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) transferases 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-
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 carbboxamide, 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 carbboxamide 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), benzothiazine (19), benimidazole (20), pyridazine (21) and pyrazole (22) or anticancer activity depending on other mechanisms such as pyrrolopyrimidine (23, 24), oxadiazole (25), triazole (26) and thia diazole (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 MGLTools 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 (Merek) 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). 1H NMR and 13C NMR spectra were deter-
A novel class of substituted spiro [quinazoline-2',1'-cyclohexane] derivatives...

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. 'H NMR (300 MHz, CDCl₃, δ, ppm): 1.20 (s, 10H, spiro cyclohexyl), 5.21 (s, 2H, NH₂, exchangeable with D₂O), 6.91-7.62 (m, 4H, aromatic H), 10.13 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3452-3277 (NH₂, NH), 3077 (CH aromatic), 1640 (CO). MS: (EI, 70 eV) m/z (%): 231 (10.17), 119 (100). Analysis: for C₁₃H₁₇N₃O (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/petroleum ether to yield compound 7a-c, respectively.

7a (from xylose), was obtained as brown powder, yield: 60%, m.p. 117-119°C. 'H NMR (300 MHz, CDCl₃, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.39-3.60 (m, 5H, aldoses), 4.25-4.30 (m, 4H, 4OH, exchangeable with D₂O), 7.10-8.03 (m, 5H, aromatic H -N=CH), 10.21 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3580-3481 (OH), 3365 (NH), 3070 (CH aromatic), 2932 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M⁺ 363 (12.07), 119 (100). Analysis: for C₁₈H₂₅N₃O₅ (m.w. 363.41) calcd.: C, 59.49; H, 6.93; N, 11.56%; found: C, 59.73; H, 7.31; N, 11.85%.

7b (from arabinose), was obtained as light brown crystals, yield: 65%, m.p. 81-83°C. 'H NMR (300 MHz, CDCl₃, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.39-3.66 (m, 5H, aldoses), 4.35-4.30 (m, 4H, 4OH, exchangeable with D₂O), 7.10-8.03 (m, 5H, aromatic H -N=CH), 10.21 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3587-3481 (OH), 3365 (NH), 2915 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M⁺ 363 (10.14), 119 (100). Analysis: for C₁₈H₂₅N₃O₅ (m.w. 363.41) calcd.: C, 59.49; H, 6.93; N, 11.56%; found: C, 59.73; H, 7.31; N, 11.85%.

7c (from mannose), was obtained as pale yellow powder, yield: 70%, m.p. 73-75°C. 'H NMR (300 MHz, CDCl₃, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 3.03-3.21 (m, 6H, aldoses), 4.51-4.73 (m, 5H, 5OH, exchangeable with D₂O), 7.52-8.64 (m, 5H, aromatic H -N=CH), 10.21 (s, 1H, NH, exchangeable with D₂O). 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 C₁₉H₂₇N₃O₆ (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 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.

8a-c (from xylose), was obtained as pale yellow powder, yield: 70%, m.p. 73-75°C. 'H NMR (300 MHz, CDCl₃, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 3.39-3.66 (m, 5H, aldoses), 4.35-4.30 (m, 4H, 4OH, exchangeable with D₂O), 7.52-8.64 (m, 5H, aromatic H -N=CH), 10.21 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3680-3480 (OH), 3360 (NH), 3077 (CH aromatic), 2937 (CH aliphatic), 1710 (CO). MS: (EI, 70 eV) m/z (%): M⁺ 363 (12.07), 119 (100). Analysis: for C₁₈H₂₅N₃O₅ (m.w. 363.41) calcd.: C, 59.49; H, 6.93; N, 11.56%; found: C, 59.73; H, 7.31; N, 11.85%.
8a (from xylose), was obtained as dark brown powder, yield: 60%, m.p. 180-182°C. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.04 (s, 1H, spiro cyclohexyl), 3.39-3.51 (m, 5H, aldoses), 3.80 (s, 2H, CH₂, thiazolidinone ring), 4.13-4.20 (m, 4H, 4OH, exchangeable with D₂O), 5.91 (s, 1H, S-CH, thiazolidinone ring), 7.41-7.68 (m, 4H, aromatic H), 8.54 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (300 MHz, CDCl₃, δ, ppm): 39.15-39.86 (spiro cyclohexyl carbons), 43.79 (spiro head carbon), 70.50 (spiro head carbon), 75.62 (2' carbon of xylose), 71.89 (3' carbon of xylose), 73.16 (1' carbon of xylose), 114.47-148.39 (aromatic carbons), 158.26 (CH, quinazoline), 170.03 (CO, thiazolidinone). IR (KBr, cm⁻¹): 3545-3430 (OH), 3320 (NH), 3092 (CH aromatic), 1679, 1660 (2CO). MS: (EI, 70 eV) m/z (%): M⁺ 437 (12.03), 436 (100). Analysis: for C₂₅H₃₀N₃O₆S (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, 6.82; S, 6.21%.

