SYNTHESIS, CYTOTOXIC EVALUATION AND MOLECULAR DOCKING STUDY OF NOVEL QUINAZOLINE DERIVATIVES AS PARP-1 INHIBITORS

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Abstract: Novel series of spiro[(2H,3H)-quinazoline-2,1'-cyclohexane] derivatives (**I-XVI**) were synthesized and biologically evaluated as cytotoxic agents against human breast carcinoma cell lines (MCF-7) using dox-orubicin as a reference drug. Most of the tested compounds displayed promising cytotoxic activity, especially derivatives **V, VIb** and **XIb**. The most active compounds were docked into the PARP-1 enzyme binding site to predict the ligand–protein binding modes. Lipinski rule of five and ADME profile suggested strongly that compounds **V** and **VIb** are promising agents as breast cancer inhibitors with drug likeness approach that have PARP-1 inhibitory activity. The structures of all newly synthesized compounds were confirmed by micro-analysis and IR, ^{1}H-NMR and mass spectral data.

Keywords: spiro quinazoline-cyclohexane derivatives, cytotoxic activity, molecular docking, PARP-1 enzyme, drug-likeness

Quinazoline derivatives are of considerable chemical and pharmacological importance as thera-peutical agents (1-3). They are being extensively uti-lized as drug like scaffold in medicinal chemistry (4) especially as anticancer agents through various mechanisms. Some of quinazoline derivatives are considered as antifolate thymidylate synthase inhibitors such as nolatrexed (**1**) (5), while 2-substi-tuted-1,3-dihydro-quinazolinone derivatives are associated with inhibitory effects on tubulin poly-merization such as GMC-5-193 (**2**) (6, 7). Also, some 4-anilinoquinazolines represent a new class of antitumor drugs (8). They were found to inhibit the epidermal growth factor receptor (EGFR) tyrosine kinase overexpression through the inhibition of EGFR autophosphorylation such as gefitinib (**3**) (9) and erlotinib (**4**) (10), in addition to 2-trichloromethyl-aminoquinazoline derivative (**5**) which exhibits a potent cyclin dependant kinase inhibitory effect (11). Furthermore, many quinazo-lines exert their antitumor activity through inhibition of poly (ADP-ribose) polymerase-1 enzyme which is involved in a variety of physiological functions including DNA replication and repair (12). As PARP-1 promotes DNA repair, there is a strong

rationale to confirm that its inhibition may increase the efficiency of certain cytotoxic treatments (13). So, PARP-1 inhibitors have been recently used as an adjuvant in cancer therapy in combination to other alkylating agents and/or radiation. For example, the potent and novel PARP-1 inhibitor NU1025 (**6**) (14) enhances the cytotoxicity of DNA-methylating agents and ionizing radiation through its role in inhi-bition of DNA repair. Moreover, many literature data have confirmed that cells deficient in BRCA1 and BRCA2, key proteins that involved in DNA double strand break repair, are highly sensitive to PARP-1 inhibitors (15). However, a new study showed that phenanthridone derived PARP-1 inhibitors promote cell death in breast cancer cells lacking BRCA-1 and BRCA-2 mutations (MCF-7 and MDA-MB-231) (16). They cause cell cycle arrest and subsequent cell death in non-hereditary breast cancer cells suggest-ing a potential broader utilization of PARP-1 inhibitors as single agents in treating certain muta-tions of breast cancer (17), and also as chemo-sensi-tizers in combination with DNA damaging agents as well as in several other therapeutic areas (18). Therefore, PARP-1 is regarded as a valuable target in the exploration of new cancer treatment regimens. In

a view of aforementioned facts and in continuation of our previous work on PARP-1 enzyme inhibitors (19), we were encouraged to synthesize a new series of quinazoline scaffolds with structural similarities to nicotinamide adenine dinucleotide (NAD^+), the natural substrate of PARP-1 enzyme, aiming to obtain potent PARP-1 inhibitors suitable for the clinical development through incorporation with different heterocyclic functionalities of reported anticancer activity either by PARP-1 inhibiting activity such as pyridazinone (20) and pyrazole rings (21) or anticancer activity depending on other mechanisms such as triazole (22) and thiadiazole (23) moieties. Also, the aim of this work is to study the interactions of these novel derivatives with NAD^+ binding sites of the target PARP-1 enzyme. Since literature survey exhibited that PARP-1 inhibitors can be considered as single drugs in treating certain mutations of breast cancer (16, 17, 24), most of the newly synthesized derivatives in this study were *in-vitro* evaluated as cytotoxic agents against breast adenocarcinoma (MCF-7) cell lines. Moreover, the biologically active compounds were subjected to molecular docking and drug likeness studies to rationalize and identify the structural features required for the antitumor properties.
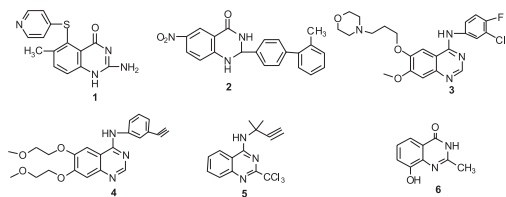
MATERIALS AND METHODS

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 Electrothermal IA9100 apparatus (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were determined in National Research Centre (NRC), Cairo, Egypt on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and the chemical shifts are expressed in δ ppm relative to TMS as an internal reference. IR spectra (KBr) were recorded in NRC on a Perkin-Elmer 1650 spectrophotometer. Mass spectra were recorded at

70 eV on EI Ms-QP 1000 EX (Shimadzu, Japan) at Faculty of Science, Cairo University, Egypt. Microanalytical data were performed in NRC by Vario El-Mentar apparatus (Shimadzu, Japan). The found values were within ±0.4% of the theoretical values.

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

This compound was prepared from the starting benzoxazine derivative according to a reported method (19). M.p. 75°C.

Spiro[(2H, 3H)-quinazoline-2,1'-cyclohexan]-4(1H)-thiol (II)

A solution of the chloro-quinazoline derivative (I) (2.34 g, 10 mmol) and thiourea (1.52 g, 20 mmol) in absolute ethanol (20 mL) was refluxed for 4 h. The reaction solution was left to cool and poured onto 2M sodium hydroxide solution (50 mL). The residue was filtered off and crystallized from ethyl acetate to obtain light brown crystals of the derivative (II).

Yield: 75%, m.p. 114-116°C. IR (KBr, cm^{-1}): 3395 (NH), 3078 (CH aromatic), 2856 (CH aliphatic), 1301 (CS). ^{1}H NMR (CDCl$_3$, δ, ppm): 1.20 (s, 10H, spiro cyclohexyl), 6.91-7.62 (m, 4H, aromatic-H), 9.62, 10.13 (2s, 2H, SH, NH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 38.71, 39.18, 40.06 (spiro cyclohexyl carbons), 70.04 (spiro head carbon), 120.53, 123.34, 125.21, 125.67, 133.77, 149.37 (aromatic carbons), 185.83 (C=S). MS (m/z): M$^+$ 232 (8.38%), 146 (100%). Analysis: for C$_{13}$H$_{16}$N$_2$S, m.w. (232.34): calcd. C, 67.20; H, 6.94; N, 12.06; S, 13.80%; found C, 67.16; H, 7.31; N, 11.84; S, 13.69%.

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

This compound was prepared according to the reported method (19). M.p. 143°C.

1-[Spiro[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl]-3-methyl-1H-pyrazol-5(4H)-one (IV)

Ethyl acetoacetate (0.13 mL, 1 mmol) was added to a solution of the hydrazine compound III (0.23 g, 1 mmol) in glacial acetic acid (10 mL), then, the mixture was refluxed for 8 h. The reaction mixture was cooled and poured into ice/H$_2$O. The formed precipitate was filtered, dried and crystallized from methanol to obtain pale yellow crystals of derivative IV.

Yield: 82%, m.p. >300°C. IR (KBr, cm^{-1}): 3226 (NH), 3057 (CH aromatic), 2927 (CH

aliphatic), 1667 (C=O). ^{1}H NMR (CDCl$_3$, δ, ppm): 1.96 (s, 10H, spiro cyclohexyl), 2.07 (s, 3H, CH$_3$), 3.34 (s, 2H, CH$_2$ of pyrazolidinone ring), 6.46-7.40 (m, 4H, H aromatic), 10.13 (s, 1H, NH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 23.84 (CH$_3$), 38.71, 39.67, 40.06 (spiro cyclohexyl carbons), 42.81 (CH$_2$, pyrazole), 68.56 (spiro head carbon), 117.68, 123.13, 126.34, 127.21, 127.67, 149.77 (aromatic carbons), 154,65, 160.82 (2C=N), 164.05 (C=O). MS: (m/z): M$^+$ 296 (13.33%), 230 (100%). Analysis: for C$_{17}$H$_{20}$N$_4$O, m.w. (296.37): calcd. C, 68.89; H, 6.80; N, 18.90%; found C, 69.32; H, 6.53; N, 19.48%.

Spiro[1,2,6,7-tetrahydro-11bH-(1,2,4)triazino[4,3-c]quinazoline-6,1'-cyclohexan]-3,4-dione (V)

A mixture of hydrazine compound **III** (2.30 g, 10 mmol) and diethyl oxalate (1.46 mL, 10 mmol) in absolute ethanol was refluxed for 8 h. The precipitate formed on cooling was filtered off, dried and crystallized from isopropanol to obtain light brown crystals.

Yield: 75%, m.p. 196-198°C. IR (KBr, cm^{-1}): 3323-3159 (3NH), 2919 (CH aliphatic), 1702, 1667 (2C=O). ^{1}H-NMR (CDCl$_3$, δ, ppm): 1.07 (s, 10H, spiro cyclohexyl), 2.65 (s, 1H, NH, exchangeable with D$_2$O), 5.84 (s, 1H, -CH of triazine ring), 7.49-8.08 (m, 4H, H aromatic), 8.09, 12.24 (2s, 2H, 2NH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 38.71, 39.88, 40.06 (spiro cyclohexyl carbons), 65.53 (spiro head carbon), 78.52 (CH), 123.07, 126.36, 127.33, 127.67, 134.91, 145.93 (aromatic carbons), 160.82, 161.35 (2C=O). MS (m/z): M$^+$ 286 (10.86%), 146 (100%). Analysis: for C$_{15}$H$_{18}$N$_4$O$_2$, m.w. (286.33): calcd. C, 62.92; H, 6.34; N, 19.57%; found C, 62.51; H, 5.98; N, 19.25%.

