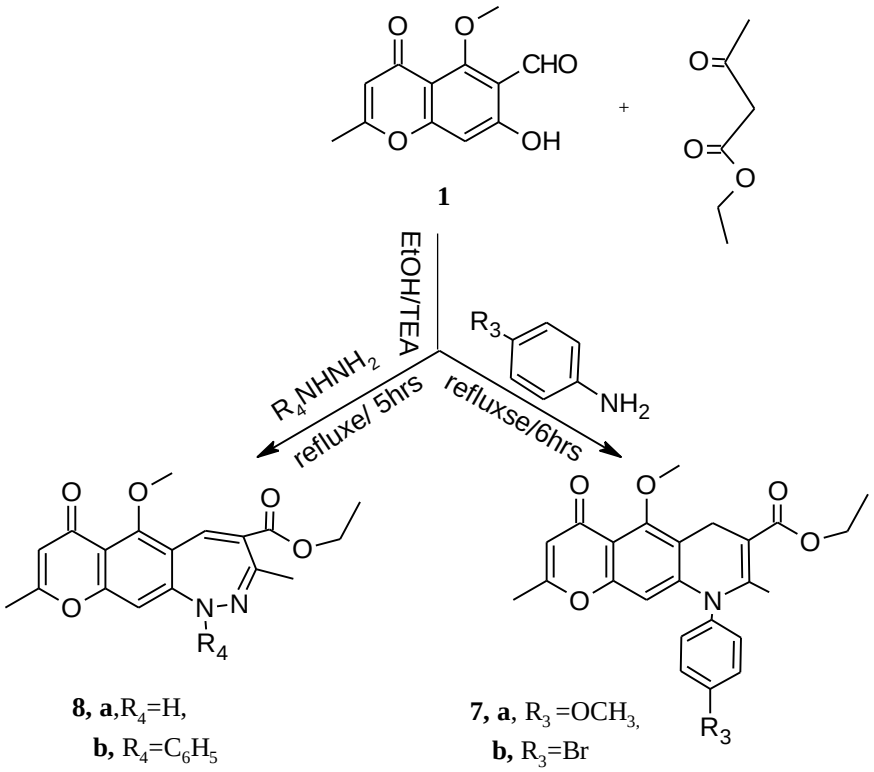


Fig 4: synthesis of compounds **7a, b and 8a, b**

Bioactivity

Anticancer Activity

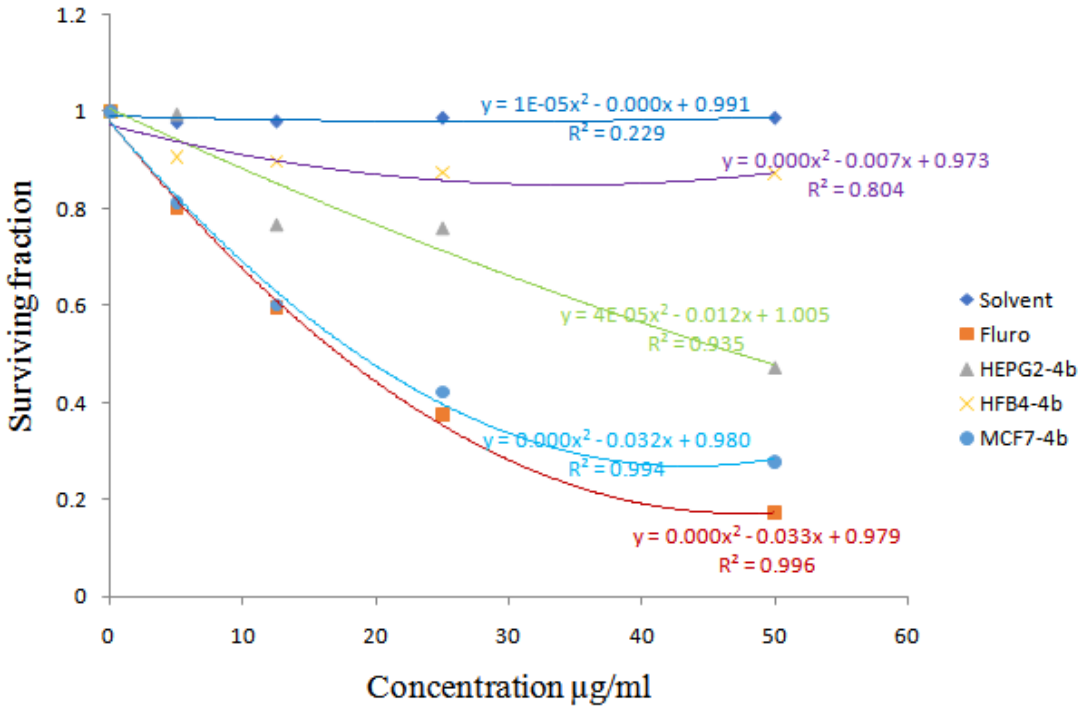
The IC_{50} determination was carried out to determine the cytotoxic potential of all compounds. The obtained viabilities were used to plot the dose-dependent response. The IC_{50} for each compound was determined and the values are shown in **Table 2.**

