Full Paper

Synthesis and Biological Evaluation of a Novel Series of 6,8-Dibromo-4(3*H*)quinazolinone Derivatives as Anticancer Agents

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Three novel series of 6,8-dibromo-4(3*H*)quinazolinone derivatives were synthesized. Some of the novel quinazolinone derivatives were tested for their antitumor activity against the human breast carcinoma cell line MCF-7. Compounds **XIIIb**, **IX**, **XIVd**, **XIVb**, **XIVe**, **XIIIa**, **XIVc**, **XVc**, and **XIVa** exerted powerful cytotoxic effects against the MCF7 cells, with very low IC_{50} values compared to doxorubicin (positive control). The IC_{50} values were 1.7, 1.8, 1.83, 5.4, 6.84, 10.8, 13.9, 15.7, and 29.6 μg/mL, respectively.

Keywords: 6,8-Dibromo-4(3*H*)quinazolinone / Human breast carcinoma / Sulfa drugs

Received: April 29, 2013; Revised: May 24, 2013; Accepted: June 12, 2013

DOI 10.1002/ardp.201300158

Introduction

It is well-known that cancer is continuing to be a major health problem in developing as well as undeveloped countries [1–6]. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important.

In the last few years, the attention was oriented towards the synthesis and biological evaluation of quinazoline derivatives as they exhibit a broad spectrum of biological activities. Indeed, several quinazoline derivatives have been reported to possess anticonvulsant [7, 8], sedative, antihypertensive [9, 10], vasodilatory [11], anti-inflammatory [12], antibiosis [13], phosphodiesterase inhibitory [14], fibrinogen receptor antagonistic [15], and antitumor activity [16–18]. Anilinoquinazoline containing compounds were recently approved for the treatment of HER2-positive metastatic breast cancer [19–27]. On the other hand, diverse chemotherapeutic agents contain pharmacophores like Br [28], sulfonamides [29], aminothiazol [30], pyrazoline [31], *N*-phenylpyrazoline [32], tetrahydropyrimidinone [33], and tetrahydropyrimidine-thione [34] functions are known to contribute to the enhancement of the antitumor activity. In view of the aforementioned facts, it seemed most interesting to synthesize a new series of anilinoquinazoline compounds in such a way to accommodate Br, sulfonamides, aminothiazol, pyrazoline, *N*-phenylpyrazoline, tetrahydropyrimidinone, or tetrahydropyrimidine-thione moiety with the aim to evaluate their antitumor activities.

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Results and discussion

Chemistry

The desired 6,8-dibromo-2-phenyl-3-(4-acetylphenyl)-4(3*H*)quinazolinone (**II**) was obtained by condensation of 6,8-dibromo-2-phenyl-4*H*-3,1-benzoxazin-4-one (**I**) with *p*-aminoacetophenone to give the corresponding key intermediate (**II**). Reaction of (**II**) with bromine in presence of glacial acetic acid afforded 6,8-dibromo-3-(4-(2-bromoacetyl)phenyl)-2-phenylquinazolin-4(3*H*)-one (**III**). Reaction of (**III**) with malononitrile in absolute ethanol gave 2-(4-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4*H*)-yl)benzoyl)malononitrile (**IV**). Cyclocondensation of (**III**) by thiourea in absolute ethanol furnished the target aminothiazol derivative (**V**) (Scheme 1).

Synthesis of 4-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4*H*)-yl)-*N*-(substituted) benzenesulfonamides (**VI–XI**) was obtained by aromatic aminolysis [35] of the known benzoxazine derivative (**I**), upon fusion with the appropriate sulfa drugs, namely: sulfamethoxazole, sulfaguanidine, sulfacetamide sodium, sulfamerazine, sulfapyridine, and sulfanilamide (Scheme 2).

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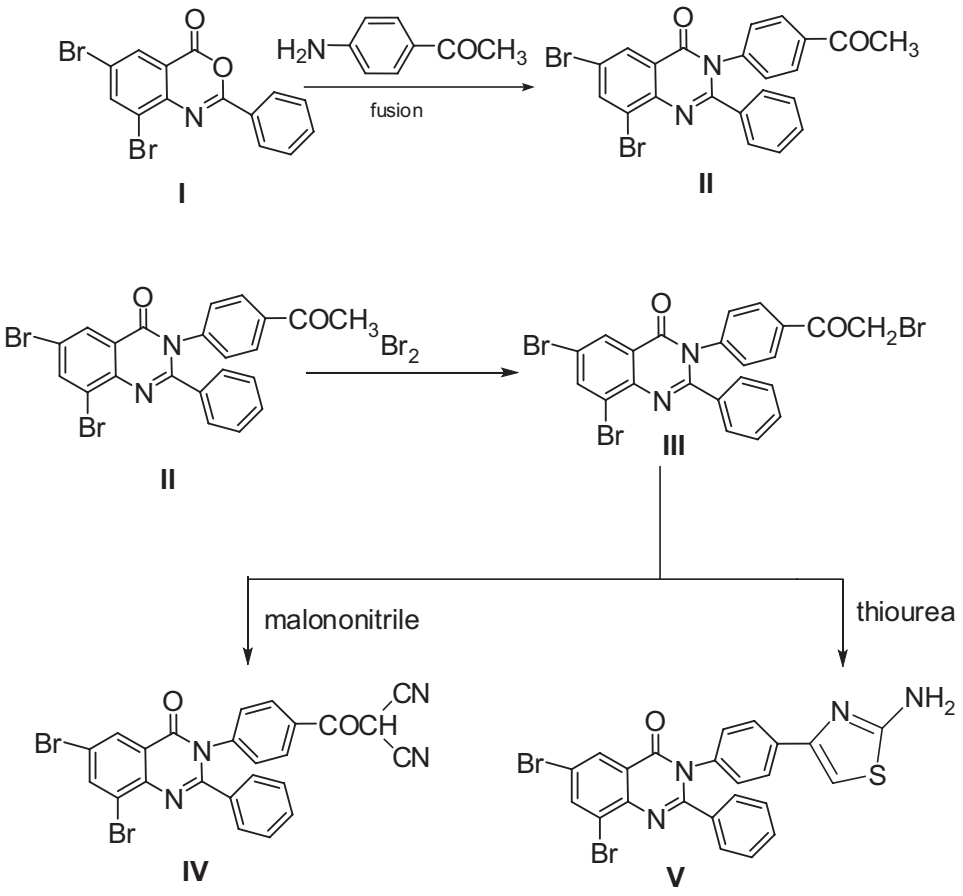