General procedure for preparation of 2-{[spiro[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}substituted acids (VIa-c)

Amino acids (10 mmol) and Na$_2$CO$_3$ (0.53 g, 5 mmol) were dissolved in water (15 mL) and the pH of the solution was adjusted to 9-9.5. Then, the chloro-quinazoline derivative (1.17 g, 50 mmol) was added and the reaction mixture was heated with continuous stirring at 100°C for 8 h at the controlled pH. The reaction was left overnight at room temperature then, treated with cold formic acid. The solid obtained was filtered off, washed with H$_2$O and crystallized from the proper solvent to give the corresponding derivatives (**VIa-c**).

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-3-phenyl propionic acid (VIa)

(From phenylalanine): crystallized from methanol to give pale yellow crystals, yield: 75%, m.p. 210-212°C. IR (KBr, cm^{-1}): 3447 (OH), 3119 (NH), 3033 (CH aromatic), 2934 (CH aliphatic), 1710 (C=O), 1298 (COOH). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.31 (s, 10H, spiro cyclohexyl), 3.04 (d, 2H, J = 7.4 Hz, β-CH$_2$), 4.13 (t, 1H, J = 3.4 Hz α-CH), 7.20-7.26 (m, 9H, aromatic-H), 8.20, 8.69, 10.13 (3s, 3H, 2NH, OH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 38.81 (CH$_2$), 38.98, 39.15, 39.32 (spiro cyclohexyl carbons), 54.12 (CH), 71.96 (spiro head carbon), 112.12, 114.42, 116.70, 119.00, 127.58, 128.92, 129.84, 132.07, 135.13, 149.18 (aromatic carbons), 159.25 (C=N), 170.87 (C=O). MS (m/z): M$^+$ 363 (10.18%), 91 (100%). Analysis: for C$_{22}$H$_{25}$N$_3$O$_2$, m.w. (363.45): calcd. C, 72.70; H, 6.93; N, 11.56%; found C, 72.42; H, 7.21; N, 11.18%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-3-methyl pentanoic acid (VIb)

(From isoleucine): crystallized from methanol to give white crystals, yield: 65%, m.p. 183-185°C. IR (KBr, cm^{-1}): 3441 (broad, OH), 3376 (NH), 2963 (CH aliphatic) 1685 (C=O), 1265 (COOH). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 0.93 (t, 3H, J = 4.1 Hz, CH$_3$), 1.15 (d, 3H, J = 7.2 Hz, CH$_3$), 1.21 (s, 10H, spiro cyclohexyl), 1.51-1.57 (m, 2H, γ-CH$_2$), 1.61-1.68 (m, 1H, β-CH), 2.92 (d, 1H, J = 7.4 Hz, α-CH), 6.84-7.03 (m, 4H, H aromatic), 8.20, 8.69, 10.13 (3s, 3H, 2NH, OH, exchangeable with D$_2$O). MS (m/z): M$^+$ 329 (5.12%), 86 (100%). Analysis: for C$_{19}$H$_{27}$N$_3$O$_2$, m.w. (329.44): calcd.: C, 69.27; H, 8.26; N, 12.76%; found C, 69.53; H, 8.65; N, 13.05%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-4-(methylthio)-butanoic acid (VIc)

(From methionine): crystallized from ethyl acetate to give light brown crystals, yield: 74%, m.p. 190-192°C. IR (KBr, cm^{-1}): 3429 (broad, OH), 3370 (NH), 2953 (CH aliphatic), 1670 (C=O), 1274 (COOH). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.31 (s, 10H, spiro cyclohexyl), 2.09 (s, 3H, CH$_3$), 2.15 (t, 2H, J = 3.8 Hz, γ-CH$_2$), 2.41-2.44 (m, 2H, β-CH$_2$), 3.49 (t, 1H, J = 4.3 Hz, α-CH), 7.31-7.52 (m, 4H, H aromatic), 8.32, 8.73, 10.23 (3s, 3H, 2NH, OH, exchangeable with D$_2$O). MS: (m/z): M$^+$ 347 (6.21%), 61 (100%). Analysis: for C$_{18}$H$_{25}$N$_3$O$_2$S,

m.w. (347.48): calcd. C, 62.22; H, 7.25; N, 12.09; S, 9.23%; found C, 61.82; H, 6.91; N, 12.41; S, 8.85%.

General procedure for preparation of 2-{[spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}acid chlorides (VIIa-c)

The quinazoline derivatives (**VIa-c**) (1 mmol) were 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 the proper solvent to yield the corresponding derivatives (**VIIa-c**).

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-3-phenyl propionyl chloride (VIIa)

(From phenylalanine): crystallized from methanol to give grayish white crystals, yield: 73%, m.p. 125-127°C. IR (KBr, cm^{-1}): 3440, 3210 (2NH), 3050 (CH aromatic), 2963 (CH aliphatic), 1670 (C=O). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.31 (s, 10H, spiro cyclohexyl), 3.22 (d, 2H, J = 7.4 Hz, β-CH$_2$), 4.27 (t, 1H, J = 3.8 Hz, α-CH), 7.20-7.26 (m, 9H, H aromatic), 8.20, 8.69 (2s, 2H, 2NH, exchangeable with D$_2$O). MS (m/z): (M$^+$ + 2) 283 (1.16%), M$^+$ 281 (3.51%) (1 : 3). Analysis: for C$_{22}$H$_{24}$ClN$_3$O, m.w. (381.90): calcd. C, 69.19; H, 6.33; N, 11.00%; found C, 68.82; H, 5.98; N, 11.18%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-3-methyl pentanoyl chloride (VIIb)

(From isoleucine): crystallized from iso-propanol to give dark brown crystals, yield: 63%, m.p. 168-170°C. IR (KBr, cm^{-1}): 3433, 3304 (2NH), 2970 (CH aliphatic), 1657 (C=O). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 0.93 (t, 3H, J = 4.1 Hz, CH$_3$), 1.35 (d, 3H, J = 7.2 Hz, CH$_3$), 1.25 (s, 10H, spiro cyclohexyl), 1.51-1.57 (m, 2H, γ-CH$_2$), 1.61-1.68 (m, 1H, β-CH), 2.92 (d, 1H, J = 6.8 Hz, α-CH), 6.84-7.03 (m, 4H, H aromatic), 8.20, 10.13 (2s, 2H, 2NH, exchangeable with D$_2$O). MS (m/z): (M$^+$ + 2) 349 (1.78%), M$^+$ 347 (5.34%) (1 : 3). Analysis: for C$_{19}$H$_{26}$ClN$_3$O, m.w. (347.88): calcd. C, 65.60; H, 7.53; N, 12.08%; found C, 65.31; H, 7.85; N, 11.73%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-4-(methylthio)-butanoyl chloride (VIIc)

(From methionine): crystallized from iso-propanol to give dark red crystals, yield: 79%, m.p. 142-144°C, IR (KBr, cm^{-1}): 3431, 3199 (2NH), 2958 (CH aliphatic), 1710 (C=O). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 2.16 (s, 3H, CH$_3$), 2.24 (t, 2H, J = 3.8 Hz, γ-CH$_2$), 2.41-2.44 (m, 2H, β-CH$_2$), 4.08 (t, 1H, J = 4.3 Hz, α-CH), 7.31-7.84 (m, 4H, H aromatic), 8.74, 10.23 (2s, 2H, 2NH, exchangeable with D$_2$O). MS (m/z): (M$^+$ + 2) 367 (1.03%), (M$^+$) 365 (3.09%) (1 : 3). Analysis: for C$_{18}$H$_{24}$ClN$_3$OS, m.w. (365.92): calcd. C, 59.08; H, 6.61; N, 11.48; S, 8.76%; found C, 58.95; H, 6.72; N, 11.13; S, 8.91%.

General procedure for preparation of 2-{[spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]-amino}substituted carbohydrazides (VIIIa-c)

Hydrazine hydrate (99%) (1.6 mL, 50 mmol) was added to the chloro compound (**VIIa-c**) (10 mmol) in absolute ethanol and refluxed for 3 h. The solid separated after concentration and cooling was crystallized from dioxane to give the corresponding derivatives (**VIIIa-c**).

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-3-phenyl propane hydrazide (VIIIa)

(From phenylalanine): yellowish white crystals, yield: 67%, m.p. 210-212°C. IR (KBr, cm^{-1}): 3434-3210 (NH$_2$, 3NH), 3050 (CH aromatic), 2963 (CH aliphatic), 1655 (C=O). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.21 (s, 10H, spiro cyclohexyl), 3.15 (d, 2H, J = 7.4 Hz, β-CH$_2$), 4.53 (t, 1H, J = 3.8 Hz, α-CH), 5.32 (s, 2H, NH$_2$), 6.54 (s, 1H, NH), 7.56-7.96 (m, 9H, H aromatic), 8.91, 10.21 (2s, 2H, 2NH, exchangeable with D$_2$O). MS (m/z): M$^+$ 377 (10.02%), 76 (100%). Analysis: for C$_{22}$H$_{27}$N$_5$O, m.w. (377.48): calcd. C, 70.00; H, 7.21; N, 18.55%; found C, 69.63; H, 7.53; N, 18.92%.

2-{[Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl]amino}-3-methyl pentane hydrazide (VIIIb)

(From isoleucine): yellow crystals, yield: 73%, m.p. 231-233°C, IR (KBr, cm^{-1}): 3433-3110 (NH$_2$, 3NH), 3057 (CH aromatic), 2970 (CH aliphatic), 1647 (C=O). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 0.75 (t, 3H, J = 4.3 Hz, CH$_3$), 1.21 (d, 3H, J = 7.3 Hz, CH$_3$), 1.28 (s, 10H, spiro cyclohexyl), 1.67-1.70, (m, 2H, γ-CH$_2$), 1.61-1.68 (m, 1H, β-CH), 5.15 (d, 1H, J = 7.4 Hz, α-CH), 5.64 (s, 2H, NH$_2$), 6.31 (s,1H, NH), 6.84-7.03 (m, 4H, H aromatic), 8.65, 10.12 (2s, 2H, 2NH, exchangeable with D$_2$O). MS: (m/z): (M$^+$ + 1) 344 (4.32%), 86 (100%). Analysis: for C$_{19}$H$_{29}$N$_5$O, m.w. (343.47): calcd. C, 66.44; H, 8.51; N, 20.39%; found C, 66.83; H, 8.75; N, 19.98%.