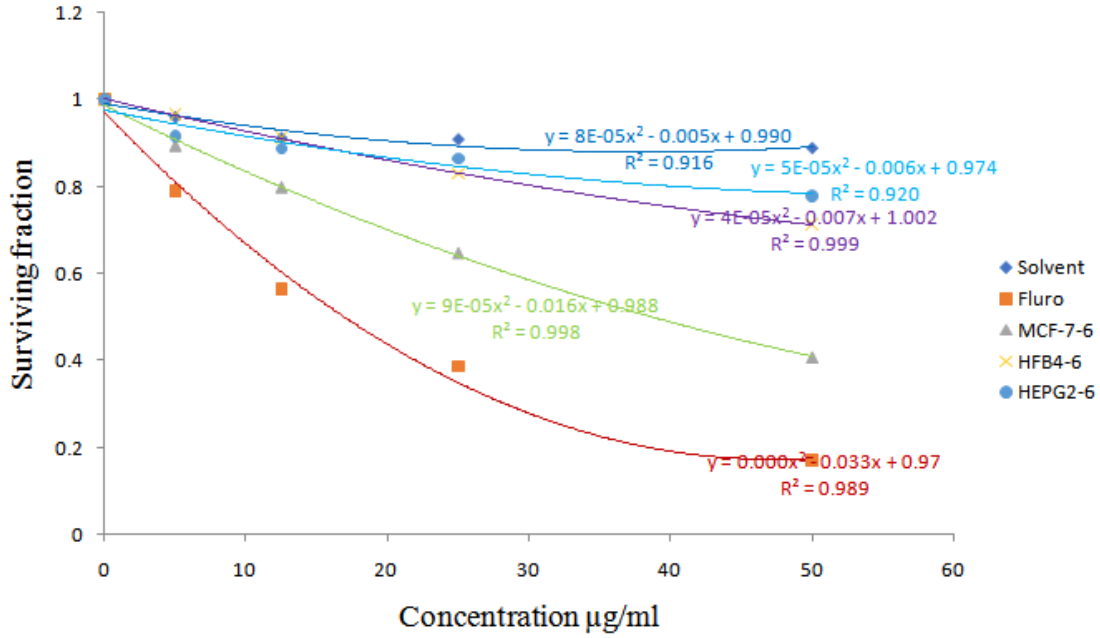
Some of the tested compounds (**4b, 6**, and **8a)** showed remarkable anticancer activity against MCF-7 cancer cells**(Figures 5-7)**. While compounds **2a**, **2b**, **3a**, **3b**, **4b**, **5a**, **5b**, **7a**, **7b** and **8b** had no effect on the breast cancer cells. In the same sense, evaluation the anticancer effect of the tested compounds against hepatic cell line (HEPG2) revealed that although compounds **2a**, **2b**, **3a**, **4a**, **4b**, **5a**, **6**, **7a**, **8a** and **8b** had no antiproliferative effect, compounds **3b, 5b**, and **7b(Figures 8-10)**showed anticancer activity closed to the reference drug.

