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Synthesis and Cytotoxic Evaluation of New 6,7,8,9-Tetrahydropyrido[3',2':4,5]thieno[3,2-*b*] quinoline Derivatives.

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ABSTRACT

A novel series of 10-Amino-6,7,8,9-tetrahydro-2-phenyl-4-substituted-pyrido[3',2':4,5]thieno[3,2-*b*] quinoline derivatives **3a-c** were synthesized. Condensation of **3a-c** with different iso(thio)cyanates afforded the corresponding substituted urea/thiourea derivatives **4a-e**. Coupling of the diazonium salts of **3a-c** with acetyl acetone and different hydrazines furnished the corresponding analogues **5a-c** and the pyrazole derivatives **6a-c**. Moreover, **3a-c** were condensed with various arylsulfonyl chlorides to afford the corresponding sulfonamides **7a-c**, while their treatment with different sugar and/or aromatic aldehydes gave the corresponding Schiff bases **8a-d**, which in turn were reacted with thioglycolic acid to furnish the corresponding thiazolidinone derivatives **9a-c.** Cytotoxic evaluation exhibited that the derivatives **3a**, **6a,7a,7b, 8b, 9a** are more potent as cytotoxic agents against breast carcinoma cell line MCF-7 comparable to Doxorubicin as a reference drug.

Keywords: 5,6,7,8-Tetrahydroquinolines; thieno[2,3-*b*]pyridines; diazonium salts; sulfonamides; thiazolidinone, breast carcinoma cell lines MCF-7.

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INTRODUCTION

Cancer still remains a potentially life threatening disease and the number of cancer related deaths are increasing alarmingly. Literature clearly indicated that more than 90% of cancer patients die due to chronic tumor metastasis with spread and invasion of other organs. The effectiveness of many existing anticancer drugs is limited by their toxicity to normal rapidly growing cells and may develop resistance to that drug. Another drawback is that the majority of the drugs currently in the market are not specific. Different classes of heterocyclic and fused heterocyclic compounds have been identified through molecular biology, empirical screening and rational drug development in the search of anticancer agents during the recent times [1,2].Tetrahydroquinolines are important structural subunit of various natural products and numerous recent reports have proved that compounds containing tetrahydroquinoline motif elicit potent biological responses leading to analgesic and anti-inflammatory [3], antinephritic[4], treating Alzheimer's disease [5], antitumor [6,7] and antiallergenic [8] activities. In addition, many tetrahydroquinoline derivatives appeared as anti-HIV [9,10], antimalarial [11,12]cholesteryl ester transfer protein inhibitors [13], anti-diabetic [14,15] and antioxidant[16]agents. An important group of antitumor spirocyclopropindoles classified under the broad name duocarmycins include among them several 1,2,3,4-tetrahydroquinoline congeners [16]. Recently, the 5,6,7,8-tetrahydroquinolines have drawn considerable attention due to their interesting pharmacological applications as tyrosine kinase inhibitors and anticancer candidates [17]. At the same time, recent studies showed that novel 5,6,7,8-tetrahydroquinoline derivatives have shown remarkable cytotoxic activity against human colon carcinoma HT29, hepatocellular carcinoma HepG2 and Caucasian breast adenocarcinoma MCF7 cell lines [18].On the other hand, the thieno[2,3-*b*]pyridine derivatives occupy special place in medicinal chemistry field due their broad pharmacological activities, including anticancer [19-25], antiviral [26-29], anti-inflammatory [30-33], antimicrobial [34-36**],** antidiabetic [37], antihypertensive [38] and osteogenic [39,40] activities, in addition to treatment of CNS disorders [41,42].

In view of these points, it was thought worthwhile to study the synthesis of new derivatives of thieno[2,3-*b*]pyridine nucleus fused with tetrahydroquinoline ring system in a single molecular framework with the hope of getting agents of synergistic anticancer activity and lower toxicity towards the normal cells. Therefore, a number of novel 6,7,8,9-tetrahydro-2-phenyl-4-substituted -pyrido[3',2':4,5]thieno[3,2-b] quinoline derivatives were synthesized and screened for their *in vitro* cytotoxic activities against breast carcinoma cell lines MCF-7.

EXPERIMENTAL

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, using VarioElementar and were found within ±0.5% of the theoretical values.Infrared spectra were recorded on a FT/IR-4100 Jasco-Japan, Fourier transform, Infrared spectrometer 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 Varian Gemini-300 MHZ NMR spectrometer at Central Services Laboratory, Cairo University, Cairo, Egypt, chemical shifts are expressed in δ (ppm) downfield from TMS as an internal standard. The mass spectra were measured with a GC MS-Qp1000EX Shimadzu, Cairo University, 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) using chloroform/ methanol (5:1, v/v) and the spots were detected by exposure to UV lamp at λ_{254}nanometer for few seconds and by iodine vapor.
The chemical names given for the prepared compounds are according to the IUPAC system.

General procedure for synthesis of 2a-c.

To a solution of compounds **1a-c** (0.01mol) in glacial acetic (20 mL), cyclohexanone (2 mL, 0.02 mol) was added. The reaction solution was refluxed on water-bath for 3h. Upon cooling, the formed yellow precipitate was collected by filtration, washed with ethanol and recrystallized from acetic acid to give the corresponding derivatives **2a-c**.

3-Cyclohexylideneamino-6-phenyl-4-thiophen-2-yl-thieno[2,3-b]pyridine-2-carboxylic acid amide(2a)

Yield 74%, mp > 300 °C. IR(KBr) ν, cm^{-1}: 3406, 3236 (NH_2), 3050(CH aromatic), 2932, 2856 (CH aliphatic), 1699 (C═O), 1614 (C=N).^{1}H NMR spectrum(DMSO, δ ppm): 1.83 (m, 6H, $3CH_2$, cyclohexanimine ring-C_3,C_4, C_5), 2.51, 2.78 (m, 4H, $2CH_2$, cyclohexanimine ring-C_2,C_6), 6.62 (s, 2H, NH_2, D_2O exchangeable), 7.21-8.10 (m, 9H, Ar-H). MS m/z: $M^{+\cdot}$ 431 (30 %). Anal. Calcd. for$C_{24}H_{21}N_3OS_2$(431.57): C, 66.79; H, 4.90; N, 9.74; S, 14.86; found: C, 66.56; H, 4.52; N, 10.01; S, 15.0.

3-Cyclohexylideneamino-4-furan-2-yl-6-phenyl-thieno[2,3-b]pyridine-2-carboxylic acid amide (2b)

Yield 72%, mp 285 °C. IR (KBr) ν, cm^{-1}: 3438, 3279 (NH_2), 3054 (CH aromatic), 2927,2855 (CH aliphatic), 1685 (C═O), 1612 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.80 (m, 6H, $3CH_2$, cyclohexanimine ring-C_3, C_4, C_5), 2.57, 2.89 (m, 4H, $2CH_2$, cyclohexanimine ring-C_2, C_6), 6.51 (s, 2H, NH_2, D_2O exchangeable), 7.25-8.14 (m, 9H, Ar-H). MS m/z: $M^{+\cdot}$ 415 (37 %).Anal. Calcd. for$C_{24}H_{21}N_3O_2S$ (415.51): C, 69.37; H, 5.09; N, 10.11; S, 7.72; found: C, 69.09; H, 5.32; N, 10.57; S, 7.43.