2-{[[Spiro-[(1H,2H)-quinazoline-2,1'-cyclo-hexan)]-4-yl]amino}-4-(methylthio)-butane hydrazide (VIIIc)

(From methionine): light brown crystals, yield: 71%, m.p. 198-200°C. IR (KBr, cm⁻¹): 3440-3152 (NH₂, 3NH), 2958 (CH aliphatic), 1650 (C=O). ¹H NMR (DMSO-d₆, δ, ppm): 1.82 (s, 10H, spiro cyclohexyl), 2.09 (s, 3H, CH₃), 2.32 (t, 2H, J = 4.2 Hz, γ-CH₂), 2.63-2.66 (m, 2H, β-CH₂), 3.64 (t, 1H, J = 3.8 Hz, α-CH), 6.02 (s, 2H, NH₂), 6.46 (s, 1H, NH), 7.31-7.52 (m, 4H, H aromatic), 8.71, 10.21 (2s, 2H, 2NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 19.04 (CH₃), 30.82 (CH₂), 33.06 (CH₂), 38.98, 39.00, 39.15 (spiro cyclohexyl carbons), 54.12 (CH), 71.96 (spiro head carbon), 110.82, 113.34, 117.57, 130.31, 132.07, 149.18 (aromatic carbons), 160.04 (C=N), 170.87 (C=O). MS (m/z): M⁺ 361 (9.98%), 61 (100%). Analysis: for C₁₈H₂₇N₅OS, m.w. (361.50): calcd. C, 59.80; H, 7.53; N, 19.37; S, 8.87%; found C, 59.42; H, 7.71; N, 18.98; S, 8.91%.

General procedure for preparation of 1-{[[spiro-[(1H, 2H)-quinazoline-2,1'-cyclohexan]-4-yl]amino]substitutedcarbonyl}-4-methylthiosemi-carbazide (IXa-c)

To a solution of the hydrazide derivative (VIIIa-c) (25 mmol) 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 solid obtained was dried under vacuum and crystallized from the proper solvent to give the corresponding derivatives IXa-c.

1-{[[Spiro-[(1H,2H)-quinazoline-2,1'-cyclo-hexan)]-4-yl]amino]3-phenylpropionyl}-4-methylthiosemicarbazide (IXa)

(From phenylalanine): crystallized from iso-propanol to give white crystals, yield: 65%, m.p. 125-127°C. IR (KBr, cm⁻¹): 3450-3210 (5NH), 3010 (CH aromatic), 2980 (CH aliphatic), 1645 (C=O), 1100 (C=S). ¹H NMR (DMSO-d₆, δ, ppm): 2.01 (s, 10H, spiro cyclohexyl), 2.31 (s, 3H, CH₃), 2.85 (d, 2H, J = 6.8 Hz, β-CH₂), 3.15, 3.46, 4.28 (3s, 3H, 3NH, exchangeable with D₂O), 4.53 (t, 1H, J = 3.8 Hz, α-CH), 6.54 (s, 1H, NH, exchangeable with D₂O), 7.56-7.96 (m, 9H, H aromatic), 10.21 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 32.07 (CH₃), 38.81 (CH₂), 38.98, 39.15, 39.32 (spiro cyclohexyl carbons), 54.12 (CH), 68.98 (spiro head carbon), 108.05, 112.12, 114.42, 116.70, 119.00, 132.07, 128.92,

129.84, 135.13, 149.18 (aromatic carbons), 155.65 (C=N), 168.63 (C=O), 170.87 (C=S). MS (m/z): M⁺ 450 (15.47%), 127 (100%). Analysis: for C₂₄H₃₀N₆OS, m.w. (450.60): calcd. C, 63.97; H, 6.71; N, 18.65; S, 7.12%; found C, 64.32; H, 7.07; N, 18.24; S, 6.83%.

1-{[[Spiro-[(1H,2H)-quinazoline-2,1'-cyclo-hexan)]-4-yl]amino]-3-methylpentanoyl}-4-methylthiosemicarbazide (IXb)

(From isoleucine): crystallized from iso-propanol/petroleum ether to give light brown crystals, yield: 62%, m.p. 85-87°C. IR (KBr, cm⁻¹): 3443-3150 (5NH), 3060 (CH aromatic), 2970 (CH aliphatic), 1633(C=O), 1150 (C=S). ¹H NMR (DMSO-d₆, δ, ppm): 1.05 (t, 3H, J = 4.1 Hz, CH₃), 1.18 (d, 3H, J = 6.8 Hz, CH₃), 1.35 (s, 10H, spiro cyclohexyl), 1.67-1.70, (m, 2H, γ-CH₂), 1.61-1.68 (m, 1H, β-CH), 2.35 (s, 3H, CH₃), 5.15 (d, 1H, J = 7.2 Hz, α-CH), 5.72, 6.04, 6.31 (3s, 3H, 3NH, exchangeable with D₂O), 6.84-7.03 (m, 4H, H aromatic), 8.65, 10.12 (2s, 2H, 2NH, exchangeable with D₂O). MS (m/z): M⁺ 416 (10.58%), 69 (100%). Analysis: for C₂₁H₃₂N₆OS, m.w. (416.58): calcd.: C, 60.55; H, 7.74; N, 20.17; S, 7.70%; found C, 60.92; H, 7.31; N, 19.82; S, 8.09%.

1-{[[Spiro-[(1H,2H)-quinazoline-2,1'-cyclo-hexan)]-4-yl]amino]-4-(methylthio) butanoyl}-4-methylthiosemicarbazide (IXc)

(From methionine): crystallized from iso-propanol/petroleum ether to give dark orange crystals, yield: 74%, m.p. 207-209°C. IR (KBr, cm⁻¹): 3430-3168 (5NH), 2958 (CH aliphatic), 1650 (C=O), 1210 (CS). ¹H NMR (DMSO-d₆, δ, ppm): 2.05 (s, 10H, spiro cyclohexyl), 2.20 (s, 3H, CH₃), 2.32 (t, 2H, J = 3.8 Hz, γ-CH₂), 2.63-2.66 (m, 2H, β-CH₂), 3.06 (s, 3H, CH₃), 3.64 (t, 1H, J = 7.1 Hz, α-CH), 5.85, 6.02, 6.41 (3s, 3H, 3NH, exchangeable with D₂O), 7.31-7.52 (m, 4H, H aromatic), 8.71, 10.21 (2s, 2H, 2NH, exchangeable with D₂O). MS (m/z): M⁺ 434 (17.05%), 62 (100%). Analysis: for C₂₀H₃₀N₆OS₂, m.w. (434.62): calcd. C, 55.27; H, 6.96; N, 19.34; S, 14.76%; found C, 54.89; H, 6.62; N, 18.97; S, 15.02%.

General procedure for preparation of 5-{[[spiro[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl]amino]substituted}-4-methyl-4H-1,2,4-triazole-3-thiol (Xa-c)

To a corresponding thiosemicarbazide derivative (IXa-c) (14 mmol), a solution of 2 M sodium hydroxide solution (0.8 g, 10 mL) (25 mL) was added portionwise. The reaction mixture was

refluxed for 3 h. The mixture was then allowed to cool to room temperature. It was filtered and then the filtrate was acidified with 2 M hydrochloric acid. The precipitated solid was filtered, washed thoroughly with water, dried and recrystallized from methanol to give the triazolo derivatives **Xa-c**.

5-{1-[(Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl)amino]-2-phenylethyl}-4-methyl-4H-1,2,4-triazole-3-thiol (Xa)

(From phenylalanine): yield: 67%, m.p. 286-288°C. IR (KBr, cm^{-1}): 3210, 3119 (2NH), 3010 (CH, aromatic), 2976 (CH, aliphatic), 2606 (SH stretching). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.17 (s, 10H, spiro cyclohexyl), 2.29 (d, 2H, J = 7.3 Hz, β-CH$_2$), 2.66 (s, 3H, CH$_3$), 3.35 (t, 1H, J = 4.1 Hz, α-CH), 6.51 (s, 1H, NH, exchangeable with D$_2$O), 7.30-8.02 (m, 9H, H aromatic), 8.39, 12.67 (2s, 2H, NH, SH, exchangeable with D$_2$O). MS (m/z): (M$^+$ + 1) 433 (53.92%), 64 (100 %). Analysis: for C$_{24}$H$_{28}$N$_6$S, m.w. (432.58): calcd. C, 66.64; H, 6.52; N, 19.43; S, 7.41%; found C, 67.08; H, 6.72; N, 19.78; S, 7.08%.

5-{1-[(Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl)amino]-2-methylbutayl} -4-methyl-4H-1,2,4-triazole-3-thiol (Xb)

(From isoleucine): yield: 73%, m.p. 273-275°C. IR (KBr, cm^{-1}): 3250, 3140 (2NH), 2976 (CH aliphatic), 2598 (SH stretching). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 0.87 (t, 3H, J = 4.1 Hz, CH$_3$), 1.08 (s, 10H, spiro cyclohexyl), 1.23 (d, 3H, J = 7.4 Hz, CH$_3$), 1.42-1.48 (m, 2H, γ-CH$_2$), 2.30-2.37 (m, 1H, β-CH), 2.87 (s, 3H, CH$_3$), 3.75 (d, 1H, J = 6.8 Hz, α-CH), 7.09-7.29 (m, 4H, H aromatic), 8.24, 8.57, 10.48 (3s, 3H, 2NH, SH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 12.27 (CH$_3$), 16.08 (CH$_3$), 20.37 (N-CH$_3$), 38.98, 39.15, 39.32 (spiro cyclohexyl carbons), 42.64 (CH), 50.62 (NH-CH), 68.98 (spiro head carbon), 110.45, 112.65, 117.32, 130.04, 132.46, 149.18 (aromatic carbons), 151.37, 155.65, 160.06 (3C=N). MS (m/z): M$^+$ 398 (10.19%), 86 (100%). Analysis: for C$_{21}$H$_{30}$N$_6$S, m.w. (398.57): calcd. C, 63.28; H, 7.59; N, 21.09; S, 8.09%; found C, 62.92; H, 7.92; N, 20.93; S, 7.73%.