Scheme 1. Synthesis of 2-(4-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4*H*)-yl)benzoyl)malononitrile (**IV**) and aminothiazol derivative (**V**).

Claisen–Schmidt condensation of the acetyl derivative (**II**) with different aldehydes namely, benzaldehyde, *p*-anisaldehyde, *p*-chlorobenzaldehyde, *p*-hydroxy benzaldehyde, and/or furan-2-carboxaldehyde in ethanolic sodium hydroxide solution afforded the corresponding α,β-unsaturated ketones (chalcones) (**XIIa–e**) [36], respectively. These chalcones are considered to be useful intermediates in several cyclization reactions to produce different types of heterocyclic compounds of diverse biological importance, according to the reactants used and the reactions conditions [37].

Cyclocondensation of the unsaturated ketone (**XIId**) by hydrazine hydrate in absolute ethanol afforded the corresponding pyrazoline derivatives (**XIIIa**).

Also, cyclocondensation of the key ketones (**XIIa**,**b**) by phenylhydrazine in absolute ethanol furnished the target *N*-phenylpyrazoline derivatives (**XIIIb**,**c**).

Further, the α,β-unsaturated ketones (**XIIa–e**) were allowed to react with hydroxylamine hydrochloride in ethanolic sodium hydroxide solution, affording the corresponding isoxazolines (**XIVa–e**), respectively. Cyclocondensation of the chalcones (**XIId**,**e**) with urea in presence of HCl or with thiourea in presence of NaOH according to a reported method [27] afforded the corresponding tetrahydropyrimidinone or tetrahydropyrimidine-thione derivatives (Scheme 3).

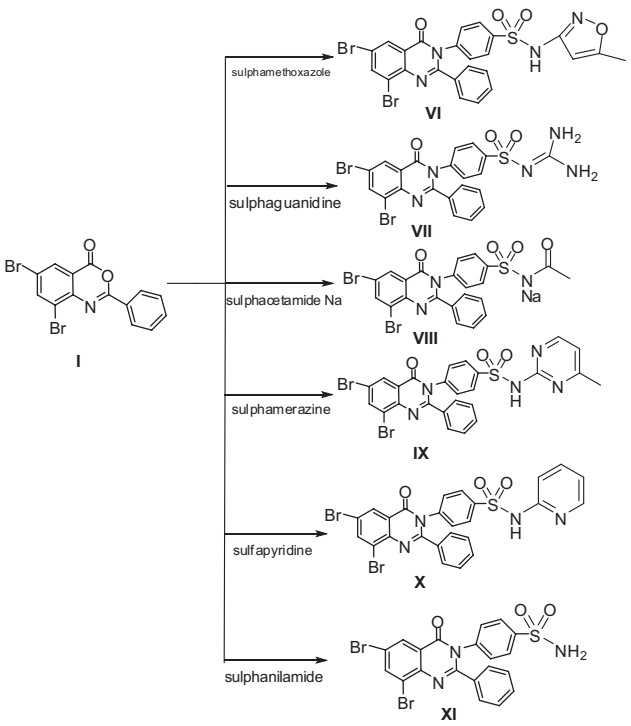
Conclusion

We have synthesized a novel series of quinazolin-4(3*H*)-ones compounds. Some of the synthesized compounds were tested in order to determine the possible anticancer activity. Most tested compounds have shown promising anticancer activities against the human breast cancer cell line MCF-7 at very low concentrations.

Experimental

Chemistry

All melting points are uncorrected, elemental analyses were carried out in the microanalytical unit of the National Research Centre and Cairo University, Egypt. IR spectra were recorded on FT-IR spectrophotometer-Nexus 670-Nicolet (USA) and Perkin Elmer-9712 spectrophotometer. ^{1}H NMR spectra were determined on a Varian-Gemmini-300 MHz and Joel-Ex 270 MHz NMR



Scheme 2. Synthesis of 4-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4*H*)-yl)-*N*-(substituted) benzenesulfonamides (**VI**–**XI**).

spectrometer using TMS as an internal standard. Mass spectra were determined on Finnigan Mat SSQ 7000, mode EI 70 eV (Thermo Inst. Sys., Inc., USA). Thin layer chromatography was carried out on silica gel 60 F254 (Merck) plates.