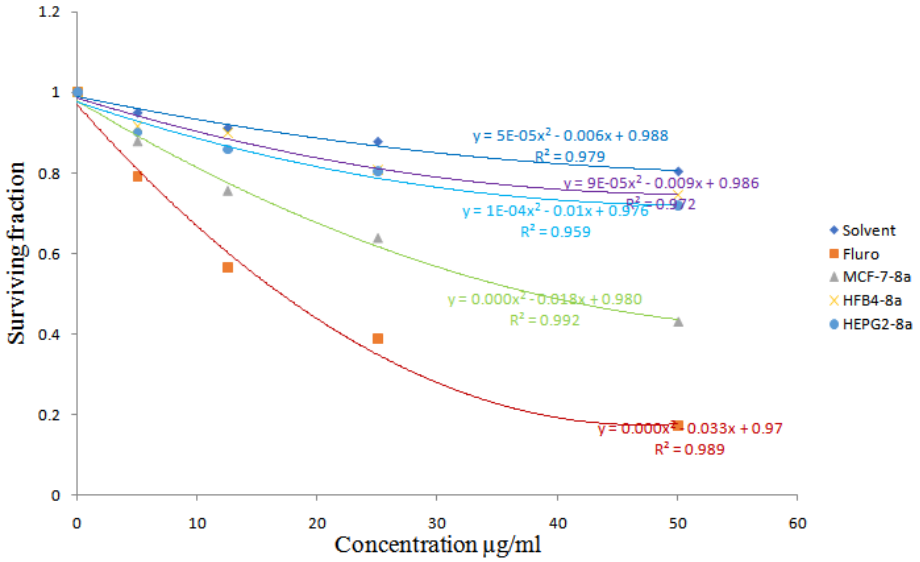
Table 2: IC_{50} value by MTT assay of hepatic cancer cell line (HEPG-2) and breast cancer cell line (MCF-7)

Compound	**Cell Lines**	
	MCF-7	**HEPG-2**
Solvent	73.74	73.74
1	53	50
2a	39	38
2b	38	36
3a	50	33
3b	43	20
4a	37	43
4b	14	39
5a	38	42
5b	40	18
6	19	35
7a	38	49
7b	53	21
8a	18	40
8b	37	44
5 flurouracil	13.35	ND*
Doxorubicin	ND*	14.70

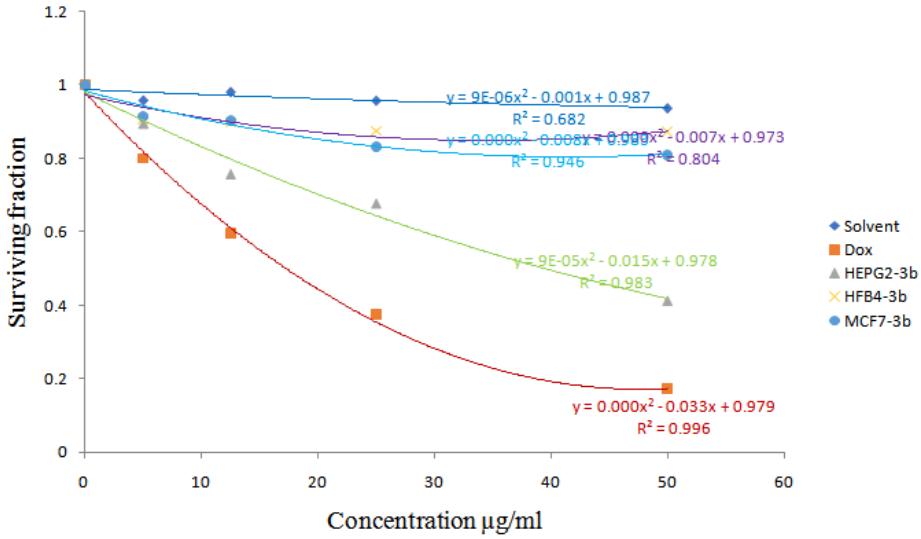
*ND: not detected
Our results also showed that most of the compounds exhibited no activity against the growth of normal HFB4 cells.

