Quinazoline derivatives are of considerable chemical and pharmacological importance as therapeutic agents (1-3). They are being extensively utilized 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-substituted-1,3-dihydro-quinazolinone derivatives are associated with inhibitory effects on tubulin polymerization 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 quinazolines 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 inhibition 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 suggesting a potential broader utilization of PARP-1 inhibitors as single agents in treating certain mutations of breast cancer (17), and also as chemo-sensitizers 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.

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

KAMELIA M. AMIN1, MANAL M. ANWAR*, MOHSEN M. KAMEL2, EMAD M. M. KASSEM2, YASMIN M. SYAM2 and SAMIA A. ELSEGINY3

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Egypt
2Department of Therapeutical Chemistry, National Research Centre, Dokki, Cairo, Egypt
3Welsh School of Pharmacy, Cardiff University, UK

**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 doxorubicin 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 microanalysis and IR, 1H-NMR and mass spectral data.

**Keywords:** spiroquinazoline-cyclohexane derivatives, cytotoxic activity, molecular docking, PARP-1 enzyme, drug-likeness

---

* Corresponding author: e-mail: manalhassan232@ymail.com. phone.: (20) 01223956970; fax: + (202) 337-0931f
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). 1H NMR and 13C 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).1H NMR (CDCl₃, 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 D₂O). 13C NMR (300 MHz, DMSO-d₆, 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 C₁₃H₁₆N₂S, 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/H₂O. The formed precipitate was filtered, dried and crystallized from methanol to obtain pale yellow crystals of the derivative (IV).

Yield: 82%, m.p. >300°C. IR (KBr, cm⁻¹): 3226 (NH), 3057 (CH aromatic), 2927 (CH
aliphatic), 1667 (C=O). 1H NMR (CDCl3, δ, ppm): 1.96 (s, 10H, spiro cyclohexyl), 2.07 (s, 3H, CH3), 3.34 (s, 2H, CH, of pyrazoline ring), 6.46-7.40 (m, 4H, H aromatic), 10.13 (s, 1H, NH, exchangeable with D2O), 5.84 (s, 1H, -CH of triazine ring), 7.49-8.10 (m, 4H, H aromatic), 8.32, 8.73, 10.23 (3s, 3H, 2NH, OH, exchangeable with D2O). 13C NMR (300 MHz, DMSO-d6, δ, ppm): 23.84 (CH3), 38.71, 39.67, 40.06 (spiro cyclohexyl carbons), 42.81 (CH2, 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 C17H20N4O, 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-11H-(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 (C=O). 1H NMR (CDCl3, δ, ppm): 1.07 (s, 10H, spiro cyclohexyl), 2.65 (s, 1H, NH, exchangeable with D2O), 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 D2O). 13C NMR (300 MHz, DMSO-d6, δ, ppm): 38.71, 39.88, 40.06 (spiro cyclohexyl carbons), 65.53 (spiro head carbon), 1.51-1.57 (m, 2H, β-CH2), 4.13 (t, 1H, γ-CH2), 2.41-2.44 (m, 2H, α-CH), 7.31-7.52 (m, 4H, H aromatic). MS (m/z): M+ 286 (5.12%), 146 (100%). Analysis: for C19H27N3O2, m.w. (286.33): calcd. C, 69.27; H, 6.93; N, 11.56%; found C, 69.53; H, 8.65; N, 11.18%.

General procedure for preparation of 2-[[spiro[(1H,2H)-quinazoline-2,1’-cyclohexan]-4-yl]amino]-3-phenyl propionic acid (VIa)

Amino acids (10 mmol) and Na2CO3 (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 chloroquinazoline 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 H2O 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). 1H NMR (DMSO-d6, δ, ppm): 2.31 (s, 10H, spiro cyclohexyl), 3.04 (d, 2H, J = 7.4 Hz, β-CH2), 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 D2O). 13C NMR (300 MHz, DMSO-d6, δ, ppm): 38.81 (CH3), 39.89, 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 C19H27N3O2S, m.w. (363.45): calcd. C, 69.53; H, 8.65; N, 11.18%.

