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Research Article

Synthesis and evaluation of antioxidant activities of novel quinazolin derivatives

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ABSTRACT

Different acid chlorides **2a-c** are used as starting material of a number of 2-(substituted)-4*H*-3,1-benzoxazin-4-one derivatives **3a-c**, Some of the newly synthesized analogues were chosen to evaluate their cytotoxic activity against human carcinoma cell lines (MCF7-HePG2–HCT116).

Keywords: Quinazolin; benzoxazin; cytotoxic activity

INTRODUCTION

The chemistry of quinazolinone system has received an increasing interest because of its biological significance. Many derivatives of this system showed antifungal (Tiwari et al., 2007), antibacterial (Grover et al., 2006), antitumor (Cao et al., 2005), anti-inflammatory (Giri et al., 2009), anticonvulsant (El-Helby et al., 2003, Kadi et al., 2006 and Jatav et al., 2008) and analgesic (Van Zyl , 2001 and Kumar et al., 2003) activities.

Benzoxazine heterocyclic compounds are potent non-steroidal progesterone receptor agonists (Zhang et al ., 2002) having many other activities such as anticancer, antiangiogenic (La et al ., 2008), antidiabetic and hypolipidemic (Madhavan et al ., 2006), antidepressant (Zhou et al ., 2006) and antiplatelet aggregation activity (Pritchard et al ., 2007).

MATERIAL AND METHODS

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer (Japan) at cm^{-1} scale using KBr disc technique at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^1H NMR spectra were determined by using a JEOl EX-270 NMR spectrometer (Japan) at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, Cairo University, Giza, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV analysis lamp at λ 254/366 nm for few seconds.

General method for the synthesis of 2-(substituted)-4*H*-3,1-benzoxazin-4-one (3 a-c)

A solution of acid chloride **(2a-c)** (0.01 mol) and anthranilic acid (0.01 mol) in dry pyridine (30 mL) was refluxed for 3 h, the reaction mixture was cooled and poured into cold diluted HCl. The solid that separated was filtered off and recrystallized from a proper solvent to give **(3a-c).** Spectroscopic data for all the compounds are given below.

2-[2-(Phenylamino) phenyl]-4*H*-3, 1-benzoxazin-4-one (3a): Yellow crystals, recrystallization solvent: ethanol, yield: 85%, MP ≈ 235-240 ^0C, analysis for $C_{20}H_{14}N_2O_2$ (314.33): C, 76.42; H, 4.49; N, 8.91%. Found: C, 76.03; H, 4.20; N, 8.34%. IR (KBr, cm^{-1}): 1690 (C=O) and 3170 (NH). ^1H NMR (DMSO-d_6, δ ppm): 7.20-8.20 (m, 13H, ArH), 11.72 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 314 (5%).

2-(Pyridin-3-yl)-4*H*-3,1-benzoxazin-4-one (3b): Yellow white crystals, recrystallization solvent: methanol, yield: 75%, MP ≈ 210-215 ^0C, analysis for $C_{13}H_8N_2O_2$ (224.21): C, 69.64; H, 3.60; N, 12.49%. Found: C, 69.43; H, 3.44; N, 12.14%. IR (KBr, cm^{-1}): 1700 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 7.50-9.03 (m, 8H, ArH). MS: (m/z) ≈ 224 (10%).

2-(Pyridin-4-yl)-4*H*-3,1-benzoxazin-4-one (3c): Yellow crystals, recrystallization solvent: ethanol, yield 85%. MP >300 ^0C, analysis for $C_{13}H_8N_2O_2$ (224.21): C, 69.64; H, 3.60; N, 12.49%. Found: C, 69.55; H, 3.51; N, 12.25%.

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IR (KBr, cm^{-1}): 1692 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 7.42-9.21 (m, 8H, ArH). MS: (m/z) ≈ 224 (15%).

General method for the synthesis of 3-hydroxy-2-[2-substituted] quinazolin-4(3*H*)-one (4a, b)

An equimolar mixture of **(3a, b)** (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) in 30 mL of dry pyridine was heated under reflux for 6 h, left to cool and then poured into cold water with constant stirring. The solid product that separated out was filtered off; thoroughly washed with water, dried and then recrystallized from the appropriate solvent to give **(4a, b)**

3-Hydroxy-2-[2-(phenylamino)phenyl]quinazolin-4(3*H*)-one (4a): Color: pale yellow powder, recrystallization solvent: ethanol, yield: 80%, MP >300 ^0C; analysis for $C_{20}H_{15}N_3O_2$ (329.35); Calcd: C, 72.94; H, 4.59; N, 12.76; found: C, 72.81; H, 4.26; N, 12.69. IR (KBr, cm^{-1}): 1637 (C=O), 3281 (NH) and 3358 (OH). ^1H NMR (DMSO-d_6, δ ppm): 7.61-8.81 (m, 13H, ArH), 10.12 (s, 1H, OH, exchangeable with D_2O) and 12.31 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 329 (5 %)

3-Hydroxy-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (4b): Color: White powder, recrystallization solvent: benzene, yield: 70%, MP ≈ 215-220 ^0C; analysis for $C_{13}H_9N_3O_2$ (239.22); Calcd: C, 65.27; H, 3.79; N, 17.56; found: C, 65.11; H, 3.66; N, 17.49. IR (KBr, cm^{-1}): 1635 (C=O) and 3347 (OH). ^1H NMR (DMSO-d_6, δ ppm): 7.32-8.51 (m, 8H, ArH) and 10.31 (s, 1H, OH, exchangeable with D_2O). MS: (m/z) ≈ 241 ([M^+ +2], 8.93 %)

General method for the synthesis of ethoxymethyl-4-oxo-2-(2-substituted) quinazoline-3(4*H*)-carboxylate (5a, b)

To a solution of **(4a, b)** (0.01 mol) in 50 mL of dry acetone were added ethyl chloroacetate (0.04 mol) and anhydrous potassium carbonate (0.04 mol). The reaction mixture was heated under reflux for 24 h. The excess acetone was removed by distillation and the residue was poured into cold water with stirring. The solid that separated out was filtered by suction, washed with cold water, dried and purified by recrsytallization from suitable solvent to afford products **(5a, b)**

Ethoxymethyl-4-oxo-2-(2-(phenylamino)phenyl)quinazoline-3(4*H*)-carboxylate (5a): Color: Yellow powder, recrystallization solvent: butanol, yield: 70%, MP ≈ 230-235 ^0C; analysis for $C_{24}H_{21}N_3O_4$ (415.44); Calcd: C, 69.39; H, 5.10; N, 10.11; found: C, 69.29; H, 4.96; N, 10.09. IR (KBr, cm^{-1}): 1633, 1831 (2C=O) and 3272 (NH). ^1H NMR (DMSO-d_6, δ ppm): 1.23 (t, 3H, CH_3), 3.40 (q, 2H, CH_2), 4.36 (s, 2H, CH_2), 7.23-8.25 (m, 13H, ArH) and 11.76 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 415 (21 %)

Ethyl{[4-oxo-2-(pyridin-3-yl)quinazolin-3(4*H*)-yl]oxy} acetate (5b): Color: Brown powder, recrystallization solvent: benzene, yield: 65%, MP >300 ^0C; analysis for $C_{17}H_{15}N_3O_4$ (325.31); Calcd: C, 62.76; H, 4.65; N, 12.92; found: C, 62.67; H, 4.59; N, 12.87. IR (KBr, cm^{-1}): 1637, 1731 (2C=O). ^1H NMR (DMSO-d_6, δ ppm): 1.31 (t, 3H, CH_3), 3.52 (q, 2H, CH_2), 5.11 (s, 2H, CH_2) and 7.23-8.25 (m, 8H, ArH). MS: (m/z) ≈ 325 (12 %)