8b (from arabinose), was obtained as dark yellow powder, yield: 60%, m.p. 189-191°C. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 5.85 (s, 1H, NH, exchangeable with D₂O), 10.00 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3523-3475 (OH), 3375 (NH), 3061 (CH aromatic), 2963 (CH aliphatic), 1679, 1665 (2C=O). MS: (EI, 70 eV) m/z (%): (M - 1)+ 438 (58.33), 437 (75.00), 419 (80.77), 55 (100). Analysis: for C₂₀H₂₇N₃O₆S (m.w. 437.51) calcd.: C, 54.90; H, 6.22; N, 9.43; S, 7.65%.

8c (from mannose), was obtained as brown powder, yield: 65%, m.p. 208-210°C. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.41-3.63 (m, 6H, aldoses), 3.85 (s, 2H, CH₂, thiazolidinone ring), 4.25-4.27 (m, 5H, 5OH, exchangeable with D₂O), 5.93 (s, 1H, S-CH, thiazolidinone ring), 7.41-7.68 (m, 4H, aromatic H), 8.54 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3674-3486 (OH), 3380 (NH), 2928 (CH aliphatic), 1676, 1665 (CO). MS: (EI, 70 eV) m/z (%): M⁺ 467 (10.07), 220 (100.00). Analysis: for C₃₂H₃₄N₄O₇S (m.w. 549.66) 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-[(E)-polyhydroxalkylidene]-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-[(1,2,3,4-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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.04 (s, 1H, spiro cyclohexyl), 2.68-2.82 (m, 5H, aldoses), 4.03-4.25 (m, 4H, 4OH, exchangeable with D₂O), 6.94 (s, 1H, CH, benzothiazine ring), 7.29-7.98 (m, 8H, aromatic H), 9.03 (s, 1H, NH, exchangeable with D₂O). ¹C NMR (300 MHz, DMSO-d₆, 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, quinazoline), 160.07 (CO, benzothiazine). IR (KBr, cm⁻¹): 3530-3415 (OH), 3323 (NH), 2961 (CH aromatic), 2963 (CH aliphatic), 1679, 1665 (CO). MS: (EI, 70 eV) m/z (%): M⁺ 499 (40.68), 225 (100). Analysis: for C₂₅H₂₉N₃O₆S (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, 6.82; S, 6.21%.

9b (from arabinose) was isolated as light brown powder, yield: 63%, m.p. 168-170°C. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.00 (s, 10H, spiro cyclohexyl), 2.60-2.92 (m, 5H, aldoses), 4.11-4.30 (m, 4H, 4OH, exchangeable with D₂O), 6.54 (s, 1H, CH, benzothiazine ring), 7.30-7.96 (m, 8I, aromatic H), 9.13 (s, 1H, NH, exchangeable with D₂O). 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 C₂₅H₂₉N₃O₆S (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, 6.82; S, 6.21%.

9c (from mannose), was obtained as dark brown powder, yield: 65%, m.p. 207-209°C.
A novel class of substituted spiro [quinazoline-2,1'-cyclohexane] derivatives...

NMR (300 MHz, CDCl₃, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 3.34-3.51 (m, 6H, aldoses), 4.07-4.11 (m, 5H, 5OH, exchangeable with D₂O), 6.44 (s, 1H, CH, benzothiazine ring), 6.69-7.99 (m, 8H, aromatic H), 8.19 (s, 1H, NH, exchangeable with D₂O). 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 C₂₆H₃₁N₃O₇S (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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.24 (s, 10H, spiro cyclohexyl), 3.85 (s, 3H, OCH₃), 4.12 (s, 1H, -N=CH), 6.94-7.86 (m, 8H, aromatic H), 8.61 (s, 1H, NH, exchangeable with D₂O). 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 C₂₁H₂₃N₃O₂ (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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.21 (s, 10H, spiro cyclohexyl), 4.71 (s, 1H, -N=CH), 7.12-7.85 (m, 8H, aromatic H), 8.65 (s, 1H, NH, exchangeable with D₂O). 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 C₂₀H₂₀FN₃O (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. 160-162°C. ¹H NMR (300 MHz, CDCl₃, 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 D₂O). 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 C₁₈H₁₉N₃OS (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-2-yl)-methyleneamino]-quinazoline-2,1'-cyclohexan]-4(1H)-one (10d)

The product was obtained as light brown powder, yield: 74%, m.p. 120-122°C. ¹H NMR (300 MHz, CDCl₃, 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 D₂O). IR (KBr, cm⁻¹): 3422, 3212 (2NH), 2928 (CH aliphatic), 1671 (CH aliphatic), 1630 (CO), 1544 (CO, quinazolinone), 1682 (CO, thiazolidinone). MS: (EI, 70 eV) m/z (%): M⁺ 308 (10.07), 77 (100).