2-[[spiro[(1H,2H)-quinazoline-2,1’-cyclohexan]-4-yl]amino]-3-methyl pentanoic acid (VIIb)

(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). 1H NMR (DMSO-d6, δ, ppm): 0.93 (t, 3H, J = 4.1 Hz, CH3), 1.15 (d, 3H, J = 7.2 Hz, CH3), 1.21 (s, 10H, spiro cyclohexyl), 1.51-1.57 (m, 2H, γ-CH2), 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 D2O). MS (m/z): M+ 329 (5.12%), 86 (100%). Analysis: for C19H19N3O2, m.w. (329.44): calcd. C, 69.27; H, 8.65; N, 13.05%.

2-[[spiro[(1H,2H)-quinazoline-2,1’-cyclohexan]-4-yl]amino]-4-(methylthio)-butanoic acid (VIIc)

(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). 1H NMR (DMSO-d6, δ, ppm): 2.31 (s, 10H, spiro cyclohexyl), 2.09 (s, 3H, CH3), 2.15 (t, 2H, J = 3.8 Hz, γ-CH2), 2.41-2.44 (m, 2H, β-CH), 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 D2O). MS: (m/z): M+ 347 (6.21%), 61 (100%). Analysis: for C19H27N3O2S,
m.w. (347.48): calcd. C, 65.60; H, 5.98; N, 11.18%; found C, 66.83; H, 8.75; N, 19.98%.

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

The quinazoline derivatives (VIIa-c) (1 mmol) were dissolved in dry chloroform, then, thionyl chloride (2.56 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), methanol to give grayish white crystals, yield: 73%, m.p. 168-170°C. IR (KBr, cm⁻¹): 3433, 3304 (2NH), 3057 (CH aromatic), 2958 (CH aliphatic), 1710 (C=O). 1H NMR (DMSO-d₆, δ, ppm): 1.86 (s, 10H, spiro cyclohexyl), 2.16 (s, 3H, CH₃), 2.24 (t, 2H, J = 3.8 Hz, γ-CH₂), 2.41-2.44 (m, 2H, β-CH), 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 D₂O). MS (m/z): (M⁺ + 2) 367 (1.03%), (M⁺) 365 (3.09%) (1 : 3). Analysis: for C₁₉H₂₆ClN₃O, m.w. (347.88); calcd. C, 65.60; H, 5.98; N, 11.18%; found C, 66.83; H, 8.75; N, 19.98%.

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. 210-212°C. IR (KBr, cm⁻¹): 3434-3210 (NH₂, 3NH), 3050 (CH aromatic), 2963 (CH aliphatic), 1670 (C=O). 1H NMR (DMSO-d₆, δ, ppm): 3.21 (s, 10H, spiro cyclohexyl), 3.22 (d, 2H, J = 6.6 Hz, γ-CH₂), 4.27 (t, 2H, J = 3.8 Hz, α-CH), 7.20-7.26 (m, 9H, H aromatic), 8.20, 8.69 (2s, 2H, 2NH, exchangeable with D₂O). MS (m/z): (M⁺ + 2) 283 (1.16%), M⁺ 281 (381.90): calcd. C, 69.63; H, 7.53; N, 18.92%; found C, 68.82; H, 5.98; N, 11.18%.

2-[(spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl]amino]-3-phenyl propane hydrazide (VIIa)

(From phenylalanine): yellowish white crystals, yield: 67%, m.p. 210-212°C. IR (KBr, cm⁻¹): 3434-3210 (NH₂, 3NH), 3050 (CH aromatic), 2963 (CH aliphatic), 1655 (C=O). 1H NMR (DMSO-d₆, δ, ppm): 3.21 (s, 10H, spiro cyclohexyl), 3.22 (d, 2H, J = 6.6 Hz, γ-CH₂), 4.27 (t, 2H, J = 3.8 Hz, α-CH), 7.20-7.26 (m, 9H, H aromatic), 8.91, 10.21 (2s, 2H, 2NH, exchangeable with D₂O). MS (m/z): (M⁺ + 2) 283 (1.16%), M⁺ 281 (381.90): calcd. C, 69.63; H, 7.53; N, 18.92%.