*6,8-Dibromo-2-phenyl-3-(4-acetylphenyl)-4(3H)-quinazolinone (**II**)*

It was prepared according to reported literatures [36].

*6,8-Dibromo-3-(4-(2-bromoacetyl)phenyl)-2-phenylquinazolin-4(3H)-one (**III**)*

To a solution of the ketone **II** (0.99 g, 0.002 mol) in glacial acetic acid was added 4 mL bromine in 5 mL glacial acetic acid dropwise and left over night, then poured into crushed ice. The separated material was filtered, air dried, and crystallized from ethanol to give **III**. m.p. 160°C, yield 65%. Analysis for $C_{22}H_{13}Br_3N_2O_2$, M.wt. (577.06) calcd.: % C, 45.79; H, 2.27; N, 4.85; Found: % C, 45.72; H, 2.21; N, 4.79. IR (KBr, cm^{-1}): 3028 (CH, aromatic), 1700 (C=O) 1672 (C=O), and 1635 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 4.5 (s, 1H, CH_2) and 7.60–8.1 (m, 12H, aromatic-H). MS (*m*/*z*, R.I.): M^+. 573.80, 575.82, 577.80 (34%, 100%, 97%).

*2-(4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-benzoyl)malononitrile (**IV**)*

A mixture of **III** (5.6 g; 10 mmol) and malononitrile (0.66 g, 10 mmol) in absolute ethanol (15 mL) was treated with NaOH (4 g in 10 mL H_2O) dropwise with stirring, then diluted with 30 mL H_2O; the precipitated solid formed was filtered and crystallized from ethanol to give **IV**. m.p. 220°C, yield 70%. Analysis for $C_{24}H_{12}Br_2N_4O_2$, M.wt. (548.19) calcd.: % C, 52.58; H, 2.21; N, 10.22; Found: % C, 52.50; H, 2.18; N, 10.10. IR (KBr, cm^{-1}): 3032 (CH, aromatic), 2200 (CN), 2220 (CN), 1700 (C=O), 1672 (C=O), and 1635 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 5.1 (s, 1H, CH) and 7.30–8.4 (m, 11H, aromatic-H). MS (*m*/*z*, R.I.): M^+. 546.02, 548.00, 549.00 (51%, 100%, 54%).

*6,8-Dibromo-3-(4-(2-aminothiazol-4-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**V**)*

A mixture of **III** (5.6 g; 10 mmol) and thiourea (0.76 g, 10 mmol) in absolute ethanol (20 mL) was refluxed for 7 h. The precipitated solid formed upon cooling was filtered and crystallized from ethanol to give **V**. m.p. 190°C, yield 70%. Analysis for $C_{23}H_{14}Br_2N_4OS$, M.wt. (554.26) calcd.: % C, 49.84; H, 2.55; N, 10.11; Found: % C, 49.74; H, 2.41; N, 10.00. IR (KBr, cm^{-1}): 3380 (NH_2), 3028 (CH, aromatic), 1672 (C=O) and 1635 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 7.1 (s, NH_2, exchangeable with D_2O) and 7.50–8.28 (m, 11H, aromatic-H). MS (*m*/*z*, R.I.): M^+. 551.92, 553.92, 555.90 (50%, 100%, 52%).

*General method for the preparation of 4-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-N-(substituted)-benzenesulfonamides (**VI**–**XI**)*

A mixture of the benzoxazine I (3.8 g, 0.01 mol) and appropriate sulfa drug, namely, sulfamethoxazole, sulfaguanidine, sulfacetamide sodium, sulfamerazine, sulfapyridine, and sulfanilamide (0.01 mol), was heated together upon fusion at 150°C on a sand bath for 2 h. After cooling, the crude mass was crystallized from the proper solvent.