3-Cyclohexylideneamino-4-(4-methoxyphenyl)-6-phenyl-thieno[2,3-b]pyridine-2-carboxylic acid amide (2c)

Yield 78%, mp>300 °C. IR (KBr) ν, cm^{-1}: 3415, 3262 (NH_2), 3069(CH aromatic),2920, 2855 (CH aliphatic), 1690 (C═O), 1608 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm):1.71 (m, 6H, $3CH_2$, cyclohexanimine ring-C_3, C_4, C_5), 2.55, 2.79 (m, 4H, $2CH_2$, cyclohexanimine ring-C_2,C_6), 3.85 (s, 3H, OCH_3), 6.40 (s, 2H, NH_2, D_2O exchangeable), 6.9.0-8.11 (m, 10H, Ar-H). MS m/z: $M^{+\cdot}$455 (41%).Anal. Calcd. for$C_{27}H_{25}N_3O_2S$ (455.57): C, 71.18; H, 5.53; N, 9.22; S, 7.04; found: C, 70.81; H, 4.26; N, 9.55; S, 7.40.

General procedure for synthesis of 3a-c.

A solution of compounds **2a-c** (0.01 mol) in phosphorous oxychloride (25 mL) was heated on a water-bath for 4h. Upon cooling, the reaction solution was poured onto ice/water and treated with ammonia solution. The obtained solid was filtered, washed with water and recrystallized from ethanol/chloroform to give the corresponding free amines **3a-c**.

10-Amino-6,7,8,9-tetrahydro-2-phenyl-4-thiophen-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinolone (3a)

Yield 61%, mp208-210°C. IR (KBr) ν, cm^{-1}: 3393, 3269 (NH_2), 3086(CH aromatic), 2920,2856 (CH aliphatic), 1632 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.88 (m, 4H, $2CH_2$, C_7, C_8), 2.66, 2.76 (m, 4H, $2CH_2$, C_6, C_9), 6.11 (s, 2H, NH_2, D_2O exchangeable), 6.87-8.24 (m, 9H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 22.80, 22.81, 23.78, 31.30 (4 CH_2, C_6, C_7, C_8, C_9),118.01, 121.36, 123.14, 125.48, 127.56, 127.81, 129.30, 129.41, 131.20, 132.12, 138.24, 144.21, 147.97, 154.11, 155.21 (aromatic-C), 159.38, 160.60 (2C=N). MS m/z: $M^{+\cdot}$ 413(25 %). Anal. Calcd. For $C_{24}H_{19}N_3S_2$ (413.56):C, 69.70; H, 4.63; N, 10.16; S, 15.51; found: C, 69.81; H, 4.26; N, 9.75; S, 15.40.

10-Amino-6,7,8,9-tetrahydro-2-phenyl-4-furan-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinolone (3b)

Yield 59%, mp200-202°C. IR (KBr) ν, cm^{-1}: 3399, 3300 (NH_2), 3029(CH aromatic), 2929, 2806 (CH aliphatic), 1652 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.74 (m, 4H, $2CH_2$, C_7, C_8), 2.56, 2.75 (m, 4H, $2CH_2$, C_6, C_9), 5.99 (s, 2H, NH_2, D_2O exchangeable), 7.0-8.11 (m, 9H, Ar-H). MS m/z: $M^{+\cdot}$397(36 %). Anal. Calcd. For $C_{24}H_{19}N_3OS$ (397.49): C, 72.52; H, 4.82; N, 10.57; S, 8.07; found: C, 72.31; H, 4.36; N, 10.95; S, 7.80.

10-Amino-6,7,8,9-tetrahydro-2-phenyl-4-(4-methoxyphenyl)-pyrido[3',2':4,5]thieno[3,2-b] quinolone (3c)

Yield 65%, mp228-230°C. IR (KBr) ν, cm^{-1}: 3390, 3233 (NH_2), 3032(CH aromatic), 2920 ,2860 (CH aliphatic), 1607 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.82 (m, 4H, $2CH_2$, C_7, C_8), 2.55, 2.75 (m, 4H, $2CH_2$, C_6, C_9), 3.75 (s, 3H, OCH_3), 6.22 (s, 2H, NH_2, D_2O exchangeable), 7.0-8.23 (m,10H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 22.59, 22.67,

23.78, 31.90 (4 CH_2, C_6, C_7, C_8, C_9),55.75(OCH_3), 113.01, 113.36, 114.64, 119.24, 123.48, 127.56, 129.36, 129.57, 130.10, 132.12, 138.24, 146.50, 147.97, 147.47, 148.24, 154.11, 155.77 (aromatic-C), 160.38, 162.60 (2C=N). MS m/z: $M^{+\cdot}$437 (30 %). Anal. Calcd. for$C_{27}H_{23}N_3OS$ (437.56): C, 74.11; H, 5.30; N, 9.60; S, 7.33; found: C, 74.43; H, 5.46; N, 9.25; S, 7.60.

General procedure for synthesis of 4a-e.

A mixture of the amine derivatives **3a-c** (0.01 mol) and different iso(thio)cyanate namely: isopropyl isocyanate, phenyl isothiocyanate, benzoyl isothiocyanate (0.01 mol) in DMF (20 mL) was heated under reflux for 8h. After reaction completion, the solvent was evaporated under reduced pressure and the obtained solid was collected and crystallized from ethanol/H_2O to give the substituted (thio)urea compounds **4a-e**.

1-Phenyl-3-(6,7,8,9-tetrahydro-2-phenyl-4-thiophen-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinolin-10-yl)thiourea (4a).

Yield 73%, mp133-135°C. IR (KBr) ν, cm^{-1}: 3324, 3287 (2NH), 3058(CH aromatic),2920, 2850 (CH aliphatic), 1577 (C=N), 1311 (C=S). ^{1}H NMR spectrum (DMSO, δ ppm): 1.83 (m, 4H, 2CH_2, C_7, C_8), 2.56, 2.75 (m, 4H, 2CH_2, C_6, C_9), 7.21-8.10 (m, 14H, Ar-H), 8.31, 8.45 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO, δ ppm): 22.43, 22.51, 23.91, 32.00 (4 CH_2, C_6, C_7, C_8, C_9),113.51, 115.44, 115.94, 119.24, 121.36, 125.60, 126.51, 127.66, 129.19, 129.54, 132.10, 132.78, 139.94, 148.20, 148.60, 153.12, 155.93 (aromatic-C), 157.26, 160.58, 169.68 (2C=N, C=S). MS m/z: $M^{+\cdot}$548 (20 %). Anal. Calcd. for $C_{31}H_{24}N_4S_3$ (548.74): C, 67.85; H, 4.41; N, 10.21; S, 17.53; found: C, 67.56; H, 4.52; N, 10.01; S, 17.05.