2-[(spiro-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-yl]amino]-3-methyl pentane hydrazide (VIIb)

(From isoleucine): yellow crystals, yield: 73%, m.p. 231-233°C. IR (KBr, cm⁻¹): 3434-3210 (NH₂, 3NH), 3050 (CH aromatic), 2963 (CH aliphatic), 1670 (C=O). 1H NMR (DMSO-d₆, δ, ppm): 3.21 (s, 10H, spiro cyclohexyl), 3.22 (d, 2H, J = 6.6 Hz, γ-CH₂), 4.27 (t, 2H, J = 3.8 Hz, α-CH), 7.20-7.26 (m, 9H, H aromatic), 8.91, 10.21 (2s, 2H, 2NH, exchangeable with D₂O). MS (m/z): (M⁺ + 2) 283 (1.16%), M⁺ 281 (381.90): calcd. C, 69.63; H, 7.53; N, 18.92%.
2-[[Spiro-[(1H,2H)-quinazoline-2,1’-cyclohexan]-4-yl][amino]-4-(methylthio)-butane hydrazide (VIIc)

(From methionine): light brown crystals, yield: 71%, m.p. 198-200°C. IR (KBr, cm^-1): 3440-3152 (NH3, 3NH, 2NH, exchangeable with D2O), 2958 (CH aromatic), 2920 (CH aliphatic), 1650 (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-methylthiosemicarbazide (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 portionwise. 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’-cyclohexan]-4-yl][amino][3-phenylpropionyl]-4-methylthiosemicarbazide (IXa)

(From phenylalanine): crystallized from isopropanol/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). 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%.

General procedure for preparation of 5-[[spiro[(1H,2H)-quinazoline-2,1’-cyclohexan]-4-yl][amino][3-phenylpropionyl]-4-methylthiosemicarbazide (IXb)

(From isoleucine): crystallized from isopropanol/petroleum ether to give dark orange crystals, yield: 74%, m.p. 207-209°C. IR (KBr, cm^-1): 3430-3168 (5NH), 3010 (CH aromatic), 2980 (CH aliphatic), 1645 (C=O), 1100 (C=S). MS (m/z): M+ 450 (15.47%), 127 (100%). Analysis: for C24H30N6OS, m.w. (450.60): calcd. C, 63.97; H, 7.25; N, 18.65; S, 7.12%; found C, 64.32; H, 7.07; N, 18.24; S, 6.83%.
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 methanol to give the triazole derivatives Xa-c.

5-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-y1amino]-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 aliphatic), 2976 (CH aliphatic), 2606 (SH stretching). ¹H NMR (DMSO-d₆, δ, ppm): 1.17 (s, 10H, spiro cyclohexyl), 2.29 (d, 2H, J = 7.3 Hz, β-CH₂), 2.66 (s, 3H, CH₃), 3.35 (t, 1H, J = 4.1 Hz, α-CH₂), 6.51 (s, 1H, NH, exchangeable with D₂O), 7.30-8.02 (m, 9H, H aromatic), 8.39, 12.67 (2s, 2H, NH, exchangeable with D₂O). MS (m/z): (M⁺ + 7.30-8.02 (m, 9H, H aromatic), 8.39, 12.67 (2s, 2H, NH, exchangeable with D₂O). MS (m/z): (M⁺ + 1) 433 (53.92%), 64 (100%). Analysis: for C₂₀H₂₈N₆S₂, m.w. (416.61): calcd. C, 66.64; H, 7.59; N, 21.09; S, 8.09%; found C, 66.76; H, 6.81; N, 19.72; S, 7.08%.