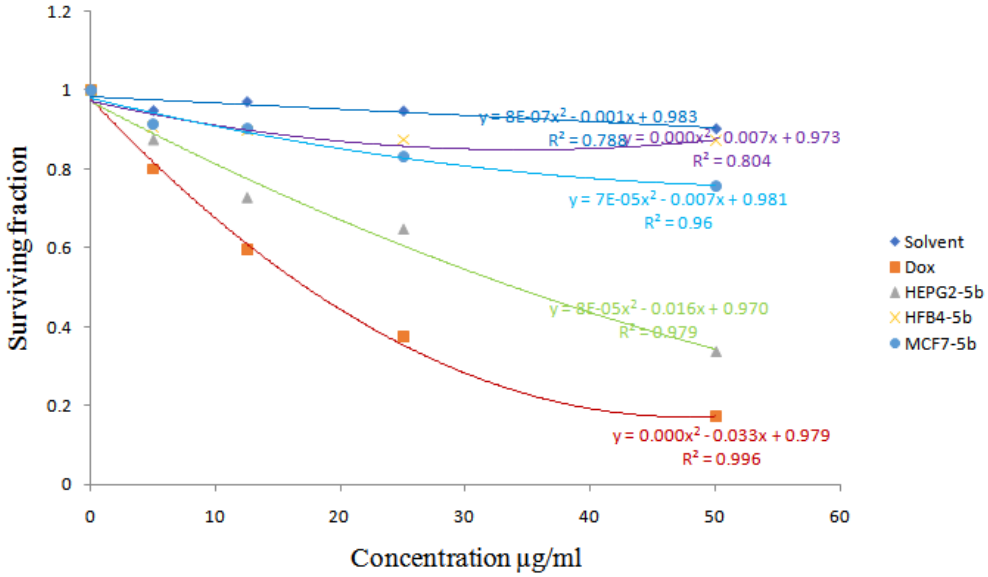


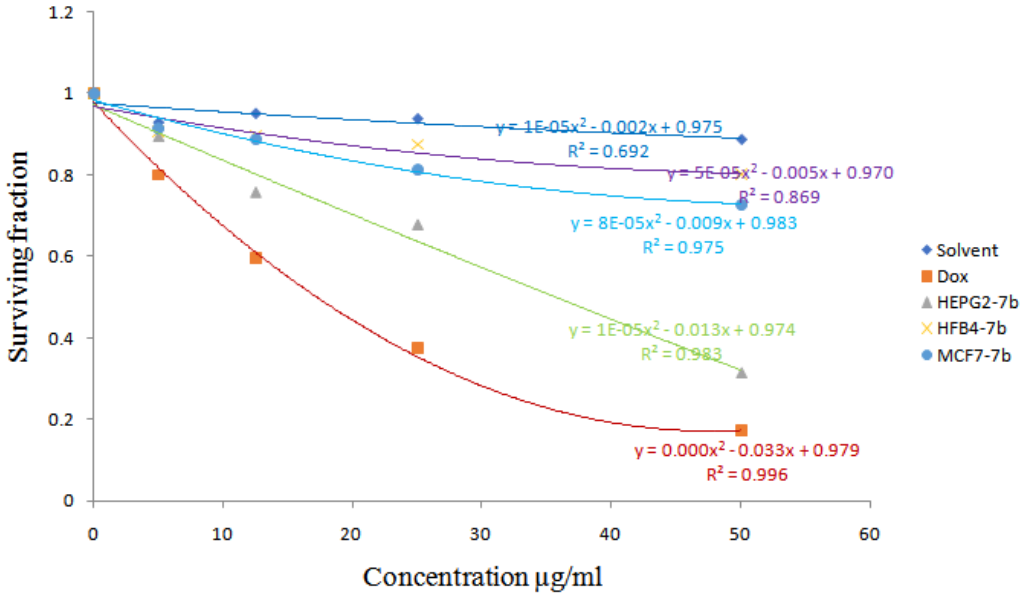




Figures: 5-7: Dose-dependent response of MCF-7 cells treated with newly derivatives determined by MTT assay







Figures 8-10: Dose-dependent response of HEPG-2 cells treated with newly derivatives determined by MTT assay

Molecular Docking results

To test our docking proposal and to ensure that the binding poses of the docked ligands represented favorable and valid potential binding modes, the docking parameters and methods were validated by redocking of the cocrystal ligand, in order to determine the ability of Auto Dock vina to reproduce the orientation and position of the ligand observed in the crystal structure. The redocking of cocrystal ligands to their respective molecular targets exhibited an RMSD value of <2Å between the original cocrystal ligand position and the docked poses, as the RMSD was 1.047Å for 1DI8 receptor and was 1.343Å for 2O2F receptor **(Figure 11)**. This confirmed that the ligands were closely bound to the true conformation of their targets indicating the reliability of the docking protocols and parameters [16].