1-Benzoyl-3-(6,7,8,9-tetrahydro-2-Phenyl-4-thiophen-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinolin-10-yl)thiourea (4b).

Yield 65%, mp113-115°C. IR (KBr) ν, cm^{-1}: 3379, 3331 (2NH), 3058(CH aromatic), 2920.2859 (CH aliphatic), 1630 (C=O), 1569 (C=N), 1329 (C=S). ^{1}H NMR spectrum (DMSO, δ ppm): 1.81 (m, 4H, 2CH_2, C_7, C_8), 2.56, 2.78 (m, 4H, 2CH_2,C_6, C_9), 7.21-8.10 (m, 14H, Ar-H), 8.51, 8.73 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO, δ ppm): 22.53, 22.42, 23.60, 32.20 (4 CH_2, C_6, C_7, C_8, C_9),113.41, 113.95, 114.31, 118.43, 119.56, 124.26, 125.12, 127.30, 129.58, 129.90, 131.10, 132.02, 138.94, 148.00, 148.33, 153.06, 155.63 (aromatic-C), 157.98, 162.38, 165.11, 170.01 (2C=N, C=O,C=S). MS m/z: $M^{+\cdot}$576 (15 %). Anal. Calcd. for $C_{32}H_{24}N_4OS_3$ (576.75): C, 66.64; H, 4.19; N, 9.71; S, 16.68; found: C, 67.06; H, 4.52; N, 10.01; S, 17.05.

1-(2-Propyl)-3-(6,7,8,9-tetrahydro-4-furan-2-yl-2-phenyl-pyrido[3',2':4,5]thieno[3,2-b] quinolin-10-yl) urea (4c).

Yield 75%, mp162-164°C. IR (KBr) ν, cm^{-1}: 3383, 3257 (2NH), 3057 (CH aromatic), 2928, 2858(CH alipatic), 1642 (C═O), 1576 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.25 (d, 6H, *J*= 6.6 Hz, 2CH_3, isopropyl), 1.82 (m, 4H, 2CH_2,C_7, C_8), 2.57-2.73 (m, 4H, 2CH_2, C_6, C_9, 1H, CH, isopropyl), 7.21-8.10 (m, 9H, Ar-H), 8.59, 8.74 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$482(37 %). Anal. Calcd. For $C_{28}H_{26}N_4O_2S$ (482.6): C, 69.69; H, 5.43; N, 11.61; S, 6.64; found: C, 69.56; H, 5.52; N, 11.21; S, 7.05.

1-Phenyl-3-(6,7,8,9-tetrahydro-4-furan-2-yl-2-phenyl-pyrido[3',2':4,5]thieno[3,2-b] quinolin-10-yl)thio urea (4d)

Yield 72%, mp148-150°C. IR (KBr) ν, cm^{-1}: 3323, 3283 (2NH), 3058(CH aromatic), 2928,2854(CH aliphatic), 1592 (C=N), 1335 (C=S). ^{1}H NMR spectrum (DMSO, δ ppm): 1.80 (m, 4H, 2CH_2, C_7, C_8), 2.56, 2.73 (m, 4H, 2CH_2, C_6, C_9), 7.21-8.10 (m, 14H, Ar-H),8.62, 8.83 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$532(45%). Anal. Calcd. For $C_{31}H_{24}N_4OS_2$ (532.68): C,69.90; H, 4.54; N, 10.52; S, 12.04; found: C, 69.56; H, 4.22; N, 10.21; S, 11.85.

1-(2-Propyl)-3-(6,7,8,9-tetrahydro-4-(4-methoxyphenyl)-2-phenyl-pyrido[3',2':4,5] thieno[3,2-b] quinolin-10-yl) urea (4e)

Yield 78%, mp152-154°C. IR (KBr) ν, cm^{-1}: 3340, 3255 (2NH), 3056(CH aromatic), 2965,2850 (CH aliphatic), 1648 (C═O), 1570 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.27 (d, 6H, *J*= 6.2 Hz, $2CH_3$, isopropyl), 1.86 (m, 4H, $2CH_2$, C_7, C_8), 2.58- 2.85 (m, 4H, $2CH_2$, C_6, C_9, 1H, CH, isopropyl), 3.75 (s, 3H, OCH_3), 7.0-7.81 (m, 10H, Ar-H),8.20, 8.41 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO, δ ppm): 22.54, 22.63, 23.82, 32.20 ($4CH_2$, C_6, C_7, C_8, C_9, $2CH_3$), 40.55, 55.98 (CH, isopropyl group,OCH_3), 113.01, 113.44, 114.66, 119.24, 123.36, 127.66, 129.36, 129.54, 130.10, 132.02, 138.24, 148.00, 148.33, 152.92, 155.93 (aromatic-C), 157.34, 160.38, 162.60 (2C=N, C=O). MS m/z: $M^{+\cdot}$522 (36 %). Anal. Calcd. For $C_{31}H_{30}N_4O_2S$ (522.66):C, 71.24; H, 5.79; N, 10.72; S, 6.13; found: C, 71.56; H, 5.52; N, 11.01; S, 6.55.

General procedure for synthesis of 5a-c.

A cold solution of sodium nitrite (10 mL, 20%) was added portion-wisely with continuous stirring to an ice cold solution of the amine derivatives **3a-c** (0.002 mol) in concentrated hydrochloric acid (10 mL) and distilled water (5 mL). Stirring was continued at 0-5°C for 15 min. Then a solution of acetylacetone(0.004 mol) in acetone (25 mL) was added and the pH of the reaction solution was adjusted at 6.5 using sodium acetate solution (10%) with continuous stirring for 1h. The obtained solid was separated by filtration and recrystallized from ethanol/H_2O to get the desired derivatives **5a-c**, respectively.

4-Hydroxy-3-(2-phenyl-4-thiophen-2-yl-6,7,8,9-tetrahydro-pyrido[3',2':4,5]thieno[3,2-*b*] quinoline-10-ylazo)-pent-3-en-2-one (5a)

Yield 75%, mp255-257°C. IR (KBr) ν, cm^{-1}: 3430-3230 (br., OH), 3058(CH aromatic), 2928, 2856 (CH aliphatic), 1651 (C═O), 1590 (C=N), 1424 (N=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.75-2.00 (m, 4H, $2CH_2$, C_7, C_8, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.58, 2.80 (m, 4H, $2CH_2$, C_6, C_9),7.21-8.20 (m, 9H, Ar-H), 11.57 (s, 1H, OH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 524 (25 %). Anal. Calcd. for$C_{29}H_{24}N_4O_2S_2$(524.66): C,66.39; H, 4.61; N, 10.68; S, 12.22; found: C, 66.56; H, 4.52; N, 11.01; S, 12.55.