Analysis: for C₁₈H₂₀N₄O (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 Na₂CO₃ solution, then filtered off and crystallized from isopropanol to obtain the desired products 11a-d, respectively.

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

The product was obtained as light brown powder, yield: 80%, m.p. 165-167°C. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.04 (s, 10H, spiro cyclohexyl), 3.48 (s, 2H, CH₂, thiazolidinone ring), 3.73 (s, 3H, OCH₃), 4.31 (s, 1H, S-CH, thiazolidinone ring), 6.84-7.35 (m, 8H, aromatic H), 10.21 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 36.32 (CH₂, thiazolidinone), 39.15-39.47 (spiro cyclohexyl carbons), 47.36 (CH, thiazolidinone), 55.39 (CH₃), 71.55 (spiro head carbon), 113.46-153.51 (aromatic carbons), 163.47 (CO, quinolinone), 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 C₂₃H₂₅N₃O₃S (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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.24 (s, 10H, spiro cyclohexyl), 3.77 (s, 2H, CH₂, 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 D₂O).

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 C₂₂H₂₂FN₃O₂S (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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.08 (s, 10H, spiro cyclohexyl), 3.65 (s, 2H, CH₂, 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 D₂O).

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 C₂₀H₂₁N₃O₂S₂ (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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 3.52 (s, 2H, CH₂, 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 D₂O).

IR (KBr, cm⁻¹): 3355 (NH), 2924 (CH aliphatic), 1695, 1650 (2CO). MS: (EI, 70 eV) m/z (%): M+ 473 (7.67), 119 (100). Analysis: for C₂₀H₂₂N₄O₂S (m.w. 473.48) 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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.24 (s, 10H, spiro cyclohexyl), 3.85 (s, 3H, OCH₃), 5.21 (s, 1H, S-CH, benzothiazine ring), 6.95-8.12 (m, 12H, aromatic H), 8.62 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 39.15-39.47 (spiro cyclohexyl carbons), 55.59 (CH₃), 60.16 (CH, benzothiazine), 71.55 (spiro head carbon), 112.03-153.51 (aromatic carbons), 160.13 (CO, quinazoline), 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 C₂₈H₂₇N₃O₃S, (m.w. 485.60) calcd.: C, 69.25; H, 5.60; N, 8.65% 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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.18 (s, 10H, spiro cyclohexyl), 5.25 (s, 1H, S-CH, benzothiazine ring), 7.12-7.84 (m, 12H, aromatic H) 8.62 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 34.45 (NH), 2927 (CH aliphatic), 1710, 1673 (2CO). MS: (EI, 70 eV) m/z (%): M+ 485 (6.39), 77 (100). Analysis: for C₂₇H₂₄FN₃O₂S (m.w. 473.56) calcd.: C, 69.25; H, 5.60; N, 8.65% found: C, 68.86; H, 5.98; N, 9.01; S, 6.38%.

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 (12c)

The product was obtained as white powder, yield: 82%, m.p. 121-123°C. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 3.52 (s, 2H, CH₂, 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 D₂O). IR (KBr, cm⁻¹): 3355 (NH), 2924 (CH aliphatic), 1695, 1650 (2CO). MS: (EI, 70 eV) m/z (%): M+ 473 (7.67), 119 (100). Analysis: for C₂₈H₂₇N₃O₃S, (m.w. 473.56) 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%
A novel class of substituted spiro [quinazoline-2,1'-cyclohexane] derivatives...

**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, CDCl₃, 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 D₂O). IR (KBr, cm⁻¹): 3440 (NH), 3048 (CH aromatic), 2923 (CH aliphatic), 1700, 1665 (2CO). MS: (EI, 70 eV) m/z (%): 445 (M⁺ + 1)+ (12.65), 444 (10.55), 186 (100).

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

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.

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

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.

**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 (16b)**

A solution 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.

**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)**

2-Amino-1-[spiro {(2H,3H)-4(H)oxo-quinazoline-2,1'cyclohexan}-3-y]-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.