General method for the synthesis of 3-(4-acetylphenyl)-2-(2-(substituted)quinazolin-4(3*H*)-one (6a, b)

To a solution of **(3a, b)** (0.01mol) in ethyl alcohol (30 mL) 4-amino acetophenone (0.01 mol) was added and the reaction mixture was heated under reflux for 6 h and left to cool after distilling off the excess solvent. The solid product which deposited was filtered off, dried and recrystallized from suitable solvent to yield compounds **(6a, b).**

3-(4-Acetylphenyl)-2-(2-(phenylamino)phenyl) quinazolin-4(3*H*)-one (6a): Color: Black powder, recrystallization solvent: butanol, yield: 77%, MP >300 ^0C; analysis for $C_{28}H_{21}N_3O_2$ (431.48); Calcd: C, 77.94; H, 4.91; N, 9.74; found: C, 77.89; H, 4.86; N, 9.68. IR (KBr, cm^{-1}): 1633, 1700 (2C=O) and 3230 (NH). ^1H NMR (DMSO-d_6, δ ppm): 2.61 (s, 3H, CH_3), 7.41-8.32 (m, 17H, ArH) and 12.12(s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 431 (31 %)

3-(4-Acetylphenyl)-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (6b): Color: Brown powder, recrystallization solvent: benzene,, yield: 65%, MP ≈ 155-160 ^0C; analysis for $C_{21}H_{15}N_3O_2$ (341.36); Calcd: C, 73.89; H, 4.43; N, 12.31; found: C, 73.79; H, 4.36; N, 12.24. IR (KBr, cm^{-1}): 1640, 1723 (2C=O). ^1H NMR (DMSO-d_6, δ ppm): 2.52 (s, 3H, CH_3) and 7.21-8.45 (m, 12H, ArH). MS: (m/z) ≈ 341 (7.71 %)

General method for the synthesis of 3-(2-aminophenyl)-2-(2-(substituted) quinazolin-4(3*H*)-one (7a, b)

An equimolar mixture of **(3a, b)** (0.01 mol) and o-phenylenediamine (0.01 mol) in 30 mL of ethyl alcohol was heated under reflux for 8 h. The excess alcohol was distilled off and the reaction solution was left to cool. The solid so obtained was filtered off, dried and recrystallized from proper solvent to afford **(7a, b)**.

3-(2-Aminophenyl)-2-(2-(phenylamino)phenyl)quinazolin-4(3*H*)-one (7a): Color: Brown powder, recrystallization solvent: ethanol, yield: 85%, MP >300 ^0C; analysis for $C_{26}H_{20}N_4O$ (404.46); Calcd: C, 77.21; H, 4.98; N, 13.85; found: C, 77.14; H, 4.87; N, 13.77. IR (KBr, cm^{-1}): 1645 (C=O), 3227 (NH) and 3355, 3469 (NH_2). ^1H NMR (DMSO-d_6, δ ppm): 6.31 (s, 2H, NH_2, exchangeable with D_2O), 6.91-7.89 (m, 17H, ArH) and 12.11(s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 404 (14 %)

3-(2-Aminophenyl)-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (7b): Color: Black powder, recrystallization solvent: benzene, yield: 60%, MP ≈ 105-110 ^0C; analysis for $C_{19}H_{14}N_4O$ (314.34); Calcd: C, 72.60; H, 4.49; N, 17.82; found: C, 72.56; H, 4.34; N, 17.79. IR (KBr, cm^{-1}): 1636 (C=O) and 3430, 3478 (NH_2). ^1H NMR (DMSO-d_6, δ

ppm): 6.54 (s, 2H, NH_2, exchangeable with D_2O) and 7.21-8.12 (m, 12H, ArH). MS: (m/z) ≈ 314 (10 %)

General method for the synthesis of 4-(4-oxo-2-(2-(substituted)quinazolin-3(4*H*)-yl)benzenesulfonamide (8a, b)

A mixture of **(3a, b**) (0.01 mol) and sulfanilamide (0.01 mol) was heated under reflux in 50 mL of dry pyridine for 8 h. The excess solvent was removed by distillation and the reaction solution was left to cool, then poured into crushed ice with stirring to obtain the crude products which were filtered off, thoroughly washed with cold water, dried and recrystallized from the proper solvent to afford products **(8 a, b)**.

4-(4-Oxo-2-(2-(phenylamino)phenyl)quinazolin-3(4*H*)-yl) benzene sulfonamide (8a): Color: Brown powder, recrystallization solvent: butanol, yield: 70%, MP ≈ 280-285 ^{0}C; analysis for $C_{26}H_{20}N_4O_3S$ (468.52); Calcd: C, 66.65; H, 4.30; N, 11.96; found: C, 66.58; H, 4.24; N, 11.88. IR (KBr, cm^{-1}): 1633 (C=O), 3317 (NH) and 3343, 3487 (NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 6.14 (s, 2H, NH_2, exchangeable with D_2O), 7.31-8.54 (m, 17H, ArH) and 11.87 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 468 (15 %)

4-[4-Oxo-2-(pyridin-3-yl)quinazolin-3(4*H*)-yl]benzenesulfonamide (8b): Color: Brown powder, recrystallization solvent: toluene, yield: 65%, MP ≈ 175-180 ^{0}C; analysis for $C_{19}H_{14}N_4O_3S$ (378.40); Calcd: C, 60.31; H, 3.73; N, 14.81; found: C, 60.27; H, 3.69; N, 14.76. IR (KBr, cm^{-1}): 1638 (C=O) and 3332, 3454 (NH_2). ^{1}H NMR (DMSO-d_6, δ ppm): 6.56 (s, 2H, NH_2, exchangeable with D_2O) and 7.43-8.76 (m, 12H, ArH). MS: (m/z) ≈ 378 (34 %)

General method for the synthesis of 5-[2-substituted][1,2,4]triazolo[1,5-c] quinazolin-2(3*H*)-one (9a, b)

To a solution of **(3a, b)** (0.01 mol) in 30 mL of pyridine semicarbazide hydrochloride (0.01 mol) was added and the reaction mixture was heated under reflux for 6 h, left to cool, poured into cold water with stirring. The solid crude product that separated out was filtered off by suction, washed with cold water, dried and recrystallized from the appropriate solvent to give compounds **(9a, b)**.

5-[2-(Phenylamino)phenyl][1,2,4]triazolo[1,5-c] quinazolin-2(3*H*)-one (9a): Color: White powder, recrystallization solvent: ethanol, yield: 90%, MP ≈ 250-255 ^{0}C; analysis for $C_{21}H_{15}N_5O$ (353.37); Calcd: C, 71.38; H, 4.28; N, 19.82; found: C, 71.30; H, 4.16; N, 19.76. IR (KBr, cm^{-1}): 1645 (C=O) and 3250, 3321 (2NH). ^{1}H NMR (DMSO-d_6, δ ppm): 7.11-8.76 (m, 13H, ArH), 10.87 (s, 1H, NH, exchangeable with D_2O) and 11.67(s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 353 (12 %)

5-(Pyridin-3-yl)[1,2,4]triazolo[1,5-c]quinazolin-2(3*H*)-one (9b): Color: pale yellow powder, recrystallization solvent: xylene, yield: 70%, MP ≈ 255-260 ^{0}C; analysis for $C_{14}H_9N_5O$ (263.25); Calcd: C, 63.87; H, 3.45; N, 26.60; found: C, 63.82; H, 3.39; N, 26.54. IR (KBr, cm^{-1}): 1637 (C=O) and 3278 (NH). ^{1}H NMR (DMSO-d_6, δ ppm): 7.54-8.87 (m, 8H, ArH) and 10.97 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 263 (24 %)

General method for the synthesis of 3-(2-hydroxyethyl)-2-[2-(substituted)]quinazolin-4(3*H*)-one (10a, b)

A solution of **(3a, b)** (0.01 mol) in ethanolamine (20 mL) was refluxed for 3 h. Most of the solvent was removed, the solid that formed was collected, washed with petroleum, dried and recrsytallized from appropriate solvent to afford compounds **(10 a, b)**.