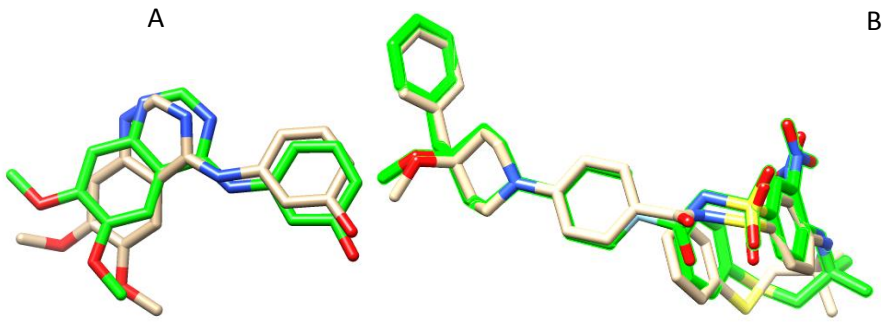


Figure 11: A.-B. Docking validation by redocking the cocrystal ligands to their corresponding receptors. The original conformation of each cocrystal ligands is displayed in green stick, while docked poses are represented in gray stick. The root mean square deviation (RMSD) was calculated between the original and docked poses of the cocrystal ligands. **A**. RMSD: 1.047Å (PDB ID: 1DI8); **B**. RMSD: 1.343Å (PDB ID: 2O2F) using Chimera software.

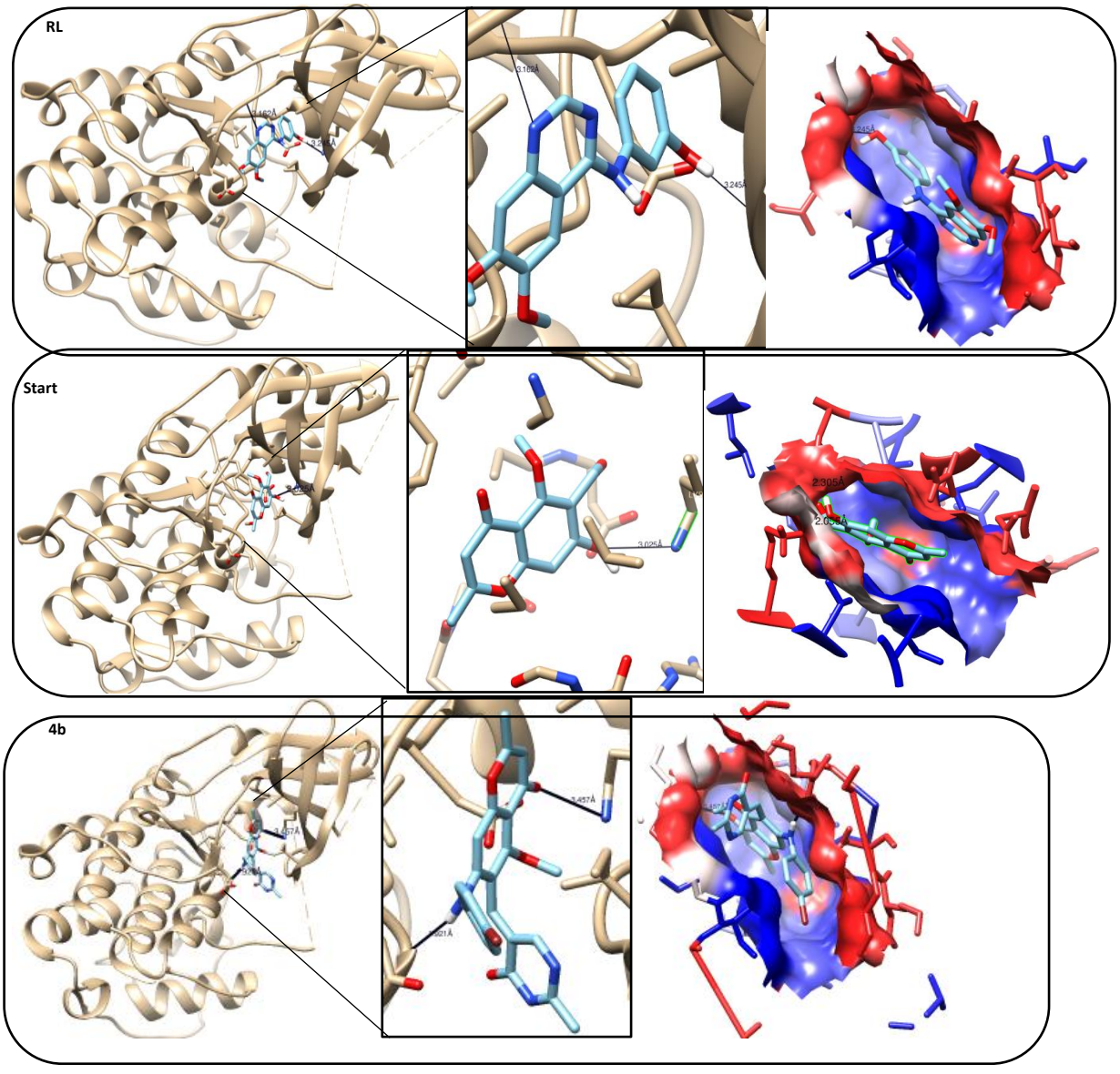
The molecular docking studies revealed that the compound **4b**, **7b**, and **8a** were the most promising compounds, which is explained by lowest binding energy (-8.5, -9.3, and -8.6 kcal/mol, respectively), hydrogen bonding and hydrophobic interactions with the active site residues of CDK2 (**Tables 3 and 4**), and (**Figure 12**).The compounds **3b** and **4b** were the most active compounds against BCL2, which is depicted by lowest binding energy (-8.1, and -8.2 kcal/mol, respectively) (**Tables 5 and 6**), and (**Figure 13**). Our molecular analysis revealed that compound 4bis the most active compounds against both proteins and that might be one of the reasons for the good activities shown by this compound in the *in vitro* assay.

