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A NOVEL CLASS OF SUBSTITUTED SPIRO [QUINAZOLINE-2,1'-CYCLOHEXANE] DERIVATIVES AS EFFECTIVE PPAR-1 INHIBITORS: MOLECULAR MODELING, SYNTHESIS, CYTOTOXIC AND ENZYME ASSAY EVALUATION

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Abstract: Molecular docking simulation study was carried out to design a novel series of spiro [(2H, 3H)quinazoline-2,1'-cyclohexan]-4(1H)-one derivatives as a new class of effective PARP-1 inhibitors. Spiro [2H-3,1-benzoxazine-2,1'-cyclohexan]-4(1H)-one (**5**) was the starting compound to synthesize the target proposed analogues. The derivatives that showed the top scores and had the best fitting in the binding sites of the target protein were selected to evaluate their *in vitro* anti-proliferative activity against the cultured human breast carcinoma cell line (MCF-7) using doxorubicin as a standard drug. Additionally, the compounds that exhibited the highest cytotoxic efficiency were further subjected to PARP-1 enzyme assay taking 3-aminobenzamide as the reference drug. The structures of the novel derivatives were confirmed on the bases of microanalytical and spectral data.

Keywords: anti-proliferative activity, molecular docking, PARP-1 inhibitors, spiro [(2H, 3H)quinazoline-2,1'-cyclohexan]-4(1H)-one

In the field of cancer therapy, it is well known that poly (ADP-ribose) polymerases (PARPs enzymes), also identified as poly (ADP-ribose) synthetases and poly (ADP-ribose) transeferases are abundant nuclear enzymes. They constitute a family of cell signaling enzymes present in eukaryotes, which catalyze poly (ADP-ribosylation) of DNA binding proteins (1, 2). Poly (ADP-ribose) polymerase-1 (PARP-1) is the first characterized and the best known member of the PARP family. It is activated by DNA strand breaks induced by several events including oxidative stress or binding of cytotoxic drugs to DNA. Subsequently, the activated PARP-1 cleaves nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and ADP-ribose moieties, then polymerizes the latter through surface accessible glutamate residue onto either on itself or on a variety of nuclear target proteins such as histones, topoisomerases, DNA polymerases and DNA ligases, in a process called poly-(ADP) ribosylation. When DNA is mildly damaged, PARP-1 is activated and participates in DNA repair process so that the cell survives. However, in excessive DNA damage, PARP-1 is overactivated and induces depletion of cellular NAD^+ and ATP levels leading to cell dysfunction or necrotic cell death (3, 4). Furthermore, extensive PARP-1 activation may also result in caspase-independent programmed cell death, mediated by the translocation of apoptosis inducing factor to the nucleus (5). So, overactivation of PARP-1 has been involved in the pathogenesis of several diseases such as stroke, myocardial infarction, diabetes, neurodegenerative disorders, allergy and several inflammatory disorders (6). Due to the dual response of PARP-1 to DNA damage and its involvement in cell death, pharmacological modulation of PARP-1 may constitute a useful tool to increase the activity of DNA binding antitumor drugs and ionizing radiation. In fact, the development of specific PARP-1 inhibitors as potential chemo and radio sensitizers will provide an important area of therapeutic potential (7). Additionally, there is increasing evidence which has linked PARP-1 to breast cancer, for example PARP-1 defi-

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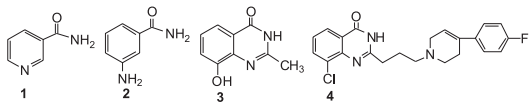
cient mice exhibit increased spontaneous mammary carcinoma formation (8). Also several PARP-1 inhibitors in clinical trials are being explored as mono therapy in cancer disease (9), they selectively kill breast cancer cells with deficiencies in DNA-repair genes such as BRCA-1 or BRCA-2 at safety administrable doses with minimal side effects (10). However, new studies showed that phenanthridone derived PARP-1 inhibitors promote cell death in breast cancer cells lacking BRCA-1 and BRCA-2 mutations (MCF-7). These results suggest a potential broader utilization of PARP-1 inhibitors as single agents in treating breast cancer beyond heredity BRCA1 or BRCA2 deficient types (11, 12).

Most PARP-1 inhibitors have been designed to imitate the substrate enzyme interaction of NAD^+ with PARP-1 and because of their structural resemblance to the substrate, these compounds act as competitive inhibitors by blocking NAD^+ binding to the catalytic domain of the enzyme (13).

Nicotinamide **1** and 3-aminobenzamide (3-AB) **2**, which are structural analogues of the nicotinamide moiety of NAD^+ are among the first discovered PARP-1 inhibitors. However, these compounds have a low potency and specificity (14). Various studies revealed that the restriction of carboxamide, which is normally free to rotate, in the bioactive anti-conformation provided an increase in the binding affinity (15). This biologically active anti-conformation can be achieved by ring closure of the carboxamide into bicyclic lactam system such as the quinazolinone derivatives NU1025 **3** and 1UK1 **4** in order to further enhance the potency and improvement of the pharmacokinetic properties (16, 17).



With the aim of finding a new class of effective PARP-1 inhibitors suitable for the clinical development, we designed and synthesized different novel quinazolinone scaffolds bearing various heterocyclic functionalities of reported either PARP-1 inhibiting activity such as thiazolidinone (18), benzothiazinone (19), benzimidazole (20), pyridazinone (21) and pyrazole (22) or anticancer activity depending on other mechanisms such as pyrrolopyrimidine (23, 24), oxadiazole (25), triazole (26) and thiadiazole (27) to study the interactions of these derivatives with NAD^+ binding sites of the target PARP-1 enzyme. Since literature survey confirmed the therapeutic potential lethality of PARP-1 inhibitors as single drugs in treating breast cancer cells rather having BRCA mutations or not (11, 12), the newly synthesized derivatives that recorded the top docking scores using Autodock Vina were *in vitro* evaluated as cytotoxic agents against breast adenocarcinoma cell lines. Then, the compounds that exhibited efficient potency were chosen to be tested in the PARP-1 enzyme assay.

MATERIALS AND METHODS

Molecular modeling

In this work Autodock Vina was used for the molecular docking. MGLTOOLS was used for preparation of both protein and ligands. SPDBV software was used for energy minimization and computing the binding free energy.

Preparation of protein and ligands

The crystal structure of PARP-1 (pdb code = 1UK1) was downloaded and loaded into MGTools window. All polar hydrogens were added and finally, the protein saved as pdbqt format. The compounds as well were built and saved as pdbqt format.

Building of the grid box

The protein was rotated and the docking site was identified at which the reported quinazoline derivative was complexed. The X, Y, and Z centers of the grid box that represents the 3 dimensions of the box were determined to be suitable for covering all the docking area. The values were found to be; center_x = 29.50, center_y = 30.6 and center_z = 17.8.

Computing of the binding free energy

As a first step for that energy minimization using SPDBV software in which 20 steps of steepest descent, all bonds, non-bonded, electrostatics, torsions and angles were included. The cutoff = 10000 Å. And finally, the total energy was computed for each protein-ligand complex.

Chemistry

Regents were purchased from Acros (Geel, Belgium) and Aldrich (St. Louis, MO, USA) and were used without purification. Analytical thin-layer chromatography was performed on silica gel 60 254F plates (Merck) using a mixture of chloroform and ethanol (5 : 1, v/v) as an eluent. UV light at λ 254 nm and iodine accomplished visualization. All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were deter-

mined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) at National Research Centre (NRC), Cairo, Egypt and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, at NRC. Mass spectra were recorded at 70 eV on EI Ms-QP 1000 EX (Shimadzu, Japan), at the Faculty of Science, Cairo University, Egypt. Microanalytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), at NRC. The values found were within ± 0.4% of the theoretical values.

Spiro [2H-3,1-benzoxazine-2,1'-cyclohexan]-4 (1H)-one (5)

This compound was prepared according to the reported method (28, 29). M.p. 143°C.

Spiro [(2H,3H)-3-aminoquinazoline-2,1'-cyclohexan]-4(1H)-one (6)

A solution of **5** (2.17 g, 10 mmol) and hydrazine hydrate 99% (1.60 mL, 50 mmol) in absolute ethanol was refluxed for 4 h. The product obtained upon cooling was filtered off and recrystallized using isopropanol/petroleum ether to yield compound **6** as pale yellow powder.

Yield: (66%), m.p. 95-97°C, ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.20 (s, 10H, spiro cyclohexyl), 5.21 (s, 2H, NH_2, exchangeable with D_2O), 6.91-7.62 (m, 4H, aromatic H), 10.13 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3452-3277 (NH_2, NH), 3077 (CH aromatic), 1640 (CO). MS: (EI, 70 eV) m/z (%): 231 (10.17), 119 (100). Analysis: for $C_{13}H_{17}N_3O$ (m.w. 231.29) calcd.: C, 67.51; H, 7.41; N, 18.17%; found: C, 67.12; H, 6.93; N, 18.31%.

General procedure for preparation of spiro {(2H,3H)-3-[(1-(E)-polyhydroxyalkylidene)-imino]quinazoline-2,1-cyclohexan}-4(1H)-ones (7a-c)

A mixture of compound **6** (2.31 g, 10 mmol) and the appropriate linear sugar namely: D-xylose, D-arabinose and D-mannose (10 mmol) in absolute ethanol in the presence of few drops of glacial acetic acid was refluxed for 6 h. The reaction mixture was cooled and the formed precipitate was filtered off and recrystallized from isopropanol to obtain the desired Schiff's bases **7a-c**, respectively.

Spiro {(2H,3H)-3-[(1-(E)-1,2,3,4-tetrahydroxy-pentylidine)-imino]quinazoline-2,1'-cyclohexan}-4(1H)-ones (7a, b)

7a (from xylose), was obtained as brown powder, yield: 60%, m.p. 117-119°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.39-3.60 (m, 5H, aldoses), 4.25-4.30 (m, 4H, 4OH, exchangeable with D_2O), 7.10-8.03(m, 5H, aromatic H + -N=CH), 10.21 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3580-3400 (OH), 3382 (NH), 3070 (CH aromatic), 2932 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M^+ 363 (10.14), 119 (100.00). Analysis: for $C_{18}H_{25}N_3O_5$ (m.w. 363.41) calcd.: C, 59.49; H, 6.93; N,11.56%; found: C, 59.41; H, 6.48; N, 11.74%.

7b (from arabinose), was obtained as light brown crystals, yield: 65%, m.p. 81-83°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.39-3.66 (m, 5H, aldoses), 4.35-4.30 (m, 4H, 4OH, exchangeable with D_2O), 6.90-8.03 (m, 5H, aromatic H + -N=CH), 10.00 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3587-3481 (OH), 3365 (NH), 2915 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M^+ 363 (12.07), 119 (100). Analysis: for $C_{18}H_{25}N_3O_5$ (m.w. 363.41) calcd.: C, 59.49; H, 6.93; N,11.56%; found: C, 59.73; H, 7.31; N, 11.85%.

Spiro {(2H,3H)-3-[(1-(E)-1,2,3,4-tetrahydroxy-hexylidine)-imino]-quinazoline-2,1'-cyclohexan} 4(1H)-one (7c)

7c (from mannose), was obtained as pale yellow powder, yield: 70%, m.p. 73-75°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 3.03-3.21 (m, 6H, aldoses), 4.51-4.73 (m, 5H, 5OH, exchangeable with D_2O), 7.52-8.64 (m, 5H, aromatic H + -N=CH), 10.21 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3680-3480 (OH), 3360 (NH), 3077 (CH aromatic), 2937 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M^+ 393 (7.56), 119 (100). Analysis: for $C_{19}H_{27}N_3O_6$ (m.w. 393.43) calcd.: C, 58.00; H, 6.92; N, 10.68%; found: C, 57.62; H, 7.31; N, 11.06%.

General procedure for preparation of spiro (2H,3H)-3-[2-[1-(E)-polyhydroxyalkylidene]-4-oxo-thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-ones (8a-c)

A mixture of Schiff bases **7a-c** (5 mmol) and thioglycolic acid (0.39 mL, 5 mmol) in dry benzene (20 mL) was refluxed for 16 h. The excess solvent was evaporated under reduced pressure and the obtained residue was neutralized using Na_2CO_3 solution, then filtered off and recrystallized from dioxane to obtain the desired products **8a-c**, respectively.

Spiro {(2H,3H)-3-[2-[1-(1,2,3,4-tetrahydroxy-butyl)]-4-oxo-thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-ones (8a,b)

8a (from xylose), was obtained as dark brown powder, yield: 60%, m.p. 180-182°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.04 (s, 10H, spiro cyclohexyl), 3.39-3.51 (m, 5H, aldoses), 3.80 (s, 2H, CH_2, thiazolidinone ring), 4.13-4.20 (m, 4H, 4OH, exchangeable with D_2O), 5.91 (s, 1H, S-CH, thiazolidinone ring), 8.06-8.44 (m, 4H, aromatic H), 10.21 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 36.92 (CH_2, thiazolidinone), 38.57-39.69 (spiro cyclohexyl carbons), 48.28 (CH, thiazolidinone),70.05 (spiro head carbon), 70.94 (4' carbon of xylose), 71.56 (2' carbon of xylose), 71.89 (3' carbon of xylose), 73.16 (1' carbon of xylose), 111.47-148.39 (aromatic carbons), 158.26 (CO, quinazolinone), 170.03 (CO, thiazolidinone). IR (KBr, cm^{-1}): 3574-3489 (OH), 3380 (NH), 3061 (CH aromatic), 2981 (CH aliphatic), 1675, 1660 (2C=O). MS: (EI, 70 eV) m/z (%): M^+ 437 (12.03), 419 (80.77), 55 (100). Analysis: for $C_{20}H_{27}N_3O_6S$ (m.w. 437.51) calcd.: C, 54.90; H, 6.22; N, 9.60; S, 7.33%; found: C, 54.32; H, 6.61; N, 9.45; S, 7.65%.