3-(2-Hydroxyethyl)-2-[2-(phenylamino)phenyl] quinazolin-4(3*H*)-one (10a): Color: pale yellow powder, recrystallization solvent: toluene, yield: 85%, MP >300 ^{0}C; analysis for $C_{22}H_{19}N_3O_2$ (357.40); Calcd: C, 73.93; H, 5.36; N, 11.76; found: C, 73.88; H, 5.28; N, 11.71. IR (KBr, cm^{-1}): 1633 (C=O), 3272 (NH) and 3422 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.42 (t, 2H, CH_2), 3.67(t, 2H, CH_2), 6.84-7.70 (m, 13H, ArH), 10.43 (s, 1H, OH, exchangeable with D_2O) and 12.21 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 357(24 %)

3-(2-Hydroxyethyl)-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (10b): Color: Black powder, recrystallization solvent: ethanol, yield: 70%, MP >300 ^{0}C; analysis for $C_{15}H_{13}N_3O_2$ (267.28); Calcd: C, 67.40; H, 4.90; N, 15.72; found: C, 67.34; H, 4.85; N, 15.69. IR (KBr, cm^{-1}): 1639 (C=O) and 3400 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.45 (t, 2H, CH_2), 3.71 (t, 2H, CH_2), 7.21-8.21 (m, 8H, ArH) and 10.65 (s, 1H, OH, exchangeable with D_2O). MS: (m/z) ≈ 267 (10 %)

General method for the synthesis of 3-(2-(2-hydroxynaphthalen-1-yl)ethyl)-2-(2-(substituted) quinazolin-4(3*H*)-one (11a, b)

A mixture of compounds **(10a, b)** (0.01 mol) and 2-naphthol (0.01 mol)was heated without solvent and 110-120^{0}C in presence of few drops of concentrated hydrochloric acid for 3 h. The solid was washed with warm light petroleum. The solid separated was filtered, dried and crystallized to afford **(11a, b)**.

3-(2-(2-Hydroxynaphthalen-1-yl)ethyl)-2-(2-(phenyl amino)phenyl)quinazolin-4(3*H*)-one (11a): Color: Black powder, recrystallization solvent: butanol, yield: 75%, MP ≈ 120-125 ^{0}C; analysis for $C_{32}H_{25}N_3O_2$ (483.55); Calcd: C, 79.48; H, 5.21; N, 8.69; found: C, 79.41; H, 5.17; N, 8.59. IR (KBr, cm^{-1}): 1644 (C=O), 3323 (NH) and 3456 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.43 (t, 2H, CH_2), 3.52 (t, 2H, CH_2), 5.21 (s, 1H, OH, exchangeable with D_2O), 7.12-8.34 (m, 19H, ArH) 12.31 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 483 (20 %)

3-[2-(3-Hydroxynaphthalen-2-yl)ethyl]-2-(pyridin-3-yl)quinazolin-4(3*H*)-one (11b): Color: Gray powder, recrystallization solvent: xylene, yield: 60%, MP ≈ 270-275 ^{0}C; analysis for $C_{25}H_{19}N_3O_2$ (393.43); Calcd: C,

76.32; H, 4.87; N, 10.68; found: C, 76.29; H, 4.81; N, 10.61. IR (KBr, cm^{-1}): 1646 (C=O) and 3377 (OH). ^1H NMR (DMSO-d_6, δ ppm): 2.50 (t, 2H, CH_2), 3.43 (t, 2H, CH_2), 4.94 (s, 1H, OH, exchangeable with D_2O) and 7.86-8.17 (m, 14H, ArH). MS: (m/z) ≈ 393 (32 %)

General method for the synthesis of 4-hydroxy-2-[2-(substituted)]quinoline-3-carbonitrile (12a, b)

Absolute ethanol (40 mL) containing sodium metal (0.01 mol) was added to malononitrile (0.01 mol). After few minutes **(3a, b)** (0.01 mol) was added. The reaction mixture was heated under reflux with stirring for 20 h. Most of the solvent was distilled off and the reaction solution was acidified with hydrochloric acid to give a crude product which was filtered off, washed several times with cold water, dried, and recrystallized to yield **(12a, b)**.

4-Hydroxy-2-[2-(phenylamino)phenyl]quinoline-3-carbonitrile (12a): Color: Black powder, recrystallization solvent: toluene, yield: 60%, MP >300 ^0C; analysis for $C_{22}H_{15}N_3O$ (337.37); Calcd: C, 78.32; H, 4.48; N, 12.46; found: C, 78.28; H, 4.41; N, 12.38. IR (KBr, cm^{-1}): 2212 (C≡N), 3208 (NH) and 3357 (OH). ^1H NMR (DMSO-d_6, δ ppm): 5.34 (s, 1H, OH, exchangeable with D_2O), 7.72-8.94 (m, 13H, ArH) and 12.43 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 337 (43 %)

4-Hydroxy-2-(pyridin-3-yl)quinoline-3-carbonitrile (12b): Color: Brown powder, recrystallization solvent: ethanol, yield: 65%, MP >300 ^0C; analysis for $C_{15}H_9N_3O$ (247.25); Calcd: C, 72.87; H, 3.67; N, 16.99; found: C, 72.82; H, 3.58; N, 16.90. IR (KBr, cm^{-1}): 2210 (C≡N) and 3364 (OH). ^1H NMR (DMSO-d_6, δ ppm): 5.21 (s, 1H, OH, exchangeable with D_2O) and 7.54-8.76 (m, 8H, ArH) . MS: (m/z) ≈ 247 (11 %)

General method for the synthesis of 3-amino-2-(substituted)quinazolin-4(3*H*)-one (13a, c)

A solution of **(3a, c)** (0.01 mol) in dry benzene (30 mL) and hydrazine hydrate (0.015 mol) was heated under reflux for 4 h. Then the mixture was poured into water. The precipitate was collected by filtration, dried and crystallized from the proper solvent to give **(13a, c).** Spectroscopic data for all the compounds are given below.

3-Amino-2-[2-(phenylamino)phenyl]quinazolin-4(3*H*)-one (13a): Yellow crystals, recrystallization solvent: butanol, yield 85%. MP≈ 260-265 ^0C, analysis for $C_{20}H_{16}N_4O$ (328.36): C, 73.15; H, 4.91; N, 17.06%. Found: C, 73.01; H, 4.75; N, 16.90%. IR (KBr, cm^{-1}): 1700 (C=O), 3172 (NH) and 3300-3434 (NH_2). ^1H NMR (DMSO-d_6, δ ppm): 3.60 (s, 2H, NH_2, exchangeable with D_2O), 6.68-8.54 (m, 13H, ArH), 12.01 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 328 (20%).