4-Hydroxy-3-(2-phenyl-4-furan-2-yl-6,7,8,9-tetrahydro-pyrido[3',2':4,5]thieno[3,2-*b*] quinoline-10-ylazo)-pent-3-en-2-one (5b)

Yield 66%, mp215-217°C. IR (KBr) ν, cm^{-1}: 3437-3237 (br., OH), 3060(CH aromatic),2943, 2856 (CH-aliphatic), 1654 (C═O), 1591 (C=N), 1421 (N=N).^{1}H NMR spectrum (DMSO, δ ppm): 1.65-1.95 (m, 4H, $2CH_2$, C_7, C_8, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.59, 2.80 (m, 4H, $2CH_2$, C_6, C_9), 7.21-8.20 (m, 9H, Ar-H), 11.89 (s, 1H, OH, D_2O exchangeable). MS m/z: $M^{+\cdot}$508(23 %). Anal. Calcd. for $C_{29}H_{24}N_4O_3S$ (508.59): C, 68.49; H, 4.76; N, 11.02; S, 6.30; found: C, 68.56; H, 4.52; N, 11.41; S, 6.55.

4-Hydroxy-3-(2-phenyl-4-(4-methoxyphenyl)-6,7,8,9-tetrahydro-pyrido[3',2':4,5]thieno[3,2-*b*] quinoline-10-ylazo)-pent-3-en-2-one (5c)

Yield 76%, mp 265-267°C. IR (KBr) ν, cm^{-1}: 3424-3224(br., OH), 3063(CH aromatic), 2945, 2856 (CH-aliphatic)1655 (C═O), 1607 (C=N), 1429 (N=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.81-2.1 (m, 4H, $2CH_2$, C_7, C_8,3H, CH_3), 2.39 (s, 3H, CH_3), 2.58, 2.80 (m, 4H, $2CH_2$, C_6, C_9), 3.75 (s, 3H, OCH_3), 7.23-8.25 (m, 10H, Ar-H), 11.27 (s, 1H, OH, D_2O exchangeable). MS m/z: $M^{+\cdot}$548(38 %). Anal. Calcd. for$C_{32}H_{28}N_4O_3S$ (548.65): C, 70.05; H, 5.14; N, 10.21; S, 5.84; found: C, 70.46; H, 4.72; N, 10.41; S, 6.15.

General procedure for synthesis of 6a-c.

A mixture of derivatives 5a-c (0.001 mol) and hydrazine hydrate 98% and/or phenyl hydrazine (0.001 mol) in DMF(10 mL) was refluxed for 5h. The reaction mixture was concentrated under reduced pressure, poured onto

ice/water. The obtained solid was filtered, dried and recrystallized from ethanol to get the desired pyrazole derivatives 6a-c, respectively.

10-(N-(3,5-dimethylpyrazol-4-yl)diazenyl)-6,7,8,9-tetrahydro-2-phenyl-4-thiophen-2-yl-pyrido[3',2':4,5]thieno[3,2-*b*] quinoline(6a)

Yield 68%, mp 206-208°C. IR (KBr) ν, cm^{-1}: 3324 (NH), 3060(CH aromatic), 2928, 2857 (CH-aliphatic), 1625 (C=N), 1420 (N=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.81 (s, 4H, $2CH_2$, C_7, C_8), 2.43 (s, 6H, $2CH_3$, pyrazole ring), 2.59, 2.76 (2s, 4H, $2CH_2$, C_6, C_9), 6.12 (s, 1H, NH, D_2O exchangeable), 7.23-8.25 (m, 9H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 22.31, 22.47, 23.12, 32.40 (4 CH_2, C_6, C_7, C_8, C_9), 39.67, 39.81 ($2CH_3$), 112.62, 113.89, 114.40, 120.12, 123.11, 124.80, 125.14, 127.56, 129.36, 129.57, 130.10, 132.72, 138.51, 148.00, 148.49, 153.02, 155.10 (aromatic-C), 158.00, 160.38, 162.51 (3C=N). MS m/z: $M^{+\cdot}$520 (13%). Anal. Calcd. for$C_{29}H_{24}N_6S_2$(520.67): C, 66.90; H, 4.65; N, 16.14; S, 12.32; found: C, 66.56; H, 4.72; N, 16.41; S, 12.15.

10-(N-(3,5-dimethyl-1-phenylpyrazol-4-yl) diazenyl)-6,7,8,9-tetrahydro-2-phenyl-4-furan -2-yl-pyrido[3',2':4,5]thieno[3,2-*b*] quinolone (6b)

Yield 63%, mp 143–145°C. IR (KBr) ν, cm^{-1}: 3058(CH aromatic),2926, 2853(CH-aliphatic), 1595 (C=N), 1424 (N=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.82 (s, 4H, $2CH_2$, C_7, C_8), 2.48 (s, 6H, $2CH_3$, pyrazole ring), 2.58, 2.79 (2s, 4H, $2CH_2$, C_6, C_9), 7.23-8.25 (m, 14H, Ar-H). MS m/z: $M^{+\cdot}$580 (40 %). Anal. Calcd. for$C_{35}H_{28}N_6OS$ (580.7):C, 72.39; H, 4.86; N, 14.47; S, 5.52; found: C, 72.56; H, 4.72; N, 14.11; S, 5.15.

10-(N-(3,5-dimethyl-1-phenylpyrazol-4-yl)diazenyl)-6,7,8,9-tetrahydro-2-phenyl-4-(4-methoxyphenyl)-pyrido[3',2':4,5]thieno[3,2-*b*] quinolone (6c)

Yield 75%, mp 165–168°C. IR (KBr) ν, cm^{-1}: 3057 (CH aromatic), 2924, 2854 (CH-aliphatic)1608 (C=N), 1453 (N=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.71 (s, 4H, $2CH_2$, C_7, C_8), 2.55 (s, 6H, $2CH_3$, pyrazole ring), 2.65, 2.80 (2s, 4H, $2CH_2$, C_6, C_9), 3.80 (s, 3H, OCH_3), 7.23-8.25 (m, 15H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 22.54, 22.63, 23.82, 32.20 (4 CH_2, cyclic alkane), 39.67, 39.84 ($2CH_3$), 55.76 (OCH_3), 112.93, 113.06, 113.44, 114.47, 119.21, 123.36, 124.66, 127.53, 129.36, 129.54, 129.85, 130.10, 132.38, 138.51, 147.10, 147.41, 148.04, 154.33, 155.37 (aromatic-C), 157.24, 160.17, 162.71 (3C=N). MS m/z: $M^{+\cdot}$620 (28 %). Anal. Calcd. For $C_{38}H_{32}N_6OS$ (620.77):C, 73.52; H, 5.20; N, 13.54; S, 5.17; found: C, 73.26; H, 5.42; N, 13.11; S, 5.45.

General procedure for synthesis of 7a-c.