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

The pyrrole 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/2-thio-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, CDCl₃, δ, ppm): 2.01 (s, 10H, spiro cyclohexyl), 5.69, 9.03, 9.80 (3s, 4H, NH₃, 2NH, exchangeable with D₂O), 6.65-7.48 (m, 10H, aromatic H + pyrrole...
 Spiro \[(2H, 3H)-3-[4-amino-5-phenyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl]-quinazoline-2,1'-cyclohexan]-4(1H)-one (16b)\]

The product was isolated as dark brown powder, yield: 82%, m.p. 245-247 °C. 1H NMR (300 MHz, CDCl₃, δ ppm): 2.41 (s, 10H, spiro cyclohexyl), 5.00, 8.80, 9.50 (3s, 4H, NH₂, 2NH, exchangeable with D₂O), 6.95-7.60 (m, 10H, aromatic H + pyrrole H). IR (KBr, cm⁻¹): 3460-3181 (NH₂, 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 C₂₅H₂₄N₆O₅ (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 \[(1H, 2H)-4-chloroquinazoline-2,1'-cyclohexane\] (19)

A suspension of compound 18 (2.16 g, 10 mmol) and PCl₅ (0.5 g) in POCl₃ (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. 1H NMR (300 MHz, CDCl₃, δ ppm): 6.98-7.50 (m, 4H, aromatic H), 8.50 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3418 (NH), 3050 (CH aromatic), 2856 (CH aliphatic), MS: (EI, 70 eV) m/z (%): M + 236 (13.20), M + 234 (4.40), 146 (100). Analysis: for C₁₃H₁₅ClN₂ (m.w. 234.72) calcd.: C, 66.41; 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/H₂O. 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. 1H NMR (300 MHz, CDCl₃, δ ppm): 7.00-7.50 (m, 4H, aromatic H), 8.92 (s, 1H, NH, exchangeable with D₂O). IR (KBr, cm⁻¹): 3418 (NH), 3050 (CH aromatic), 2977 (CH aliphatic), MS: (EI, 70 eV) m/z (%): M + 241 (100). Analysis: for C₁₃H₁₅N₅ (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. ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.22 (s, 10H, spiro cyclohexyl), 3.80-5.70 (m, 4H, aromatic H), 6.80-7.60 (m, 4H, aromatic H), 8.11 (s, 1H, NH), exchangeable with D₂O). IR (KBr, cm⁻¹): 3423, 3320 (NH, OH), 2929 (CH aliphatic), 1709 (CO). MS: (EI, 70 eV) m/z (%): (M + 1)+ 274 (10.62), M+ 273 (4.61), 230 (100). Analysis: for C₁₅H₁₉N₃O₂ (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 30 min at 70°C. After cooling, the solvent was evaporated under reduced pressure and the obtained crude product was crystallized from methanol to give the chloroquinazoline derivative 25 as white powder.

Yield: (61%), m.p. 146-148 °C. ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 1.91 (s, 10H, spiro cyclohexyl), 3.93 (d, 2H, J = 7.8 Hz, -CH₂), 6.70 (t, 1H, J = 4.0 Hz, NH), 7.12-7.74 (m, 4H, aromatic H), 8.11 (s, 1H, NH, exchangeable with D₂O). 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 C₁₅H₁₆ClN₂O (m.w. 291.78) calcd.: C, 61.75; H, 6.22; N, 14.04%; found: C, 61.42; H, 5.85; N, 14.04%.
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 dioxiane to get the hydrazide compound 26 as light brown powder.

Yield: (65%), m.p. 180-182°C. 'H NMR (300 MHz, DMSO-d6, δ, ppm): 1.21 (s, 10H, spiro cyclo-hexyl), 3.91 (d, 2H, J = 7.8 Hz, α-CH2), 5.21 (s, 2H, NH2, exchangeable with D2O), 6.21 (t, 1H, J = 3.4 Hz, NH, exchangeable with D2O), 6.99-7.51 (m, 4H, aromatic H), 8.91, 9.01 (2s, 2H, 2NH, exchangeable with D2O). IR (KBr, cm⁻¹): 3438-3150 (NH2, 3NH), 1641 (CO), 1127 (CS). MS: (EI, 70 eV) m/z (%): M⁺ 360 (50.49), 212 (100). Analysis: for C15H21N5O (m.w. 287.36) calcd.: C, 62.70; H, 7.37; N, 24.37%; found: C, 69.69; H, 6.48; N, 23.93%.

1-[[N-(1H,2H)-quinazoline-2,1'-cyclo-hexan]-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) was added portionwise to NaOH solution (2.00 g of NaOH in 25 mL of water). The reaction mixture was refluxed for 15 h. Upon pouring on crushed ice/water, the obtained product was filtered off, dried and recrystallized from ethanol to get the desired thiosemicarbazide derivative 27.