Table 3: The results of molecular docking of best conformer with CDK2(1DI8) receptor

Compounds	Binding energy (Kcal/mol)	No. of H- bonds	Length of H-bonds	Formed amino acids with H-bonds
Reference ligand (DTQ)	-8.3	2	3.162 Å 3.245 Å	LEU83A LYS33A
Start	-7.2	1	2.305 Å 2.055 Å	ASP145A LYS33A
3b	-7.4	-	-	-
4b	-8.5	2	2.750 Å 1.921 Å	LYS 33A GLN 131A
5b	-5.4	-	-	-
6	-7.8	1	3.325 Å	LYS 33A
7b	-9.3	1	3.131Å	LYS 33A
8a	-8.6	1	3.037Å	LYS33A

Table 4: The Hydrophobic interactions of best conformer with CDK2 (1DI8) receptor

Compounds	**Hydrophobic interactions**
Reference ligand	ILE10, VAL18, LEU148, VAL64, LEU134, LEU83
Start	ILE10, VAL18, LEU148, VAL64, LEU134, LEU83
4b	ILE10, VAL18, LEU148, VAL64, LEU134, LEU83, LEU133, VAL17, LEU298
7b	ILE10, VAL18, LEU148, VAL64, LEU134, LEU83, PHE82, LEU298
8a	ILE10, VAL18, LEU148, VAL64, LEU134, LEU83, PHE80