8b (from arabinose), was obtained as dark yellow powder, yield: 60%, m.p. 189-191°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.11 (s, 10H, spiro cyclohexyl), 3.39-3.45 (m, 5H, aldoses), 3.90 (s, 2H, CH_2, thiazolidinone ring), 4.53-4.80 (m, 4H, 4OH, exchangeable with D_2O), 5.82 (s, 1H, S-CH, thiazolidinone ring), 8.16-8.80 (m, 4H, aromatic H), 10.00 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3523-3475 (OH), 3375 (NH), 3093 (CH aromatic), 2960 (CH aliphatic), 1674, 1665 (2C=O). MS: (EI, 70 eV) m/z (%): $(M + 1)^+$ 438 (58.33), 437 (75.00), 67 (100). Analysis: for $C_{20}H_{27}N_3O_6S$ (m.w. 437.51) calcd.: C, 54.90; H, 6.22; N, 9.60; S, 7.33%; found: C, 55.41; H, 5.92; N, 10.05; S, 6.91%.

Spiro {(2H,3H)-3-[2-[1-(1,2,3,4-tetrahydroxypentyl)]-4-oxo-thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (8c)

8c (from mannose), was obtained as brown powder, yield: 65%, m.p. 208-210°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.41-3.63 (m, 6H, aldoses), 3.85 (s, 2H, CH_2, thiazolidinone ring), 4.25-4.27 (m, 5H, 5OH, exchangeable with D_2O), 5.93 (s, 1H, S-CH, thiazolidinone ring), 7.41-7.68 (m, 4H, aromatic H), 8.54 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3674-3486 (OH), 3380 (NH), 2928 (CH aliphatic), 1676, 1665 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 467 (10.07), 220 (100.00). Analysis: for $C_{21}H_{29}N_3O_7S$ (m.w. 467.54) calcd.: C, 53.95; H, 6.25; N, 8.99; S, 6.86%; found: C, 54.23; H, 6.43; N, 9.43; S, 6.92%.

General procedure for preparation of spiro {(2H,3H)-3-[2-[1-(E)-polyhydroxyalkylidene]-4-oxo-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan]-4(1H)-ones (9a-c)

A mixture of compounds **7a-c** (5 mmol) and thiosalicylic acid (0.77 g, 5 mmol) in dry benzene (20 mL) was refluxed for 16 h. The excess solvent was evaporated under reduced pressure and the obtained residue was treated with petroleum ether. The solid product was filtered off, washed with petroleum ether and crystallized from isopropanol to obtain the desired products **9a-c**, respectively.

Spiro {(2H,3H)-3-[2-[1-(1,2,3,4-tetrahydroxybutyl)]-4-oxo-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan]-4(1H)-ones (9a,b)

9a (from xylose), was isolated as dark red powder, yield: 60%, m.p. 210-212°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.04 (s, 10H, spiro cyclohexyl), 2.68-2.82 (m, 5H, aldoses), 4.03-4.25 (m, 4H, 4OH, exchangeable with D_2O), 6.94 (s, 1H, CH, benzothiazine ring), 7.29-7.98 (m, 8H, aromatic H), 9.03 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 39.15-39.86 (spiro cyclohexyl carbons), 53.36 (CH, benzothiazine), 70.05 (spiro head carbon), 70.94 (4' carbon of xylose), 71.56 (2' carbon of xylose), 71.89 (3' carbon of xylose), 72.16 (1' carbon of xylose), 113.48-147.39 (aromatic carbons), 158.13 (CO, quinazolinone), 160.07 (CO, benzothiazine). IR (KBr, cm^{-1}): 3530-3415 (OH), 3323 (NH), 3061 (CH aromatic), 2963 (CH aliphatic), 1679, 1665 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 499 (40.68), 225 (100). Analysis: for $C_{25}H_{29}N_3O_6S$ (m.w. 499.58) calcd.: C, 60.10; H, 5.85; N, 8.41; S, 6.42%; found: C, 59.83; H, 6.11; N, 8.62; S, 6.21%.

9b (from arabinose) was isolated as light brown powder, yield: 63%, m.p. 168-170°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.00 (s, 10H, spiro cyclohexyl), 2.60-2.92 (m, 5H, aldoses), 4.11-4.30 (m, 4H, 4OH, exchangeable with D_2O), 6.54 (s, 1H, CH, benzothiazine ring), 7.30-7.96 (m, 8H, aromatic H), 9.13 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3545-3430 (OH), 3320 (NH), 3092 (CH aromatic), 1679, 1660 (2CO). MS: (EI, 70 eV) m/z (%): $(M - 1)^+$ 498 (25.31), 133 (100). Analysis: for $C_{25}H_{29}N_3O_6S$ (m.w. 499.58) calcd.: C, 60.10; H, 5.85; N, 8.41; S, 6.42%; found: C, 59.72; H, 5.52; N, 8.26; S, 6.03%.

Spiro {(2H,3H)-3-[2-[1-(1,2,3,4-tetrahydroxypentyl)]-4-oxo-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (9c)

9c (from mannose), was obtained as dark brown powder, yield: 65%, m.p. 207-209°C. ^{1}H

NMR (300 MHz, $CDCl_3$, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.34-3.51 (m, 6H, aldoses), 4.07-4.11 (m, 5H, 5OH, exchangeable with D_2O), 6.44 (s, 1H, CH, benzothiazine ring), 6.69-7.99 (m, 8H, aromatic H), 8.19 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3565-3440 (OH), 3320 (NH), 2928 (CH aliphatic), 1676, 1660 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 529 (6.53), 136 (100). Analysis: for $C_{26}H_{31}N_3O_7S$ (m.w. 529.61) calcd.: C, 58.96; H, 5.90; N, 7.93; S, 6.05%; found: C, 59.43; H, 6.43; N, 7.52; S, 5.91%.

General procedure for preparation of spiro [(2H,3H)-3-(substituted enamino)-quinazoline-2,1'-cyclohexan]-4(1H)-ones (10a-d)

A mixture of compound **6** (2.31 g, 10 mmol) and the appropriate aromatic aldehyde namely: anisaldehyde, *p*-fluorobenzaldehyde, 2-thiophenaldehyde and pyrrolo-2-carboxaldehyde (10 mmol) in absolute ethanol containing few drops of glacial acetic acid was refluxed for 12 h. The reaction mixture was cooled and the formed precipitate was filtered off and recrystallized from dioxane to give the desired Schiff's bases **10a-d**.

Spiro [(2H,3H)-3-(4-methoxybenzylideneamino)-quinazoline-2,1'-cyclohexan]-4(1H)-one (10a)

The product was isolated as bright yellow crystals, yield: 80%, m.p. 160-162°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.24 (s, 10H, spiro cyclohexyl), 3.85 (s, 3H, OCH_3), 4.12 (s, 1H, -N=CH), 6.94-7.86 (m, 8H, aromatic H), 8.61 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3432 (NH), 2967 (CH aliphatic), 1717 (CO). MS: (EI, 70 eV) m/z (%): $(M - 1)^+$ 348 (15.13), 91 (100). Analysis: for $C_{21}H_{23}N_3O_2$ (m.w. 349.43) calcd.: C, 72.18; H, 6.63; N, 12.03%; found: C, 71.93; H, 6.48; N, 11.81%.

Spiro [(2H,3H)-3-(4-fluorobenzylideneamino)-quinazoline-2,1'-cyclohexan]-4(1H)-one (10b)

The product was obtained as light yellow crystals, yield: 85%, m.p. 128-130°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.21 (s, 10 H, spiro cyclohexyl), 4.71 (s, 1H, -N=CH), 7.12-7.85 (m, 8H, aromatic H), 8.65 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3371 (NH), 3030 (CH aromatic), 2927 (CH aliphatic), 1671 (CO). MS: (EI, 70 eV) m/z (%): M^+ 337 (12.26), 76 (100). Analysis: for $C_{20}H_{20}FN_3O$ (m.w. 337.39) calcd.: C, 71.20; H, 5.97; N, 12.45%; found: C, 71.65; H, 6.26; N, 12.01%.

Spiro [(2H,3H)-3-[(thiophen-2-yl)-methyleneamino] -quinazoline-2,1'-cyclohexan]-4(1H)-one (10c)

The product was obtained as light green powder, yield: 82%, m.p. 151-153°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.50 (s, 10H, spiro cyclohexyl), 4.21 (s, 1H, -N=CH), 6.78-7.43 (m, 7H, aromatic H), 8.65 (s, 1H, NH exchangeable with D_2O). IR (KBr, cm^{-1}): 3455 (NH), 3089 (CH aromatic), 2955 (CH aliphatic), 1741 (CO). MS: (EI, 70 eV) m/z (%): M^+ 325 (9.00), 78 (100). Analysis: for $C_{18}H_{19}N_3OS$ (m.w. 325.43) calcd.: C, 66.43; H, 5.88; N, 12.91; S, 9.85%; found: C, 66.72; H, 5.39; N, 13.28; S, 9.49%.

Spiro [(2H,3H)-3-[(1H-pyrrol-2yl)-methyleneamino]-quinazoline-2,1'-cyclohexan]-4(1H)-one (10d)

The product was obtained as light brown powder, yield: 74%, m.p. 120-122°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.35 (s, 10H, spiro cyclohexyl), 4.17 (s, 1H, -N=CH), 7.13-7.52 (m, 7H, aromatic H), 8.65, 10.21 (2s, 2H, 2NH exchangeable with D_2O). IR (KBr, cm^{-1}): 3422, 3212 (2NH), 2928 (CH aliphatic), 1670 (CO). MS: (EI, 70 eV) m/z (%): M^+ 308 (10.07), 77 (100). Analysis: for $C_{18}H_{20}N_4O$ (m.w. 308.38) calcd.: C, 70.11; H, 6.54; N, 18.17%; found: C, 69.83; H, 6.94; N, 17.81%.

General procedure for preparation of spiro {(2H,3H)-3-[2-substituted-4-oxo-thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-ones (11a-d)

A mixture of Schiff's bases **10a-d** (5 mmol) and thioglycolic acid (0.39 mL, 5 mmol) in dry benzene (20 mL) was refluxed for 16 h. The excess solvent was evaporated under reduced pressure and the obtained residue was neutralized using Na_2CO_3 solution, then filtered off and crystallized from isopropanol to obtain the desired products **11a-d**, respectively.

Spiro {(2H,3H)-3-[2-(4-methoxyphenyl)-4-oxo-thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (11a)

The product was isolated as light brown powder, yield: 80%, m.p. 165-167°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.04 (s, 10H, spiro cyclohexyl), 3.48 (s, 2H, CH_2, thiazolidinone ring), 3.73 (s, 3H, OCH_3), 4.31 (s, 1H, S-CH, thiazolidinone ring), 6.84-7.35 (m, 8H, aromatic H), 10.21 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 36.32 (CH_2, thiazolidinone), 39.15-39.47 (spiro cyclohexyl carbons), 47.36 (CH, thiazolidinone), 55.59 (CH_3), 71.55 (spiro head carbon), 113.46-153.51 (aromatic carbons), 163.47 (CO, quinazolinone), 168.26 (CO, thiazolidinone).

IR (KBr, cm^{-1}): 3355 (NH), 3036 (CH aromatic), 2926 (CH aliphatic), 1710, 1665 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 423 (12.63), 77 (100). Analysis: for $C_{23}H_{25}N_3O_3S$ (m.w. 423.53) calcd.: C, 65.23; H, 5.95; N, 9.92; S, 7.57%; found: C, 64.93; H, 6.38; N, 10.26; S, 7.21%.

Spiro {(2H,3H)-3-[2-(4-fluorophenyl)-4-oxo-thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (11b)

The product was obtained as white powder, yield: 83%, m.p. 111-113°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.24 (s, 10H, spiro cyclohexyl), 3.77 (s, 2H, CH_2, thiazolidinone ring), 5.45 (s, 1H, S-CH, thiazolidinone ring), 6.68-7.53 (m, 8H, aromatic H), 8.61 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3344 (NH), 3123 (CH aromatic), 2923 (CH aliphatic), 1715, 1686 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 411 (12.14), 410 (10.63), 317 (100). Analysis: for $C_{22}H_{22}FN_3O_2S$ (m.w. 411.49) calcd.: C, 64.21; H, 5.39; N, 10.21; S, 7.79%; found: C, 63.98; H, 5.64; N,10.68; S, 7.91%.

Spiro {(2H,3H)-3-[4-oxo-2-(thiophen-2-yl)thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (11c)

The product was isolated as white powder, yield: 82%, m.p. >300°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.08 (s, 10H, spiro cyclohexyl), 3.65 (s, 2H, CH_2, thiazolidinone ring), 5.31 (s, 1H, S-CH, thiazolidinone ring), 6.74-8.32 (m, 7H, aromatic H), 10.31 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3375 (NH), 2956 (CH aliphatic), 1715, 1675 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 399 (28.49), 72 (100). Analysis: for $C_{20}H_{21}N_3O_2S_2$ (m.w. 399.53) calcd.: C, 60.12; H, 5.30; N, 10.52; S, 16.05%; found: C, 59.84; H, 5.73; N, 10.71; S, 16.48%.