*4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (**VI**)*

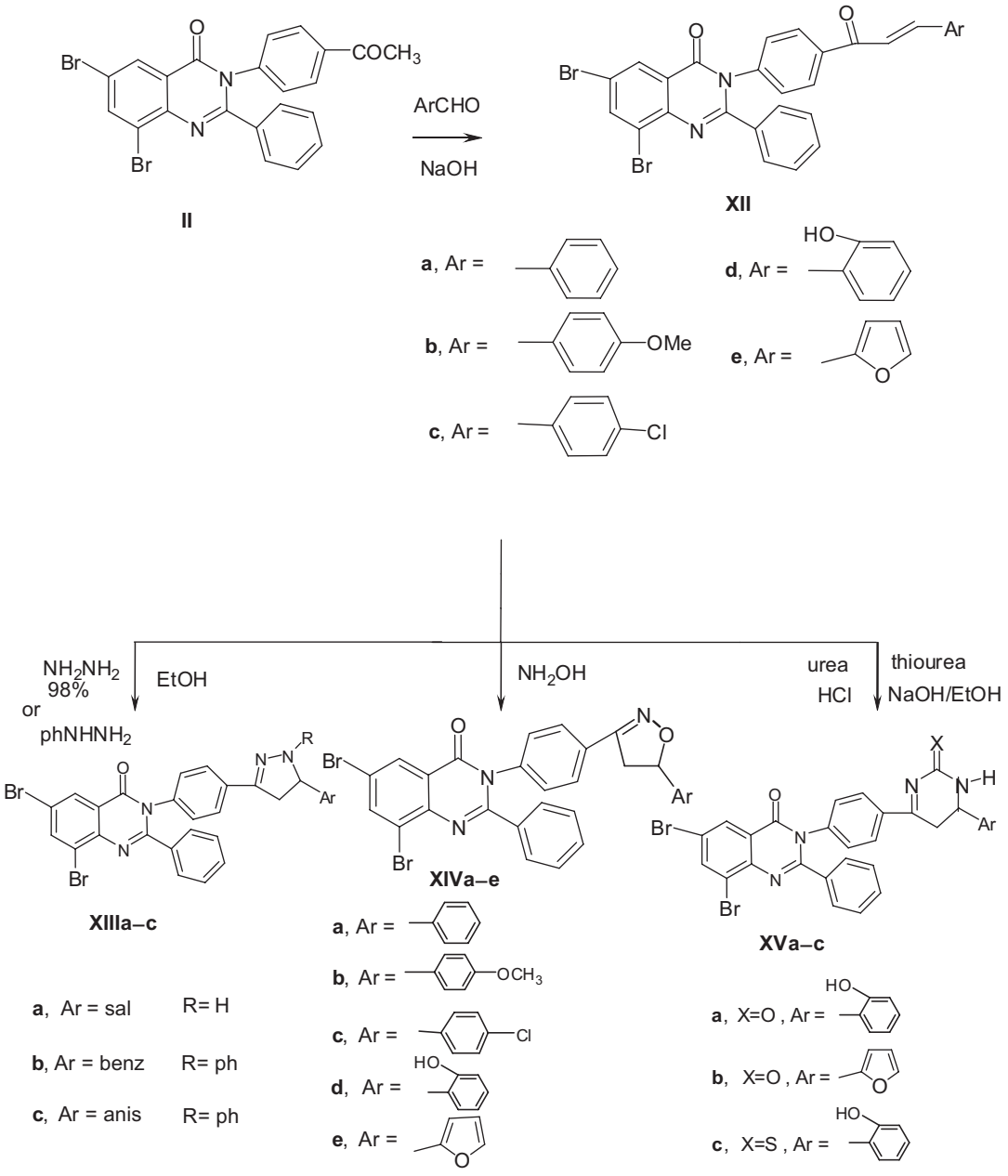
Crystallized from ethanol to give **VI**. m.p. 210°C, yield 60%. Analysis for $C_{24}H_{16}Br_2N_4O_4S$, M.wt. (613.93) calcd.: % C, 46.77; H, 2.62; N, 9.09; Found: % C, 46.72; H, 2.58; N, 9.00. IR (KBr, cm^{-1}): 3380 (NH), 3040 (CH, aromatic), 1678 (C=O), and 1635 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.3 (s, 1H, CH_3), 6.50–8.5 (m, 12H, aromatic-H) and 11.01 (s, NH, exchangeable with D_2O). MS (*m*/*z*, R.I.): M^+. 613.93, 615.92, 617.93 (51%, 100%, 49%).

*4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-N-(diaminomethylene)benzenesulfonamide (**VII**)*

Crystallized from ethanol to give **VII**. m.p. 250°C, yield 65%. Analysis for $C_{21}H_{15}Br_2N_5O_3S$, M.wt. (577.25) calcd.: % C, 43.69; H, 2.62; N, 12.13; Found: % C, 43.61; H, 2.58; N, 12.01. IR (KBr, cm^{-1}): 3380 (NH_2), 3370 (NH_2), 3040 (CH, aromatic), 1678 (C=O), and 1635 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 6.61 (s, 4H, NH_2, exchangeable with D_2O) and 6.50–8.5 (m, 11H, aromatic-H). MS (*m*/*z*, R.I.): M^+. 574.91, 576.92, 578.93 (53%, 100%, 50%).

*4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-sodiumacetyl(phenylsulfonyl)amide (**VIII**)*

Crystallized from ethanol to give **VIII**. m.p. 290°C, yield 75%. Analysis for $C_{22}H_{14}Br_2N_3NaO_4S$, M.wt. (599.23) calcd.: % C, 44.10; H, 2.35; N, 7.01; Found: % C, 43.99; H, 2.31; N, 6.98. IR (KBr, cm^{-1}): 3040 (CH, aromatic), 1700 (C=O), 1678 (C=O), and 1635 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.1 (s, 1H, CH_3) and 6.50–8.5 (m, 11H, aromatic-H). MS (*m*/*z*, R.I.): M^+. 596.91, 598.92, 600.93 (50%, 100%, 49%).



Scheme 3. Synthesis of pyrazoline derivative (**XIIIa**), *N*-phenylpyrazolines (**XIIIb**,**c**), isoxazolines (**XIVa**–**e**), tetrahydropyrimidinones (**XVa**,**b**), and tetrahydropyrimidine-thione derivative (**XVc**).

*4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**IX**)*

Crystallized from ethanol to give **IX**. m.p. 280°C, yield 60%. Analysis for $C_{25}H_{17}Br_2N_5O_3S$, M.wt. (627.31) calcd.: % C, 47.87; H, 2.73; N, 11.16; Found: % C, 47.85; H, 2.70; N, 11.10. IR (KBr, cm^{-1}): 3380 (NH), 3040 (CH, aromatic), 1678 (C=O), and 1630 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 2.3 (s, 1H, CH_3), 7.50–8.5 (m, 13H, aromatic-H) and 11.01 (s, NH, exchangeable with D_2O). MS (*m*/*z*, R.I.): M^+. 625.01, 627.02, 629.03 (49%, 100%, 51%).