5-{1-[(Spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan)]-4-yl)amino]-3-(methylthio) propyl} -4-methyl-4H-1,2,4-triazole-3-thiol (Xc)

(From methionine): yield: 71%, m.p. 265-267°C. IR (KBr, cm^{-1}): 3330, 3220 (2NH), 2985 (CH aliphatic), 2603 (SH, stretching). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.25 (s, 10H, spiro cyclohexyl), 1.87 (s,

3H, CH$_3$), 2.52 (t, 2H, J = 4.2 Hz, γ-CH$_2$), 2.58-2.66 (m, 2H, β-CH$_2$), 3.06 (s, 3H, CH$_3$), 3.64 (t, 1H, J = 3.8 Hz, α-CH), 4.85 (1s, 1H, NH, exchangeable with D$_2$O), 7.31-7.52 (m, 4H, H aromatic), 8.71, 10.21 (2s, 2H, NH, SH, exchangeable with D$_2$O). MS (m/z): M$^+$ 416 (6.22%), (M$^+$ - 1) 415 (31.09), 68 (100%). Analysis: for C$_{20}$H$_{28}$N$_6$S$_2$, m.w. (416.61): calcd. C, 57.66; H, 6.77; N, 20.17; S, 15.39%; found C, 57.83; H, 7.01; N, 20.41; S, 15.72%.

General procedure for preparation of spiro{(1H, 2H)-N-[(5-(methylamino)-1,3,4-thiadiazol-2-yl)substituted]quinazoline-2,1'-cyclohexane}-4-amine (XIa-c)

To the thiosemicarbazide derivative **IXa-c** (6 mmol), conc. H$_2$SO$_4$ (1 mL) was added under continuous stirring. The reaction mixture was stirred at room temperature for 3 h, then added dropwise to cold H$_2$O. The obtained solid was filtered off, dried and crystallized from ethanol to give the desired thiadiazole derivatives **XIa-c**.

Spiro{(1H, 2H)-N-[1-(5-(methylamino)-1,3,4-thiadiazol-2-yl)-2-phenylethyl]quinazoline-2,1'-cyclohexane}-4-amine (XIa)

(From phenylalanine): yield: 67%, m.p. 262-264°C. IR (KBr, cm^{-1}): 3410-3212 (3NH), 3010 (CH aromatic), 2966 (CH aliphatic). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 2.11 (s, 3H, CH$_3$), 3.34 (d, 2H, J = 7.2 Hz, β-CH$_2$), 4.26 (t, 1H, J = 4.1 Hz, α-CH), 6.45 (s, 1H, NH), 7.56-7.97 (m, 9H, aromatic-H), 8.91, 10.31 (2s, 2H, 2NH, exchangeable with D$_2$O). MS (m/z): M$^+$ 432 (30.31%), 76 (100%). Analysis: for C$_{24}$H$_{28}$N$_6$S, m.w. (432.58): calcd. C, 66.64; H, 6.52; N, 19.43; S, 7.41%; found C, 66.76; H, 6.81; N, 19.72; S, 7.08%.

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

(From isoleucine): yield: 73%, m.p. 185-187°C. IR (KBr, cm^{-1}): 3415-3120 (3NH), 2966 (CH aliphatic). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 0.81 (s, 10H, spiro cyclohexyl), 0.88 (t, 3H, J = 3.8 Hz, CH$_3$), 1.26 (d, 3H, J = 7.1 Hz, CH$_3$), 1.42-1.67 (m, 2H, γ-CH$_2$), 1.84 (s, 1H, NH, exchangeable with D$_2$O), 2.28-2.31 (m, 1H, β-CH), 3.00 (s, 3H, CH$_3$), 3.90 (d, 1H, J = 6.8 Hz, α-CH), 7.09-7.29 (m, 4H, H aromatic), 8.24, 8.57 (2s, 2H, 2NH, exchangeable with D$_2$O). MS (m/z): M$^+$ 398 (20.03%), 86 (100%). Analysis: for C$_{21}$H$_{30}$N$_6$S, m.w. (398.57): calcd. C, 63.28; H, 7.59; N, 21.09; S, 8.09%; found C, 63.73; H, 7.35; N, 21.42; S, 7.81%.

Spiro{(1H, 2H)-N-[1-(5(-methylamino)-1,3,4-thia-diazol-2-yl)-3-(methylthio)-propyl] quinazoline-2,1'-cyclohexane}-4-amine (XIc)

(From methionine): yield: 71%, m.p. 180-182°C. IR (KBr, cm^{-1}): 3410-3225 (3NH), 3106 (CH aromatic), 2968 (CH aliphatic). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.08 (s, 10H, spiro cyclohexyl), 2.15 (s, 3H, -S-CH$_3$), 2.35 (t, 2H, J = 3.8 Hz, γ-CH$_2$), 2.52-2.58 (m, 2H, β-CH$_2$), 3.06 (s, 3H, CH$_3$), 3.64 (t, 1H, J = 4.1 Hz, α-CH), 7.83-8.07 (m, 4H, H aromatic), 8.71, 9.32, 10.21 (3s, 3H, 3NH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 19.04 (CH$_3$), 30.76 (NH-CH$_3$), 33.26 (CH$_2$), 36.07 (CH$_2$), 38.98, 39.15, 39.32 (spiro cyclohexyl carbons), 54.12 (CH), 71.96 (spiro head carbon), 108.93, 116.32, 119.27, 131.90, 133.06, 149.18 (aromatic carbons), 154.52, 160.04 (2C=N), 164.35 (NH-C=N). MS (m/z): (M$^+$ - 1) 415 (40.09%). 68 (100%). Analysis: for C$_{20}$H$_{28}$N$_6$S$_2$, m.w. (416.61): calcd. C, 57.66; H, 6.77; N, 20.17; S, 15.39; found C, 57.28; H, 6.53; N, 20.09; S, 15.28%.

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

This compound was prepared from the starting benzoxazine derivative according to a reported method (19). M.p. 264°C.

Spiro{(2H, 3H)-3-[4-(3-methyl-1H-5(4H)-oxopy-razol-1-yl)carbonylmethoxy phenyl] quinazoline-2,1'-cyclohexan}-4(1H-)one (XIII)

Ethyl acetoacetate (1.95 mL, 15 mmol) was added to the hydrazine derivative (XII) (3.08 g, 10 mmol) in glacial acetic acid, and then was refluxed for 8 h. The reaction mixture was cooled, poured onto ice/H$_2$O, filtered, dried and crystallized from methanol to give light brown crystals.

Yield: 73%, m.p. 140-142°C. IR (KBr, cm^{-1}): 3436 (NH), 3027 (CH aromatic), 2931 (CH aliphatic), 1720-1667 (3C=O). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 1.58 (s, 10H, spiro cyclohexyl), 2.37 (s, 3H, CH$_3$), 2.87 (s, 2H of pyrazolidinone ring), 5.74 (s, 2H, CH$_2$), 6.81-8.11 (m, 8H, H aromatic), 8.51 (s, 1H, NH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 25.31 (CH$_3$), 38.71, 39.73, 40.06 (spiro cyclohexyl carbons), 42.81 (CH$_2$, pyrazolidinone ring), 68.56 (spiro head carbon), 70.03 (O-CH$_2$), 117.68, 120.97, 121.38, 127.08, 128.93, 129.54, 130.14, 135.16, 147.77, 154.65 (aromatic carbons), 159.04 (C=N), 164.05, 170.08 (2C=O). MS (m/z): (M$^+$ + 1) 447 (12.02%), 146 (100%). Analysis: for C$_{25}$H$_{26}$N$_4$O$_4$, m.w. (446.50): calcd. C, 67.25; H, 5.87; N, 12.55%; found C, 67.63; H, 6.27; N, 12.86%.

1-{[4-[Spiro((2H, 3H)-4(1H)-oxo-quinazoline-2,1'-cyclohexan)-3-yl]oxyphenyl]acetyl}4-phenyl thio-semicarbazide (XIV)

A solution of phenyl isothiocyanate (1.35 mL, 10 mmol) in methanol was added to a solution of the hydrazine derivative (XII) (3.80 g, 10 mmol) in methanol (10 mL). The reaction mixture was heated under reflux for 6 h. The reaction mixture was left to attain room temperature and the product was filtered, washed with ethanol, dried and crystallized from dioxane to yield white crystals.

Yield: 83%, m.p. 116-118°C. IR (KBr, cm^{-1}): 3461-3158 (4NH), 2985 (CH aliphatic), 1710, 1630 (2C=O), 1130 (C=S). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 2.46, 3.29, 3.41 (3s, 3H, 3NH, exchangeable with D$_2$O), 5.10 (s, 2H, CH$_2$), 6.86-7.52 (m, 13H, H aromatic), 10.13 (s, 1H, NH, exchangeable with D$_2$O). MS (m/z): M$^+$ 515 (9.27%), 77 (100%). Analysis: for C$_{28}$H$_{29}$N$_5$O$_3$S, m.w. (515.63): calcd. C, 65.22; H, 5.67; N, 13.58; S, 6.22%; found C, 64.84; H, 6.05; N, 13.85; S, 6.60%.

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

To a corresponding thiosemicarbazide deriva-tive (XIV) (0.72 g, 14 mmol), a solution of 2 M sodium hydroxide solution (0.8 g, 10 mL) (25 mL) was added portionwise. The reaction mixture was refluxed for 3 h. The mixture was then allowed to cool to room temperature. It was filtered and then the filtrate was acidified with 2 M hydrochloric acid. The precipitated solid was filtered, washed thoroughly with water, dried and recrystallized from methanol to give the triazolo derivative (XV).

Yield: 75%, m.p. 217-219°C. IR (KBr, cm^{-1}): 3433 (NH), 2923 (CH aliphatic), 1672 (C=O), 2550 (SH-stretching). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 1.20 (s, 10H, spiro cyclohexyl), 4.82 (s, 2H, CH$_2$), 6.86-7.63 (m, 13H, H aromatic), 9.32, 10.13 (2s, 2H, NH, SH, exchangeable with D$_2$O). ^{13}C NMR (300 MHz, DMSO-d$_6$, δ, ppm): 38.71, 39.73, 40.06 (spiro cyclohexyl carbons), 68.56 (spiro head carbon), 70.03 (-O-CH$_2$), 117.68, 121.39, 122.38, 127.47, 128.93, 130.14, 132.56, 135.16, 149.77, 156.46 (aromatic carbons), 159.04 (C=N), 160.35 (C=O), 168.03 (-N=C-SH). MS (m/z): M$^+$ 497 (11.40%), 256 (100%). Analysis: for C$_{28}$H$_{27}$N$_5$O$_2$S, m.w. (497.61): calcd. C, 67.58; H, 5.47; N, 14.07% S, 6.44%; found C, 67.89; H, 5.31; N, 13.78; S, 6.82%.