Yield: 69%, m.p. 160-162°C. 'H NMR (300 MHz, DMSO-d6, δ, ppm): 1.18 (s, 10H, spiro cyclo-hexyl), 2.05 (s, 3H, CH3), 3.12 (s, 1H, 1NH, exchangeable with D2O), 3.35 (d, 2H, J =7.5 Hz, CH2), 5.81, 6.00 (2s, 2H, NH, exchangeable with D2O), 6.12 (t, 1H, J = 4.1 Hz, NH, exchangeable with D2O), 9.61-10.37 (m, 4H, aromatic H), 10.73 (s, 1H, 1NH, exchangeable with D2O). 13C NMR (300 MHz, DMSO-d6, δ, ppm): 30.18 (CH3), 38.78-40.23 (spiro cyclohexyl carbons), 46.09 (CH2), 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), 192 (100). Analysis: for C15H19N5SO (m.w. 360.48) calcd.: C, 56.64; H, 6.71; N, 23.31; S, 9.80%; found: C, 57.04; H, 7.04; N, 22.83; S, 9.31%.

5-[[N-(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 triazole-compound 28 as yellowish white crystals.

Yield: 65%, m.p. 269-271°C. 'H NMR (300 MHz, DMSO-d6, δ, ppm): 1.21 (s, 10H, spiro cyclo-hexyl), 2.10 (s, 3H, CH3), 3.91 (d, 2H, J = 7.6 Hz, α-CH2), 6.43 (t, 1H, J = 3.9Hz, NH, exchangeable with D2O), 6.99-7.51 (m, 4H, aromatic H), 8.39, 9.63 (2s, 2H, SH, NH, exchangeable with D2O). 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 C15H19N5S (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-thia-diazol-2-yl)methyl]quinazoline-2,1'-cyclohexan]-4-amine (29)

To the thiosemicarbazide derivative 27 (2.16 g, 6 mmol), conc. H2SO4 (1 mL) was added under continuous stirring. The reaction mixture was stirred at room temperature for 3 h, then added dropwise to cold H2O. The obtained solid was filtered off, dried and crystallized from ethanol to get the desired thia-diazole derivative 29 as light brown powder.

Yield: 69%, m.p. 160-162°C. 'H NMR (300 MHz, DMSO-d6, δ, ppm): 1.18 (s, 10H, spiro cyclohexyl), 2.31 (s, 3H, CH3), 3.91 (d, 2H, J = 7.6 Hz, α-CH2), 5.76 (t, 1H, J = 4.1 Hz, NH, exchangeable with D2O), 7.38-7.42 (m, 4H, aromatic H), 10.73 (s, 1H, 1NH, exchangeable with D2O). 13C NMR (300 MHz, DMSO-d6, δ, ppm): 30.18 (CH3), 38.78-40.23 (spiro cyclohexyl carbons), 46.09 (CH2), 70.81 (spiro head carbon), 104.32-154.53 (aromatic carbons), 170.36 (CO), 186.06 (CS), IR (KBr, cm⁻¹): 3413-3110 (3NH), 3003 (CH aromatic), 1640 (CO, amide), 1127 (CS). MS: (EI, 70 eV) m/z (%): M⁺ 342 (50.49), 212 (100). Analysis: for C15H19N5S (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)-quinozaine-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.
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washed with water and recrystallized from methanol to give 30 as light brown crystals.

Yield: 73%, m.p. 230-232°C. 1H NMR (300 MHz, DMSO-d6, 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 D2O). 13C NMR (300 MHz, DMSO-d6, 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 + 1)+ 421 (49.09), M+ 420 (175 (100). Analysis: for C24H29N3O3 (m.w. 407.51) calcd.: C, 70.74; H, 7.17; N, 10.31%; found: C, 71.10; H, 6.89; N, 9.92%.

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 mixture of the hydroxyl quinazolinone derivative 30 (3.08 g, 10 mmol) and the appropriate amine (15 mmol) was added to a solution of the quinazolinone derivative 31a-c

A solution of paraformaldehyde (0.90 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 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. 1H NMR (300 MHz, CDCl3, δ, ppm): 1.17 (t, 3H, J = 7.1 Hz, CH3 of ethyl group), 1.84 (s, 10H, spiro cyclohexyl), 4.14 (s, 2H, CH2), 4.67 (q, 2H, J = 7.1 Hz, CH2 of ethyl group), 6.80-7.43 (m, 8H, aromatic H), 10.13 (1s, 1H, NH, exchangeable with D2O). 13C NMR (300 MHz, DMSO-d6, δ, ppm): 15.23 (CH3), 38.88-40.06 (spiro cyclohexyl carbons), 61.37 (OCH3), 65.74 (CH2CO), 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 C21H24N4O3 (CH aromatic), 1670, 1631 (2CO). MS: (EI, 70 eV) m/z (%): M+ 422 (10.03), 97 (100). Analysis: for C23H26N2O4 (m.w. 394.46) calcd.: C, 70.03; H, 6.64; N, 12.92%. Found: C, 70.81; H, 6.78; N, 12.76%.