5-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-y1amino]-2-methylbutyl]-4-methyl-4H-1,2,4-triazole-3-thiol (Xb)

(From isoleucine): yield: 73%, m.p. 262-264°C. IR (KBr, cm⁻¹): 3410-3212 (3NH), 3010 (CH aromatic), 2966 (CH aliphatic), 2976 (CH aliphatic), 2598 (SH stretching). ¹H NMR (DMSO-d₆, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 1.25 (s, 1H, CH₃), 1.87 (s, 1H, NH, exchangeable with D₂O), 2.11 (s, 3H, CH₃), 3.34 (d, 2H, J = 7.4 Hz, β-CH₂), 4.27 (t, 1H, J = 7.1 Hz, α-CH₂), 7.09-7.29 (m, 4H, H aromatic), 8.24, 8.57, 10.48 (3s, 3H, NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 12.27 (CH₃), 16.08 (CH), 20.37 (N-CH₃), 38.98, 39.15, 39.32 (spiro cyclohexyl carbons), 42.64 (CH), 50.62 (NH-CH), 68.98 (spiro head carbon), 78.81, 87.01, 112.45, 112.65, 117.32, 130.04, 132.46, 149.18 (aromatic carbons), 151.37, 155.65, 160.06 (3C=CN). MS (m/z): M⁺ 398 (10.19%), 86 (100%). Analysis: for C₂₂H₂₄N₆S₃, m.w. (432.58): calcd. C, 66.76; H, 6.64; H, 6.52; N, 19.43; S, 7.41%; found C, 66.76; H, 6.81; N, 19.72; S, 7.08%.

5-[(1H,2H)-quinazoline-2,1'-cyclohexan]-4-y1amino]-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). ¹H NMR (DMSO-d₆, δ, ppm): 1.25 (s, 10H, spiro cyclohexyl), 1.87 (s, 3H, CH₃), 2.52 (t, 2H, J = 4.2 Hz, γ-CH₂), 3.06 (s, 3H, CH₃), 3.64 (t, 1H, J = 3.8 Hz, α-CH₂), 4.85 (1s, 1H, NH, exchangeable with D₂O), 7.31-7.52 (m, 4H, H aromatic), 8.71, 10.21 (2s, 2H, NH, SH, exchangeable with D₂O). MS (m/z): M⁺ 415 (6.22%), (M⁺ - 1) 415 (31.09), 68 (100%). Analysis: for C₂₉H₂₉NₓS₅, m.w. (461.61): 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-amino (Xla-c)

To the thiosemicarbazide derivative IXa-c (6 mmol), conc. HSO₃ (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 give the desired thia diazole derivatives Xla-c.

Spiro((1H, 2H)-N-[(5-(methylamino)-1,3,4-thiadiazol-2-yl)quinazoline-2,1'-cyclohexane]-4-amino (Xla)

(From phenylalanine): yield: 67%, m.p. 262-264°C. IR (KBr, cm⁻¹): 3410-3212 (3NH), 3010 (CH aromatic), 2966 (CH aliphatic). ¹H NMR (DMSO-d₆, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 2.11 (s, 3H, CH₃), 3.34 (d, 2H, J = 7.2 Hz, β-CH₂), 4.26 (t, 1H, J = 4.1 Hz, α-CH₂), 6.45 (s, 1H, NH), 7.56-7.97 (m, 9H, aromatic-H), 8.91, 10.31 (2s, 2H, 2NH, exchangeable with D₂O). MS (m/z): M⁺ 430 (32.31%), 76 (100%). Analysis: for C₂₅H₂₅N₆S₃, m.w. (432.58): calcd. C, 66.76; H, 6.64; 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-amino (Xlb)