Spiro{(2H, 3H)-3-[4-(4-anilino-1,3,4-thiadiazol-2-yl)methoxyphenyl]quinazoline-2,1'-cyclohexan}-4(1H)-one (XVI)

To a corresponding thiosemicarbazide derivative (**XIV**) (3.09 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 dried and the product was crystallized from isopropanol to yield yellow crystals of the thiadiazolo derivative (**XVI**).

Yield: 75%, m.p. 250-252°C. IR (KBr, cm⁻¹): 3411, 3236 (2NH), 3127 (CH aromatic), 2922 (CH aliphatic), 1651 (CO). ¹H-NMR (DMSO-d₆, δ, ppm): 1.08 (s, 10H, spiro cyclohexyl), 5.21 (s, 2H, CH_2), 7.20-7.83 (m, 13H, H aromatic), 9.32, 10.13 (2s, 2H, 2NH, exchangeable with D_2O). MS (m/z): M^+ 497 (10.28%), 77 (100%). Analysis: for $C_{28}H_{27}N_5O_2S$, m.w. (497.61): calcd. C, 67.58; H, 5.47; N, 14.07; S, 6.44%; found C, 67.31; H, 5.73; N, 14.32; S, 6.08%.

Cytotoxic activity screening

Preliminary anticancer experiments were done using the breast carcinoma cell line to identify the potential cytotoxicity of nineteen newly synthesized compounds (**II**, **IV**, **V**, **VIa**, **VIb**, **VIc**, **VIIa**, **VIIb**, **VIIc**, **VIIIc**, **Xa**, **Xb**, **Xc**, **XIa**, **XIb**, **XIc XIII**, **XV** and **XVI**) in comparison to the known anticancer drug - doxorubicin by SRB using the method of Skehan et al. (31) as follows: Cells were plated in 96-multiwell plate (10^4 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 (0.01, 0.1, 1, 10, 100 µM) 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.

Molecular modeling

All molecular modeling studies were performed on a RM Innovator Pentium IV2.4 GHz running Linux Fedora Core 3. The protein crystal structure of Poly (ADP-ribose) polymerase-1 (PARP-1) was downloaded from (http://www.rcsb.org/ -pdb code: 1UK1). Hydrogen atoms were added to the protein using the protentate 3D option in MOE 2010.10 (Molecular Operating Environment); (http://www.chemcomp.com.). Ligand structures were built with MOE and energy minimized using the MMFF94x force field until a RMSD gradient of 0.05 Kcal mol⁻¹ Å⁻¹ was reached. Docking procedure was done using Flex-X-Leadit.2.1.2 program (http;//www. BioSolveIt GmbH-leadit.).

RESULTS AND DISCUSSION

Chemistry

In this investigation, the key starting material 4-chloroquinazoline derivative **I**, prepared according to reported method (19), was refluxed with thiourea in absolute ethanol to obtain the quinazoline-thiol derivative **II** according to reported method (25). IR, ¹H-NMR, mass spectra and elemental analyses were used for determination and identification of the structures of all the new compounds. IR spectrum of derivative **II** revealed the presence of absorption bands at 3395 cm⁻¹ and 1301 cm⁻¹ corresponding to NH and C=S groups respectively. 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 (19). Accordingly, reaction of 4-chloroquinazoline compound **I** with an excess of hydrazine hydrate in ethanol under reflux afforded the hydrazino derivative **III** in a high yield. Cyclocondensation of **III** with ethyl acetoacetate in glacial acetic acid resulted in the formation of 3-methylpyrazolidinone derivative **IV**. ¹H-NMR (CDCl₃, δ, ppm) spectrum of **IV** showed two singlet signals at δ 2.07 and δ 3.34 ppm representing the protons of CH_3 and CH_2 of pyrazolidinone ring. Moreover, the mass spectrum of compound **IV** exhibited the molecular ion peak at m/z 296 (67.35%). In order to obtain 1,2,4-triazino[4,3-c]quinazoline-3,4-dione derivative **V**, the hydrazino derivative **III** was allowed to react with diethyl oxalate in refluxing absolute ethanol containing a catalytic amount of sodium ethoxide (26). IR spectrum of compound **V** exhibited an absorption band at the range of 3323-3159 cm⁻¹ attributed to three NH groups and two characteristic bands at 1702 and 1667 cm⁻¹ due to the presence of two C=O groups. ¹H NMR (CDCl₃, δ ppm) of the same derivative displayed a singlet signal at δ 5.84 ppm corresponding to the methine proton of HN-C*H*-N- of the triazine ring, in addition to other three exchangeable singlets at δ 2.65, 8.09 and 12.24 ppm representing the protons of the three NH groups.

The α-carboxyl and α-amino groups of all amino acids exhibit characteristic chemical reactivi-

ty. Thus in our investigation, the chloro quinazoline derivative **I** was allowed to react with different amino acids in the presence of Na_2CO_3 as a catalytic base at pH 9-9.5 (27) to get the quinazoline amino acid derivatives **VIa-c**. ^{1}H-NMR (DMSO-d_6, δ, ppm) of **VIa** showed the methine proton of α-C*H* as a triplet signal at δ 4.13 ppm while the two protons of β-C*H$_2$* of the amino acid side chain appeared as a doublet at δ 3.04 ppm. ^{1}H NMR (DMSO-d_6, δ, ppm) of **VIb** showed the methine proton of α-C*H* as a triplet signal at δ 4.13 ppm, the three protons of –C*H*-C*H$_2$*- appeared as a multiplet at δ 1.82 ppm, while the six protons of 2C*H$_3$* represented another multiplet at δ 1.11 ppm. Also, ^{1}H NMR (DMSO-d_6, δ, ppm) of **VIc** showed a singlet signal at δ 2.09 ppm attributed to the three protons of the C*H$_3$* group, while the four protons of -C*H$_2$*-C*H$_2$*-S group appeared as multiplet and triplet signals at δ 2.15 and δ 2.41 ppm, respectively. The methine proton of the α-C*H* group was represented as a triplet signal at δ 3.49 ppm.

The acid chloride derivatives **VIIa-c** were synthesized by the reaction of the corresponding amino-acid analogues with thionyl chloride in chloroform at 70ºC (28). Further condensation with excess hydrazine hydrate in refluxing absolute ethanol led to obtain the hydrazide analogues **VIIIa-c** which were allowed to react with methyl isothiocyanate in refluxing methanol to get the target thiosemicarbazide derivatives **IXa-c**. Mass spectra of the obtained derivatives represented the molecular ion peaks at m/e 450 (15.47%), 416 (10.58%) and 434 (17%), respectively.

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 (29), 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 (30). Thus, since the scope of this study was 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 **Xa-c** and **XIa-c**, respectively. IR spectra of **Xa-c** represented stretching bands at the region of 2598-2606 cm^{-1} due to the thiol group, in addition to other two stretching bands at the regions 3330-3119 cm^{-1} contributing to 2NH groups. Also, IR spectra of **XIa-c** exhibited three stretching absorption bands in the regions 3415-3120 cm^{-1} representing 3NH groups (Scheme 1).

Another trend in this investigation was to prepare different quinazolinone derivatives to compare the effect of the structural modifications of the quinazolinone derivatives with that of the quinazolines on the cytotoxic activity. So, the acetohydrazide quinazolinone derivative **XII**, prepared according to reported method (19), was reacted with ethyl acetoacetate in glacial acetic acid to obtain the pyrazolidinone derivative **XIII**. The structure of this derivative was confirmed on the basis of different microanalytical and spectral data. IR spectrum exhibited the presence of three absorption bands due to 3 C=O groups at the range of 1720-1670 cm^{-1} and an absorption band at 3436 cm^{-1} indicating NH group. ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) displayed the presence of four singlet signals at δ 1.58, 2.37, 2.87 and 5.74 ppm assigned for ten protons of the spiro cyclohexyl ring, C*H$_3$*, -C*H$_2$*-CO of pyrazolidinone ring and -O-C*H$_2$*- groups, respectively. The aromatic protons appeared as multiplet signals at δ 6.81-8.11 ppm, while NH appeared as an exchangeable signal at δ 8.51 ppm.

Further, reaction of the methanolic solution of the hydrazide derivative **XII** with phenyl isothiocyanate was performed to obtain the corresponding phenyl thiosemicarbazide derivative **XIV** which was further subjected to intramolecular cyclization using either 2 M NaOH or conc. H_2SO_4 to obtain triazolo and thiadiazolo-quinazolinone derivatives, respectively. Microanalyses and spectral data confirmed the structures of the obtained compounds. IR spectrum of compound **XV** revealed the presence of an absorption band at 2550 cm^{-1} attributed to SH group, while its ^{1}H NMR (DMSO-d_6, δ, ppm) exhibited two singlet signals at δ 1.20, 4.82 ppm corresponding to 10 H of the spiro cyclohexyl ring and the two methylene protons of –O-C*H$_2$* group, respectively. Two exchangeable singlets were present at δ 9.32 and 10.13 ppm due to NH and SH groups, respectively. IR spectrum of the thiadiazolo quinazolinone derivative **XVI** represented the absorption bands of two NH groups at 3411, 3236 cm^{-1} and C=O group at 1651 cm^{-1}. Mass spectrum of the same derivative revealed the molecular ion peak at m/z 497 (10.28%) (Scheme 2).