\[ \text{IC}_{50} = \frac{K_d}{[D]^n} \\text{ED}_{50} + [D]^n \]

\[ \text{IC}_{50}^r = \text{IC}_{50} \times \left(1 - \frac{[D]^n}{K_d + [D]^n}\right) + R \]

where \( K_d \) 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., \( K_d = \text{IC}_{50} \) when \( R = 0 \) and \( E_{\text{max}} = 100 - R \) (31).

**Enzyme assay**

The procedure was done according to the supplied protocol of HT Universal Colorimetric PARP assay.
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 IC50 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.
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 and 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Affinity kcal/mol</th>
<th>Binding free energy kJ/mol</th>
<th>Main residue from PARP-1</th>
<th>Main atoms from the compound</th>
<th>Distance in Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-7.52</td>
<td>-26.51</td>
<td>Lys 903</td>
<td>C=O of quinazoline ring</td>
<td>2.79</td>
</tr>
<tr>
<td>7a</td>
<td>-3.89</td>
<td>-26.76</td>
<td>Glu 763</td>
<td>-OH of sugar</td>
<td>2.41</td>
</tr>
<tr>
<td>7c</td>
<td>-4.50</td>
<td>-26.76</td>
<td>Tyr 907</td>
<td>-OH of sugar</td>
<td>2.98</td>
</tr>
<tr>
<td>8a</td>
<td>-6.45</td>
<td>-37.31</td>
<td>Lys 903</td>
<td>-OH of sugar</td>
<td>3.54</td>
</tr>
<tr>
<td>8c</td>
<td>-7.00</td>
<td>-37.31</td>
<td>Asp 770</td>
<td>C=O of quinazoline ring</td>
<td>3.20</td>
</tr>
<tr>
<td>9a</td>
<td>-3.78</td>
<td>-37.31</td>
<td>Gly 863</td>
<td>C=O of quinazoline ring</td>
<td>2.37</td>
</tr>
<tr>
<td>9c</td>
<td>-5.46</td>
<td>-27.49</td>
<td>Glu 863</td>
<td>-OH of sugar</td>
<td>2.54</td>
</tr>
<tr>
<td>10b</td>
<td>-4.50</td>
<td>-25.79</td>
<td>Tyr 907</td>
<td>C=N</td>
<td>2.47</td>
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<tr>
<td>11b</td>
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<td>-35.95</td>
<td>Tyr 907</td>
<td>C=O of quinazoline ring</td>
<td>2.5</td>
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<tr>
<td>15</td>
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<td>-29.05</td>
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<td>16a</td>
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<td>Asn 767</td>
<td>C=O of quinazoline ring</td>
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<tr>
<td>17</td>
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<td>-28.77</td>
<td>Lys 903</td>
<td>C=O of quinazoline ring</td>
<td>2.92</td>
</tr>
<tr>
<td>18</td>
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<td>-36.85</td>
<td>Lys 908</td>
<td>C=O of quinazoline ring</td>
<td>3.08</td>
</tr>
<tr>
<td>20</td>
<td>-6.90</td>
<td>-36.92</td>
<td>Lys 903</td>
<td>C=N of tetrazole ring</td>
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</tr>
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<td>-27.33</td>
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<td>C=O of triazole ring</td>
<td>2.85</td>
</tr>
<tr>
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<td>-25.05</td>
<td>Asp 770</td>
<td>C=O of -COO</td>
<td>2.87</td>
</tr>
<tr>
<td>25</td>
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<td>-28.81</td>
<td>Gly 863</td>
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<td>3.60</td>
</tr>
<tr>
<td>30</td>
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<td>-28.81</td>
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<td>p-OH group</td>
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<tr>
<td>31a</td>
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<td>-28.81</td>
<td>Glu 763</td>
<td>-NH of quinazoline ring</td>
<td>2.76</td>
</tr>
<tr>
<td>34</td>
<td>-4.86</td>
<td>-37.11</td>
<td>Arg 878</td>
<td>-NH of quinazoline ring</td>
<td>2.80</td>
</tr>
</tbody>
</table>
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, which has been found to be the most active compound with lowest IC50 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 IC50 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, 1H 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 and OH groups. The IR spectrum of derivative 9a-c, respectively, 1H NMR (DMSO-d6, 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-methoxybenzaldehyde, p-fluorobenzaldehyde, 2-thiophenaldehyde and pyrrolo-2-carboxaldehyde. Cyclocondensation reaction of 10a-d with thiglycolic 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 C=O groups of both quinazolinone and thiazolidinone or benzothiazine rings. At the same time, 1H NMR (CDCl3, 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 –CH2 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 amino-quinazolinone derivative 6 with 2-chloromethylbenzimidazole (39) in refluxing ethanol using K2CO3 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 1H NMR (CDCl3, δ ppm) spectrum of compound 13 is the appearance of a singlet signal at δ 4.63 ppm corresponding to 2H of HN-CH2 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 aminquinazolinone 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 (NH₂, 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 3435-3239 cm⁻¹ related to NH₂ 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⁺ - 1)⁺ 439 (10.19), 456 (10.21) and (M⁺ + 1)⁺ 425 (11.63), respectively (Scheme 1).