(From isoleucine): yield: 73%, m.p. 185-187°C. IR (KBr, cm⁻¹): 3415-3120 (3NH), 2966 (CH aliphatic). ¹H NMR (DMSO-d₆, δ, ppm): 0.81 (s, 10H, spiro cyclohexyl), 0.88 (t, 3H, J = 3.8 Hz, CH₃), 1.26 (d, 3H, J = 7.1 Hz, CH₃), 1.42-1.67 (m, 2H, γ-CH₂), 1.84 (s, 1H, NH, exchangeable with D₂O), 2.28-2.31 (m, 1H, β-CH₂), 3.00 (s, 3H, CH₃), 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 D₂O). MS (m/z): M⁺ 398 (20.03%), 86 (100%). Analysis: for C₂₅H₂₅N₆S₃, m.w. (398.57): calcd. C, 66.76; H, 6.64; H, 6.52; N, 19.43; S, 7.41%; found C, 63.73; H, 7.35; N, 21.42; S, 7.81%.
Spiro((1H, 2H)-N-[1-(5-methylamino)-1,3,4-thiadiazol-2-yl)-3-(methylthio)-propyl] quinazoline-2,1'-cyclohexane]-4-amine (Xc)

(from methionine): yield: 71%, m.p. 180-182°C. IR (KBr, cm⁻¹): 3440-3225 (3NH), 3106 (CH aromatic), 2968 (CH aliphatic). ¹H NMR (DMSO-d₆, δ, ppm): 1.08 (s, 10H, spiro cyclohexyl), 2.15 (s, 3H, -S-CH₃), 2.35 (t, 2H, J = 3.8 Hz, γ-CH₂), 2.52-2.58 (m, 2H, β-CH₂), 3.06 (s, 3H, CH₃), 3.64 (t, 1H, J = 4.1 Hz, α-CH₂), 7.83-8.07 (m, 4H, H aromatic), 8.71, 9.32, 10.21 (3s, 3H, 3NH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 19.04 (CH₃), 30.76 (NH-CH₃), 33.26 (CH₂), 36.07 (CH₂), 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=O), 164.35 (NH-C=N). MS (m/z): (M+ - 1) 415 (40.09%).

yield: 83%, m.p. 116-118°C. IR (KBr, cm⁻¹): 3461-3158 (4NH), 2985 (CH aliphatic), 1710, 1630 (2C=O), 1130 (C=S). ¹H NMR (DMSO-d₆, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 2.46, 3.29, 3.41 (3s, 3H, 3NH, exchangeable with D₂O), 5.10 (s, 2H, H aromatic), 10.13 (s, 1H, NH, exchangeable with D₂O). MS (m/z): M+ 515 (92.7%), 77 (100%). Analysis: for C₁₈H₁₇N₃O₂S, m.w. (515.63): calcd. C, 67.58; H, 5.47; N, 14.07% S, 6.82%; found C, 64.84; 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-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.

Yield: 83%, m.p. 116-118°C. IR (KBr, cm⁻¹): 3461-3158 (4NH), 2985 (CH aliphatic), 1710, 1630 (2C=O), 1130 (C=S). ¹H NMR (DMSO-d₆, δ, ppm): 1.23 (s, 10H, spiro cyclohexyl), 2.46, 3.29, 3.41 (3s, 3H, 3NH, exchangeable with D₂O), 5.10 (s, 2H, H aromatic), 10.13 (s, 1H, NH, exchangeable with D₂O). MS (m/z): M+ 515 (92.7%), 77 (100%). Analysis: for C₁₈H₁₇N₃O₂S, m.w. (515.63): calcd. C, 67.58; H, 5.47; N, 14.07% S, 6.82%; 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 derivative (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 8 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 light brown crystals.