In vitro cytotoxic activity

In this work, nineteen newly synthesized compounds **II**, **IV**, **V**, **VIa**, **VIb, VIc**, **VIIa**, **VIIb**, **VIIc**, **VIIIc**, **Xa**, **Xb**, **Xc**, **XIa**, **XIb**, **XIc**, **XIII**, **XV** and **XVI** that represented different classes of the newly synthesized quinazolines, were selected to evaluate their growth inhibitory activity against breast carcinoma cell line (MCF-7) using the sulforhodamine B stain (SRB) assay in a trial to correlate between both structural variations and cytotoxic activity of the
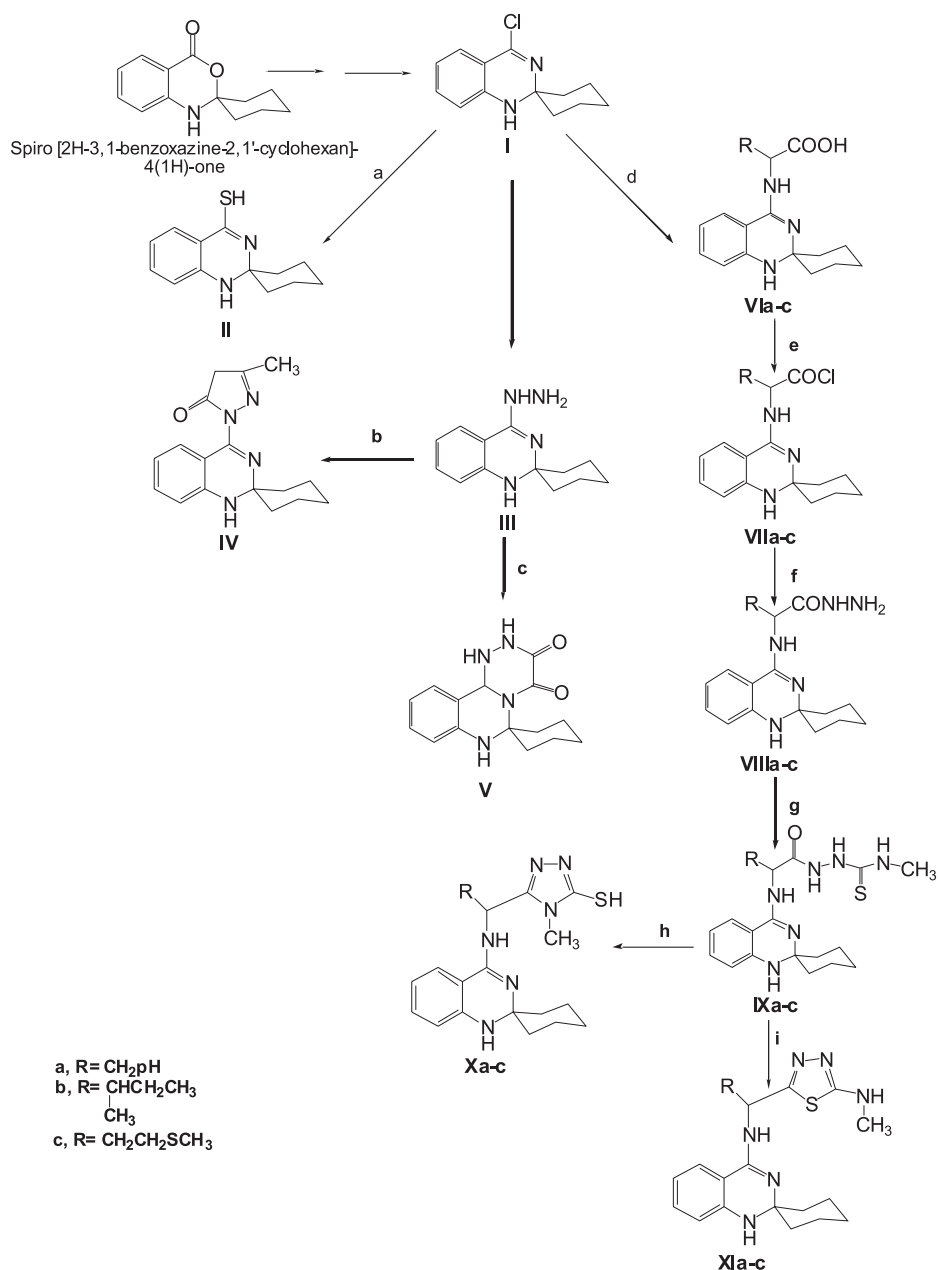
Scheme 1. (a) H$_2$NCSNH$_2$, abs. EtOH, reflux 4 h. (b) CH$_3$COCH$_2$COOEt, glacial AcOH, reflux 8 h. (c) (COOEt)$_2$, abs. EtOH, reflux 8 h. (d) different amino acids, Na$_2$CO$_3$, reflux at 100°C for 8 h. (e) SOCl$_2$, CHCl$_3$, 70°C, 30 min. (f) NH$_2$NH$_2$, EtOH reflux 3 h. (g) S=C=N-CH$_3$, MeOH, reflux 70-80°C, 3 h. (h) 2 M NaOH, reflux 80°C, 3 h. (i) conc. H$_2$SO$_4$, room temp., stirring 3 h

synthesized compounds. IC$_{50}$ was determined for each compound using doxorubicin as a reference standard (31).

With respect to the group of the 4-substituted quinazoline derivatives, the anticancer evaluation showed a wide variation according to the different substituents or the ring systems conjugated to 4-position of quinazoline ring. Marked efficiency has been gained by the derivatives having quinazoline ring fused to pyridazine ring **V** or attached to the

thiadiazole moiety of amino acid isoleucine **XIb** (IC$_{50}$ = 1.3, 11.2 µM). Gradual reduction in the efficacy was detected by the analogue bearing free ioleucine amino acid **VIb**, the chloro derivatives of phenylalanine amino acid **VIIa** and methionine amino acid **VIIc** (IC$_{50}$ = 14.2, 23.4, 26.3 µM), in addition to the thiadiazole analog of phenylalanine amino acid **XIa** (IC$_{50}$ = 33.4 µM).

Further cytotoxic activity reduction was observed by the hydrazide-methionine amino acid derivative **VIIIc** (IC$_{50}$ = 52.2 µM), the pyrazolidinone derivative **IV** (IC$_{50}$ = 68.6 µM) and the triazole methionine amino acid analogue **Xc** (IC$_{50}$ = 50.6 µM). The thioquinazoline **II** and phenyl alanine quinazoline **VIa** derivatives displayed lower cytotoxic activity (IC$_{50}$ = 78.9, 76.0 µM). The quinazoline derivatives bearing free methionine amino acid **VIc**, the chloro analogue of isoleucine amino acid **VIIb** and the triazolo derivatives of either phenyl alanine **Xa** or isoleucine **Xb** amino acids were completely inactive (IC$_{50}$ = >100 µM) (Table 1).

Furthermore, the cytotoxic activity of a number of 3-(*p*-substituted phenyl) quinazolinones derivatives was also studied. The attachment of thiadiazole ring *via* an ether linkage to the phenyl ring **XVI** exhibited good activity (IC$_{50}$ = 14.7 µM), while the triazolo quinazolinone derivative **XV** displayed lower cytotoxic activity (IC$_{50}$ = 62.8 µM). Unfortunately, the analogue bearing the pyrazolidinone ring **XIII** was completely inactive (Table 1).

Molecular docking studies

The resultant antitumor activity of the tested compounds, especially **V**, **VIb** and **XIb**, prompted us to perform molecular docking studies to understand the ligand–protein interactions in details. The most active compounds **V**, **VIb**, **VIIa**, **VIIc**, **VIIIc**, **Xc**, **XIa**, **XIb**, **XV** and **XVI** were docked with FlexX-Leadit.2.1.2. The active site of PARP-1 was defined to include residues within at 6.5 Å and the docking scores were calculated by the same program. Visualization inside the pocket site was done with MOE 2010.10.