Spiro {(2H,3H)-3-[4-oxo-2-(1H-pyrrol-2yl)thiazolidin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (11d)

The product was isolated as light brown powder, yield: 74%, mp >300°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 3.52 (s, 2H, CH_2, thiazolidinone ring), 4.85 (s, 1H, S-CH, thiazolidinone ring), 7.84-8.33 (m, 7H, aromatic H), 8.91, 10.23 (2s, 2H, 2NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3355 (NH), 2924 (CH aliphatic), 1695, 1650 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 382 (11.37), 119 (100). Analysis: for $C_{20}H_{22}N_4O_2S$ (m.w. 382.48) calcd.: C, 62.80; H, 5.80; N, 14.65; S, 8.38%; found: C, 63.41; H, 5.47; N, 14.31; S, 7.98%

General procedure for preparation of spiro {(2H,3H)-3-[2-substituted-4-oxo-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-ones(12a-d)

A mixture of Schiff's bases **10a-d** (5 mmol) and thiosalicylic acid (0.77 g, 5 mmol) in dry benzene (20 mL) was refluxed for 16 h. The excess solvent was evaporated under reduced pressure and the obtained residue was treated with petroleum ether. The solid product was filtered off, washed with petroleum ether and recrystallized from isopropyl alcohol/petroleum ether to obtain the desired products **12a-d**, respectively.

Spiro {(2H,3H)-3-[2-(4-methoxyphenyl)-4-oxo-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (12a)

The product was obtained as light brown powder, yield: 80%, m.p. 181-183°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.24 (s, 10H, spiro cyclohexyl), 3.85 (s, 3H, OCH_3), 5.21 (s, 1H, S-CH, benzothiazine ring), 6.95-8.12 (m, 12H, aromatic H), 8.62 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 39.15-39.47 (spiro cyclohexyl carbons), 55.59 (CH_3), 60.16 (CH, benzothiazine), 71.55 (spiro head carbon), 112.03-153.51 (aromatic carbons), 160.13 (CO, quinazolinone), 168.15 (CO, benzothiazine). IR (KBr, cm^{-1}): 3475 (NH), 2927 (CH aliphatic), 1710, 1673 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 485 (6.39), 77 (100). Analysis: for $C_{28}H_{27}N_3O_3S$, (m.w. 485.60) calcd.: C, 69.25; H, 5.60; N, 8.65; S, 6.60%; found: C, 68.86; H, 5.98; N, 9.01; S, 6.24%.

Spiro {(2H,3H)-3-[2-(4-fluorophenyl)-4-oxo-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (12b)

The product was obtained as white powder, yield: 85%, m.p. 157-159°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.18 (s, 10H, spiro cyclohexyl), 5.25 (s, 1H, S-CH, benzothiazine ring), 7.12-7.84 (m, 12H, aromatic H) 8.83 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3445 (NH), 3069 (CH aromatic), 2928 (CH aliphatic), 1700, 1671 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 473 (7.67), 119 (100). Analysis: for $C_{27}H_{24}FN_3O_2S$ (m.w. 473.56) calcd.: C, 68.48; H, 5.11; N, 8.87; S, 6.77%; found: C, 68.04; H, 5.49; N, 8.48; S, 6.38%.

Spiro {(2H,3H)-3-[4-oxo-2-(thiophen-2-yl)-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (12c)

The product was isolated as brown powder, yield: 82%, m.p. 121-123°C. ^{1}H NMR (300 MHz,

$CDCl_3$, δ, ppm): 1.02 (s, 10H, spiro cyclohexyl), 5.39 (s, 1H, S-CH, benzothiazine ring), 6.84-7.54 (m, 11H, aromatic H), 9.62 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3375 (NH), 3085 (CH aromatic), 2956 (CH aliphatic), 1700, 1655 (2CO). MS: (EI, 70 eV) m/z (%): $(M^{\cdot} + 1)^+$ 462 (28.07), 72 (100). Analysis: for $C_{25}H_{23}N_3O_2S_2$ (m.w. 461.60) calcd.: C, 65.05; H, 5.02; N, 9.10; S, 13.89%; found: C, 65.35; H, 5.39; N, 9.51; S, 14.38%.

Spiro {(2H,3H)-3-[4-oxo-2-(1H-pyrrol-2-yl)-2H,4H-benzo[e][1,3]thiazin-3-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (12d)

The product was obtained as light brown powder, yield: 74%, m.p. 202-204°C. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.17 (s, 10H, spiro cyclohexyl), 5.42 (s, 1H, S-CH, benzothiazine ring), 7.56-8.44 (m, 11H, aromatic H), 8.91, 10.31 (2s, 2H, 2NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3440 (NH), 3048 (CH aromatic), 2923 (CH aliphatic), 1700, 1665 (2CO). MS: (EI, 70 eV) m/z (%): 445 $(M^{\cdot} + 1)^+$ (12.65), 444 (10.55), 186 (100). Analysis: for $C_{25}H_{24}N_4O_2S$ (m.w. 444.55) calcd.: C, 67.54; H, 5.44; N, 12.60; S, 7.21%; found: C, 67.92; H, 4.98; N, 13.09; S, 7.47%.

Spiro {(2H,3H)-3-[(1H-benzo[d]imidazol-2-yl)-methylamino]-quinazoline-2,1'-cyclohexan}-4(1H)-one (13)

A solution mixture the amino derivative **6** (2.31 g, 10 mmol) and 2-chloromethylbenzimidazole (1.66 g, 10 mmol) in absolute ethanol containing anhydrous K_2CO_3 (1.38 g, 10 mmol) was refluxed for 16 h. The product obtained upon pouring onto ice/H_2O was filtered off and recrystallized from dioxane. The product was obtained as yellow powder.

Yield: 85%, m.p. 116-118°C. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.02 (s, 10H, spiro cyclohexyl), 4.63 (s, 2H, -NH-CH_2), 6.57-7.80 (m, 8H, aromatic H), 8.02, 8.76, 10.13 (3s, 3H, 3NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 38.71-40.03 (spiro cyclohexyl carbons), 55.89 (CH_2), 71.02 (spiro head carbon), 112.17-151.96 (aromatic carbons), 170.64 (CO). IR (KBr, cm^{-1}): 3422-3147 (3NH), 3031 (CH aromatic), 2955 (CH aliphatic), 1741 (CO). MS: (EI, 70 eV) m/z (%): M^+ 361 (10.03), 131 (100). Analysis: for $C_{21}H_{23}N_5O$ (m.w. 361.44) calcd.: C, 69.78; H, 6.41; N, 19.38%; found: C, 70.31; H, 6.23; N, 18.82%.

Spiro [(2H,3H)-3-(benzoylmethylamino)-quinazoline-2,1'-cyclohexan]-4(1H)-one (14)

A mixture of the amino derivative **6** (2.31 g, 10 mmol) and phenacyl bromide (1.99 g, 10 mmol) was refluxed in ethanol for 3 h. The obtained solid was filtered off and crystallized from dioxane to obtain red powder.

Yield: 68%, m.p. 106-108°C. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.01 (s, 10H, spiro cyclohexyl), 2.98 (s, 2H, -NH-CH_2), 6.65-7.48 (m, 9H, aromatic H), 7.91, 10.13 (2s, 2H, 2NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3439, 3242 (2NH), 3043 (CH aromatic), 2978 (CH aliphatic), 1712, 1673 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 349 (11.27), 77 (100). Analysis: for $C_{21}H_{23}N_3O_2$ (m.w. 349.43) calcd.: C, 72.18; H, 6.63; N, 12.03% found: C, 71.98; H, 6.18; N, 11.65%.

2-Amino-1-[spiro [(2H,3H)-4(1H)oxo-quinazoline-2,1'-cyclohexan)]-3-yl]-4-phenyl-1H-pyrrole-3-carbonitrile (15)

A solution mixture of the derivative **14** (3.49 g, 10 mmol) and malononitrile (0.66 mL, 10 mmol) in ethanol containing sodium ethoxide (10 mmol) was refluxed for 3 h. The reaction mixture was cooled and acidified with HCl. The obtained solid was filtered off and recrystallized from isopropanol to get the desired product **15** as light brown powder.

Yield: 80% m.p. 130-132°C. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.01 (s, 10H, spiro cyclohexyl), 5.49, 9.00 (2s, 3H, NH_2, NH, exchangeable with D_2O), 6.65-7.48 (m, 10H, aromatic H + pyrrole H). IR (KBr, cm^{-1}): 3407-3240 (NH_2, NH), 3089 (CH aromatic), 2917 (CH aliphatic), 2208 (C=N), 1651 (CO). MS: (EI, 70 eV) m/z (%): M^+ 397 (10.32), 119 (100). Analysis: for $C_{24}H_{23}N_5O$ (m.w. 397.47) calcd.: C, 72.52; H, 5.83; N, 17.62%; found: C, 72.14; H, 6.18; N, 17.97%.

Spiro {(2H, 3H)-3-[4-amino-1,2-dihydro-2-oxo/thio-5-phenylpyrrolo[2,3-d]pyrimidin-7-yl]-quinazoline -2,1'-cyclohexan}-4(1H)-ones (16a,b).

The pyrrolo derivative **15** (3.97 g, 10 mmol) was fused with urea/thiourea (10 mmol) at 220°C for 20 min. Then, the reaction product was treated with petroleum ether and recrystallized from ethanol to give compounds **16a,b**.

Spiro {(2H, 3H)- 3-[4-amino-1,2-dihydro-2-oxo-5-phenylpyrrolo[2,3-d]pyrimidin-7-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (16a)

The product was obtained as dark red powder, yield: 76%, m.p. 236-238°C. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.01 (s, 10H, spiro cyclohexyl), 5.69, 9.03, 9.80 (3s, 4H, NH_2, 2NH, exchangeable with D_2O), 6.65-7.48 (m, 10H, aromatic H + pyrrole

H). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 38.78-40.07 (spiro cyclohexyl carbons), 70.81 (spiro head carbon), 115.23-150.68 (aromatic carbons), 160.79 (CO, pyrimidinone), 168.17 (CO, quinazolinone). IR (KBr, cm^{-1}): 3455-3239 (NH_2, 2NH), 3050 (CH aromatic), 2925 (CH aliphatic), 1672, 1665 (2CO), 1620 (C=N). MS: (EI, 70 eV) m/z (%): $(M - 1)^+$ 439 (10.19), 438 (26.49), 105 (100). Analysis: for $C_{25}H_{24}N_6O_2$ (m.w. 440.50) calcd.: C, 68.17; H, 5.49; N, 19.08%; found: C, 67.63; H, 5.29; N, 19.42%.

Spiro {(2H, 3H)-3-[4-amino-1,2-dihydro-5-phenyl-2-thioxopyrrolo[2,3-d]pyrimidin-7-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (16b).

The product was isolated as dark brown powder, yield: 82%, m.p. 245-247°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.41 (s, 10H, spiro cyclohexyl), 5.00, 8.80, 9.50 (3s, 4H, NH_2, 2NH, exchangeable with D_2O), 6.95-7.60 (m, 10H, aromatic H + pyrrole H). IR (KBr, cm^{-1}): 3460-3181 (NH_2, 2NH), 3012 (CH aromatic), 1670 (CO), 1590 (C=N), 1273 (C=S). MS: (EI, 70 eV) m/z (%): M^+ 456 (10.21), 121 (100). Analysis: for $C_{25}H_{24}N_6OS$ (m.w. 456.56) calcd.: C, 65.77; H, 5.30; N, 18.41; S, 7.02%; found: C, 64.98; H, 5.64; N, 18.68; S, 7.43%.

Spiro {(2H, 3H)-3-[4-amino-5-phenyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (17)

A solution mixture of the pyrrolo derivative **15** (3.97 g, 10 mmol) in formamide (30 mL) was refluxed for 5 h. Then, the reaction mixture was cooled and poured onto ice/water. The obtained precipitate was filtered off and recrystallized from isopropanol to give compound **17**.

Yield: 80%, m.p. 225-227°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.11 (s, 10H, spiro cyclohexyl), 5.50, 8.90 (2s, 3H, NH_2, NH, exchangeable with D_2O), 6.65-7.60 (m, 11H, aromatic H + pyrrole H). IR (KBr, cm^{-1}): 3414-3339 (NH_2, 2NH), 3050 (CH aromatic), 2892 (CH aliphatic), 1690 (CO), 1515 (C=N). MS: (EI, 70 eV) m/z (%): $(M + 1)^+$ 425 (11.63), 424 (4.89), 105 (100). Analysis: for $C_{25}H_{24}N_6O$ (m.w. 424.50) calcd.: C, 70.73; H, 5.70; N, 19.80%; found: C, 70.51; H, 6.95; N, 20.17%.

Spiro [(2H, 3H)-quinazoline-2,1'-cyclohexan]-4(1H)-one (18)

The benzoxazine derivative **5** (4.34 g, 20 mmol) was dissolved in formamide (15 mL) and refluxed for 3 h. The reaction mixture was cooled and then diluted with excess water. The separated solid was filtered off, dried and recrystallized from dioxane to yield compound **18** as white powder.