Moreover, this study reports the reaction of benzoxazine derivative 5 with formamide to get the desired intermediate quinazolone derivative 18, which was converted to its chloro analogue 19 via its exposure to refluxing POCl₃/PCl₅.

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 hydratino-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. 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 Na₂CO₃ as a catalytic base at pH 9-9.5 (40) to get the quinazolino amino acid derivative 24. H NMR of the derivative showed the 2H and 1H of α-CH₂ 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 (acyl 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°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. H NMR spectrum of 27 revealed a singlet signal at δ 2.05 ppm due to 3H of CH₃ group and three singlets at δ 3.12, 5.81, 6.00 ppm corresponding to 3H of 3NH groups of the thiosemicarbazide 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. H₂SO₄ 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-thiadiazolo-quinazoline 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...
A novel class of substituted spiro [quinazoline-2,1'-cyclohexane] derivatives... 703

derivative with p-formaldehyde and the appropriate secondary amines, namely: N-methylpiperazine, morpholine and p-methylpiperidine afforded the corresponding Mannich bases 31a-c, respectively.

1H NMR spectra of these derivatives exhibited 2 protons of N-CH2 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 K2CO3 as an acid scavenger to afford the desired corresponding ester 32. Its 1H 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-CH2) appeared as a singlet signal at δ 4.14 ppm. The ester derivative 32 was employed to synthesize the key intermediate acetoxyhydrazide 33 by its treatment with excess hydrazine hydrate in absolute ethanol. Further treatment of the ethanolic solution of 33 either with CS2 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

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

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>IC50 µM</th>
<th>R fraction %</th>
<th>Compound no.</th>
<th>IC50 µM</th>
<th>R fraction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>138.9</td>
<td>-</td>
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<td>16.15</td>
<td>0.0</td>
</tr>
<tr>
<td>7a</td>
<td>12.17</td>
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<td>18</td>
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<tr>
<td>7c</td>
<td>21.8</td>
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<tr>
<td>8a</td>
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<td>0.0</td>
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<td>8c</td>
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<tr>
<td>16a</td>
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<td>0.0</td>
<td>Doxorubicin</td>
<td>0.13</td>
<td>0.0</td>
</tr>
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</table>

IC50 = 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.

<table>
<thead>
<tr>
<th>Compounds/conc</th>
<th>IC50 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>18.90</td>
</tr>
<tr>
<td>7c</td>
<td>2.20</td>
</tr>
<tr>
<td>9a</td>
<td>1.45</td>
</tr>
<tr>
<td>9c</td>
<td>21.16</td>
</tr>
<tr>
<td>10b</td>
<td>18.32</td>
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<tr>
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<td>n.d. a</td>
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<td>1.81</td>
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<td>30</td>
<td>2.12</td>
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<td>31a</td>
<td>&gt;100</td>
</tr>
<tr>
<td>34</td>
<td>1.67</td>
</tr>
<tr>
<td>3-AB</td>
<td>2.08</td>
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</tbody>
</table>

a IC50 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.

1H NMR spectra of these derivatives exhibited 2 protons of N-CH2 bridge as a singlet signal at δ 5.21-5.46 ppm, besides the other expected protons at their expected regions.