In general, the most active compound **V** formed four H-bonds with the residues of the pocket site, NH of 1,2,4-triazine-5,6-dione moiety donated (H) to C=O group of Asp770 in distance 1.65 Å and C=O group of triazine moiety formed two H-bonds with NH of His862 and OH of Ser 864 in distance 2.85, 2.98 Å, respectively, while Arg878 accepted (H) from NH of quinazoline ring to form H-bond in distance 1.85 Å (Figs. 1-3). Compound **V** exhibited good fitting inside the pocket site of the residue, it embedded completely inside the protein molecular surface (Fig. 4). The H-bonds formation between **V** and residues of the pocket site was in good distance for interaction and perfect fitting inside the molecular surface in addition to reasonable docking score (-20.60). This compound displayed the best docking score which correlates with the biological results (Table 2).
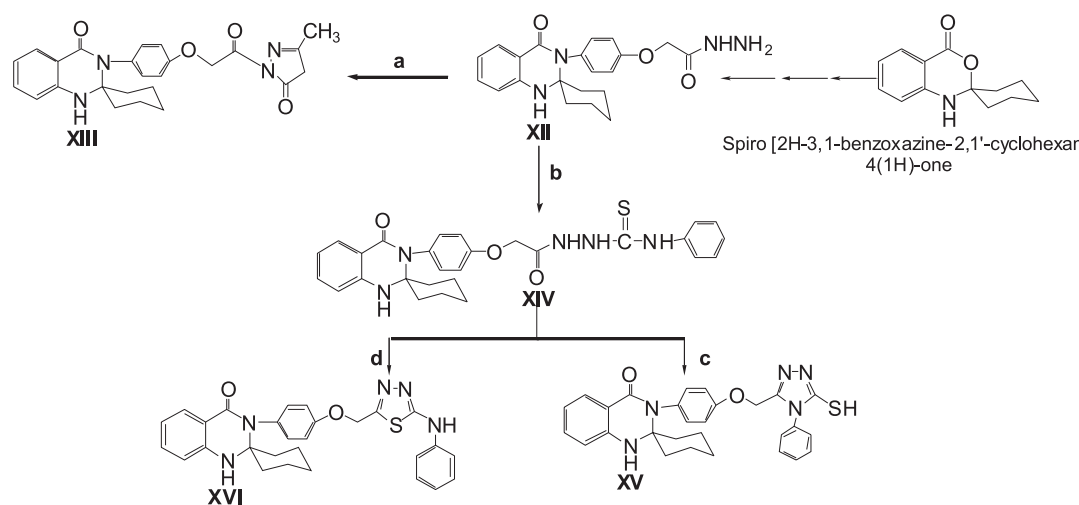
Scheme 2. (a) CH$_3$COCH$_2$COOEt, glacial AcOH, reflux 8 h. (b) S=C=N-Ph, MeOH, reflux, 6 h. (c) 2 M NaOH, reflux 80°C, 3 h. (d) conc. H$_2$SO$_4$, room temp., stirring 3 h
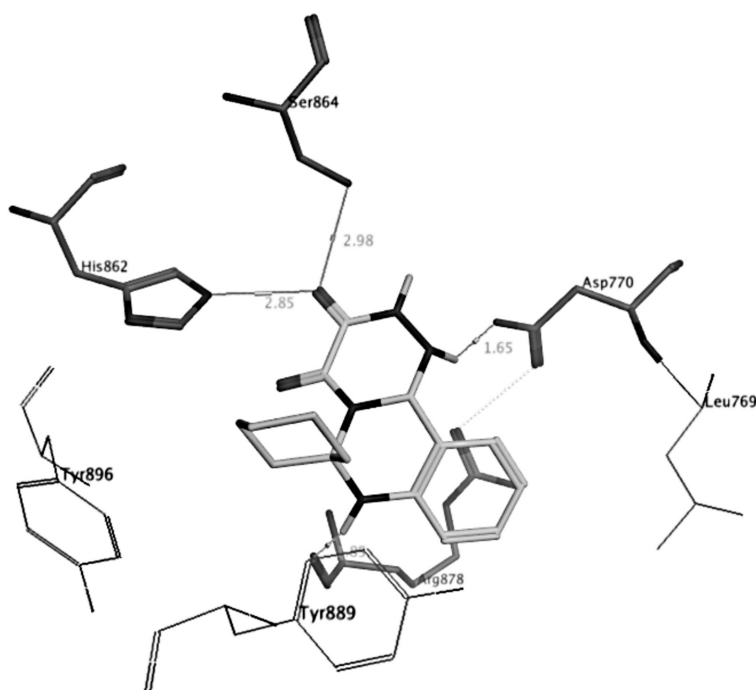
Figure 1. Interaction of compound **V** (stick, black) with amino acid residues (stick, dark grey) of the pocket site of PARP-1 enzyme. 1,2,4-triazine-5,6-dione moiety formed three H-bonds, NH with Asp770 in distance 1.65 Å and C=O group formed two H-bonds with His 862, Ser 864 in distance 2.85, 2.98 Å, respectively.
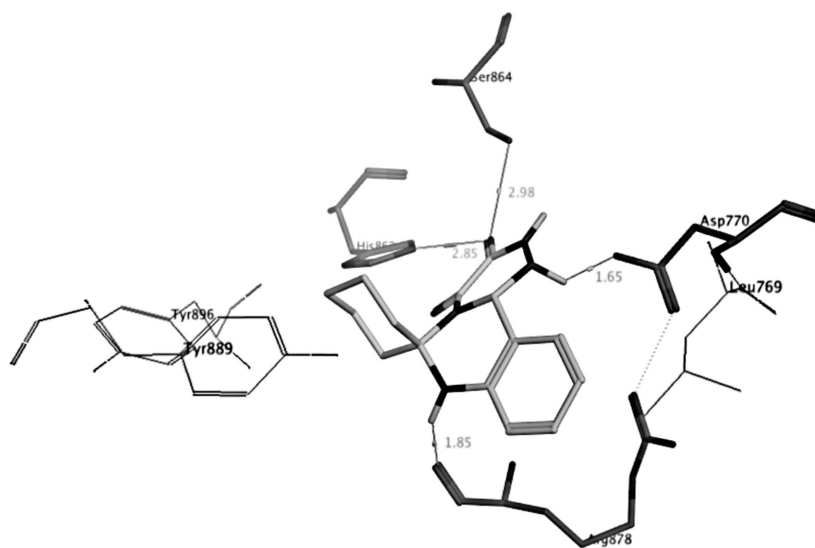

Figue 2. Compound **V** measured distance between NH of quinazoline ring and C=O group of Arg 878 (1.85 Ĺ)

Replacement of 1,2,4-triazine-5,6-dione moiety by NH-CH linker space in compound **XIb** and the presence of thiadiazole moiety kept the good fitting of **XIb** in the binding site of the enzyme (Fig. 5), but the number of H-bonds between **XIb** and the residues decreased to three, as NH interacted with Glu763 in distance 1.88 Å, Ser864 formed H-bond with NH of quinazoline in distance 1.85 Å and Tyr896 accepted hydrogen from NH moiety and formed H-bond in distance 1.91 Å. Docking score of **XIb** was -20.2 (Table 2).
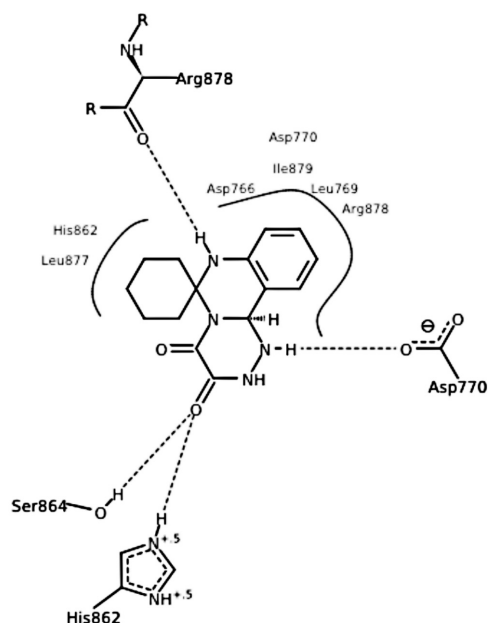
Figure 3. The possible interaction between **V** and His862, Ser864, p770 and Arg878

Figure 4. Compound **V** (grey, stick) deeply embedded into PARP-1 binding domain**,** forming H-bonds with different residues (light grey, stick). Molecular surface of PARP-1 colored as hydrophobic (light grey), hydrophilic (dark grey) and neutral (white)

The carboxylic acid of **VIb** formed four H-bonds with His862, Ser864 and Arg 865. Moreover, the NH moiety of quinazoline ring also interacted with Glu763 and Asp766 in a distance 1.71, 2.06 Å), which indicates the importance of free NH group in the activity of our synthesized compounds. Docking score of **VIb** was -19.6 (Table 2).

NH of quinazoline ring of **XVI** kept the interaction with the pocket site residue. As Arg878 accepted hydrogen from NH at a good distance 1.72 Å (Table 2).

The two analogues **VIIa** and **VIIc** exhibited nearly the same interaction with the amino acids, except the phenyl group **VIIa** formed arene-H bond with His862 and **VIIc** formed by its sulfur atom H-bond with Gly863. **VIIa** and **VIIc** had docking scores -17 and -16, respectively (Table 2).

The sulfur group in **Xc** was observed to have an interaction with His862 and Ser864, also NH group in **XIa** interacted with Gly894 (data not shown).

Compounds **XV** and **VIIIc** that displayed low cytotoxic activity, had two conformations either forming one H-bond with one amino acid or no bonding or interaction (Table 2).

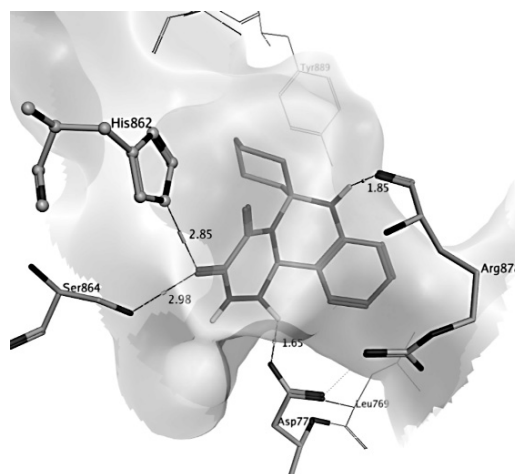It is clear that the molecular docking results are in agreement with the biological assay data.

Lipinski rule of five and ADME profile

We assessed the most active compounds (**V, VIb, VIIa, VIIc XIb, XVI**) using ADME (adsorption, distribution, metabolism, elimination) method. In particular, we calculated the compliance of compounds to the Lipinski's rule of five (32). Briefly, this simple rule is based on the observation that most orally administered drugs have a molecular weight (m.w.) of 500 or less, a log P no higher than 5, five or fewer hydrogen bond donor sites and 10 or fewer hydrogen bond acceptor sites. In addition, we calculated the polar surface area (PSA), since it is another key property that has been linked to drug bioavailability (33). Thus, passively absorbed molecules with a PSA > 140 Å^2 are thought to have low oral bioavilabilities (34).

Our results showed that most of the active compounds fulfilled Lipinski rule (molecular weight = 286.3-497.6, log P = 1.3-4.9, number of hydrogen bond acceptors = 2-4, number of hydrogen bond donors 2-3) (Table 3). In addition, our active compounds fulfilled the topological descriptors and fingerprints of molecular drug-likeness structure keys as PSA, log S.

We can conclude that biological results supported by the docking results (Table 2) and ADME profile (Table 3) suggested strongly that compounds **V**, and **VIb** are promising agents as breast cancer inhibitors with drug likeness approach that have PARP-1 inhibitory activity.

Table 1. Cytotoxicity assessment of the tested derivatives against MCF-7 breast adenocarcinoma cell line.