Yield: 75%, m.p. 193-195°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.04 (s, 10H, spiro cyclohexyl), 7.05-7.60 (m, 4H, aromatic H), 8.90, 9.82 (2s, 2H, 2NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 38.78-40.12 (spiro cyclohexyl carbons),71.82 (spiro head carbon), 123.13-145.94 (aromatic carbons), 161.32 (CO). IR (KBr, cm^{-1}): 3444, 3312 (2NH), 2924 (CH aliphatic), 1698 (CO). MS: (EI, 70 eV) m/z (%): M^+ 216 (13.39), 119 (100). Analysis: for $C_{13}H_{16}N_2O$ (m.w. 216.28) calcd.: C, 72.19; H, 7.46; N, 12.95%; found: C, 72.09; H, 6.93; N, 13.32%.

Spiro [(1H, 2H)-4-chloroquinazoline-2,1'-cyclohexane] (19)

A suspension of compound **18** (2.16 g, 10 mmol) and PCl_5 (0.5 g) in $POCl_3$ (8 mL) was heated under reflux for 2 h on a water bath. After cooling, the reaction mixture was poured slowly on crushed ice (30 g) then neutralized with NaOH solution. The solid formed was filtered off, washed with cold water and dried to give the chloro derivative **19** as light brown powder.

Yield: 75%, m.p. 75-77°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.99 (s, 10H, spiro cyclohexyl), 6.98-7.50 (m, 4H, aromatic H), 8.50 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3418 (NH), 3050 (CH aromatic), 2856 (CH aliphatic). MS: (EI, 70 eV) m/z (%): $(M + 2)^+$ 236 (13.20), M^+ 234 (4.40), 146 (100). Analysis: for $C_{13}H_{15}ClN_2$ (m.w. 234.72) calcd.: C, 66.52; H, 6.44; N, 11.93%; found: C, 66.41; H, 6.85; N, 11.59%.

Spiro [(5H,6H)-tetrazolo(1,5c)quinazoline-5,1'-cyclohexane] (20)

A mixture of the chloroquinazoline derivative **19** (0.47 g, 2 mmol) and sodium azide (0.13 g, 2 mmol) in glacial acetic acid was refluxed for 6 h. The reaction mixture was cooled and poured onto ice/H_2O. The formed precipitate was filtered off, dried and recrystallized from ethyl acetate to give the desired compound **20** as white powder.

Yield: 80%, m.p. 160-162°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.12 (s, 10H, spiro cyclohexyl), 7.00-7.50 (m, 4H, aromatic H), 8.92 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3454 (NH), 3050 (CH aromatic), 2977 (CH aliphatic), 1630 (C=N). MS: (EI, 70 eV) m/z (%): M^+ 241 (100). Analysis: for $C_{13}H_{15}N_5$ (m.w. 241.29) calcd.: C, 64.71; H, 6.27; N, 29.02%; found: C, 65.01; H, 5.82; N, 29.47%.

1-{Spiro [(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl}hydrazine (21)

Hydrazine hydrate (99%) (1.6 mL, 50 mmol) was added to the chloro compound **19** (2.34 g, 10 mmol) dissolved in absolute ethanol (20 mL) and refluxed for 8 h. The solid separated after concentration and cooling was filtered off and then recrystallized from isopropanol to yield compound **21** as white powder.

Yield: 80%, m.p. 143-145°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.22 (s, 10H, spiro cyclohexyl), 6.90-7.67 (m, 4H, aromatic H), 5.38, 8.92, 9.11 (3s, 4H, NH_2, 2NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3447-3132 (NH_2, 2NH), 3081 (CH aromatic), 2926 (CH aliphatic). MS: (EI, 70 eV) m/z (%): M^+ 230 (13.51), 119 (100). Analysis: for $C_{13}H_{18}N_4$ (m.w. 230.31) calcd.: C, 67.80; H, 7.88; N, 24.33%; found: C, 67.54; H, 8.31; N, 23.93%.

1-{Spiro [(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl}pyrazolidin-3,5-dione (22)

A mixture of the hydrazinyl compound **21** (2.30 g, 10 mmol) and diethylmalonate (1.60 mL, 10 mmol) in glacial acetic acid (10 mL) was refluxed for 8 h. The reaction mixture was cooled and poured onto ice/H_2O. Then, the formed solid was filtered off and recrystallized from dioxane to give compound **22** as light brown powder.

Yield: 65%, m.p. 180-182°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.51 (s, 10H, spiro cyclohexyl), 3.60 (s, 2H, CH_2 pyrazoline ring), 4.53 (s, 1H, NH, exchangeable with D_2O), 6.85-7.61 (m, 4H, aromatic H), 10.13 (s, 1H, NH exchangeable with D_2O). IR (KBr, cm^{-1}): 3432, 3201 (2NH), 2928 (CH aliphatic), 1690, 1650 (2CO), 1530 (C=N). MS: (EI, 70 eV) m/z (%): $(M + 1)^+$ 299 (5.56), 298 (7.22), 230 (100). Analysis: for $C_{16}H_{18}N_4O_2$ (m.w. 298.34) calcd.: C, 64.41; H, 6.08; N, 18.78%; found: C, 64.78; H, 5.97; N, 18.57%.

Spiro [5,6-dihydro-(1,2,4)triazolo[4,3-c]quinazoline-5,1'-cyclohexan]-3(2H)-one (23)

A mixture of the hydrazinyl compound **21** (2.30 g, 10 mmol) and ethyl chloroformate (1.08 mL, 10 mmol) in pyridine (10 mL) was refluxed for 6 h. The reaction mixture was cooled, poured onto ice/cold water containing few drops of HCl. Then, the filtered product was washed with water, dried and recrystallized from isopropanol to give light brown powder.

Yield: 73%, m.p. 162-164°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.91 (s, 10H, spiro cyclohexyl), 6.95-7.51 (m, 4H, aromatic H), 9.13, 10.00 (2s, 2H, 2NH exchangeable with D_2O). IR (KBr, cm^{-1}): 3423-3220 (2NH), 2928 (CH aliphatic), 1709 (CO). MS: (EI, 70 eV) m/z (%): M^+ 256 (11.03), 146 (100). Analysis: for $C_{14}H_{16}N_4O$ (m.w. 256.32) calcd.: C, 65.61; H, 6.29; N, 21.86%; found: C, 65.85; H, 6.43; N, 21.63%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}acetic acid (24)

Amino acid (glycine) (0.75 g, 10 mmol) and Na_2CO_3 (0.53 g, 5 mmol) were dissolved in water (15 mL), and the pH was adjusted to 9-9.5. Then the chloro-quinazoline derivative **19** (1.17 g, 50 mmol) was added and the reaction mixture was stirred at 100°C for 8 h at the controlled pH. The reaction mixture was left overnight at room temperature then treated with cold formic acid. The solid obtained was filtered off, washed with H_2O and crystallized from methanol to yield yellowish white crystals.

Yield: (70%), m.p. 166-168 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 3.81 (d, 2H, J = 7.6 Hz, α-CH_2), 6.91 (t, 1H, J = 3.4 Hz, NH), 7.02-7.61 (m, 4H, aromatic H), 8.11 (s, 1H, NH, exchangeable with D_2O), 10.13 (s, 1H, OH, exchangrable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 38.98-39.32 (spiro cyclohexyl carbons), 44.56 (CH_2), 70.81 (spiro head carbon), 104.32-154.53 (aromatic carbons), 173.24 (CO). IR (KBr, cm^{-1}): 3423, 3320 (NH, OH), 2929 (CH aliphatic), 1677 (CO), 1254 (COOH). MS: (EI, 70 eV) m/z (%): $(M+1)^+$ 274 (10.62), M^+ 273 (4.61), 248 (100). Analysis: for $C_{15}H_{19}N_3O_2$ (m.w. 273.33) calcd.: C, 65.91; H, 7.01; N, 15.37%; found: C, 66.21; H, 6.73; N, 15.84%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}acetyl chloride (25)

The quinazoline derivative **24** (0.27 g, 1 mmol) was dissolved in dry chloroform, then thionyl chloride (2.36 mL, 20 mmol) was added dropwise and the reaction mixture was stirred for 30min at 70°C. After cooling, the solvent was evaporated under reduced pressure and the obtained crude product was crystallized from methanol to give the chloro-quinazoline derivative **25** as white powder.

Yield: (61%), m.p. 146-148 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.01 (s, 10H, spiro cyclohexyl), 3.93 (d, 2H, J = 7.8 Hz, α-CH_2), 6.70 (t, 1H, J = 4.0 Hz, NH), 7.12-7.74 (m, 4H, aromatic H), 8.11 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3435, 3217 (2NH), 3071 (CH aromatic), 2975 (CH aliphatic), 1715 (CO). MS: (EI, 70 eV) m/z (%): $(M+2)^+$ 293 (4.60), M^+ 291 (13.80), 146 (100). Analysis: for $C_{15}H_{18}ClN_3O$ (m.w. 291.78) calcd.: C, 61.75; H, 6.22; N, 14.40%; found: C, 61.42; H, 5.85; N, 14.04%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}acetohydrazide (26)

Hydrazine hydrate (99%) (1.6 mL, 50 mmol) was added to the chloro-compound **25** (2.91 g, 10 mmol) dissolved in absolute ethanol (20 mL) and the reaction mixture was refluxed for 3 h. The solid separated after concentration and cooling was filtered off and then recrystallized from dioxane to get the hydrazide compound **26** as light brown powder.

Yield: (65%), m.p. 180-182°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 3.91 (d, 2H, *J* = 7.8 Hz, α-CH_2), 5.21 (s, 2H, NH_2, exchangeable with D_2O), 6.21 (t, 1H, *J* = 3.4 Hz, NH, exchangeable with D_2O), 6.99-7.51 (m, 4H, aromatic H), 8.91, 9.01 (2s, 2H, 2NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3438-3150 (NH_2, 3NH), 2981 (CH aliphatic), 1641 (CO)**.** MS: (EI, 70 eV) m/z (%): M^+ 287 (9.27), 118 (100). Analysis: for $C_{15}H_{21}N_5O$ (m.w. 287.36) calcd.: C, 62.70; H, 7.37; N, 24.37%; found: C, 62.94; H, 6.84; N, 23.93%.

1-{[[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl]amino]acetyl}-4-methylthiosemicarbazide (27)

To a solution of the hydrazide-derivative **26** (7.17 g, 25 mmol) dissolved in methanol (10 mL) a solution of methyl isothiocyanate (1.83 g, 25 mmol) in methanol (10 mL) was added. The reaction mixture was heated at 70-80°C for 3 h. After cooling the solvent was evaporated under reduced pressure and the obtained solid was filtered off, dried under vacuum at room temperature and recrystallized from ethanol to get clear white crystals of the thiosemicarbazide derivative **27**.

Yield: (65%), m.p. 120-122°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.17 (s, 10H, spiro cyclohexyl), 2.05 (s, 3H, CH_3), 3.12 (s, 1H, 1NH, exchangeable with D_2O), 3.35 (d, 2H, *J*=7.5 Hz, CH_2), 5.81, 6.00 (2s, 2H, 2NH, exchangeable with D_2O), 6.12 (t, 1H, *J* = 4.1 Hz, NH, exchangeable with D_2O), 9.61-10.37 (m, 4H, aromatic H), 10.73 (s, 1H, 1NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 30.18 (CH_3), 38.78-40.23 (spiro cyclohexyl carbons), 46.09 (CH_2), 70.81 (spiro head carbon), 104.32-154.53 (aromatic carbons), 170.36 (CO), 186. 06 (CS). IR (KBr, cm^{-1}): 3451-3150 (5NH), 2967 (CH aliphatic), 1640 (CO, amide), 1127 (CS)**.** MS: (EI, 70 eV) m/z (%): M^+360 (10.01), 80 (100). Analysis: for $C_{17}H_{24}N_6OS$ (m.w. 360.48) calcd.: C, 56.64; H, 6.71; N, 23.31; S, 8.90%; found: C, 57.04; H, 7.04; N, 22.83; S, 9.31%.

5-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl]aminomethyl}-4-methyl-4H-1,2,4-triazol-3-thiol (28)

The thiosemicarbazide derivative **27** (0.50 g, 14 mmol) was added portionwise to NaOH solution (2.00 g of NaOH in 25 mL of water). The reaction mixture was refluxed for 3 h, then allowed to cool to room temperature. It was filtered and then the filtrate was acidified with hydrochloric acid. The precipitated solid was filtered, washed thoroughly with water, dried and recrystallized from methanol to give the triazolo-compound **28** as yellowish white crystals.

Yield: 65%, m.p. 269-271°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 2.10 (s, 3H, CH_3), 3.91 (d, 2H, *J* = 7.6 Hz, α-CH_2), 6.43 (t, 1H, *J* = 3.8Hz, NH, exchangeable with D_2O), 6.99-7.51 (m, 4H, aromatic H), 8.39, 9.63 (2s, 2H, SH, NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3413, 3220 (2NH), 2982 (CH aliphatic), 2600 (SH stretching). MS: (EI, 70 eV) m/z (%): M^+ 342 (23.18), 211 (100.00). Analysis: for $C_{17}H_{22}N_6S$ (m.w. 342.46) calcd.: C, 59.62; H, 6.48; N, 24.54; S, 9.36%; found: C, 60.01; H, 6.72; N, 24.93; S, 8.82%.