3-Amino-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (13c): Black crystals, recrystallization solvent: ethanol, yield 75%. MP≈ 150-155 ^0C, analysis for $C_{13}H_{10}N_4O$ (238.24): C, 65.54; H, 4.23; N, 23.52%. Found: C, 65.32; H, 4.18; N, 23.40%. IR (KBr, cm^{-1}): 1685 (C=O) and 3311-3420 (NH_2). ^1H NMR (DMSO-d_6, δ ppm): 7.68-8.66 (m, 8H, ArH), 10.08 (s, 2H, NH_2, exchangeable with D_2O). MS: (m/z) ≈ 238 (15%).

General method for the synthesis of (*E*)-3-(2-hydroxybenzylideneamino)-2-(2-(substituted) quinazolin-4(3*H*)-one 14 (a, c), 15 (a, c)

A mixture of **(13a, c)** (0.01 mol) and the appropriate aldehyde, namely salicaldehyde and anisaldehyde (0.01 mol) in ethyl alcohol (50 mL) was heated under reflux for 4 h in presence of catalytic amount of piperidine. The excess alcohol was distilled off and the reaction solution was left to cool to obtain the crude product which was recrystallized from suitable solvents to give **14 (a, c), 15 (a, c)**

(*E*)-3-(2-hydroxybenzylideneamino)-2-(2-(phenyl amino)phenyl)quinazolin-4(3*H*)-one (14a): Color: Black powder, recrystallization solvent: butanol, yield: 90%, MP >300^0C; analysis for $C_{27}H_{20}N_4O_2$ (432.47); Calcd: C, 74.98; H, 4.66; N, 12.95; found: C, 74.90; H, 4.61; N, 12.89. IR (KBr, cm^{-1}): 1639 (C=O), 3376 (NH) and 3455 (OH). ^1H NMR (DMSO-d_6, δ ppm): 5.44 (s, 1H, OH, exchangeable with D_2O), 6.87-8.12 (m, 17H, ArH), 8.43 (s, 1H, CH) and 12.21 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 432 (17 %)

3-[(*E*)-(2-hydroxybenzylidene)amino]-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (14c): Color: pale yellow powder, recrystallization solvent: toluene, yield: 75%, MP ≈ 245-250 ^0C; analysis for $C_{20}H_{14}N_4O_2$ (342.35); Calcd: C, 70.17; H, 4.12; N, 16.37; found: C, 70.01; H, 3.99; N, 16.31. IR (KBr, cm^{-1}): 1642 (C=O) and 3512 (OH). ^1H NMR (DMSO-d_6, δ ppm): 5.23 (s, 1H, OH, exchangeable with D_2O), 6.98-8.23 (m, 12H, ArH) and 8.21 (s, 1H, CH). MS: (m/z) ≈ 342 (23 %)

(*E*)-3-(4-methoxybenzylideneamino)-2-(2-(phenyl amino)phenyl)quinazolin-4(3*H*)-one (15a): Color: pale yellow powder, recrystallization solvent: butanol, yield: 60%, MP >300 ^0C; analysis for $C_{28}H_{22}N_4O_2$ (446.49); Calcd: C, 75.32; H, 4.97; N, 12.55; found: C, 75.29; H, 4.91; N, 12.49. IR (KBr, cm^{-1}): 1645 (C=O) and 3350 (NH). ^1H NMR (DMSO-d_6, δ ppm): 3.81 (s, 3H, OCH_3), 6.84-7.64 (m, 17H, ArH), 9.23 (s, 1H, CH) and 12.31 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 446 (56 %)

3-[(*E*)-(4-methoxybenzylidene)amino]-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (15c): Color: White powder, recrystallization solvent: ethanol, yield: 65%, MP ≈ 185-190 ^0C; analysis for $C_{21}H_{16}N_4O_2$ (356.37); Calcd: C, 70.77; H, 4.53; N, 15.72; found: C, 70.69; H, 4.48; N, 15.68. IR (KBr, cm^{-1}): 1649 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 3.56 (s, 3H, OCH_3), 6.65-7.97 (m, 12H, ArH) and 9.34 (s, 1H, CH). MS: (m/z) ≈ 356 (19 %)

General method for the synthesis of 3-((substituted) (phenylthio)methylamino)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (16c, 17c)

To a mixture of **14c** and/or **15c** (0.01 mol) and thiophenol (0.01 mol) in dry benzene (40 mL) was added few drops of piperidine and the reaction mixture is then heated under reflux for 2 h. Distilling off the excess solvent and cooling gave a crude solid which was filtered off, washed with petroleum (20 mL) and recrystallized to afford products **16c, 17c**.

3-((2-Hydroxyphenyl)(phenylthio)methylamino)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (16c): Color: White powder, recrystallization solvent: toluene, yield: 85%, MP ≈ 225-230 ^{0}C; analysis for $C_{26}H_{20}N_4O_2S$ (452.52); Calcd: C, 69.01; H, 4.45; N, 12.38; found: C, 68.88; H, 4.38; N, 12.18. IR (KBr, cm^{-1}): 1635 (C=O), 3365 (NH) and 3444 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 4.38 (s, 1H, CH), 6.91-8.81 (m, 17H, ArH), 11.07 (s, 1H, OH, exchangeable with D_2O) and 12.27 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 452 (14 %)

3-((4-Methoxyphenyl)(phenylthio)methylamino)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (17c): Color: White powder, recrystallization solvent: ethanol, yield: 80%, MP ≈ 165-170^{0}C; analysis for $C_{27}H_{22}N_4O_2S$ (466.55); Calcd: C, 69.51; H, 4.75; N, 12.01; found: C, 69.47; H, 4.58; N, 11.90. IR (KBr, cm^{-1}): 1636 (C=O) and 3343 (NH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.82 (s, 3H, OCH_3), 4.91 (s, 1H, CH), 7.03-8.73 (m, 17H, ArH) and 12.07 (s, 1H, NH, exchangeable with D_2O). MS: (m/z) ≈ 466 (46 %)

Cytotoxic effect on human cell line (HePG2 – MCF 7 - HCT116)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan (Mosmann et al ,1983).

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2- MCF7 and HCT116. The media are supplemented with 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37 ^{0}C under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ^{0}C for 24 h under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 ug/ml). After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37 ^{0}C under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37^{0}C. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions (El-Menshawi et al ., 2010 and Thabrew et al ., 1997).