Yield: 75%, m.p. 217-219°C. IR (KBr, cm⁻¹): 3433 (NH), 2923 (CH aliphatic), 1672 (C=C=O), 2550 (SH-stretching). ¹H NMR (DMSO-d₆, δ, ppm): 1.20 (s, 10H, spiro cyclohexyl), 4.82 (s, 2H, CH₂), 6.86-7.63 (m, 13H, H aromatic), 9.32, 10.13 (2s, 2H, NH, SH, exchangeable with D₂O). ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 25.31 (CH₃), 37.81, 39.73, 40.06 (spiro cyclohexyl carbons), 42.81 (CH₃, pyrazolidinone ring), 68.56 (spiro head carbon), 70.03 (O-CH₂), 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=C=N), 164.05, 170.08 (2C=O). MS (m/z): (M+ + 1) 447 (12.02%), 146 (100%). Analysis: for C₁₈H₁₇N₆O₄S, m.w. (446.60): calcd. C, 67.25; H, 5.87; N, 12.55%; found C, 67.63; H, 6.27; N, 12.86%.
To a corresponding thiosemicarbazide derivative (XIV) (3.09 g, 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 dried and the product was crystallized from iso-propanol 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), $^1$H-NMR (DMSO-d$_6$, 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$_2$O). MS (m/z): M + 497 (10.28%), 77 (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, 6.08%; found C, 67.31; H, 5.73; N, 14.32; S, 6.08%.

Cytoxic 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, Vlb, VIc, VIIa, VIIb, VIIc, VIIIc, IXa, Xc, XIa, XIIb, 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$_{50}$, model.

Molecular modeling

All molecular modeling studies were performed on a RM Innovator Pentium IV 2.4 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 an 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, $^1$H-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. $^1$H-NMR (CDCl$_3$, 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 3323 and 1667 cm$^{-1}$ due to the presence of two C=O groups. $^1$H NMR (CDCl$_3$, δ ppm) of the same derivative displayed a singlet signal at δ 5.84 ppm corresponding to the methine proton of HN-CH-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 Na₂CO₃ as a catalytic base at pH 9-9.5 (27) to get the quinazoline amino acid derivatives V₁a-c. ¹H-NMR (DMSO-d₆, δ, ppm) of V₁a showed the methine proton of α-CH as a triplet signal at δ 4.13 ppm while the two protons of β-CH₂ of the amino acid side chain appeared as a doublet at δ 3.04 ppm. ¹H NMR (DMSO-d₆, δ, ppm) of V₁b showed the methine proton of α-CH as a triplet signal at δ 4.13 ppm, the three protons of -CH₂-CH₂- appeared as a multiplet at δ 1.82 ppm, while the six protons of 2CH₂ represented another multiplet at δ 1.11 ppm. Also, ¹H NMR (DMSO-d₆, δ, ppm) of V₁c showed a singlet signal at δ 2.09 ppm attributed to the three protons of the CH₃ group, while the four protons of -CH₂-CH₂-S group appeared as multiplet and triplet signals at δ 2.15 and δ 2.41 ppm, respectively. The methine proton of the α-CH group was represented as a triplet signal at δ 3.49 ppm.

The acid chloride derivatives VⅡⅠa-c were synthesized by the reaction of the corresponding aminoacid analogues with thionyl chloride in chloroform at 70°C (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 IXⅠa-c. Mass spectra of the obtained derivatives represented the molecular ion peaks at m/z 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 triazole derivatives (29), but intramolecular dehydrative cyclization can be carried out by their treatment with conc. H₂SO₄ to obtain triazolo and thiaazolinoquinazoline 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 ¹H NMR (DMSO-d₆, 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-CH₂ 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 thiaazolinoquinazoline 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 (SRB) assay in a trial to correlate between both structural variations and cytotoxic activity of the
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 isoleucine 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 Xla (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 triazole 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 triazole 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, Xla, 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 Ser864 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.

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

Replacement of 1,2,4-triazine-5,6-dione moiety by NH-CH linker space in compound X1b and the presence of thiadiazole moiety kept the good fitting of X1b in the binding site of the enzyme (Fig. 5), but the number of H-bonds between X1b 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 X1b was -20.2 (Table 2).
The carboxylic acid of \textbf{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 \textgreek{Å} (Table 2), which indicates the importance of free NH group in the activity of our synthesized compounds. Docking score of \textbf{VIb} was -19.6 (Table 2).