Spiro {(1H, 2H)-N-[(5-(methylamino)-1,3,4-thiadiazol-2-yl)methyl]quinazoline-2,1'-cyclohexan}-4-amine (29)

To the thiosemicarbazide derivative **27** (2.16 g, 6 mmol), conc. H_2SO_4 (1 mL) was added under continuous stirring. The reaction mixture was stirred at room temperature for 3 h, then added dropwise to cold H_2O. The obtained solid was filtered off, dried and crystallized from ethanol to get the desired thiadiazole derivative **29** as light brown powder.

Yield: 69%, m.p. 160-162°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.18 (s, 10H, spiro cyclohexyl), 2.31 (s, 3H, CH_3), 3.91 (d, 2H, *J* = 7.6 Hz, α-CH_2), 5.76 (t, 1H, *J* = 4.1 Hz, NH, exchangeable with D_2O), 7.38-7.42 (m, 4H, aromatic H), 9.63, 10.31 (2s, 2H, 2NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3413-3110 (3NH), 3003 (CH aromatic), 2950 (CH aliphatic)**.** MS: (EI, 70 eV) m/z (%): M^+ 342 (50.49), 212 (100). Analysis: for $C_{17}H_{22}N_6S$ (m.w. 342.46) calcd.: C, 59.62; H, 6.48; N, 24.54; S, 9.36%; found: C, 59.42; H, 5.92; N, 24.81; S, 9.46%.

Spiro [(2H, 3H)-3-(4-hydroxyphenyl)-quinazoline-2,1'-cyclohexan]-4(1H)-one (30)

A solution mixture of the benzoxazine derivative **5** (2.17 g, 10 mmol,) and *p*-hydroxyaniline (1.09 g, 10 mmol) in glacial acetic acid (20 mL) containing anhydrous sodium acetate (1.64 g, 20 mmol) was refluxed for 15 h. Upon pouring on crushed ice/water, the obtained product was filtered off,

washed with water and recrystallized from methanol to give **30** as light brown crystals.

Yield: 73%, m.p. 230-232°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.12 (s, 10H, spiro cyclohexyl), 7.00-7.90 (m, 8H, aromatic H), 8.92, 10.11 (2s, 2H, NH, OH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 39.91-40.23 (spiro cyclohexyl carbons), 71.31 (spiro head carbon), 116.54-155.79 (aromatic carbons), 162.11 (CO). IR (KBr, cm^{-1}): 3426 (OH), 3400 (NH), 3119 (CH aromatic), 2964 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M^+ 308 (12.41), 119 (100). Analysis: for $C_{19}H_{20}N_2O_2$ (m.w. 308.37) calcd.: C, 74.00; H, 6.54; N, 9.08%; found: C, 74.42; H, 6.22; N, 8.78%.

General procedure for preparation of spiro {(2H, 3H)-3-[4-hydroxy-3-[substituted methyl]phenyl] quinazoline-2,1'-cyclohexan}-4-(1H)-one (31a-c)

A solution of paraformaldehyde (0.90 g, 10 mmol) and the appropriate amine (15 mmol) was refluxed in absolute ethanol (20 mL) for 30 min till complete solubilization of *para*-formaldehyde. Then, a solution of the quinazolinone derivative **30** (3.08 g, 10 mmol) in absolute ethanol (10 mL) was added to the previous mixture and refluxed for 8 h. The product obtained upon cooling the reaction solution was filtered off and recrystallized from dioxane to give the corresponding Mannich bases **31a-c**.

Spiro {(2H, 3H)-3-[4-hydroxy-3-(4-methylpiperazinoylmethyl)phenyl]quinazoline-2,1'-cyclohexan}-4-(1H)-one (31a)

31a (from N-methylpiperazine), the product was obtained as dark red powder, yield: 75%, m.p. 210-212°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.30 (s, 10H, spiro cyclohexyl), 2.42 (s, 3H, CH_3), 2.46 (s, 8H, piperazine), 5.46 (s, 2H, CH_2), 6.85-7.26 (m, 7H, aromatic H), 9.13 (s, 1H, NH, exchangeable with D_2O), 10.32 (s, 1H, OH, exchangeable with D_2O).^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 37.36-39.79 (spiro cyclohexyl carbons), 45.27 (CH_3), 46.36 (CH_2), 49.91, 52.03 ($4CH_2$, piperazine), 71.31 (spiro head carbon), 113.21-154.53 (aromatic carbons), 160.36 (CO). IR (KBr, cm^{-1}): 3550 (OH), 3312 (NH), 2937 (CH aliphatic), 1670 (CO). MS: (EI, 70 eV) m/z (%): $(M + 1)^+$ 421 (49.09), M^+ 420 (11.82), 58 (100). Analysis: for $C_{25}H_{32}N_4O_2$ (m.w. 420.55) calcd.: C, 71.40; H, 7.67; N, 13.32%; found: C, 71.73; H, 7.33; N, 12.93%.

Spiro {(2H, 3H)-3-[4-hydroxy-3-(morpholinomethyl)phenyl]quinazoline-2,1'-cyclohexan}-4-(1H)-one (31b)

31b (from morpholine): the product was obtained as dark brown powder, yield: 73%, m.p. 134-136°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.80 (s, 10H, spiro cyclohexyl), 2.75 (m, 4H, $-N(CH_2)_2$, morpholine ring), 3.71 (m, 4H, $-O(CH_2)_2$, morpholine ring), 5.21 (s, 2H, CH_2), 7.23-7.47 (m, 7H, aromatic H), 8.48, 10.48 (2s, 2H, NH, OH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3455 (OH), 3223 (NH), 3010 (CH aromatic), 2966 (CH aliphatic), 1673 (CO). MS: (EI, 70 eV) m/z (%): $(M + 1)^+$ 408 (11.04), M^+ 407 (5.21), 100 (100). Analysis: for $C_{24}H_{29}N_3O_3$ (m.w. 407.51) calcd.: C, 70.74; H, 7.17; N, 10.31%; found: C, 71.10; H, 6.89; N, 9.92%.

Spiro {(2H, 3H)-3-[4-hydroxy-3-(4-methylpiperidinoylmethyl)phenyl]quinazolin-2,1'-cyclohexan}-4-(1H)-one (31c)

31c (from 4-methylpiperidine): the product was obtained as light brown powder, yield: 73%, m.p. 140-142°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.30 (s, 10H, spiro cyclohexyl), 2.42 (s, 3H, CH_3), 2.46 (s, 9H, piperidine ring), 5.46 (s, 2H, CH_2), 6.85-7.26 (m, 7H, aromatic H), 9.13, 10.32 (2s, 2H, NH, OH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3434 (OH), 3255 (NH), 2966 (CH aliphatic), 1663 (CO). MS: (EI, 70 eV) m/z (%): M^+ 419 (8.21), 175 (100). Analysis: for $C_{26}H_{33}N_3O_2$ (m.w. 419.56) calcd.: C, 74.43; H, 7.93; N, 10.02%; found: C, 74.21; H, 8.24; N, 10.42%.

Spiro {(2H, 3H)-3-[4-(ethoxycarbonylmethoxy)-phenyl]-quinazoline-2,1'-cyclohexan}-4(1H)-one (32)

A solution mixture of the hydroxyl quinazolinone derivative **30** (3.08 g, 10 mmol), ethyl bromoacetate (2.49 mL, 15 mmol) and potassium carbonate (2.00 g, 14 mmol) in dry acetone (20 mL) was refluxed for 12 h. While hot, the reaction mixture was filtered and the filtrate was concentrated under vacuum to give the crude product that was filtered off, washed several times with petroleum ether and recrystallized from ethyl acetate to give the ester compound **32** as dark red crystals.

Yield: 75%, m.p. 144-146°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.17 (t, 3H, J = 6.7 Hz, CH_3 of ethyl group), 1.84 (s, 10H, spiro cyclohexyl), 4.14 (s, 2H, CH_2), 4.67 (q, 2H, J = 7.1 Hz, CH_2 of ethyl group), 6.80-7.43 (m, 8H, aromatic H), 10.13 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 15.23 (CH_3), 38.88-40.06 (spiro cyclohexyl carbons), 61.37 (OCH_2), 65.74 (CH_2CO), 71.31 (spiro head carbon), 113.21-154.53 (aromatic carbons), 160.36 (CO, quinazolinone), 169.4 (CO, ester). IR (KBr, cm^{-1}): 3422 (NH), 2933

(CH aliphatic), 1734, 1673 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 394 (7.03), 77 (100). Analysis: for $C_{23}H_{26}N_2O_4$ (m.w. 394.46) calcd.: C, 70.03; H, 6.64; N, 7.10%; found: C, 69.61; H, 6.82; N, 6.73%.

Spiro {(2H, 3H)-3-[4-hydrazidomethoxyphenyl]quinazoline-2,1'-cyclohexan}-4(1H)-one (33)

Hydrazine hydrate (99%) (1.60 mL, 50 mmol) was added to a solution of the ester derivative **32** (3.94 g, 10 mmol) in absolute ethanol (20 mL) and refluxed for 3 h. The solid separated after concentration and cooling was filtered off and crystallized from isopropanol to yield **33** as brown powder.

Yield: 85%, m.p. 264-266°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.14 (s, 10H, spiro cyclohexyl), 4.14 (s, 2H, CH_2), 6.80-7.43 (m, 8H, aromatic H), 5.67, 8.23, 8.99 (3s, 4H, 2NH, NH_2, exchangeable with D_2O). IR (KBr, cm^{-1}): 3416-3158 (NH_2, 2NH), 3038 (CH aromatic), 2989 (CH aliphatic), 1670, 1631 (2CO). MS: (EI, 70 eV) m/z (%): M^+ 380 (7), 77 (100). Analysis: for $C_{21}H_{24}N_4O_3$ (m.w. 380.44) calcd.: C, 66.30; H, 6.36; N, 14.73%; found: C, 66.75; H, 6.64; N, 14.41%.

Spiro {(2H, 3H)-3-[4-(5-mercapto-1,3,4-oxadiazol-2-yl)methoxyphenyl] quinazoline-2,1'-cyclohexan}-4(1H)one (34)

A mixture of the hydrazide derivative (0.38 g, 1 mmol), KOH (0.056 g, 1 mmol) and CS_2 (1 mL) in absolute ethanol (30 mL) was heated under reflux for 12 h. The reaction mixture was concentrated, cooled and neutralized with conc. HCl. The separated solid was filtered, washed with water, dried and crystallized to give the oxadiazole compound **34** as light brown crystals.

Yield: 82%, m.p. 215-217°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.00 (s, 10H, spiro cyclohexyl), 4.30 (s, 2H, CH_2), 6.90-7.43 (m, 8H, aromatic H), 8.23, 9.97 (2s, 2H, NH, SH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3415 (NH), 3190 (CH aromatic), 2922 (CH aliphatic), 2603 (SH), 1665 (CO). MS: (EI, 70 eV) m/z (%): M^+ 422 (10.03), 97 (100). Analysis: for $C_{22}H_{22}N_4O_3S$ (m.w. 422.50) calcd.: C, 62.54; H, 5.25; N, 13.26; S, 7.59%; found: C, 62.83; H, 5.78; N, 12.92; S, 7.36%.

5-{4-[Spiro ((2H,3H)-4(1H)-oxo-quinazoline-2,1'-cyclohexan)-3-yl]oxyphenyl} pyridazine-3,4,6-trione (35)

A mixture of the hydrazide compound **33** (3.8 g, 10 mmol) and diethyl oxalate (1.46 mL, 10 mmol) and Na metal (0.23 g) in absolute ethanol was refluxed for 12 h. Upon cooling, the reaction mixture was acidified with HCl, and the obtained product was filtered off, dried and recrystallized from dioxane to give the pyridazine compound **35** as yellowish brown powder.

Yield: 65%, m.p. 205-207°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.20 (s, 10H, spiro cyclohexyl), 5.30 (s, 1H, CH), 7.00-7.73 (m, 8H, aromatic H), 8.23, 9.00, 9.97 (3s, 3H, 3NH, exchangeable with D_2O). IR (KBr, cm^{-1}): 3436-3200 (3NH), 3040 (CH aromatic), 2924 (CH aliphatic), 1720 1651 (4CO). MS: (MS: (EI, 70 eV) m/z (%): M^+ 434 (17.22), 96 (100). Analysis: for $C_{23}H_{22}N_4O_5$ (m.w. 434.44) calcd.: C, 63.59; H, 5.10; N, 12.90%; found: C, 63.86; H, 5.43; N, 13.17%.

Cytotoxic activity screening

Preliminary experiments were done using the breast carcinoma cell line to identify the potential cytotoxicity of selected twenty one newly synthesized compounds (**6**, **7a**, **7c**, **8a**, **8b**, **9a**, **9c**, **10b**, **11b**, **13**, **16a**, **17**, **18**, **20**, **22**, **23**, **24**, **25**, **30**, **31a** and **34**) in comparison to the known anticancer drug - doxorubicin, by SRB using the method of Skehan et al. (30) as follows: Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the tested compounds to allow the attachment of cells to the wall of the plate. Different concentrations of the compounds under test (0.0, 1.0, 2.5, 5.0 and 10.0 μg/mL) were added to the cell monolayer triplicate wells which were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulforhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fractions and drug concentrations is plotted to get the survival curve of each tumor cell line for the specified compound. The dose response curve of compounds was analyzed using E_{max} model.

$$\% \text{ Cell viability} = (100 - R) \times \left(1 - \frac{[D]^n}{K_d^m + [D]^n}\right) + R$$

where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., $K_d = IC_{50}$ when R = 0 and E_{max} = 100 - R) (31).