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

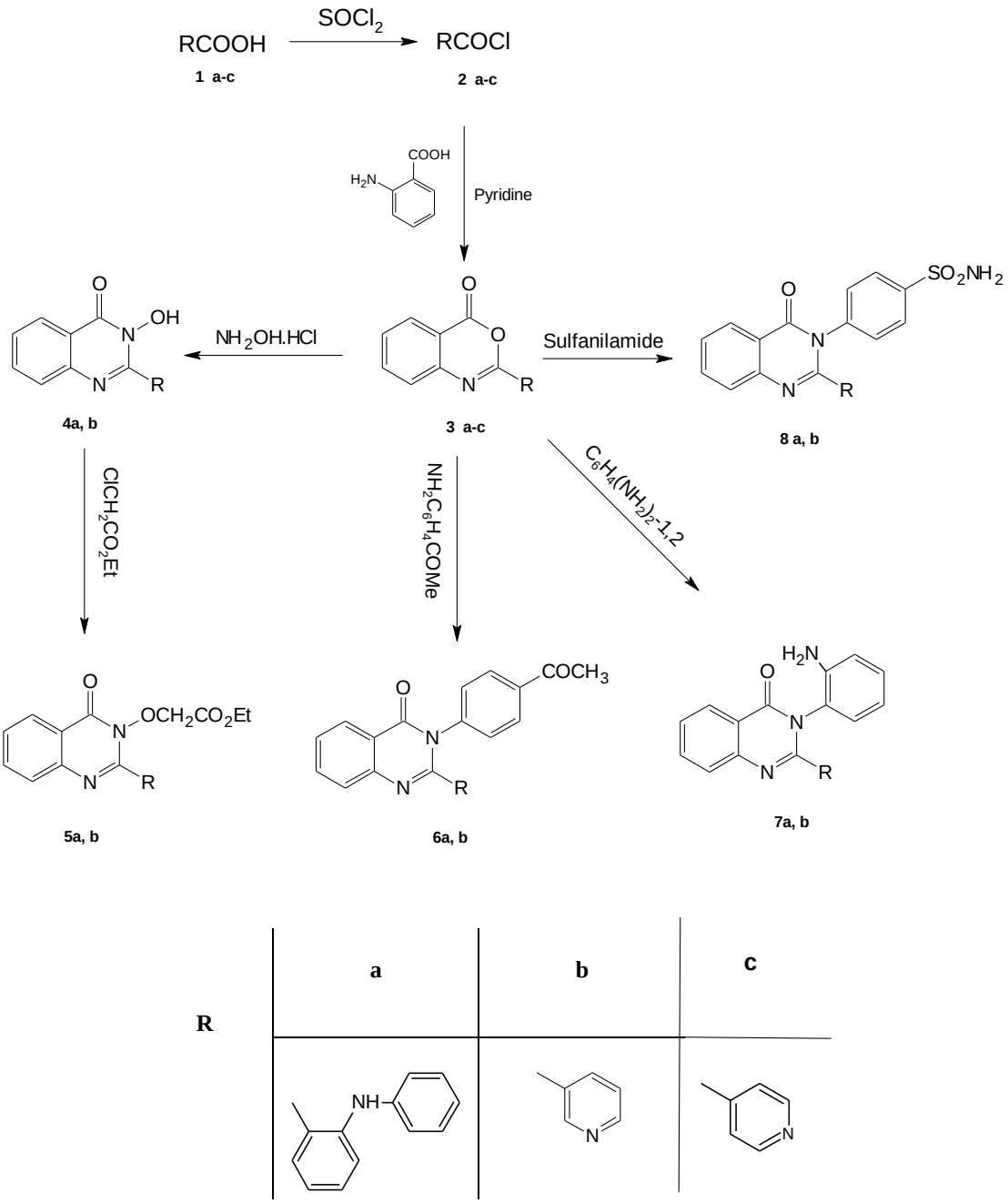
(Reading of extract / Reading of negative control) -1) x 100. A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

RESULT AND DISCUSSION

Chemistry

Different acid chlorides namely, 2-(phenylamino) benzoyl chloride , pyridine-3-carbonyl chloride and pyridine-4-carbonyl chloride **2a-c** respectively reacted with anthranilic acid to produce 2-[substituted]-4*H*-3,1-benzoxazin-4-one **3a-c** (Scheme 1). Compounds **3a, b** reacted with hydroxylamine hydrochloride to give 3-hydroxy-2-[2-substituted]quinazolin-4(3*H*)-one **4a, b** which reacted with ethyl chloroacetate to give ethoxymethyl4-oxo-2-(2-substituted)quinazoline-3(4*H*)-carboxylate **5a, b** (Scheme 1). The structures of all of the newly synthesized derivatives were established via the elemental analyses and IR, ^{1}H NMR and mass spectral data. IR spectra of the compounds **4a, b** exhibited characteristic absorption bands at the range 3358 and 3347 cm^{-1} respectively due to the respective (OH). ^{1}H NMR (DMSO-d_6) spectra of compounds **5a, b** revealed signals at δ 1.23 and 1.31 ppm representing (CH_3) of ethyl group and 3.40 and 5.11 ppm, representing (CH_2) of ethyl group, respectively.

Compounds **3a, b** reacted with 4-amino acetophenone, o-phenylenediamine, sulfanilamide, semicarbazide hydrochloride and ethanolamine to give compounds **6 (a, b)-10 (a,b)** respectively (Scheme 1, 2). Compounds **10 (a, b)** reacted with 2-naphthol to give 3-(2-(2-hydroxynaphthalen-1-yl)ethyl)-2-(2-(substituted)quinazolin-4(3*H*)-one **11a, b** (Scheme 2). IR spectra of the compounds **6a, b** exhibited characteristic absorption bands at the range 1633, 1700 and 1640, 1723 cm^{-1} respectively due to the respective (2C=O). ^{1}H NMR (DMSO-d_6) spectra of compounds **7a, b** revealed signas at δ 6.31 and 6.54 ppm representing (NH_2) group , respectively. Also ^{1}H NMR (DMSO-d_6) spectra of compounds **8a, b** revealed signals at δ 6.14 and 6.56 ppm representing (NH_2) group , respectively. ^{1}H NMR (DMSO-d_6) spectra of compounds **9a, b** re-



Scheme-1

vealed signals at δ 11.67 and 10.97 ppm representing (NH) group, respectively. IR spectra of the compounds **10a, b** exhibited characteristic absorption bands at the range 3422 and 3400 cm^{-1} respectively due to the respective (OH). The mass spectrum of compounds **11a, b** showed the molecular ion peak $[M]^+$ at m/z = 483(20%) and $[M]^+$ at m/z = 393(32%), respectively.