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

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

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

Compounds \textbf{XV} and \textbf{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.

\section*{Lipinski rule of five and ADME profile}

We assessed the most active compounds (\textbf{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 \textgreek{Å}² are thought to have low oral bioavailabilities (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 \textbf{V}, and \textbf{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.

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>IC₅₀ µM</th>
<th>Compound no.</th>
<th>IC₅₀ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>78.9</td>
<td>Xa</td>
<td>&gt;100</td>
</tr>
<tr>
<td>IV</td>
<td>68.6</td>
<td>Xb</td>
<td>&gt;100</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>Xc</td>
<td>50.6</td>
</tr>
<tr>
<td>VIa</td>
<td>76.0</td>
<td>Xla</td>
<td>33.4</td>
</tr>
<tr>
<td>VIb</td>
<td>14.2</td>
<td>Xlb</td>
<td>11.2</td>
</tr>
<tr>
<td>VIc</td>
<td>&gt;100</td>
<td>Xlc</td>
<td>111.1</td>
</tr>
<tr>
<td>VIIa</td>
<td>23.4</td>
<td>XIII</td>
<td>&gt;100</td>
</tr>
<tr>
<td>VIIb</td>
<td>&gt;100</td>
<td>XV</td>
<td>62.8</td>
</tr>
<tr>
<td>VIIc</td>
<td>26.3</td>
<td>XVI</td>
<td>14.7</td>
</tr>
<tr>
<td>VIIIc</td>
<td>52.2</td>
<td>Doxorubicin</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*IC₅₀ - compound concentration required to inhibit tumor cell line proliferation by 50%. * Values are the means of three experiments.

**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, Xla, Xlb, 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 3. Solubility and calculated Lipinski’s rule of five for the most active compounds over breast cancer (MCF-7) cell line.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>log S</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>01.3</td>
<td>-2.79</td>
<td>log P</td>
</tr>
<tr>
<td>VIlb</td>
<td>14.2</td>
<td>-3.81</td>
<td>PSA</td>
</tr>
<tr>
<td>VIIa</td>
<td>23.4</td>
<td>-6.08</td>
<td>MW</td>
</tr>
<tr>
<td>VIIc</td>
<td>26.3</td>
<td>-5.44</td>
<td>nH-acc</td>
</tr>
<tr>
<td>XIb</td>
<td>11.2</td>
<td>-6.25</td>
<td>nH-don</td>
</tr>
<tr>
<td>XVI</td>
<td>14.7</td>
<td>-7.92</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>log S</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>01.3</td>
<td>-2.79</td>
<td>log P</td>
</tr>
<tr>
<td>VIlb</td>
<td>14.2</td>
<td>-3.81</td>
<td>PSA</td>
</tr>
<tr>
<td>VIIa</td>
<td>23.4</td>
<td>-6.08</td>
<td>MW</td>
</tr>
<tr>
<td>VIIc</td>
<td>26.3</td>
<td>-5.44</td>
<td>nH-acc</td>
</tr>
<tr>
<td>XIb</td>
<td>11.2</td>
<td>-6.25</td>
<td>nH-don</td>
</tr>
<tr>
<td>XVI</td>
<td>14.7</td>
<td>-7.92</td>
<td></td>
</tr>
</tbody>
</table>

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 cyclohexylquinazoline 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

The authors would thank Dr. Ahmed Mohammed Al-Abd, Pharmacology Department, National Research Center, for performing the biological section of this study. In addition, the authors would thank the Microanalytical and Spectral Unit, National Research Centre and Cairo University, Egypt for microanalytical, IR, 1H NMR, 13C NMR and mass spectral data.

Competing interests

The authors declare no conflict of interest.

REFERENCES


Received: 07. 02. 2013