*4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-N-(pyridin-2-yl)benzenesulfonamide (**X**)*

Crystallized from ethanol to give **X**. m.p. 232°C, yield 65%. Analysis for $C_{25}H_{16}Br_2N_4O_3S$, M.wt. (612.29) calcd.: % C, 49.04; H, 2.63; N, 9.15; Found: % C, 49.00; H, 2.59; N, 9.05. IR (KBr, cm^{-1}): 3385 (NH), 3045 (CH, aromatic), 1670 (C=O), and 1630 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 7.50–8.5 (m, 15H, aromatic-H) and 11.2 (s, NH, exchangeable with D_2O). MS (*m*/*z*, R.I.): M^+. 609.93, 611.92, 613.93 (50%, 100%, 53%).

*4-(6,8-Dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-benzenesulfonamide (**XI**)*

Crystallized from ethanol to give **XI**. m.p. 254°C, yield 70%. Analysis for $C_{20}H_{13}Br_2N_3O_3S$, M.wt. (535.21) calcd.: % C, 44.88; H, 2.45; N, 7.85; Found: % C, 44.81; H, 2.40; N, 7.80. IR (KBr, cm^{-1}): 3380 (NH_2), 3045 (CH, aromatic), 1675 (C=O) and 1632 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 7.2 (s, NH_2, exchangeable with D_2O) and 7.60–8.3 (m, 11H, aromatic-H). MS (*m/z*, R.I.): M^+. 532.93, 534.90, 536.93 (51%, 100%, 54%).

*General method for the synthesis of **XIIa–e***

6,8-Dibromo-2-phenyl-3-{4-[(*E*)-3-substituted arylacryloyl]-phenyl}-3*H*-quinazolin-4-ones (**XIIa–e**) (chalcones) were prepared according to reported literatures [36].

*6,8-Dibromo-2-phenyl-3-{4-[5-(4-hydroxy phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-3H-quinazolin-4-one (**XIIIa**)*

A mixture of the chalcone **XIId** (0.005 mol) and hydrazine hydrate (2.5 mL, 0.005 mol, 98%) in absolute ethanol (25 mL) was heated under reflux for 10 h. After cooling, the separated material was filtered, air dried, and crystallized from ethanol to give **XIIIa**. m.p. 120°C, yield 60%. Analysis for $C_{29}H_{22}Br_2N_4O_2$, M. wt. (616.3) calcd.: % C, 56.52; H, 3.27; N, 9.09; Found: % C, 56.42; H, 3.10; N, 9.00. IR (KBr, cm^{-1}): 3380 (OH), 3385 (NH), 3030 (CH, aromatic), 1670 (C=O), and 1635 (C=N). ^1H NMR (DMSO-d_6, δ ppm):, 3.1 (d, d, 2H, CH_2, pyrazoline ring, $J = 5.6$ Hz), 3.90 (t, 1H, CH of pyrazoline), 7.21 (s, NH, exchangeable with D_2O), 7.50–8.28 (m, 16H, aromatic-H) and 9.80 (s, 1H, OH). MS (*m/z*, R.I.): M^+. 614.00, 616.08, 618.00 (49%, 100%, 50%).

*6,8-Dibromo-2-phenyl-3-{4-[1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-3H-quinazolin-4-one (**XIIIb**)*

A mixture of the chalcone **XIIa** (0.003 mol) and phenylhydrazine (0.32 g, 0.003 mol, 98%) in absolute ethanol (25 mL) was heated under reflux for 10 h. After cooling, the separated material was filtered, air dried, and crystallized from ethanol to give **XIIIb**. m.p. 180°C, yield 75%. Analysis for $C_{35}H_{24}Br_2N_4O$, M.wt. (674.03) calcd.: % C, 62.15; H, 3.58; N, 8.28; Found: % C, 62.00; H, 3.52; N, 8.19. IR (KBr, cm^{-1}): 3388 (NH), 3038 (CH, aromatic), 1675 (C=O), and 1635 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.6 (d, d, 2H, CH_2, pyrazoline ring), 5.1 (t, 1H, CH of pyrazoline), and 7.50–8.5 (m, 21H, aromatic-H). MS (*m/z*, R.I.): M^+. 674.03, 676.00, 678.03 (51%, 100%, 49%).

*6,8-Dibromo-2-phenyl-3-{4-[5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-3H-quinazolin-4-one (**XIIIc**)*

A mixture of the chalcone **XIIb** (0.003 mol) and phenylhydrazine (0.32 g, 0.003 mol, 98%) in absolute ethanol (25 mL) was heated under reflux for 10 h. After cooling, the separated material was filtered, air dried, and crystallized from ethanol to give **XIIIc**. m.p. 215°C, yield 65%. Analysis for $C_{36}H_{26}Br_2N_4O_2$, M.wt. (706.04) calcd.: % C, 61.21; H, 3.71; N, 7.93; Found: % C, 61.18; H, 3.62; N, 7.89. IR (KBr, cm^{-1}): 3390 (NH), 3040 (CH, aromatic), 1673 (C=O), and 1638 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.8 (d, d, 2H, CH_2, pyrazoline ring), 3.80 (s, 1H, OCH_3), 5.31 (t, 1H, CH of pyrazoline), and 7.60–8.54 (m, 20H, aromatic-H). MS (*m/z*, R.I.): M^+. 704.03, 706.00, 708.00 (52%, 100%, 49.7%).