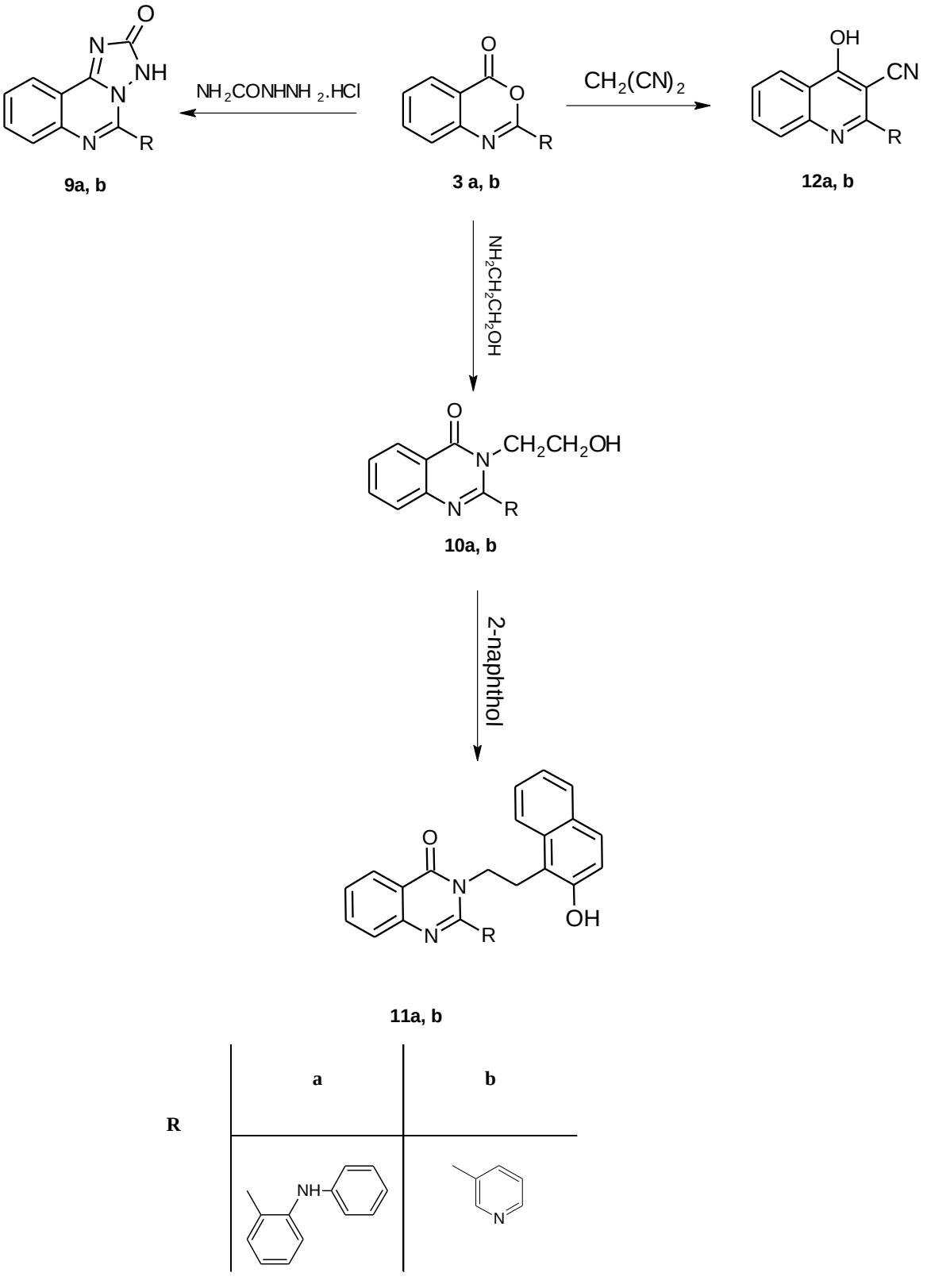
Also compounds **3a, b** reacted with malononitrile to give compounds 4-hydroxy-2-[2-(substituted] quinoline-3-carbonitrile **12a, b** (Scheme 2). IR spectra of the compounds **12a, b** exhibited characteristic absorption bands at the range 2212 and 2210 cm^{-1} respectively due to the respective (C≡N).

Compounds **3a, c** reacted with hydrazine hydrate to give compounds 3-amino-2-(substituted)quinazolin-4(3*H*)-one **13a, c** (Scheme 3) which reacted with appropriate aldehyde, namely salicaldehyde and anisaldehyde to give **14 (a, c), 15 (a, c)** (Scheme 3).

Finally, Compounds **14c, 15c** reacted with thiophenol to give compounds 3-((substituted)(phenylthio)methylamino)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one

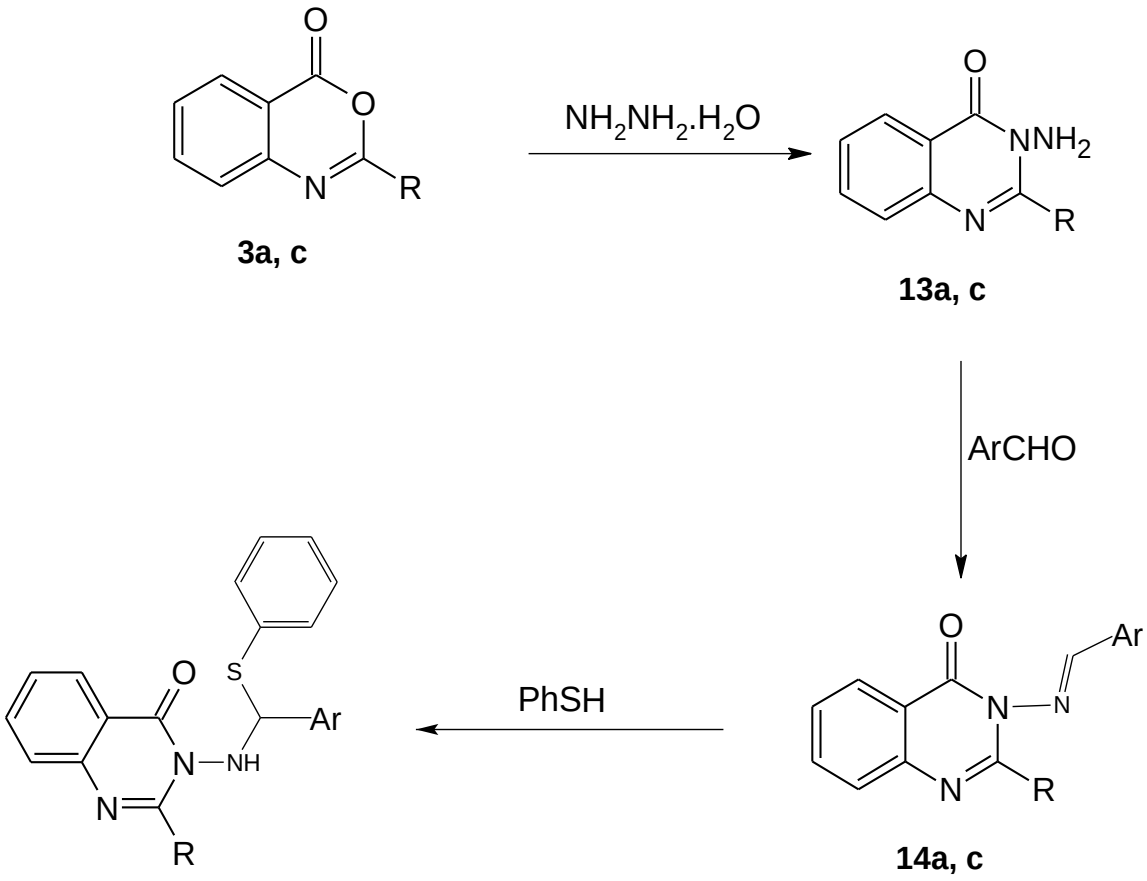
16c, 17c (Scheme 3)

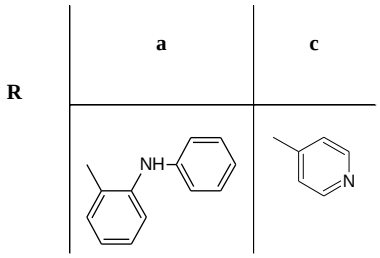
In-vitro Antitumor Screening against HePG2– MCF7 and HCT116 cell lines



Scheme-2

The cytotoxic potencies of compounds **4a-4b-6a-6b-7a-7b-8a-8b-9a-9b-10a-11a-11b-12a-12b-14a-14c-15a-15c** against a panel of three human tumor cell lines were investigated and compared with the reference drug doxorubicin. The human tumor cell line panel consisted of breast carcinoma (**MCF7**), liver carcinoma (**HePG2**) and colon carcinoma (**HCT116**) using MTT assay. Tumor cells were incubated either alone (negative control) or with different concentrations of the test compounds (100–50–25–12.5–6.25–3.125–0.78 and 1.56 µM). With regard to sensitivity against individual cell lines, this class is more effective on colon carcinoma more than other two cell lines. from the results the most active compounds are listed below:





Scheme-3

(1) Compound **4b** showed selective potency against **MCF7** cell line (IC_{50} = 71.6) as shown in Table 1.

(2) Compound **9a** showed selective potency against **HePG2** cell line with (IC_{50}= 58) µg/ml as shown in Table 2.

(3) Compound **6a** for **HCT116** cell line with (IC_{50}= 75.2) as shown in Table 3.