To a solution of the amine derivatives **3a,b** (0.001 mol) in dry acetone (20 mL) containing few drops of triethyl amine, the appropriate aryl sulfonyl chloride namely:benzenesulfonyl chloride and *p*-toluenesulfonylchloride (0.001 mol) was added. The reaction mixture was refluxed for 4h. Upon reaction completion, the excess solvent was evaporated under reduced pressure, the obtained residue was treated by $CHCl_3$/pet. ether, collected by filtration, dried and recrystallized by $CHCl_3$ to get the desired sulfonamide derivatives **7a-c**, respectively.

10-(N-benzenesulphonyl)amino-6,7,8,9-tetrahydro-2-Phenyl-4-thiophen-2-yl-pyrido [3',2':4,5]thieno[3,2-*b*] quinolone (7a)

Yield 69%, mp> 300°C. IR (KBr) ν, cm^{-1}: 3325 (NH), 3087(CH aromatic), 2927,2856 (CH-aliphatic), 1595 (C=N), 1370, 1186 (SO_2). ^{1}H NMR spectrum (DMSO, δ ppm): 1.83 (s, 4H, $2CH_2$, C_7, C_8), 2.59, 2.82 (2s, 4H, $2CH_2$, C_6, C_9), 7.03-8.11 (m, 13H, Ar-H), 8.35(s, 1H, NH, D_2O exchangeable).^{13}C NMR (DMSO, δ ppm): 22.73, 22.80, 23.61, 31.33 (4 CH_2, C_6, C_7, C_8, C_9), 120.70, 121.38, 123.45, 125.51, 127.57, 127.80, 129.30, 129.30, 131.50, 132.12, 138.23, 144.50, 147.97, 154.69, 155.31 (aromatic-C), 159.31, 163.60 (2C=N).MS m/z: $M^{+\cdot}$553 (50 %). Anal. Calcd. for $C_{30}H_{23}N_3O_2S_3$ (553.72):C, 65.07; H, 4.19; N, 7.59; S, 17.37; found: C, 65.26; H, 4.42; N, 7.11; S, 17.45.

10-(N-4-toluenesulphonyl)amino-6,7,8,9-tetrahydro-2-Phenyl-4-thiophen-2-yl-pyrido [3',2':4,5]thieno[3,2-*b*] quinolone (7b)

Yield 65%, mp> 300°C. IR (KBr) ν, cm^{-1}: 3369 (NH), 3075(CH aromatic), 2938, 2860(CH-aliphatic), 1592 (C=N), 1364, 1198 (SO_2). ^{1}H NMR spectrum (DMSO, δ ppm): 1.83 (s, 4H, C_7, C_8), 2.28 (s, 3H, CH_3), 2.59, 2.82 (2s, 4H, $2CH_2$, C_6, C_9), 7.03-8.35 (m, 13H, Ar-H), 11.62 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 567 (55 %). Anal. Calcd. for $C_{31}H_{25}N_3O_2S_3$ (567.74): C, 65.58; H, 4.44; N, 7.40; S, 16.94; found: C, 65.26; H, 4.12; N, 7.11; S, 17.25.

10-(N-4-toluenesulphonyl)amino-6,7,8,9-tetrahydro-2-Phenyl-4-furan-2-yl-pyrido [3',2':4,5]thieno[3,2-*b*] quinoline (7c)

Yield 60%, mp275°C. IR (KBr) ν, cm^{-1}: 3395 (NH), 3075(CH aromatic),2971, 2853(CH_2, CH-aliphatic), 1569 (C=N), 1351, 1192 (SO_2). ^{1}H NMR spectrum (DMSO, δ ppm): 1.80 (s, 4H, $2CH_2$, C_7, C_8), 2.31 (s, 3H, CH_3), 2.57, 2.81 (2s, 4H, $2CH_2$, C_6, C_9), 7.13-8.35 (m, 13H, Ar-H), 11.21 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO, δ ppm): 22.70, 22.81, 23.62, 31.37 (4 CH_2, C_6, C_7, C_8, C_9), 24.80 (CH_3), 120.71, 122.40, 124.40, 125.35, 127.69, 127.81, 129.36, 129.60, 131.40, 132.11, 138.51, 144.50, 147.53, 154.70, 155.30 (aromatic-C), 160.02, 162.78 (2C=N). MS m/z: $M^{+\cdot}$ 551 (66 %). Anal. Calcd. for $C_{31}H_{25}N_3O_3S_2$ (551.68): C, 67.49; H, 4.57; N, 7.62; S, 11.62; found: C, 67.26; H, 4.12; N, 7.21; S, 11.25.

General procedure for synthesis of 8a-d.

A mixture of the amine compounds **3a-c** (0.001 mol) and the appropriate sugars namely: D-ribose and D-mannose and/or the appropriate aromatic aldehydes namely:4-methoxybenzaldehyde, 2-thiophencarboxaldehyde (0.001 mol) in glacial acetic acid (10 mL) was refluxed for 6-8h. After reaction completion, the excess solvent was evaporated under reduced pressure. The obtained residue was treated with diluted ethanol (20 mL) and the obtained solid was filtered, dried and recrystallized from ethanol to get the required Schiff bases **8a-d**, respectively.

10-(2,3,4.5-Tetrahydroxypentylidene)amino-6,7,8,9-tetrahydro-2-Phenyl-4-thiophen-2-yl-pyrido[3',2':4,5]thieno[3,2-*b*] quinolone (8a)

Yield 72%, mp 225-227°C. IR (KBr) ν, cm^{-1}: 3428-3380 (br., OH), 3093 (CH aromatic),2919, 2851 (CH_2, CH-aliphatic), 1626 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.80 (s, 4H, $2CH_2$, C_7, C_8), 2.57, 2.81 (2s, 4H, $2CH_2$, C_6, C_9), 3.39-3.60 (m, 5H, pentylidene-C*H*), 6.12 (m, 4H, 4OH, exchangeable with D_2O), 7.22-8.23 (m, 10H, Ar-H, -N=CH). ^{13}C NMR (DMSO, δ ppm): 22.81, 22.83, 23.50, 31.24 (4CH_2, C_6, C_7, C_8, C_9), 62.21, 69.01, 72.31, 74.45 (pentylidene-4C), 121.70, 122.27, 125.50, 125.59, 127.61, 128.39, 129.40, 129.60, 131.39, 132.21, 136.21, 145.38, 148.71, 154.70 (aromatic-C), 158.33,160.67, 162.71 (3C=N). MS m/z: $M^{+\cdot}$545 (25 %). Anal. Calcd. for$C_{29}H_{27}N_3O_4S_2$(545.67): C, 63.83; H, 4.99; N, 7.70; S, 11.75; found: C, 64.12; H, 5.23; N, 8.01; S, 11.39.