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assay in a trial to correlate both docking and cytotoxic evaluation studies. Two parameters, IC\textsubscript{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 \textit{6} was inactive with IC\textsubscript{50} = 138.9 µM and has high percentage resistant fraction. The data showed that the C-nucleoside Shiff’s bases \textit{7a}, \textit{7c} exhibited promising cytotoxic activity against breast carcinoma cell line comparable to doxorubicin (IC\textsubscript{50} = 12.17, 21.8 µM, respectively) with 0% resistant fraction. The benzothiazine analogue \textit{9a} appeared to be a less effective cytotoxic agent with IC\textsubscript{50} = 26.7 µM, while the other thiazolidinone and benzothiazine analogues \textit{8a}, \textit{9c} did not exhibit distinctive differences from their starting precursors (IC\textsubscript{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 \textit{8c} (IC\textsubscript{50} = 2.36 µM) with approximate 0% resistant fraction. Unexpectedly, \textit{p}-fluoro Shiff’s base \textit{10b} exhibited a decreased cytotoxic potency to reach IC\textsubscript{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 \textit{11b} with IC\textsubscript{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 \textit{15}, or fused pyrrolo-pyrimidine ring system as in compounds \textit{16a}, \textit{17} led to a remarkable decrease in the anticancer activity (IC\textsubscript{50} = 15.11, 22.6, 16.15 µM, respectively) with complete absence of cell resistance (R fraction = 0%). The quinazolinone derivative \textit{18} with no ring attachment at 3-nitrogen position exhibited a slight increase in the cytotoxic activity (IC\textsubscript{50} = 10.04 µM, R fraction = 0%).

\begin{scheme}
\begin{equation*}
\text{a = NH, NH}_2, \text{EtOH, reflux 4 h; b = aldoses, EtOH/drops glacial AcOH, reflux 6 h; c = HSCH}_2\text{COOH, dry C}_6\text{H}_6, \text{reflux 16 h; d = thiosalicylic acid, dry C}_6\text{H}_6, \text{reflux 16 h; e = aromatic aldehyde, EtOH/glacial AcOH, reflux 12 h; f = 2-chlorobenzimidazole, EtOH, anhyd. K}_2\text{CO}_3, \text{dry C}_6\text{H}_6, \text{reflux 16 h; g = PhCOCH}_2\text{Br, EtOH, reflux 3 h; h = CH}_3\text{(CN)}_2, \text{EtOH/EtONa, reflux 3 h; i = urea/thiourea, fusion 220°C, 20 min; j = HCHO, reflux 5 h.}
\end{equation*}
\end{scheme}
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\textsubscript{50} = 3.44 µM, R fraction = 0.12%), then the efficacy started to reduce by the analogue bearing fused triazolo-quinazoline 23 (IC\textsubscript{50} = 14.3 µM, R fraction = 0%). Further reduction was observed by that bearing the pyrazolidione ring compound 22 (IC\textsubscript{50} = 41.5 µM, R fraction = 0%). It can be seen in the Table that the attachment of 

hydroxy group or oxadiazole ring via an ether link-

age to the phenyl ring as in derivatives 30 and 34 resulted in moderate activity (IC\textsubscript{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\textsubscript{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.
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 exhibi-

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Scheme 3. a = p-hydroxyaniline, glacial AcOH, anhyd. CH$_3$COONa, reflux 15 h; b = sec. amines, EtOH, reflux 8 h; c = EtCOOCH$_2$Br, anhyd. K$_2$CO$_3$, dry CH$_3$COCH$_3$, reflux 12 h; d = NH$_2$NH$_2$, EtOH, reflux 3 h; e = CS$_2$, KOH, EtOH, reflux 12 h; f = (COOEt)$_2$, EtONa, reflux 12 h.
A novel class of substituted spiro [quinazoline-2,1’-cyclohexane] derivatives... 707

...ited very high potency even greater than that of 3-AB (IC\textsubscript{50} = 1.45 µM). The derivatives carrying pyrrole ring or fused pyrrolo-pyrimidine ring system in conjunction with quinazoline core \textsubscript{15, 16a, 17} appeared to be effective PARP-1 enzyme inhibitors (IC\textsubscript{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 (\textsubscript{18}) exhibited higher potency than the reference drug (IC\textsubscript{50} = 1.78 µM).

When the quinazoline core is fused to heterocyclic rings carrying more than one nitrogen atoms such as pyrazolidine-dione \textsubscript{22} or triazolone \textsubscript{23}, the derivatives showed high inhibitory activity (IC\textsubscript{50} = 2.18, 1.71 µM). The resultant data also showed that the p-hydroxyphenyl-quinazoline derivative \textsubscript{30} was an active enzyme inhibitor similar to 3-AB (IC\textsubscript{50} = 2.12 µM) and higher inhibitory activity was gained by the oxadiazole analogue \textsubscript{34} (IC\textsubscript{50} = 1.67 µM) to be a more effective inhibitor than the standard.

An unexpected result was obtained by the thiazolidine-4-one derivative \textsubscript{11b} and the Mannich base analogue \textsubscript{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. 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).

REFERENCES


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