STRUCTURAL-ACTIVITY RELATIONSHIP

The activity of the tested compounds could be correlated to structure variation and modifications. By investigating the variation in the selectivity of the tested compounds over the three cell lines, it was revealed that: (1) nearly all of the compounds which are derivatives of nicotinic acid and *N*-phenylaniline showed

Table1 : MCF7 [Human Caucasian breast adenocarcinoma]

Sample Code	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Remarks
4a	--------	--------	22.2% at 100ppm
4b	71.6	114.1	75.6% at 100ppm
6a	--------	--------	31.5% at 100ppm
6b	--------	--------	25.3% at 100ppm
7a	--------	--------	40.3% at 100ppm
7b	--------	--------	43.1% at 100ppm
8a	--------	--------	34.1% at 100ppm
8b	--------	--------	24.3% at 100ppm
9a	--------	--------	53.7% at 100ppm
9b	--------	--------	7.6% at 100ppm
10a	71.2	115.7	75.7% at 100ppm
11a	--------	--------	3.8% at 100ppm
11b	55.7	91.8	88.3% at 100ppm
12a	--------	--------	32.8% at 100ppm
12b	--------	--------	39.6% at 100ppm
14a	--------	--------	33.6% at 100ppm
14c	--------	--------	8.6% at 100ppm
15a	--------	--------	50.3% at 100ppm
15c	--------	--------	1.3% at 100ppm
DMSO	--------	--------	3% at 100ppm
Negative control	--------	--------	0 %

Table2 : HePG 2 [Human hepatocellular carcinoma cell line]

Sample Code	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Remarks
4a	--------	--------	30.2% at 100ppm
4b	--------	--------	77.5% at 100ppm
6a	--------	--------	53.2% at 100ppm
6b	--------	--------	39.3% at 100ppm
7a	--------	--------	22.3% at 100ppm
7b	--------	--------	25.6% at 100ppm
8a	--------	--------	68.3% at 100ppm
8b	--------	--------	2.6% at 100ppm
9a	58.0	93.5	87.6% at 100ppm
9b	--------	--------	-13.2% at 100ppm
10a	52.2	88.2	88.9% at 100ppm
11a	--------	--------	17.5% at 100ppm
11b	49.2	87.8	90.7% at 100ppm
12a	--------	--------	18.5% at 100ppm
12b	--------	--------	7.3% at 100ppm
14a	--------	--------	2.1% at 100ppm
14c	--------	--------	1.5% at 100ppm
15a	--------	--------	40.5% at 100ppm
15c	--------	--------	-20.3% at 100ppm
DMSO	--------	--------	1% at 100ppm
Negative control	--------	--------	0 %

significant inhibition for the tested three cell lines. On the other hand all of the compounds which are derivatives of iso nicotinic acid didn't showed any significant inhibition for the tested three cell lines

(2) From the results of anticancer test it found that compounds **4b, 10a** showed selective potency against **HCT116** and **MCF7**, respectively and this indicated that the hydroxyl group was important for their cytotoxic activity.

Table3 : HCT116 [Colon cell line]

Sample Code	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Remarks
4a	--------	--------	50.8% at 100ppm
4b	54.8	90.6	89.7% at 100ppm
6a	75.2	119.5	70.6% at 100ppm
6b	69.8	110.6	77.4% at 100ppm
7a			8.8% at 100ppm
7b	--------	--------	20.3% at 100ppm
8a	--------	--------	54.1% at 100ppm
8b	--------	--------	2.1% at 100ppm
9a	--------	--------	21.1% at 100ppm
9b	--------	--------	1.3% at 100ppm
10a	29.9	50.7	100% at 100ppm
11a	--------	--------	3.5% at 100ppm
11b	53.6	91.6	85.4% at 100ppm
12a	--------	--------	42.4% at 100ppm
12b	--------	--------	-5.3% at 100ppm
14a	--------	--------	1.2% at 100ppm
14c	--------	--------	25.6% at 100ppm
15a	--------	--------	11.2% at 100ppm
15c	--------	--------	-11.7% at 100ppm
DMSO	--------	--------	1% at 100ppm
Negative control	--------	--------	0 %

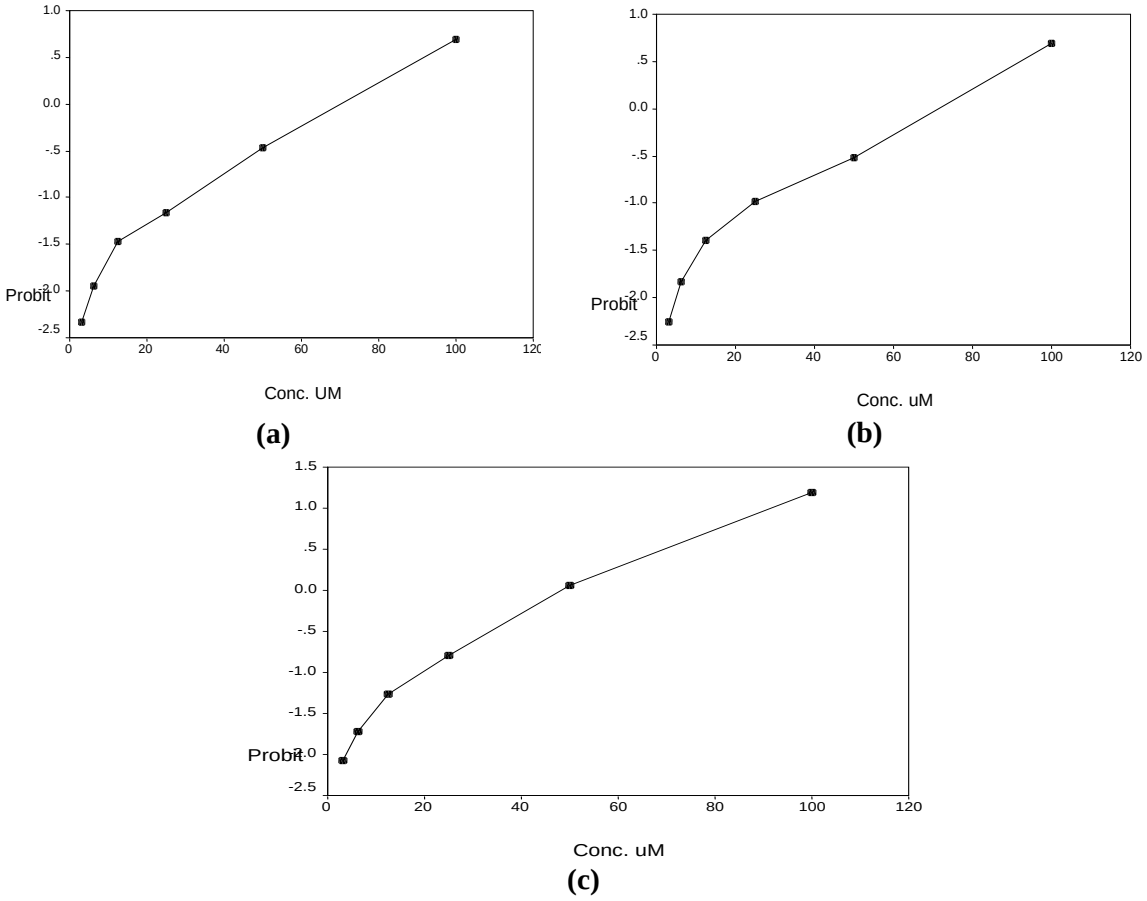


Figure 1: (a) Probit Transformed Responses 4b; (b) Probit Transformed Responses 10a; (c) Probit Transformed Responses 11b