10-(2,3,4,5,6-Pentahydroxyhexylidene)amino-6,7,8,9-tetrahydro-2-Phenyl-4-furan-2-yl-pyrido[3',2':4,5]thieno[3,2-*b*] quinolone (8b)

Yield 74%, mp> 300°C. IR (KBr) ν, cm^{-1}: 3383-3212 (br., OH), 3062(CH aromatic), 2927, 2858 (CH aliphatic), 1624 (C=N). ^{1}H NMR spectrum (DMSO, δ ppm): 1.79 (s, 4H, $2CH_2$, C_7, C_8), 2.59, 2.78 (2s, 4H, $2CH_2$, C_6, C_9), 3.32-3.65 (m, 6H, hexylidene-C*H*), 6.52 (m, 5H, 5OH, exchangeable with D_2O), 7.22-8.23 (m, 10H, Ar-H, -N=CH). MS m/z: $M^{+\cdot}$559 (37 %). Anal. Calcd. for $C_{30}H_{29}N_3O_6S$ (559.63): C, 64.39; H, 5.22; N, 7.51; S, 5.73; found: C, 64.76; H, 4.92; N, 7.11; S, 5.25.

10-(4-Methoxybenzylidene)amino-6,7,8,9-tetrahydro-2-Phenyl-4-furan-2-yl-pyrido[3',2':4,5]thieno[3,2-*b*] quinolone (8c)

Yield 68%, mp 278-280°C. IR (KBr) ν, cm^{-1}: 3065 (CH aromatic), 2943, 2871 (CH aliphatic), 1619 (C=N). ^1H NMR spectrum (DMSO, δ ppm): 1.79 (s, 4H, $2CH_2$, C_7, C_8), 2.62, 2.91 (2s, 4H, $2CH_2$, C_6, C_9), 3.87 (s, 3H, OCH_3), 6.82-8.73 (m, 14H, Ar-H, -N=CH). MS m/z: $M^{+\cdot}$515 (42 %). Anal. Calcd. for $C_{32}H_{25}N_3O_2S$ (515.62): C, 74.54; H, 4.89; N, 8.15; S, 6.22; found: C, 74.76; H, 5.02; N, 8.60; S, 6.65.

10-(Thiophen-2-methylidene)amino-6,7,8,9-tetrahydro-2-Phenyl-4-(4-methoxyphenyl)-pyrido[3',2':4,5]thieno[3,2-*b*] quinolone (8d)

Yield 75%, mp 238–240°C. IR (KBr) ν, cm^{-1}: 3059 (CH aromatic), 2962, 2832 (CH aliphatic), 1629 (C=N). ^1H NMR spectrum (DMSO, δ ppm): 1.81 (s, 4H, $2CH_2$, C_7, C_8), 2.58, 2.83 (2s, 4H, $2CH_2$, C_6, C_9), 3.85 (s, 3H, OCH_3), 6.82-8.71 (m, 14H, Ar-H, -N=CH). ^{13}C NMR (DMSO, δ ppm): 22.60, 22.82, 23.14, 32.47 ($4CH_2$, C_6, C_7, C_8, C_9), 55.51 (OCH_3), 119.54, 122.57, 125.10, 125.62, 127.11, 128.42, 129.36, 130.28, 132.31, 133.75, 135.04, 139.72, 141.16, 145.24, 147.55, 150.18, 155.7 6 (aromatic-C), 159.64, 161.70, 163.69 (3C=N).MS m/z: $M^{+\cdot}$ 515 (42 %). Anal. Calcd. for $C_{32}H_{25}N_3OS_2$ (531.69): C, 72.29; H, 4.74; N, 7.90; S, 12.06; found: C, 72.76; H, 5.02; N, 8.20; S, 12.45.

General procedure for synthesis of 9a-c.

A mixture of Schiff bases **8a,c,d** (0.001 mol) and thioglycolic acid (0.001 mol) in dry benzene (20 mL) was refluxed for 5h. The excess solvent was evaporated under reduced pressure and the obtained residue was neutralized using Na_2CO_3 solution, then filtered, dried and crystallized from isopropanol to obtain the desired thiazolidinone products **9a-c**, respectively.

2-(1,2,3,4-Tetrahydroxybutyl)-3-(6,7,8,9-tetrahydro-2-Phenyl-4-thiophen-2-yl-pyrido [3',2':4,5]thieno[3,2-*b*] quinolin-10-yl)thiazolidin-4-one (9a)

Yield 67%, mp 176-178°C. IR (KBr) ν, cm^{-1}: 3333-3221 (br., OH), 3066(CH aromatic), 2930, 2857 (CH aliphatic), 1645 (C=O), 1587 (C=N). ^1H NMR spectrum (DMSO, δ ppm): 1.81 (s, 4H, $2CH_2$, C_7, C_8), 2.57, 2.78 (2s, 4H, $2CH_2$, C_6, C_9), 3.24-3.56 (m, 5H,tetrahydroxybutyl-CH), 3.65 (s, 2H, CH_2, thiazolidinone ring), 5.21 (s, 1H, S-CH, thiazolidinone ring), 6.22 (s, 4H, 4OH, exchangeable with D_2O), 7.22-8.23 (m, 9H, Ar-H). MS m/z: $M^{+\cdot}$619 (43 %). Anal. Calcd. for $C_{31}H_{29}N_3O_5S_3$ (619.77): C, 60.08; H, 4.72; N, 6.78; S, 15.52; found: C, 60.36; H, 4.52; N, 7.11; S, 15.00.

2-(4-Methoxyphenyl)-3-(6,7,8,9-tetrahydro-2-Phenyl-4-furan-2-yl-pyrido[3',2':4,5]thieno [3,2-*b*] quinolin-10-yl)thiazolidin-4-one (9b)

Yield 64%, mp 165°C. IR (KBr) ν, cm^{-1}: 3062(CH aromatic),2932, 2856 (CH-aliphatic), 1642 (C=O), 1570 (C=N). ^1H NMR spectrum (DMSO, δ ppm): 1.82 (s, 4H, $2CH_2$,), 2.59, 2.81 (2s, 4H, $2CH_2$,C_7, C_8), 3.75 (s, 2H, CH_2, thiazolidinone ring), 3.81 (s, 3H, OCH_3), 6.13 (s, 1H, S-CH, thiazolidinone ring), 7.22-8.20 (m, 13H, Ar-H). MS m/z: $M^{+\cdot}$589 (36 %). Anal. Calcd. for $C_{34}H_{27}N_3O_3S_2$ (589.73): C, 69.25; H, 4.61; N, 7.13; S, 10.87; found: C, 69.52; H, 5.01; N, 6.81; S, 10.54.