Enzyme assay

The procedure was done according to the supplied protocol of HT Universal Colorimetric PARP

Assay Kit with Histone-coated Strip Wells, 96 well, Cat# 4677-096-K (Trevigen Inc. Gaithersburg, MD, USA) following manufacturer's recommendations. The % inhibition of each compound was calculated by measuring the absorbance of each compound at different concentrations (0, 2, 20, 200, 2000 μM) using 96-well plate ELISA reader with 450 nm filter. By interpretation of data IC_{50} of each compound can be calculated in comparison to 3-AB (provided in kit) as a standard reference (32).

RESULTS AND DISCUSSION

Molecular docking study

Molecular docking is the process that predicts the orientation of organic compounds inside a target macromolecule. Docking process will result in calculation of the affinities of the synthesized compounds toward the specified enzyme. The docking output could be used for explanation of the biological activity. In this work, PARP-1 enzyme was used as a target for our study and as a result the crystal structure of PARP-1 was downloaded from Protein Data Bank (33). PARP-1 has a lot of crystal structures complexed with different inhibitors of different chemical scaffolds. Two crystal structures were found complexed with effective inhibitors of quinazoline scaffold with pdb codes 1UK1 and 3SMI, respectively (34, 35). The two structures were downloaded and aligned in order to find out if they have different binding sites. As a result for the alignment, they were found fitted in the same site that may have different residues numbers in both enzymes due to the different chains length and different numbering of the two enzymes. For example, it was found that in 1UK1 the quinazoline ring of the inhibitor forms hydrogen bond with Gly 863 that has a Ser 904 close to it, while in 3SMI, the quinazoline ring forms hydrogen bond with Gly 1602 close to Ser 1641 (Fig. 1).

What we were looking for was the role of quinazoline ring in the interactions formed in the binding sites and to rank our compounds with different substitutions according to their affinities.

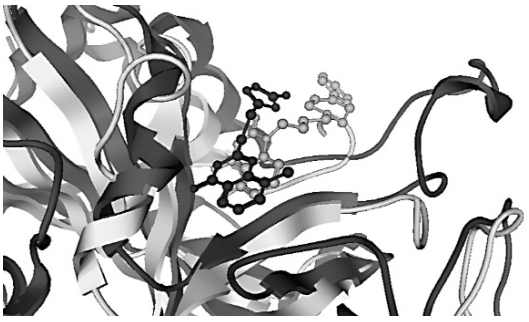


Figure 1. Molecular alignment of 1UK1 (dark grey color with black complexed ligand) and 3SMI (faint grey color with light grey complexed ligand). Both crystal structures are in cartoon representation

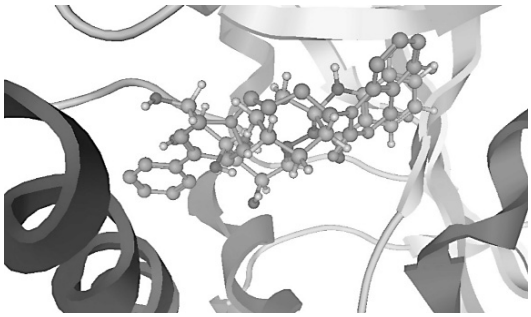


Figure 3. The main binding site represented in cartoon, showing the reported quinazoline inhibitor 1UK1 colored in dark grey aligned with the most active compound (**9a**)

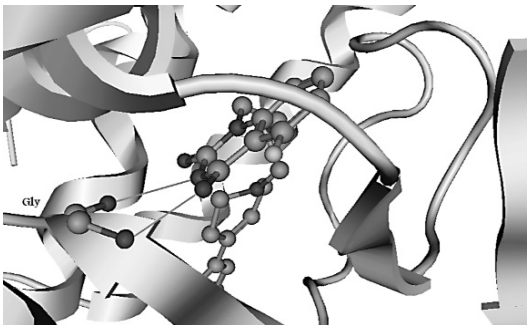


Figure 2. Two hydrogen bonds formed by the quinazoline ring of the inhibitor 1UK1 with Gly 863

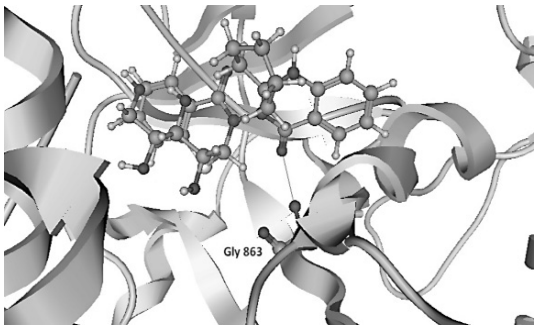


Figure 4. The formation of hydrogen bond between the –C=O group of quinazoline ring of compound (**9a**) and the –NH of Gly 863

Docking process started by using 1UK1 crystal structure and all compounds were built and saved. The molecular docking was performed using Autodock Vina (36). Autodock Vina significantly improves the average accuracy of the binding mode predictions. It includes flexible docking and enables the calculation of all affinities. All the amino acids in the binding sites were selected to be within 5.5 Å from the reported complexed ligand in building the grid box (Fig. 2).

All the synthesized compounds were built and prepared for docking. The protein was prepared as well and the grid box was designed to cover all the surrounding residues and the docking process was performed. As a result, all the resulted affinities were visualized and found that all calculated affinities were ranged from -3.17 to -20.73 kcal/mol. The top selected twenty one scores that have best fitting together calculated affinities (-3.17 to -7.52) were selected. After evaluation of their growth inhibitory activity against breast carcinoma cell line (MCF-7), the most active compounds were tested for their inhibitory activity against PARP-1 enzyme. The sixteen active compounds were further subjected to another calculation of the binding free energy using SPDBV software that was calculated in KJ/mol. The aim from calculating the binding free energy was to evaluate and correlate the docking results with the biological results. Also, to prove that compounds with high affinity values should have lower binding free energy values (Table 1).

According to the docking results, most of the residues found in the binding site were involved in the interactions including Arg 878, Glu 763, Gly 863, Tyr 907, Ser 864, Asp 770 and Lys 908. The docked compounds have different binding modes for their conformations represented by different groups in their structures. For example, the formation of hydrogen bond between the –C=O of quinazoline ring of compound **9a** and –NH group of Gly 863. The hydroxyl groups from the sugar moieties were also involved in the hydrogen bond formation

Table 1. All docking results showing main residues involved in the interactions with docked compounds.

Compound	Affinity kcal/mol	Binding free energy kJ/mol	Main residue from PARP-1	Main atoms from the compound	Distance in Å
6	-7.52	……	Lys 903	C=O of quinazoline ring	2.79
7a	-3.89	-26.51	Glu 763	-OH of sugar	2.41
7c	-4.50	-26.76	Tyr 907	-OH of sugar	2.98
8a	-6.45	……	Lys 903	-OH of sugar	3.54
8c	-7.00	……	Asp 770	C=O of quinazoline ring	3.20
9a	-3.78	-37.31	Gly 863 Ser 864	C=O of quinazoline ring C=O of quinazoline ring	2.37 2.65
9c	-5.46	-27.49	Glu 763	-OH of sugar	2.54
10b	-4.50	-25.79	Tyr 907	C=N	2.47
11b	-3.91	……	Tyr 907	C=O of quinazoline ring	2.5
15	-3.89	-29.05	Arg 878	-CN group	3.12
16a	-4.11	-35.95	Asn 767	C=O of quinazoline ring	2.90
17	-3.87	-28.77	Lys 903	C=O of quinazoline ring	2.92
18	-3.17	-36.85	Lys 908	C=O of quinazoline ring	3.08
20	-6.90	……	Lys 903	C=N of tetrazole ring	2.25
22	-4.08	-27.33	Arg 878	C=O of pyrazolidine ring	3.04
23	-4.25	-36.92	Ser 864	C=O of triazole ring	2.85
24	-4.56	-25.05	Asp 770	C=O of -COO	2.87
25	-5.85	…….	Gly 863	-NH of quinazoline ring	3.60
30	-4.56	-28.81	Gly 863	p-OH group	2.80
31a	-4.04	……	Glu 763	-NH of quinazoline ring	2.76
34	-4.86	-37.11	Arg 878	-NH of quinazoline ring	2.80

with Glu 763 as shown in **9c** and with Tyr 907 as shown in **7c**. The –C=O group of triazolo[4,3-*c*]pyrimidinone ring found in compound **23** has participated in the hydrogen bond formation with Ser 864. The most observed character here is that compound **9a**, that has been found to be the most active compound with lowest IC_{50} value in PARP-1 inhibition, had two main interactions in the binding site. One with Ser 864, and the other with Gly 863, which was interacted with the reported quinazoline inhibitor as well (Figs. 3, 4).

All compounds exhibited good fitting in the binding sites. In addition, the most active compounds with good IC_{50} values had shown their interactions either by the C=O or –NH groups of the quinazoline ring that may be an explanation for their activities, for example, compounds **9a**, **16a**, **18**, **34** as shown in Table 1.

Chemistry

In this study, the key starting benzoxazine **5**, prepared according to the reported method (28, 29), was subjected to aminolysis by its fusion with hydrazine hydrate (37) to give the 3-amino-quinazolinone derivative **6**. IR, ^{1}H NMR, mass spectra and elemental analyses were used for determination and identification of the structures of new compounds. The IR spectrum of derivative **6** revealed the presence of absorption bands in the range of 3452-3277 cm^{-1} corresponding to NH_2 and NH respectively, while the lactam C=O appeared at 1640 cm^{-1}. In order to construct new quinazolinone C-nucleoside analogues, compound **6** was allowed to react with the appropriate linear sugars namely: D-xylose, D-arabinose and D-mannose in absolute ethanol under reflux in the presence of a catalytic amount of glacial acetic acid to yield the corresponding C-nucleosides **7a-c**. Cyclocondensation reaction of **7a-c** with thioglycolic acid in dry benzene led to the formation of the corresponding thiazolidinone analogues **8a-c**. The IR spectra of compounds **7a-c** showed broad absorption bands at the range of 3680-3360 cm^{-1} corresponding to OH and NH groups, while the absorption band of C=O of quinazolinone ring appeared at 1710 cm^{-1}. The IR spectra of **8a-c** revealed broad absorption bands at 3674-3375 cm^{-1} representing OH and NH groups and two bands at 1676-1660 cm^{-1} due to the lactam C=O groups. ^{1}H NMR (DMSO-d_6, δ, ppm) spectra of compounds **8a-c** exhibited two singlets at δ 3.80-3.90 and δ 5.82-5.93 ppm corresponding to $–CH_2$ and S-CH protons of the thiazolidinone ring, while the methine and OH protons of the sugar part appeared as two multiplet signals at δ 3.39-3.63 and 4.13-4.80 ppm. The aromatic protons presented as multiplet signals at δ 7.41-8.80 ppm.

Nucleophilic substitution followed by intramolecular cyclization was carried out upon the reaction of the imino derivatives **7a-c** with thiosalicylic acid in refluxing dry benzene (38) to gain the benzothiazine analogues **9a-c**, respectively. ^{1}H NMR (DMSO-d_6, δ ppm) spectra displayed the methine proton of the benzothiazine ring as a singlet signal at δ 6.44-6.94 ppm and the aromatic protons as multiplet signals at the range δ 6.69-7.99 ppm.

The Schiff's bases **10a-d** were obtained *via* nucleophilic substitution of the amino-quinazolinone derivative **6** with the appropriate aromatic aldehydes, namely: *p*-methoxybezaldehyde, *p*-fluorobenzaldehyde, 2-thiophenaldehyde and pyrrolo-2-carboxaldehyde. Cyclocondensation reaction of **10a-d** with thioglycolic acid in dry benzene yielded the thiazolidinone derivatives **11a-d** and with thiosalicylic acid led to the formation of the benzothiazine derivatives **12a-d**. The IR spectra of **11a-d** and **12a-d** derivatives exhibited two absorption bands at 1715-1650 cm^{-1} representing the 2C=O groups of both quinazolinone and thiazolidinone or benzothiazine rings. At the same time, ^{1}H NMR ($CDCl_3$, δ, ppm) spectra of **11a-d** derivatives revealed two singlet signals in the regions of δ 3.48-3.77 ppm and δ 4.31-5.45 ppm indicating the two protons of $-CH_2$ and the methine proton of S-CH of the thiazolidinone rings, while those of **12a-d** derivatives displayed the methine proton of N-CH of benzothiazine ring at δ 5.21-5.42 ppm in addition to the other protons of the molecules in their expected regions.

Since chlorine atom is a good leaving group, thus nucleophilic substitution reaction of the aminoquinazolinone derivative **6** with 2-chloromethylbenzimidazole (39) in refluxing ethanol using K_2CO_3 as a basic catalyst led to the formation of the benzimidazole compound **13**. Micro analyses and spectral data came in agreement with the structure of this derivative. The characteristic feature of ^{1}H NMR ($CDCl_3$, δ, ppm) spectrum of compound **13** is the appearance of a singlet signal at δ 4.63 ppm corresponding to 2H of $HN-CH_2$ and other 3 singlet signals at δ 8.02, 8.76, 10.13 ppm contributing to the 3 NH protons.