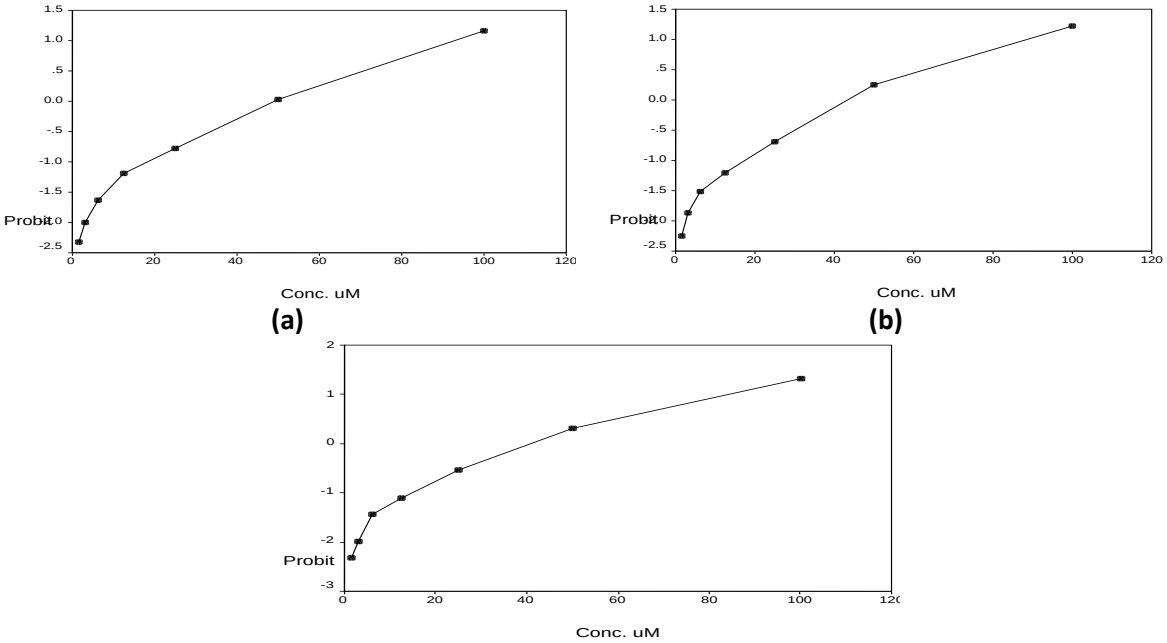


Figure 2: (a)Probit Transformed Responses 9a; (b)Probit Transformed Responses 10a; (c)Probit Transformed Responses 11b

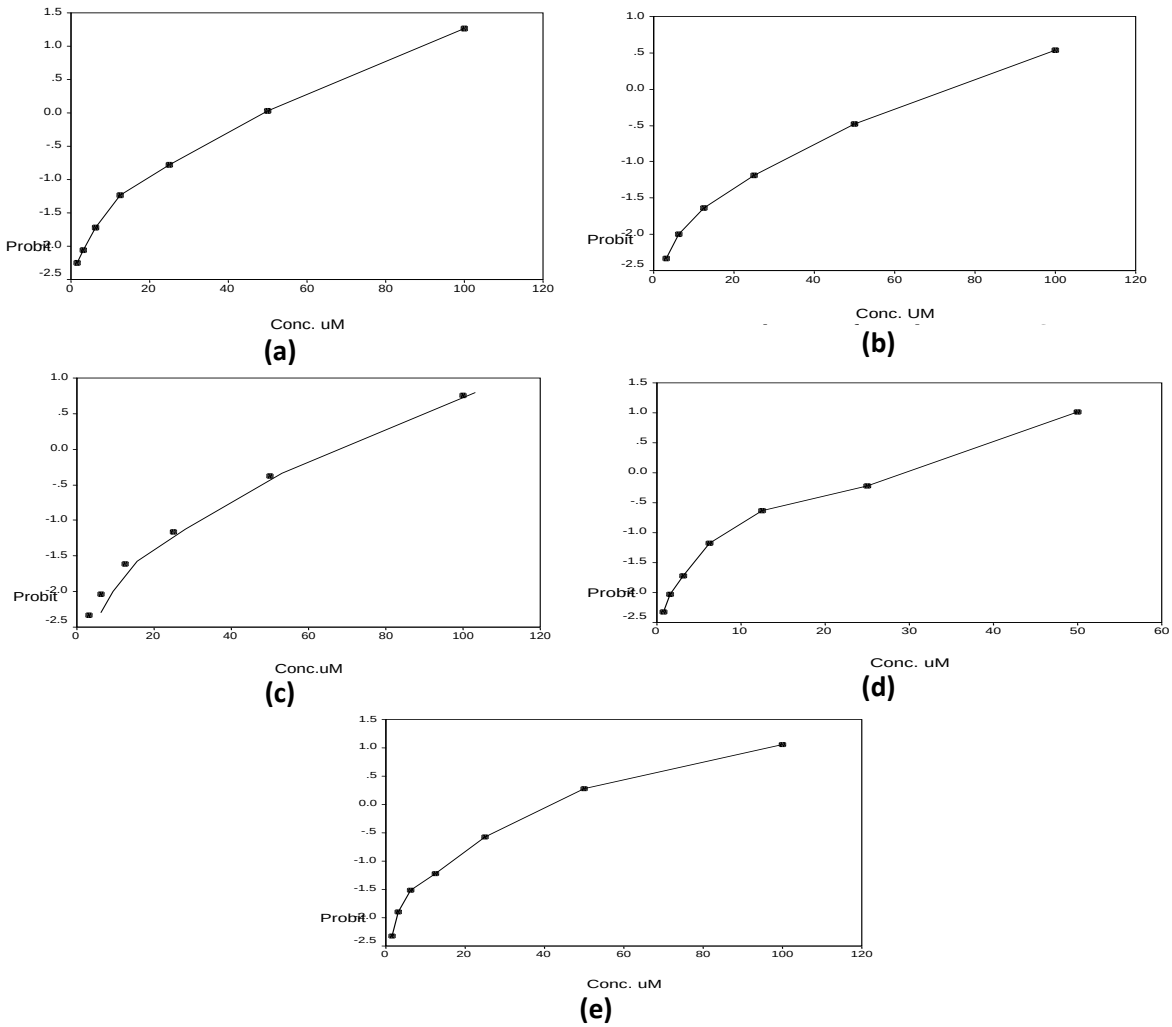


Figure 3: (a) Probit Transformed Responses 4b; (b) Probit Transformed Responses 6a; (c) Probit Transformed Responses 6b; (d) Probit Transformed Responses 10a; (e) Probit Transformed Responses 11b

(3) Compounds **6a, b** showed selective potency against **HCT116** this is may be due to the presence of acetophenone group as acetophenones exhibit wide range of biological activities like antitumor [(Ballini et al ., 2005, Bali et al ., 2010 and Kotra et al ., 2010)

CONCLUSION

A novel series of some new quinazolin derivatives were synthesized and evaluated as antitumor agents against human carcinoma cell lines (**HePG2– MCF7– HCT116**). The antitumor activity results exhibited that, compounds **4b-6a-6b-10a-11b** showed significant and selective inhibition for **HCT116** (Table 3) (Figure 3). On the other hand, compounds **9a-10a-11b** showed significant and selective inhibition for **HePG2** (Table 2) (Figure 2). Compounds **4b-10a-11b** showed significant inhibition for **MCF7** (Table 1) (Figure 1) comparing to the used reference drug Doxorubicin.

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