2-Thiophen-2-yl-3-(6,7,8,9-tetrahydro-2-Phenyl-4-(4-methoxyphenyl)-pyrido[3',2':4,5] thieno[3,2-*b*] quinolin-10-yl)thiazolidin-4-one (9c)

Yield 61%, mp 194°C. IR (KBr) ν, cm^{-1}: 3052(CH aromatic),2945, 2856 (CH-aliphatic), 1640 (C=O), 1595 (C=N). ^1H NMR spectrum (DMSO, δ ppm): 1.79 (s, 4H, $2CH_2$, cyclohexyl ring), 2.59, 2.79 (2s, 4H, $2CH_2$, C_6, C_9), 3.75 (s, 2H, CH_2, thiazolidinone ring), 3.85 (s, 3H, OCH_3), 6.01 (s, 1H, S-CH, thiazolidinone ring), 6.92-8.23 (m, 13H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 22.74, 22.80, 23.47, 32.37 ($4CH_2$, C_6, C_7, C_8, C_9), 35.02, 54.21 (CH_2, CH, thiazolidinone ring), 55.47 (OCH_3), 123.58, 126.42, 127.74, 128.05, 129.30, 131.51, 132.70, 134.60, 135.54, 141.16, 143.10,

144.64, 146.00, 153.27, 155.70 (aromatic-C), 160.15, 161.23, 164.23 (2C=N, C=O). MS m/z: $M^{+\cdot}$ 605 (34 %). Anal. Calcd. for $C_{34}H_{27}N_3O_2S_3$(605.79): C, 67.41; H, 4.49; N, 6.94; S, 15.88; found: C, 67.96; H, 4.12; N, 6.61; S,15.45.

Cytotoxic bioassay

Breast cancer cell lines (MCF-7cell lines) were obtainedfrom Cell Bank in National Cancer Institute, Cairo, Egypt.The potential toxicity of the selected newly synthesizedderivatives was done by SRB using the method Skehan*et al.*[43] as follows: cells were plated in 96-multiwell plate(104 cells/well) for 24 h before treatment with compounds toallow attachment of cell to the wall of the plate. Differentconcentrations of the compound under test (1, 2.5, 5 and10 g/mL) were added to the cell monolayer triplicate wellswhich were prepared for each individual dose. Monolayercells 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 Sulfo-Rhodamine-B stain. Excessstain was washed with acetic acid and attached stain wasrecovered with Tris-EDTA buffer. Color intensity wasmeasured in an ELISA reader. Measurements were done sixtimes (n = 6) and averaged. The relation between survivingfraction and drug concentration is plotted to get the survivalcurve of each tumor cell line after the specified compound.

RESULTS AND DISCUSSION

The routes adopted for the synthesis of the new 6,7,8,9-tetrahydro-2-phenyl-4-substituted-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinoline derivatives in this study are depicted in Schemes 1 and 2. The key starting and the intermediate materials 3-amino/cyclohexylideneamino-6-phenyl-4-substituted-2-yl-thieno[2,3-b]pyridine-2-carboxylic acid amide were prepared following the procedures reported earlier by [44-48] as illustrated in Scheme 1.

The structures of all newly synthesized compounds were established by different spectroscopic techniques (^{1}H, ^{13}C-NMR, IR, and MS) and elemental analyses. IR spectra of the derivatives 2a-c exhibited the characteristic bands of NH_2 and C=O groups at the regions 3438, 3236 cm^{-1} and 1699-1685 cm^{-1}, respectively. At the same time, ^{1}H-NMR spectra displayed a multiplet signal at the region δ 1.71-1.83 representing β $2CH_2$ and γ CH_2 groups of cyclohexanimine ring, while its α 2CH2 groups appeared as two multiplets at δ 2.51-2.89 ppm, alongside with a singlet signal at δ 6.40-6.62 ppm due to NH_2 groups. All the other aromatic protons were observed at the expected regions. In case of compound 2c revealed an additional singlet signal at δ 3.85 ppm due to OCH_3 group. Mass spectra of the derivatives showed the molecular ion peaks in agreement with their molecular formulae. Intramolecular cylization of the compounds 2a-c by their heating in phosphorous oxychloride led to the formation of the free amine derivatives 3a-c, respectively. IR spectra of the latter compounds showed the disappearance of the absorption bands of C=O groups and the presence of the characteristic absorption bands related to NH_2 groups at 3399- 3233 cm^{-1}. ^{1}H NMR spectra were consistent with the structures of the new compounds. All compounds displayed one multiplet signals in the regions δ 1.74-1.88 ppm due to β $2CH_2$, while α $2CH_2$ appeared as two other multiplets at the region δ 2.55-2.76 ppm and a singlet signal at δ 5.99- 6.22 ppm representing NH_2 groups. The other protons of the molecules were present at their expected regions. ^{13}C NMR spectrum of 3c showed four characteristic signals at δ 22.59, 22.67, 23.78, 31.90 ppm referring to the four carbons of CH_2 functionalities, another signal at δ 55.75 ppm due to the methoxy carbon, in addition to other signals at the range δ 113.01-162.60 due to the aromatic and C=N carbons.

Since, iso(thio)cyanates are pivotal intermediates in organic synthesis, especially in the synthesis of various heterocyclic compounds and unsymmetric (thio)ureas [49,50], nucleophilic addition of the free amino groups of the derivatives 3a-c to various iso(thio)cyanates was carried out in refluxing DMF to gain the desired (thio)urea derivatives 4a-e. IR spectra of the latter compounds indicated the appearance of two characteristic absorption bands at the region 3383-3255 cm^{-1} representing NH stretching vibration. IR spectra of compounds 4c,e revealed absorption bands at 1648-1630 cm^{-1} related to C=O stretching vibration, while the absorption bands of C=S groups of derivatives 4a,b,d appeared at the region 1335-1311 cm^{-1}. ^{1}H NMR spectra of the new derivatives were in agreement with their molecular structures. For example, ^{1}H NMR spectrum of compound 4c revealed, in addition to the parent protons, the isopropyl group as a douplet signal at δ 1.25 ppm due to $2CH_3$ and a multiplet

signal at δ 2.57-2.73 ppm representing the methine proton of CH group. Furthermore, ^{13}C NMR of compound 4e represented four signals at δ 22.54, 22.63, 23.82, 32.20 referring to 4 CH_2 and $2CH_3$ of the isopropyl group and other two signals appeared at δ 40.55, 55.98 ppm due to CH of isopropyl and OCH_3 groups. Different signals were present at the range 113.01-155.93 due to the aromatic-C and at 157.34, 160.38, 162.60 corresponding to 2C=N and C=O groups.