*General method for the synthesis of 6,8-dibromo-2-phenyl-3-(4-(5-(substituted)-4,5-dihydroisoxazol-3-yl)phenyl)-quinazolin-4(3H)-ones (**XIVa–e**)*

A mixture of chalcones **XIIa–e** (3 mmol) and hydroxylamine hydrochloride (5 mmol) in sodium hydroxide solution (0.5 g NaOH in 0.5 mL water) in ethanol (60 mL) was refluxed for 3 h. The product obtained upon cooling was filtered off, washed with water, and recrystalized from the proper solvents to obtain the desired compounds **XIVa–e**, respectively.

*6,8-Dibromo-2-phenyl-3-(4-(5-phenyl-4,5-dihydroisoxazol-3-yl)phenyl)quinazolin-4(3H)-one (**XIVa**)*

Crystallized from ethanol to give **XIVa**. m.p. 160°C, yield 70%. Analysis for $C_{29}H_{19}Br_2N_3O_2$, M.wt. (601.29) calcd.: % C, 57.90; H, 3.18; N, 6.99; Found: % C, 57.83; H, 3.12; N, 6.88. IR (KBr, cm^{-1}): 3036 (CH, aromatic), 1673 (C=O), and 1630 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.7–3.82 (d, d, 2H, CH_2, isoxazoline ring, $J = 5.4$ Hz), 6.34 (t, 1H, CH, isoxazoline ring), and 7.80–8.54 (m, 16H, aromatic-H). MS (*m/z*, R.I.): M^+. 598.98, 600.98, 602.98 (50%, 100%, 48.7%).

*6,8-Dibromo-3-(4-(5-(4-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**XIVb**)*

Crystallized from ethanol to give **XIVb**. m.p. 200°C, yield 65%. Analysis for $C_{30}H_{21}Br_2N_3O_3$, M.wt. (631.31) calcd.: % C, 57.07; H, 3.35; N, 6.66; Found: % C, 57.00; H, 3.29; N, 6.61. IR (KBr, cm^{-1}): 3040 (CH, aromatic), 1670 (C=O), and 1637 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.48–3.52 (d, d, 2H, CH_2, isoxazoline ring), 3.80 (s, 1H, OCH_3), 6.34 (t, 1H, CH, isoxazoline ring), and 7.50–8.34 (m, 15H, aromatic-H). MS (*m/z*, R.I.): M^+. 629.00, 631.01, 633.00 (51%, 100%, 49.0%).

*6,8-Dibromo-3-(4-(5-(2-chlorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**XIVc**)*

Crystallized from ethanol to give **XIVc**. m.p. 170°C, yield 75%. Analysis for $C_{29}H_{18}Br_2ClN_3O_3$, M.wt. (635.73) calcd.: % C, 54.79; H, 2.85; N, 6.61; Found: % C, 54.72; H, 2.79; N, 6.58. IR (KBr, cm^{-1}): 3040 (CH, aromatic), 1675 (C=O), and 1635 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.58–3.62 (d, d, 2H, CH_2, isoxazoline ring), 6.24 (t, 1H, CH, isoxazoline ring), and 7.40–8.34 (m, 15H, aromatic-H). MS (*m/z*, R.I.): M^+. 633.00, 636.03, 638.00 (44%, 100%, 70.2%).

*6,8-Dibromo-3-(4-(5-(2-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**XIVd**)*

Crystallized from ethanol to give **XIVd**. m.p. 270°C, yield 60%. Analysis for $C_{29}H_{19}Br_2N_3O_3$, M.wt. (617.29) calcd.: % C, 56.43; H, 3.10; N, 6.81; Found: % C, 56.40; H, 3.02; N, 6.76. IR (KBr, cm^{-1}): 3334 (OH), 3045 (CH, aromatic), 1675 (C=O), and 1630 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.58–3.62 (d, d, 2H, CH_2, isoxazoline ring), 6.24 (t, 1H, CH, isoxazoline ring), 7.40–8.34 (m, 15H, aromatic-H), and 9.60 (s, 1H, OH). MS (*m/z*, R.I.): M^+. 614.98, 617.00, 618.98 (50%, 100%, 52%).

*6,8-Dibromo-3-(4-(5-(furan-2-yl)-4,5-dihydroisoxazol-3-yl)-phenyl)-2-phenylquinazolin-4(3H)-one (**XIVe**)*

Crystallized from ethanol to give **XIVe**. m.p. 110°C, yield 65%. Analysis for $C_{27}H_{17}Br_2N_3O_3$, M.wt. (591.25) calcd.: % C, 54.85; H,

2.90; N, 7.11; Found: % C, 54.80; H, 2.79; N, 7.08. IR (KBr, cm^{-1}): 3042 (CH, aromatic), 1677 (C=O) and 1637 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.60–3.68 (d, d, 2H, CH_2, isoxazoline ring), 6.44 (t, 1H, CH, isoxazoline ring), and 7.60–8.5 (m, 14H, aromatic-H). MS (*m*/*z*, R.I.): M^+. 589.00, 591.02, 593.03 (48%, 100%, 50%).