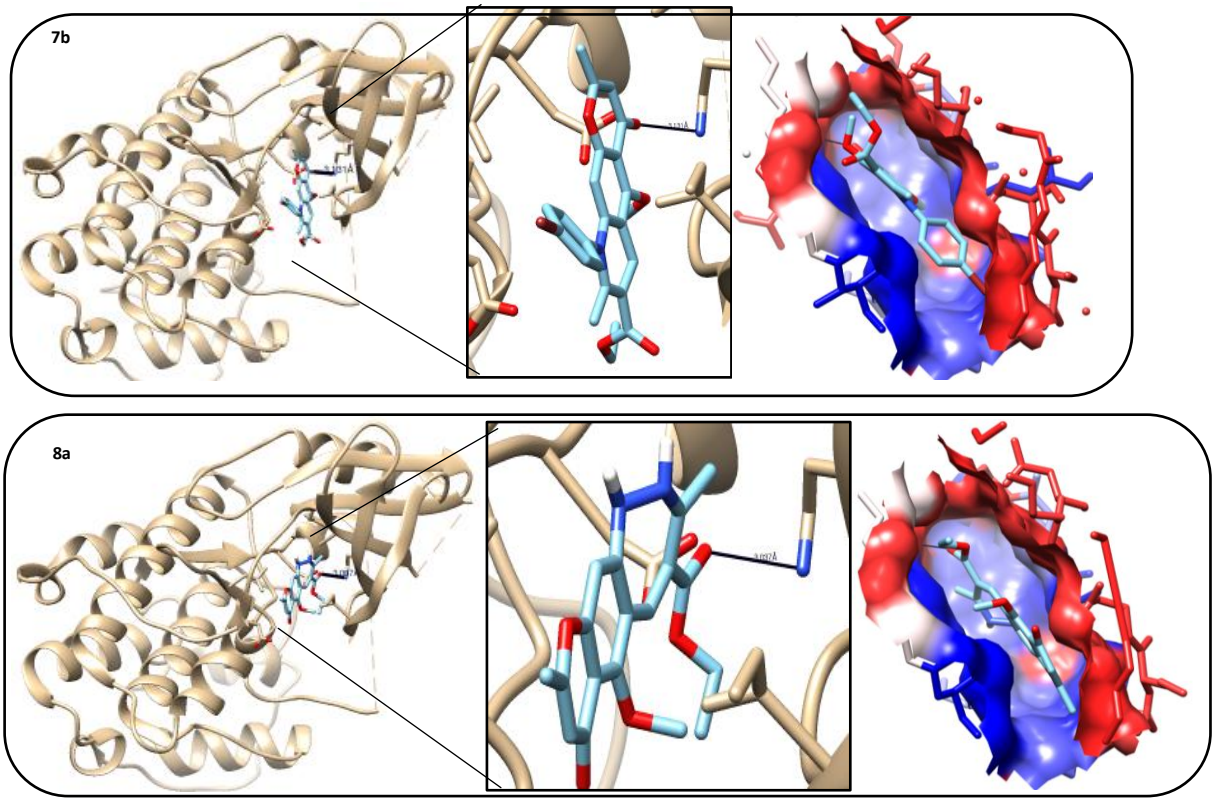


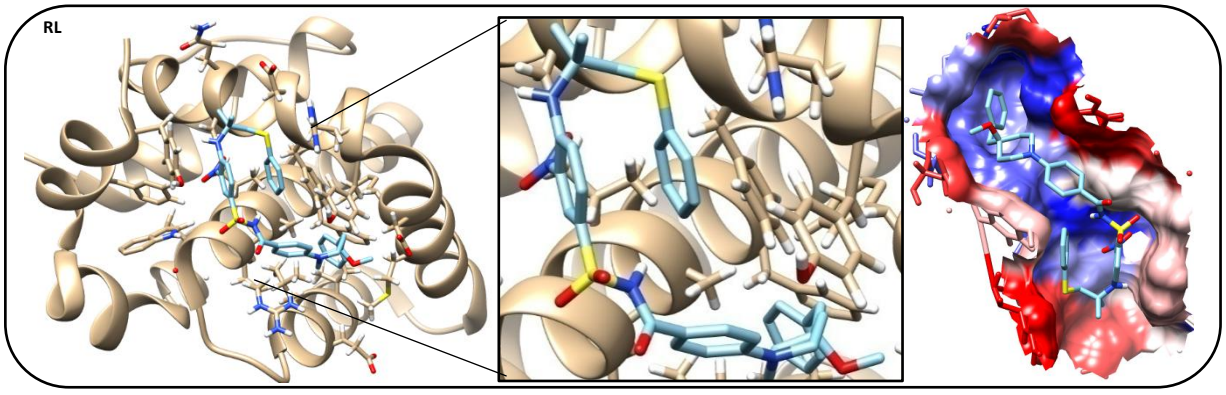
Figure 12: 3D of hydrogen bond and hydrophobic interaction between reference ligand (RL), start compound and the most promising compounds (**4b**,**7b** and **8a**) with CDK-2 protein.

Table 5: The results of molecular docking of best conformer with BCL-2 (2O2F) receptor

Compounds	Binding energy (Kcal/mol)	No. of H- bonds	Length of H-bonds	Formed amino acids with H-bonds
Reference ligand	-10.00	-	-	-
Start	-5.7	-	-	-
3b	-8.1	2	2.138 Å 2.149 Å	ASP 108A ASP 108A
4b	-8.2	1	2.424 Å	ARG 143A
5b	-6.9	-	-	-
6	-7.1	-	-	-
7b	-7.2	-	-	-
8a	-6.1	1	2.536 Å	GLY 142A

Table 6: The Hydrophobic interactions of best conformer with BCL-2 (2O2F) receptor

Compounds	**Hydrophobic interactions**
Reference ligand	MET112, VAL130, VAL145, LEU134, ALA146, PHE101, PHE109, PHE150
3b	MET112, VAL130, VAL145, LEU134, PHE101, PHE109, PHE150, PHE147
4b	MET112, VAL130, VAL145, LEU134, PHE101, PHE109, PHE150, PHE195