Compound no.	IC$_{50}$ μM	Compound no.	IC$_{50}$ μM
II	78.9	**Xa**	>100
IV	68.6	**Xb**	>100
V	1.3	**Xc**	50.6
VIa	76.0	**XIa**	33.4
VIb	14.2	**XIb**	11.2
VIc	>100	**XIc**	111.1
VIIa	23.4	**XIII**	>100
VIIb	>100	**XV**	62.8
VIIc	26.3	**XVI**	14.7
VIIIc	52.2	Doxorubicin	0.13

[a] IC$_{50}$ - compound concentration required to inhibit tumor cell line proliferation by 50%. [b] Values are the means of three experiments.
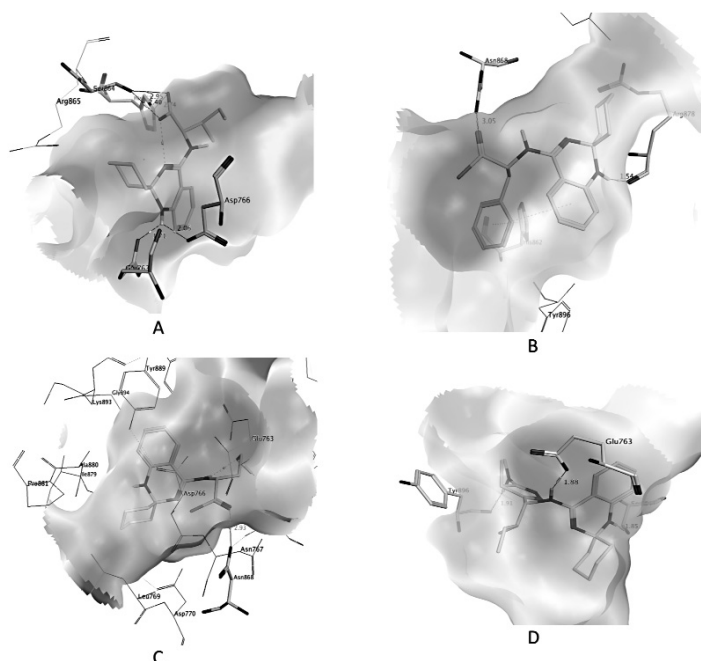

Figure 5. Fitting of compounds **VIb** (panel A), **VIIa** (panel B) and **VIIc** (panel C), **XIb** (panel D) into pocket site of PARP-1. The ligands (stick, light grey). The molecular surface of the pocket site colored as hydrophobic (light grey), hydrophilic (dark grey) and neutral (white)

CONCLUSION

The present work deals with the development of novel antitumor compounds bearing spiro[(2H,3H)-quinazoline-2,1'-cyclohexane] pharmacophore. Human breast cell line (MCF-7) was selected to evaluate the cytotoxicity effect of the synthesized compounds. Most of the derivatives exhibited promising cytotoxic activity against the carcinoma cell line. As PARP-1 inhibition may increase the efficiency of certain cytotoxic treatments, the compounds which induced the highest activity **V**, **VIb**, **VIIa**, **VIIc**, **VIIIc**, **Xc**, **XIa**, **XIb**, **XV** and **XVI** were docked with FlexX-Leadit.2.1.2. to the active site of PARP-1 enzyme to define the compounds-protein interactions in details. Visualization inside the pocket site was done with MOE 2010.10. Molecular docking stud-

Table 2. All docking results showing the main residues involved in the interactions with the docked compounds.

Compound no.	Docking scores	Main atoms From the compound	Main residue From PARP-1	Distance Å
V	-20.6	NH of triazinane-5,6-dione	C=O group of Asp770	1.65
		C=O of triazinane-dione	NH of His862 OH of Ser864	2.85 2.98
		NH of quinazoline	C=O group of Arg878	1.85
VIb	-19.60	NH of quinazoline	OH of Asp766 OH of Glu763	2.06 1.71
		Free carboxylic group	NH of Arg865 OH of Ser864 NH of Hist862	2.87 2.40 & 2.95 2.74
VIIa	-17.00	C=O group	NH of Asn868	3.05
		NH of quinazoline	C=O of Arg878	1.54
		Phenyl ring of side chain	Arene-H bond with Hist862	
VIIc	-16.2	Sulfur atom	NH of Gly863	2.09
		NH group	C=O Arg878	1.89
		C=O group	NH of Asn868	2.93
VIIIc	-25.04	S-CH₃	Ser864	1.70
Xc	-19.73	Sulfur atom	His862, Ser864	3.19 & 3.08
XIa	-19.96	NH group	Gly894	1.84
XIb	-20.2	NH group	OH of Glu763	1.88
		NH group	C=O Tyr896	1.91
		NH of quinazoline	OH of Ser864	1.85
XV	-19.70	SH group	Gly863	1.97
XVI	-26.46	NH of quinazoline phenyl ring of quinazoline thiadiazine ring	C=O group of Arg878 Arene-H bond interaction with Ala880 Arene-H bond interaction with Tyr896	1.72

Table 3. Solubility and calculated Lipinski's rule of five for the most active compounds over breast cancer (MCF-7) cell line.

Compound No.	IC₅₀	log S	Parameter				
			log P	PSA	MW	nH-acc	nH-don
V	01.3	-2.79	1.3	73.47	286.3	3	3
VIb	14.2	-3.81	4.2	76.88	331.4	4	3
VIIa	23.4	-6.08	5.9	53.49	381.0	2	2
VIIc	26.3	-5.44	4.9	92.29	351.9	2	2
XIb	11.2	-6.25	5.9	92.38	412.6	3	3
XVI	14.7	-7.92	7.2	79.38	497.6	2	4

log S - solubility parameter; MW - molecular weight; log P - calculated lipophilicity; nH-acc - number of hydrogen bond acceptors; PSA - polar surface area (Å²); nH-don - number of hydrogen bond donors

ies supported the promising inhibitory activity of the tested compounds. The most active compounds (**V**, **VIb**, **VIIa**, **VIIc**, **XIb** and **XVI**) were assessed using ADME (adsorption, distribution, metabolism, elimination) method to calculate the compliance of compounds to the Lipinski's rule of five. We can conclude that biological results were supported by the docking results (Table 2) and ADME profile (Table 3) suggesting strongly that compounds **V** and **VIb** are promising agents as breast cancer inhibitors with drug likeness approach that have PARP-1 inhibitory activity. These results indicated the importance of spiro cyclohexyl-quinazoline ring when fused with triazine ring **V** or attached to the amino acid isoleucine **VIb** to adopt conformations that have identical distances and orientations to give the greatest inhibition of breast carcinoma cell line.

Acknowledgment

Competing interests

The authors declare no conflict of interest.

REFERENCES

1. Mhaske S.B., Argade, N.P.: Tetrahedron 62, 9787 (2006).
2. Liverton N.J., Armstrong D.J., Claremon D.A., Remy D.C., Baldwin J.J., Lynch R.J., Zhang G., Gould R.J.: Bioorg. Med. Chem. Lett. 8, 483 (1998).
3. Zhang W., Mayer J.P., Hall S.E., Weigel J.A.: J. Comb. Chem. 3, 255 (2001).
4. Griffin R.J., Srinivasan S., Bowman K., Calvert A.H., Curtin N.J., Newell D.R., Pemberton L.C., Golding B.T.: J. Med. Chem. 41, 5247 (1998).
5. Hughes A.N., Rafi I., Griffin M.J., Calvert A.H., Newell D.R., Calverte J.A., Johnston A. et al.: Clin. Cancer Res. 5, 111 (1999).
6. Hamel E., Lin C.M., Plowman J., Wang H.K., Lee K.H., Paull K.D.: Biochem. Pharmacol. 51, 53 (1996).
7. Hwang S.H., Rait A., Pirollo K.F., Zhou Q., Yenugonda V.M., Chinigo G.M., Brown M. L., Chang E.H.: Mol. Cancer Ther. 7, 559 (2008).
8. Bridges A.J.: Chem. Rev. 101, 2541 (2001).
9. Kundu S.K., Mahidranate M.P.D., Quintero M.V., Bao A., Negrete G.R.: Arkivoc (ii), 33 (2008).
10. Cohen M.H., Johnson J.R., Chen Y.F., Sridhara R., Pazdur R.: Oncologist 10, 461 (2005).
11. Sielecki T.M., Johnson T.L., Liu J., Muckelbauer J.K., Grafstrom R.H., Cox S., Boylan J. et al.: Bioorg. Med. Chem. Lett. 11, 1157 (2001).
12. Hattori K., Kido Y., Yamamoto H., Ishida J., Iwashita A., Mihara K.: Bioorg. Med. Chem. Lett. 17, 5577 (2007).
13. Madhusudan S., Middleton M.R.: Cancer Treat. Rev. 31, 603 (2005).
14. De-Soto J.A., Deng, C.X.: Int. J. Med. Sci. 3(4), 117 (2006).
15. Bryant H.E., Schultz N., Thomas H.D., Parker K.M., Flower D., Lopez E., Kyle S. et al.: Nature 434, 913 (2005).
16. Inbar-Rozensal D., Castiel A., Visochek L., Castel D., Dantzer F., Izraeli S., Cohen-Armon M.: Breast Cancer Res. 11, R78 (2009).
17. Frizzell K.M., Kraus, W.L.: Breast Cancer Res. 11, 111 (2009).
18. Rodon J.; Iniesta M.D., Papadopoulos, K.: Expert Opin. Investig. Drugs. 18, 31 (2009).
19. Amin K.M., Anwar M.M., Syam Y.M., Khedr M.A., Kamel M.M., Kassem E.M.: Acta Pol. Pharm. Drug Res. 70, 687 (2013).
20. Ferrigno F., Branca D., Kinzel O., Lillini S., Bufi L., Monteagudo E., Muraglia E.M. et al.: Bioorg. Med. Chem. Lett. 20, 1100 (2010).
21. Moree W.J., Goldman P., Demaggio A.J., Christenson E., Herendeen D., Eksterowicz J., Kesicki E.A. et al.: Bioorg. Med. Chem. Lett. 18, 5126 (2008).
22. Shivarama H.B., Veerendra B., Shivananda M.K., Poojary B.: Eur. J. Med Chem. 38, 759 (2003).
23. Wei M.X., Feng L., Li X.Q., Zhou X.Z., Shao Z.H.: Eur. J. Med. Chem. 44, 3340 (2009).
24. Ashworth A.: J. Clin. Oncol. 26, 3785 (2008).
25. Postovskii Y.; Vereshchagina N.N., Mertsalov S.L.: Chem. Heterocycl. Compd. 2, 94 (1966).
26. Abdel-Megid M., Elkazak A.M., Seada M., Farouk O.: J. Appl. Sci. Res. 6, 2133 (2010).
27. Cherng Y.J.: Tetrahedron 56, 8287 (2000).
28. Varnavas A., Lassiani L., Valenta V., Berti F., Tontini A., Mennuni L., Makovec F.: Eur. J. Med. Chem. 39, 85 (2004).
29. Hejsek M., Wiedermannová I.: AUPO Chemica 40, 15 (2001).

30. Samel A.B., Pai, N.P.: J. Chin. Chem. Soc. 57, 1327 (2010).
31. Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J.T. et al.: J. Natl. Cancer Inst. 82, 1107 (1990).
32. Lipinski C.A., Lombardo F., Dominy B.W., Feeney P.J.: Adv. Drug Deliv. Rev. 46, 3 (2001).
33. Clark D.E., Pickett S.D.: Drug Discov. Today 5, 49 (2000).
34. Palm K., Stenberg P., Luthman K., Artursson, P.: Pharm. Res., 14, 568 (1997).