*General method for the synthesis of 6,8-dibromo-3-(4-(6-(substituted)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)-phenyl)-2-phenylquinazolin-4(3H)-one (**XVa**,**b**)*

A mixture of the chalcones **XIId**,**e** (0.005 mol) and urea (0.5 g, 0.005 mol) in ethanol (20 mL), and conc. HCl (5 mL) was refluxed for 7 h. The reaction mixture was concentrated to 1/2 its volume, cooled, and neutralized with NH_4OH solution. The precipitated solid was filtered off, washed with water, air dried, and crystallized from the proper solvent to give compounds **XVa**,**b**.

*6,8-Dibromo-3-(4-(6-(2-hydroxyphenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**XVa**)*

Crystallized from ethanol to give **XVa**. m.p. 115°C, yield 70%. Analysis for $C_{30}H_{20}Br_2N_4O_3$, M.wt. (644.31) calcd.: % C, 55.92; H, 3.13; N, 8.70; Found: % C, 55.88; H, 3.10; N, 8.65. IR (KBr, cm^{-1}): 3200–3600 (OH enolic of pyrimidine), 3042 (CH, aromatic), 1688 (C=O), and 1618 (C=N). ^1H NMR (DMSO-d_6, δ ppm): at 3.0 (2H, d, CH_2 of pyrimidinone), 5.1 (1H, t, CH of pyrimidinone), 7.2–8.5 (16H, m, aromatic protons including that of pyrimidone and quinazolone rings), and 9.8 (1H, S, OH). MS (*m*/*z*, R.I.): M^+. 642.00, 644.02, 646.03 (49%, 100%, 52%).

*6,8-Dibromo-3-(4-(6-(furan-2-yl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**XVb**)*

Crystallized from ethanol to give **XVb**. m.p. 180°C, yield 75%. Analysis for $C_{28}H_{18}Br_2N_4O_3$, M.wt. (618.28) calcd.: % C, 54.39; H, 2.93; N, 9.06; Found: % C, 54.34; H, 2.89; N, 9.00. IR (KBr, cm^{-1}): 3220–3610 (OH enolic of pyrimidine), 3045 (CH, aromatic), 1690 (C=O), and 1620 (C=N). ^1H NMR (DMSO-d_6, δ ppm): at 3.1 (2H, d, CH_2 of pyrimidinone), 5.4 (1H, t, CH of pyrimidinone), and 7.5–8.3 (15H, m, aromatic protons including that of pyrimidone and quinazolone rings). MS (*m*/*z*, R.I.): M^+. 615.97, 617.97, 619.96 (52%, 100%, 54%).

*6,8-Dibromo-3-(4-(6-(2-hydroxyphenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-2-phenylquinazolin-4(3H)-one (**XVc**)*

A mixture of the chalcone **XIId** (0.005 mol) and thiourea (0.005 mol) in presence of 0.5 g of NaOH in 5 mL of water. The mixture was refluxed in (25 mL) of ethanol for 6 h, then concentrated under vaccum and neutralized with dilute HCl. The precipitated material was filtered off, washed with water, dried, and crystallized from ethanol to give **XVc**. m.p. 210°C, yield 65%. Analysis for $C_{30}H_{20}Br_2N_4O_2S$, M.wt. (660.38) calcd.: % C, 54.56; H, 3.05; N, 8.48; Found: % C, 54.50; H, 3.00; N, 8.38. IR (KBr, cm^{-1}): 3220–3610 (OH enolic of pyrimidine), 3045 (CH, aromatic), 1690 (C=O), 1620 (C=N) and 1275 (C=S). ^1H NMR (DMSO-d_6, δ ppm): at 3.3 (2H, d, CH_2 0f pyrimidinone), 5.2 (1H, t, CH of pyrimidinone), 7.7–8.5 (15H, m, aromatic protons including that of pyrimidone and quinazolone rings), and 9.7 (1H, S, OH). MS (*m*/*z*, R.I.): M^+. 657.95, 659.94, 661.95 (50%, 100%, 54%).

Pharmacology

Materials and methods

Cell line and treatments

The human breast cancer cell line (MCF-7) was obtained from the American Type Culture Collection and grown in DMEM medium with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were maintained at 37° in a humidified atmosphere of 5% CO_2. Stock solutions of our compounds were prepared in DMSO and stored at −20°C. All controls were exposed to DMSO alone; DMSO concentration was always <0.1%.

Cell viability assay (sulforhodamine B assay)

To assess cellular proliferation, sulforhodamine B (SRB) assay was used according to the manufacturer's instructions. Briefly, cells were grown in tissue culture flasks, and then harvested by treating the flasks with 0.025% trypsin and 0.25 mM EDTA for 5 min. Once detached, cells were washed, counted and an aliquot (5×10^3 cells) was placed in each well of a 96-well cell culture plate in a total volume of 100 μL. Cells were allowed to attach overnight and then treated with or without increasing concentrations of curcumin. After treatment, cell medium was aspirated, and 100 mm^3/well of 10% trichloroacetic acid was added. After fixation at 4°C and washing, 50 mm^3 of 0.4% w/v sulforhodamine B (SRB; Sigma–Aldrich) was added. Plates were incubated at room temperature for 30 min. Unbound SRB was removed with 1% acetic acid. Bound SRB was solubilized with 100 mm^3 of 10 mM Tris-base solution. Absorbance was measured using a precision microplate reader (Molecular Devices, Sunnyvale, CA) at 570 and 650 nm (background). Cell viability was expressed as the percentage of cell survival relative to untreated controls.