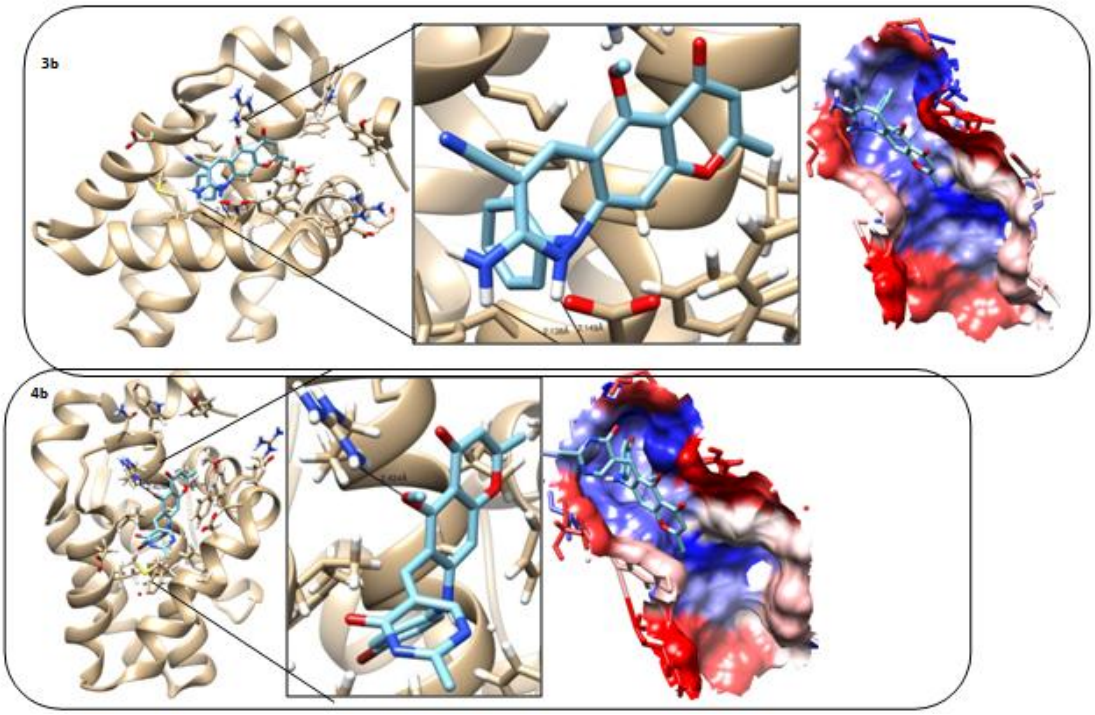


Figure 13:3D of hydrogen bond and hydrophobic interaction between reference ligand, and the most promising compounds (**3b**, and **4b**) and BCL-2 protein.

IV. Conclusion

In the present work, we have synthesisquinoline-7-carbonitrile derivatives **2a, b**. Diazepine-4-carbonitrile derivatives **3a, b** *via* one-pot three-component condensation reaction from carbaldehyde 1, substituted anisidine/ or *p*-bromoaniline/ or hydrazine/or phenyl hydrazine and malononitrile. Also, pyrimidine derivatives **5a, b, 6** were obtained at the refluxing of ethyl acetoacetate/or hexanone, carbaldehyde **1** and malononitrile. Quinoline-7-carboxylate derivatives **7a, b** diazepine-4-carboxylate compounds **8a, b** have been prepared via one pot reactions between carbaldehyde **1,** *p*-anisidine/ or *p*-Br aniline / or hydrazine hydrate/ or phenyl hydrazine. The synthesized compounds were tested, along with reference compounds,5-Flurouracil and Doxorubicin, for their cytotoxic potential against two tumor cell lines (HEPG-2 and MCF-7). Some of newly synthesized products displayed a moderate to good growth inhibition activity, where compound **7b**demonstrated the greatest activity against HEPG-2 cells, and compound **4b** demonstrated the highest activity against MCF7 cells.

Molecular-docking analyses revealed that compound **4b** was the best docked ligand against both targets, as it displayed the lowest binding energies, and critical hydrogen bonds and hydrophobic interactions with the targets were also revealed.

Reference

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