This investigation also deals with the reaction of the aminoquinazolinone derivative **6** with phenacyl bromide in refluxing ethanol to get the corresponding benzoyl methylaminoquinazoline derivative **14**, which was allowed to undergo nucleophilic substitution with malononitrile in refluxing ethanol in the presence of a catalytic amount of sodium

ethoxide as an alkaline medium to obtain the pyrrolo derivative **15**. The IR spectrum of compound **15** revealed the presence of characteristic absorption bands at 3407-3240 cm^{-1} for (NH_2, NH) and at 2208 cm^{-1} representing CN group. Upon fusion of the pyrrolo derivative **15** with urea, thiourea and formamide, nucleophilic substitution occurred followed by intramolecular cyclization to give the pyrrolo[2,3-*d*]pyrimidine derivatives **16a,b** and **17**, respectively. The IR spectrum of **16a** exhibited different absorption bands in the range of 3455-3239 cm^{-1} related to NH_2 and 2NH groups, while the 2C=O groups appeared at 1672 and 1665 cm^{-1}. Mass spectra of the compounds revealed the molecular ion peaks at m/z $(M^{\bullet}- 1)^+$ 439 (10.19), 456 (10.21) and $(M^{\bullet}+ 1)^+$ 425 (11.63), respectively (Scheme 1).

Moreover, this study reports the reaction of benzoxazine derivative **5** with formamide to get the desired intermediate quinazolinone derivative **18**, which was converted to its chloro analogue **19** *via* its exposure to refluxing $POCl_3/PCl_5$.

Tetrazolo-quinazoline derivative **20** was prepared *via* the reaction of the chloro derivative **19** with sodium azide in glacial acetic acid. The IR spectrum of **20** revealed the absence of an azide group, which indicates that the derivative has the tetrazolo structure, beside the appearance of an absorption band at 3454 cm^{-1} corresponding to NH group. Additionally, the mass spectrum exhibited the molecular ion peak of the compound at m/z 241 (100%).

The literature survey revealed that the hydrazino-quinazoline nucleus is a good precursor for synthesis of different heterocyclic ring systems either conjugated or fused to the quinazoline ring. Accordingly, hydrazinolysis of the chloro quinazoline compound **19** was carried out by its reaction with an excess of hydrazine hydrate in ethanol under reflux to gain the hydrazinyl derivative **21** in a high yield. Cyclocondensation of **21** with the bielectrophilic reagent - diethyl malonate, in refluxing glacial acetic acid led to the preparation of pyrazolidin-3,5-dione derivative **22**. The spectral data proved the structure of compound **22**. ^{1}H NMR spectrum exhibited two methylene protons of the pyrazoline ring as a singlet signal at δ 3.60 ppm. In order to obtain the fused triazolo[4,3-*c*]quinazoline **23**, the hydrazinyl derivative **21** was allowed to react with ethyl chloroformate in refluxing pyridine. The IR spectrum of **23** exhibited the presence of C=O group as an absorption band at 1709 cm^{-1} (Scheme 2).

The α-carboxyl and α-amino groups of all amino acids exhibit characteristic chemical reactivity. Thus, in our investigation, the chloroquinazoline derivative **19** was allowed to react with glycine in the presence of Na_2CO_3 as a catalytic base at pH 9-9.5 (40) to get the quinazoline amino acid derivative **24**. ^{1}H NMR of the derivative showed the 2H and 1H of α-CH_2 and NH groups of the amino acid as doublet-triplet signals at δ 3.81 and 6.91 ppm, respectively.

Thionyl chloride is a reactive chemical reagent used in chlorination reactions, converting the carboxylic acid into acid chloride (acyloyl chloride) *via* addition of a chloride ion to the carbonyl carbon followed by elimination of HCl (41). Accordingly, the derivative **24** was refluxed with thionyl chloride in dry chloroform at 70^{o}C to yield the acid chloride compound **25**. Further condensation with an excess of hydrazine hydrate in refluxing absolute ethanol led to the hydrazide analogue **26** which was allowed to react with methyl isothiocyanate in refluxing methanol to give the target thiosemicarbazide derivative **27**. The micro analyses and spectral data were in agreement with the structure of the obtained analogue. ^{1}H NMR spectrum of **27** revealed a singlet signal at δ 2.05 ppm due to 3H of CH_3 group and three singlets at δ 3.12, 5.81, 6.00 ppm corresponding to 3H of 3NH groups of the thiosemicabazide side chain.

It is documented that the intramolecular nucleophilic cyclization of different substituted thiosemicarbazides can be carried *via* their treatment with 2 M NaOH solution to furnish the triazolo derivatives (42), but intramolecular dehydrative cyclization can be carried out by their treatment with conc. H_2SO_4 to obtain compounds bearing 1,3,4-thiadiazole heterocyclic ring system (43). Thus, since the scope of this study is to synthesize new different heterocycles of expected cytotoxic activity, the same methods were used to get the 1,2,4-triazolo- and 1,3,4-thiadiazoloquinazoline derivatives **28** and **29**, respectively. The IR spectrum of **28** showed a stretching band at 2600 cm^{-1} due to the thiol group, in addition to other two stretching bands at the regions 3414-3220 cm^{-1} contributing to 2 NH groups. The IR spectrum of **29** exhibited three stretching absorption bands at the regions 3413-3110 cm^{-1} representing 3 NH groups (Scheme 2).

Another trend in this investigation was the treatment of benzoxazine **5** with the nitrogen nucleophile - *p*-hydroxyaniline in glacial acetic acid in the presence of anhydrous sodium acetate to furnish 4-hydroxyphenylquinazoline derivative **30** to be a new starting key for the synthesis of other heterocyclic functionalities. Since Mannich bases were considered as intermediates in the field of drug synthesis, accordingly, the treatment of ethanolic solution of

Table 2. Cytotoxicity assessment against MCF-7 breast adenocarcinoma cell line.

Compound no.	IC_{50} µM	R fraction %	Compound no.	IC_{50} µM	R fraction %
6	138.9	-	**17**	16.15	0.0
7a	12.17	0.0	**18**	10.04	0.0
7c	21.8	0.0	**20**	301.99	-
8a	16.5	0.0	**22**	41.5	0.0
8c	2.36	0.96	**23**	14.3	0.0
9a	26.7	0.0	**24**	3.44	0.12
9c	18.6	1.4	**25**	243.5	-
10b	34.8	0.0	**30**	17.3	0.0
11b	1.58	0.0	**31a**	2.1	1.95
15	15.11	0.0	**34**	20.5	0.0
16a	22.6	0.0	Doxorubicin	0.13	0.0

IC_{50} = compound concentration required to inhibit tumor cell line proliferation by 50%. Values are the means of three experiments. R fraction % = percentage of unaffected fraction.

Table 3. PARP-1 enzyme assessment of selected sixteen compounds.

Compounds/conc	IC_{50} [a] µM
7a	18.90
7c	2.20
9a	1.45
9c	21.16
10b	18.32
11b	n.d. [b]
15	2.09
16a	1.81
17	2.16
18	1.78
22	2.18
23	1.71
24	19.92
30	2.12
31a	>100
34	1.67
3-AB	2.08

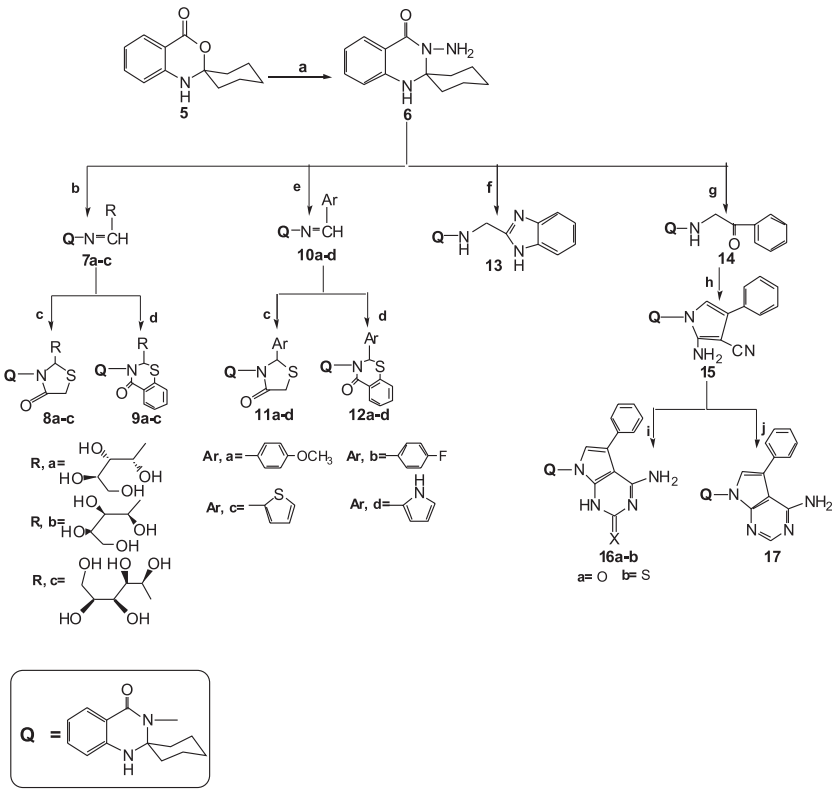
[a] IC_{50} values have been determined by using a commercially available *in vitro* PARP-1 inhibition assay kit (Trevigen, Gaithersburg, MD, USA) following manufacturer's recommendations.
[b] n.d. = not determined

derivative **30** with *p*-formaldehyde and the appropriate secondary amines, namely: N-methylpiperazine, morpholine and *p*-methylpiperidine afforded the corresponding Mannich bases **31a-c**, respectively. ^{1}H NMR spectra of these derivatives exhibited 2 protons of N-CH_2 bridge as a singlet signal at δ 5.21-5.46 ppm, besides the other expected protons at their expected regions.

Further reactions were carried out by reaction of **30** with ethyl bromoacetate in the presence of K_2CO_3 as an acid scavenger to afford the desired corresponding ester **32**. Its ^{1}H NMR spectrum exhibited the characteristic triplet-quartet pattern of ethyl group at δ 1.17, 4.67 ppm, while the 2 protons of the methylene group (O-CH_2) appeared as a singlet signal at δ 4.14 ppm. The ester derivative **32** was employed to synthesize the key intermediate acetohydrazide **33** by its treatment with excess hydrazine hydrate in absolute ethanol. Further treatment of the ethanolic solution of **33** either with CS_2 in the presence of KOH or with diethyl oxalate yielded the target 1,3,4-oxadiazole-2(3H)-thione derivative **34** and pyridazine-3,4,6-trione analogue **35** (44), respectively. Micro analyses and spectral data confirmed the structures of the obtained compounds. Mass spectra revealed the molecular ion peaks at m/e 422 (10.03) and 434 (17.22), respectively (Scheme 3).

In vitro cytotoxic activity

In this work, twenty one of newly synthesized compounds **6**, **7a**, **7c**, **8a**, **8c, 9a**, **9c**, **10b**, **11b**, **15**, **16a**, **17**, **18**, **20**, **22**, **23**, **24**, **25**, **30**, **31a** and **34** that revealed the lowest energy profile towards the target protein, were selected to evaluate their growth inhibitory activity against breast carcinoma cell line (MCF-7) using the sulforhodamine-B (SRB) stain



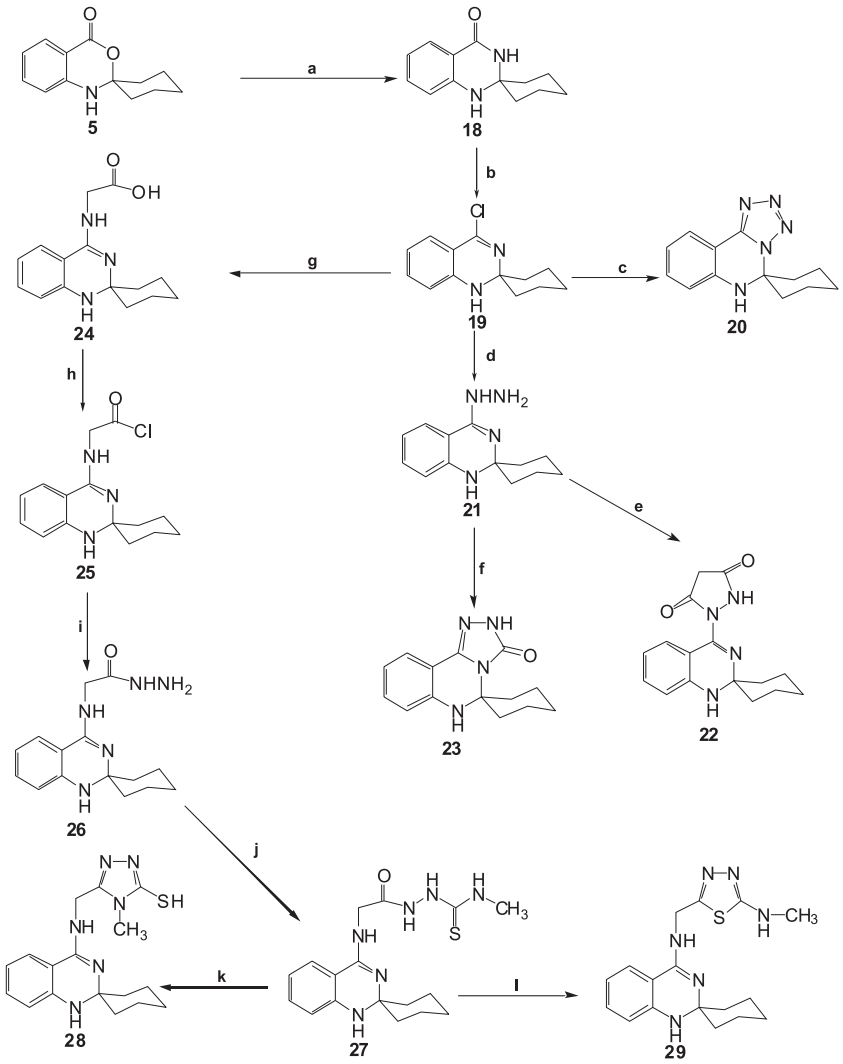
Scheme 1. **a** = NH_2NH_2, EtOH, reflux 4 h; **b** = aldoses, EtOH/drops glacial AcOH, reflux 6 h; **c** = $HSCH_2COOH$, dry C_6H_6, reflux 16 h; **d** = thiosalicylic acid, dry C_6H_6, reflux 16 h; **e** = aromatic aldehyde, EtOH/glacial AcOH, reflux 12 h; **f** = 2-chlorobenzimidazole, EtOH, anhyd. K_2CO_3, reflux 16 h; **g** = $PhCOCH_2Br$, EtOH, reflux 3 h; **h** = $CH_2(CN)_2$, EtOH/EtONa, reflux 3 h; **i** = urea/thiourea, fusion 220°C, 20 min; **j** = HCHO, reflux 5 h

assay in a trial to correlate both docking and cytotoxic evaluation studies. Two parameters, IC_{50} and resistant fraction percentage (R fraction %) were determined for each compound using doxorubicin as a reference standard (30).