Caspase-Glo 3/7 assay

Caspase-GloTM3/7 Assay (Promega) was used to detect caspase 3/7 activities of MCF-7 cancer cell lines triggered by our compounds. This test provides a proluminescent caspase 3/7 substrate, which contains the caspase 3 specific tetrapeptide sequence DEVD in a reagent optimized for cell lysis and determination of caspases and luciferase activity. MCF-7 cells cultured in DMEM were seeded in 96-well plates and treated with our compounds. Six hours after treatment, cellular caspase 3/7 activity was determined according to the manufacturer's protocol. Luminescence was measured using Mithras LB 940 (Berthold Technologies, BadWildbad, Germany). Cellular apoptosis was expressed as relative to the untreated medium control.

In this present work a novel series of quinazolin-4(3*H*)-ones compounds was synthesized. Synthetic Schemes 1–3 illustrate the way used for the synthesis of target compounds. Some of the synthesized compounds were tested in order to determine

Table 1. Cytotoxity of tested compounds against the human breast cancer cell line MCF-7 compared to doxorubicin.

IC_{50} values (μg/mL) with MCF-7 breast cancer cell line	
Compounds	**IC_{50} (μg/mL)**
XIVd	1.83 ± 0.09
XIVc	13.9 ± 2.41
XIVe	6.84 ± 0.92
XIIIc	38.5 ± 1.8
VIII	68.12 ± 3.42
XIVa	21.4 ± 1.71
IV	68.4 ± 3.83
XVb	53.1 ± 2.54
XVc	15.7 ± 0.92
XVa	>100
XIVb	5.4 ± 1.23
XIIIb	1.7 ± 0.35
XIIIa	10.8 ± 1.98
IX	1.8 ± 0.12
Doxorubicin	29.6 ± 2.78

the possible anticancer activity. MCF7 human breast cancer cells were cultured in a monolayer and treated with our compounds for 48 h. The SRB assay was performed as previously shown [38] to assess the rate of proliferation, and the resulting growth curves showed that our molecules exhibited cytotoxicity against the above-mentioned cell line with very low IC_{50} values (Table 1). Compounds **XIIIb**, **IX**, **XIVd**, **XIVb**, **XIVe**, **XIIIa**, **XIVc**, **XVc**, and **XIVa** exerted powerful cytotoxic effects against MCF7 cells with very low IC_{50} values compared to doxorubicin (positive control; IC_{50} values were 1.7, 1.8, 1.83, 5.4, 6.84, 10.8, 13.9, 15.7, and 29.6 μg/mL, respectively). Compounds **XIIIc**, **VIII**, **IV**, and **XVb** exerted moderate cytotoxic effect against MCF7 cells (IC_{50} values were 38.51, 68.12, 68.4, and 53.1 μg/mL, respectively). Compound **XVa** exerted a weak cytotoxic effect against MCF7 cells (IC_{50} values >100 μg/mL). The most potent anticancer activity exhibited by compounds **XIIIa**,**b**, **IX**, **XIVa–e**, and **XVc** might be due to the presence of *N*-phenylpyrazoline, *N*-(4-methylpyrimidin-2-yl)benzenesulfonamide, isoxazolines, and tetrahydropyrimidine-thione moiety of the 2-phenyl substituted quinazolin-4(3*H*)-one, respectively. It is interesting to note that a minor alteration in the molecular configuration of the investigated compounds may have a pronounced effect on anticancer activity, e.g., compound **XVc** having tetrahydropyrimidine-thione moiety showed more anticancer activity than **XVa** with tetrahydropyrimidinone.

It is well-established that the induction of the apoptotic cascade is one of the main mechanisms of chemotherapy-induced cell death [39], in order to determine whether the chemosensitizing effect of our compounds demonstrated above is secondary to its ability to activate the apoptotic cascade, MCF-7 cells were treated with test compounds at their IC_{50} concentrations. Six hours after treatment, the activity of caspase 3/7 was measured using the Caspase-Glo 3/7 assay. Figure 1 shows that some of the tested compounds caused significant increase in activation of caspase 3/7 (e.g., compounds **XIVd**, **VIII**, **XIVb**).

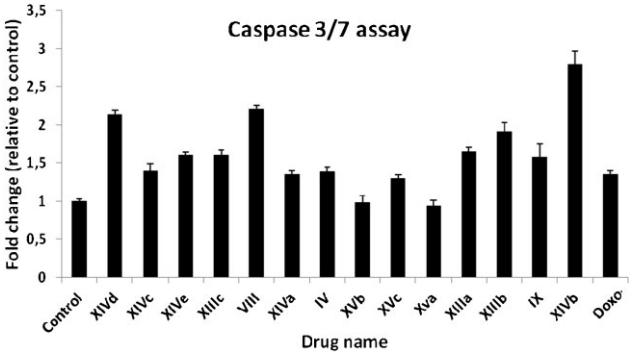


Figure 1. Caspase-Glo 3/7 activity of tested compounds relative to untreated control. Enzymatic activity of caspase 3 after 6-h treatment of MCF-7 cells. The activity is expressed as percentage relative to untreated cells.

The authors have declared no conflict of interest.

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