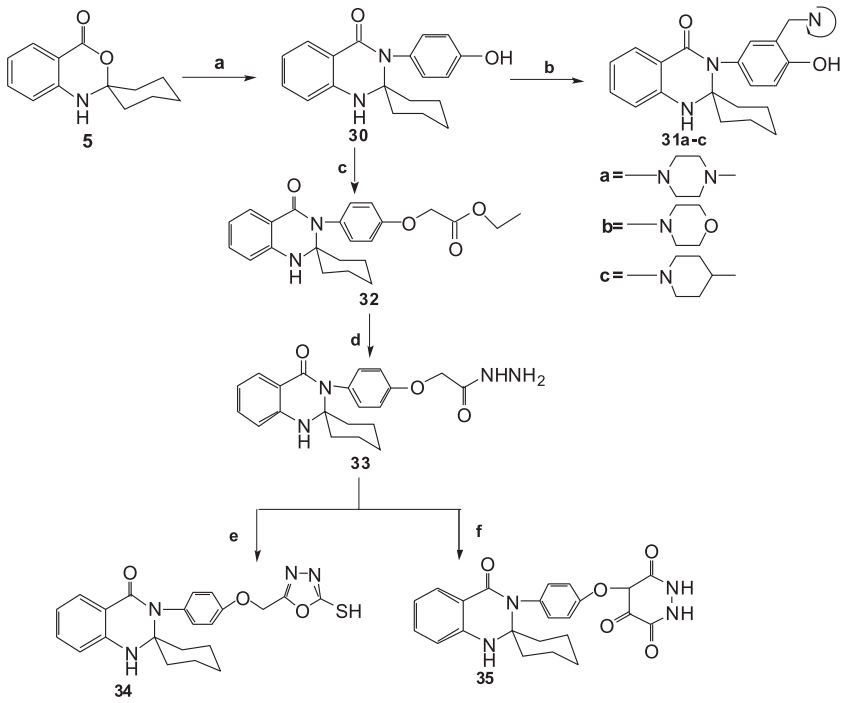
According to the obtained results (Table 2), most of the tested quinazoline analogues exhibited distinctive growth inhibitory activity against MCF-7 breast adenocarcinoma cell line. Regarding the 3-substituted quinazolinones derivatives, the starting 3-aminoquinazoline derivative **6** was inactive with IC_{50} = 138.9 µM and has high percentage resistant fraction. The data showed that the C-nucleoside Shiff's bases **7a**, **7c** exhibited promising cytotoxic activity against breast carcinoma cell line comparable to doxorubicin (IC_{50} = 12.17, 21.8 µM, respectively) with 0% resistant fraction. The benzothiazine analogue **9a** appeared to be a less effective cytotoxic agent with IC_{50} = 26.7 µM, while the other thiazolidinone and benzothiazine analogues **8a**, **9c** did not exhibit distinctive differences from their starting precursors (IC_{50} = 16.5, 18.6 µM, respectively) and the resistant fractions are still 0%. The most potency was gained by the thiazolidinone analogue of mannose sugar **8c** (IC_{50} = 2.36 µM) with approximate 0% resistant fraction. Unexpectedly, *p*-fluoro Schiff's base **10b** exhibited a decreased cytotoxic potency to reach IC_{50} = 34.8 µM but with complete disappearance of cell resistance (R fraction = 0%). Great increase in the activity to be approximately equipotent to doxorubicin was achieved by the fluoro thiazolidinone analogue **11b** with IC_{50} = 1.58 µM and 0% resistant fraction. The study also exhibited that the replacement of thiazolidinone ring with a pyrrole ring, as in compound **15,** or fused pyrrolo-pyrimidine ring system as in compounds **16a**, **17** led to a remarkable decrease in the anticancer activity (IC_{50} = 15.11, 22.6, 16.15 µM, respectively) with complete absence of cell resistance (R fraction = 0%). The quinazolinone derivative **18** with no ring attachment at 3-nitrogen position exhibited a slight increase in the cytotoxic activity (IC_{50} = 10.04 µM, R fraction = 0%).

With respect to the group of the 4-substituted quinazoline derivatives, the cytotoxic evaluation showed a wide variation according to the different substituents conjugated to the 4-position of quinazoline ring. Marked efficacy has been gained by the derivative having quinazoline ring attached to the amino acid glycine **24** (IC_{50} = 3.44 µM, R fraction = 0.12%), then the efficacy started to reduce by the analogue bearing fused triazolo-quinazoline **23** (IC_{50} = 14.3 µM, R fraction = 0%). Further reduction was observed by that bearing the pyrazolidione ring compound **22** (IC_{50} = 41.5 µM, R fraction = 0%). It can be seen in the Table that the attachment of *p*-hydroxy group or oxadiazole ring *via* an ether linkage to the phenyl ring as in derivatives **30** and **34** resulted in moderate activity (IC_{50} = 17.3, 20.5 µM and R fraction = 0%). A significant activity (slightly less than that of doxorubicin) was revealed by the Mannich base analogue **31a** (IC_{50} = 2.1 µM, R fraction = 1.95%). Unfortunately, the derivatives bearing fused tetrazolo-quinazoline ring and the chloro derivative of the glycine analogue **20**, **25** were completely inactive.

Thus, it can be concluded that, the highest cytotoxic activity that is approximately equipotent to that of the reference doxorubicin against breast carcinoma cell line was gained by the quinazoline derivative bearing *p*-fluorophenylthiazolidine-4-one



Scheme 2. **a** = formamide, reflux 3 h; **b** = $PCl_5/POCl_3$, reflux 2 h, water bath; **c** = NaN_3, glacial AcOH, reflux 6 h; **d** = NH_2NH_2, EtOH, reflux 8 h; **e** = $CH_2(COOEt)_2$, glacial AcOH, reflux 8 h; **f** = ClCOOEt, pyridine, reflux 6 h; **g** = glycine, Na_2CO_3, reflux at 100°C, 8 h; **h** = $SOCl_2$, $CHCl_3$, 70°C, 30 min; **i** = NH_2NH_2, EtOH, reflux 3 h; **j** = CH_3NCS, MeOH, reflux 70-80°C, 3 h; **k** = 2 M NaOH, reflux 80°C, 3 h; **l** = H_2SO_4, room temp. 3 h



Scheme 3. **a** = *p*-hydroxyaniline, glacial AcOH, anhyd. CH_3COONa, reflux 15 h; **b** = sec. amines, EtOH, reflux 8 h; **c** = $EtCOOCH_2Br$, anhyd. K_2CO_3, dry CH_3COCH_3, reflux 12 h; **d** = NH_2NH_2, EtOH, reflux 3 h, **e** = CS_2, KOH, EtOH, reflux 12 h; **f** = $(COOEt)_2$, EtONa, reflux 12 h

11b (IC_{50} = 1.58 µM, R fraction = 0%). The significance of the activity decreased to a lesser extent by the quinazoline derivatives carrying thiazolidinone-mannose moiety (**8c**), the Mannich base side chain **31a** and the amino acid glycine **24** (IC_{50} = 2.36, 2.1, 3.44 µM) with approximate 0% of R fraction.

The above mentioned points revealed the importance of the conjugation of quinazoline nucleus with thiazolidinone, Mannich base side chain and glycine substituents for exhibiting the desired cytotoxic activity, the ideas that can be taken in our consideration in the future designing and synthesis of novel quinazoline derivatives to get more selective and efficient anticancer agents (Table 2).

PARP-1 inhibitory activity screening

The main target of this research was to study the inhibition of PARP-1 enzyme by the novel synthesized quinazoline derivatives that showed high *in-vitro* cytotoxic activity against breast cancerous cell lines. It was very interesting to find a relationship between the biological evaluation of the synthesized quinazoline analogues and their inhibitory effect on PARP-1 enzyme, so we can deduce if the cytotoxic potency is due to PARP-1 enzyme inhibition or due to another mechanism of action. For this reason, the derivatives that exhibited the highest potency against breast carcinoma cell lines and were soluble in ethanol were chosen as representative examples to study their effects on the enzyme inhibition. Thus, compounds **7a**, **7c**, **9a**, **9c**, **10b**, **11b**, **15**, **16a**, **17**, **18**, **22**, **23**, **24**, **30**, **31a** and **34** were selected for enzyme assay performance.

According to the data of IC_{50} concentrations of the tested derivatives presented in Table 3, it can be concluded that most of the derivatives exhibited enzyme inhibition at low IC_{50} concentrations even less than that of the reference standard 3-aminobenzamide (3-AB). It is obvious that the C-nucleoside Shiff's base of mannose sugar **7c** gave high inhibitory activity that is approximately equipotent to that of 3-AB (IC_{50} = 2.2 µM), but its cyclized benzothiazine analogue **9c** exhibited low inhibitory activity (IC_{50} = 21.16 µM). At the same time, the xylose sugar analogue **7a** displayed low activity (IC_{50} = 18.9 µM), while its cyclized benzothiazine analogue **9a** exhib-

ited very high potency even greater than that of 3-AB (IC_{50} = 1.45 µM). The derivatives carrying pyrrole ring or fused pyrrolo-pyrimidine ring system in conjugation with quinazoline core **15**, **16a**, **17** appeared to be effective PARP-1 enzyme inhibitors (IC_{50} = 2.09, 1.81, 2.16 µM) and they are equipotent to 3-AB.

Also, it can be noted that the quinazoline intermediate having no substituents attached to 3-N-position (**18**) exhibited higher potency than the reference drug (IC_{50} = 1.78 µM).

When the quinazoline core is fused to heterocyclic rings carrying more than one nitrogen atoms such as pyrazolidine-dione **22** or triazolone **23**, the derivatives showed high inhibitory activity (IC_{50} = 2.18, 1.71 µM). The resultant data also showed that the *p*-hydroxyphenyl-quinazoline derivative **30** was an active enzyme inhibitor similar to 3-AB (IC_{50} = 2.12 µM) and higher inhibitory activity was gained by the oxadiazole analogue **34** (IC_{50} = 1.67 µM) to be a more effective inhibitor than the standard. Although the amino acid glycine analogue **24** exhibited high potency as cytotoxic agent, it appeared to have a lower inhibitory activity on PARP-1 enzyme (IC_{50} = 19.92 µM).

An unexpected result was obtained by the thiazolidine-4-one derivative **11b** and the Mannich base analogue **31a** which exhibited great potency as anti-breast cancer agents; they displayed complete loss of PARP-1 enzyme inhibition activity. This result can be explained that these derivatives might produce their cytotoxic activity *via* inhibition of other enzymes or affecting a specific stage of cell growth cycle (Table 3).

CONCLUSION

This study described the molecular design and the synthesis of novel series of spiro [(2H,3H) quinazoline-2,1`-cyclohexan]-4(1H)-one derivatives as PARP-1 inhibitors. Twenty one derivatives that exhibited the best fitting to the target protein were selected to evaluate their *in vitro* cytotoxic activity. Further selection was performed for the compounds that showed the highest cytotoxic activity (16 compounds) to study their inhibitory effect on PARP-1 enzyme. It has been found that most of the proposed quinazoline derivatives exhibited effective anti-proliferative activity against breast carcinoma cell lines and showed effective inhibitory effect on the target PARP-1 enzyme. The study also exhibited that although most of the derivatives showed dual potency such as **7c**, **9a**, **15**, **16a**, **17**, **18**, **23**, **30** and **34**, but some showed complete loss of enzyme inhibition such as the derivatives **11b** and **31a**.

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