Diazotization of the amino derivatives 3a-c was carried out using sodium nitrite and hydrochloric acid at 0^{o}C to get their diazonium salts which were coupled with acetyl acetone in acetone in the presence of sodium acetate to obtain the desired derivatives 5a-c respectively. IR spectra of the obtained derivatives displayed broad bands at the region 3437-3224 cm^{-1} due to OH stretching vibration of intramolecular hydrogen bonded enolic groups. Other bands appeared at 1655-1651 and 1429-1421 cm^{-1} representing C=O and -N=N- groups, respectively. The disappearance of NH_2 bands confirmed the conversion of NH_2 group into –N=N- (azo group). ^{1}H NMR spectra of the same derivatives exhibited two singlets at δ 1.65-2.39 ppm (overlapped with those of $2CH_2$; C7,C8) referring to the six protons of $2CH_3$. The other expected protons of the molecules appeared at their correct ranges. At the same time, mass spectra represented the molecular ion peaks in agreement with the molecular formulae of the compounds. Cyclocondesation reaction was carried out by refluxing the derivatives 5a-c with hydrazine hydrate and/or phenyl hydrazine in ethanol in order to obtain the corresponding 1-substituted-3,5-dimethyl-4-azopyrazole analogues 6a-c. IR spectra of the pyrazole derivatives revealed the disappearance of the absorption bands of OH and C=O groups that proved the formation of the pyrazole ring. ^{1}H NMR spectra exhibited the six protons of $2CH_3$ of the new formed pyrazole ring as a singlet signal at the range δ 2.43-2.55 ppm. ^{13}C-nmr spectrum of compound 6c displayed signals at δ 22.54, 22.63, 23.82, 32.20 due to 4CH2, 39.67, 39.84 due to $2CH_3$ of the pyrazole ring and 55.76 (OCH_3) in addition to signals at 112.93-162.71 ppm related to the aromatic and C=N carbons (scheme 1).
Moreover, condensation of the parent amines 3a,b with benzene/p-toluenesulfonyl chloride in dry acetone in the presence of a catalytic basic amount of TEA furnished the corresponding sulfonamide derivatives 7a-c. IR spectra of the latter derivatives displayed absorption bands at 3395-3325 cm^{-1} representing NH stretching vibration and two bands at the region 1364- 1186 cm^{-1} referring to SO_2 groups. Furthermore, ^{1}H NMR spectra of the derivatives 7b,c revealed singlet signals at δ 2.28-2.31 ppm attributed to CH_3 protons of the p-toulidine moiety, in addition to the other signals that were present at their expected regions. Mass spectra confirmed the molecular formulae of the compounds.

Furthermore, Schiff bases 8a-d were obtained via nucleophilic addition/elimination reaction of the of the parent amino compounds 3a-c to the carbonyl group of various aldoses such as: ribose, arabinose and mannose and other different aromatic or heterocyclic aldehydes namely; 4-methoxybenzaldehyde and 2-thiophenecarboxaldehyde in glacial acetic acid. Upon condensation of the latter derivatives 8a,c,d with thioglycolic acid in dry benzene led to the formation of the corresponding thiazolidinone derivatives 9a-c. The formed 8a-d products revealed the expected absorption bands due to OH, CH_2 and C═N at the correct ranges in their IR spectra. Also, their 1H NMR spectra showed the presence of the sugar protons and azomethine proton (CH═N) in the expected regions. At the same time, IR spectra of the thiazolidinone compounds revealed the appearance of new absorption bands at 1645-1640 cm^{-1} attributed to C=O functionalties, while their ^{1}H NMR spectra showed singlet signals at the ranges δ 3.65- 3.75 ppm and δ 5.21-5.53 representing the corresponding methylene protons (CH_2) and the methine protons (N-CH-S) of the new formed thiazolidinone ring. Mass spectra of the compounds showed the molecular ion peaks which were in agreement with their molecular formulae (scheme 2).

Cytotoxic evaluation

Breast cancer is the most common cause of cancer death among women specially in the less developed countries of the world and it now represents one in four of all cancers in women [51]. Thus, synthesis of novel fused tetra-heterocyclic compounds carrying 6,7,8,9-tetrahydro-2-phenyl-4-substituted-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinoline nucleus and evaluate their cytotoxic potency against breast carcinoma cell lines MCF-7 was the main goal of this work. Accordingly, nine of the newly synthesized compounds (**3a, 4a, 5a, 6a, 7a, 7b, 8a, 8c, 9a**) were selected as representative examples to examine their growth inhibitory activity against breast carcinoma cell lines MCF-7 using Doxorubicin as a standard drug according to the method described by

Skehan *et al.* [43]. The results were expressed as IC50 values measured in µg/mL (the concentrations of compounds that reduce the survival cells to 50%) (table 1, Fig. 1). The resultant data evidenced that all the examined compounds are cytotoxic agents against MCF-7 carcinoma cell lines producing IC50 values less than or slightly higher than those of the reference doxorubicin, indicating the biological value of the parent pyrido[3',2':4,5]thieno[3,2-b] tetrahydroquinoline ring system in producing the desired growth inhibitory activity. The results indicated that the incorporation of the parent nucleus to the pyrazole ring through an azo group (compound 6a) produced the highest cytotoxic potency that is 1.5 times as potent as that of the reference drug (IC50; 2.68, 3.70 µg/mL, respectively). The sensitivity of the breast cancer cell lines slightly decreased against the ribose Schiff base (compound **8a**) and its cyclized thiazolidinone analogue (compound **9a**) but the activity is still more potent than that of the standard drug Doxorubicin. Also, the obtained data revealed that potent cytotoxic activity higher than that of the reference Doxorubicin was also gained by the key starting amino compound **3a** and its sulfonamide derivatives **7a,b** (IC50; 3.23, 3.38 µg/mL, respectively). Lower activity was obtained by p-methoxyphenyl Schiff base **8c** and the coupled-acetyl acetone derivative **5a** (IC50; 5.03, 5.18 µg/mL, respectively). Further dramatic decrease in the activity was observed upon the attachment of the parent fused ring system with phenyl thiourea side chain (compound **4a**, IC50; 12.8 µg/mL). In the view of the aforementioned discussion, compounds 3a, 6a, 7a, 7b, 8a, 9a could be taken in consideration as lead candidates in the field of drug discovery of new anti-breast cancer agents.

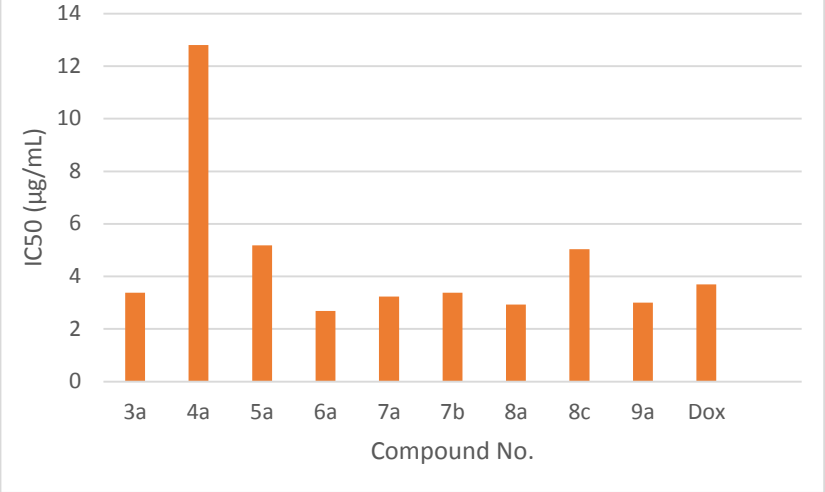

Figure 1. Cytotoxic activity of some newly synthesized compounds against human breast carcinoma cell lines (MCF-7).

Table 1. Cytotoxic activity of some newly synthesized compounds against human breast carcinoma cell lines (MCF-7) by determination of IC_{50}.

Comd. No	IC_{50} (µg/mL)
3a	3.38
4a	12.8
5a	5.18
6a	2.68
7a	3.23
7b	3.38
8a	2.93
8c	5.03
9a	3.00
Dox	3.70

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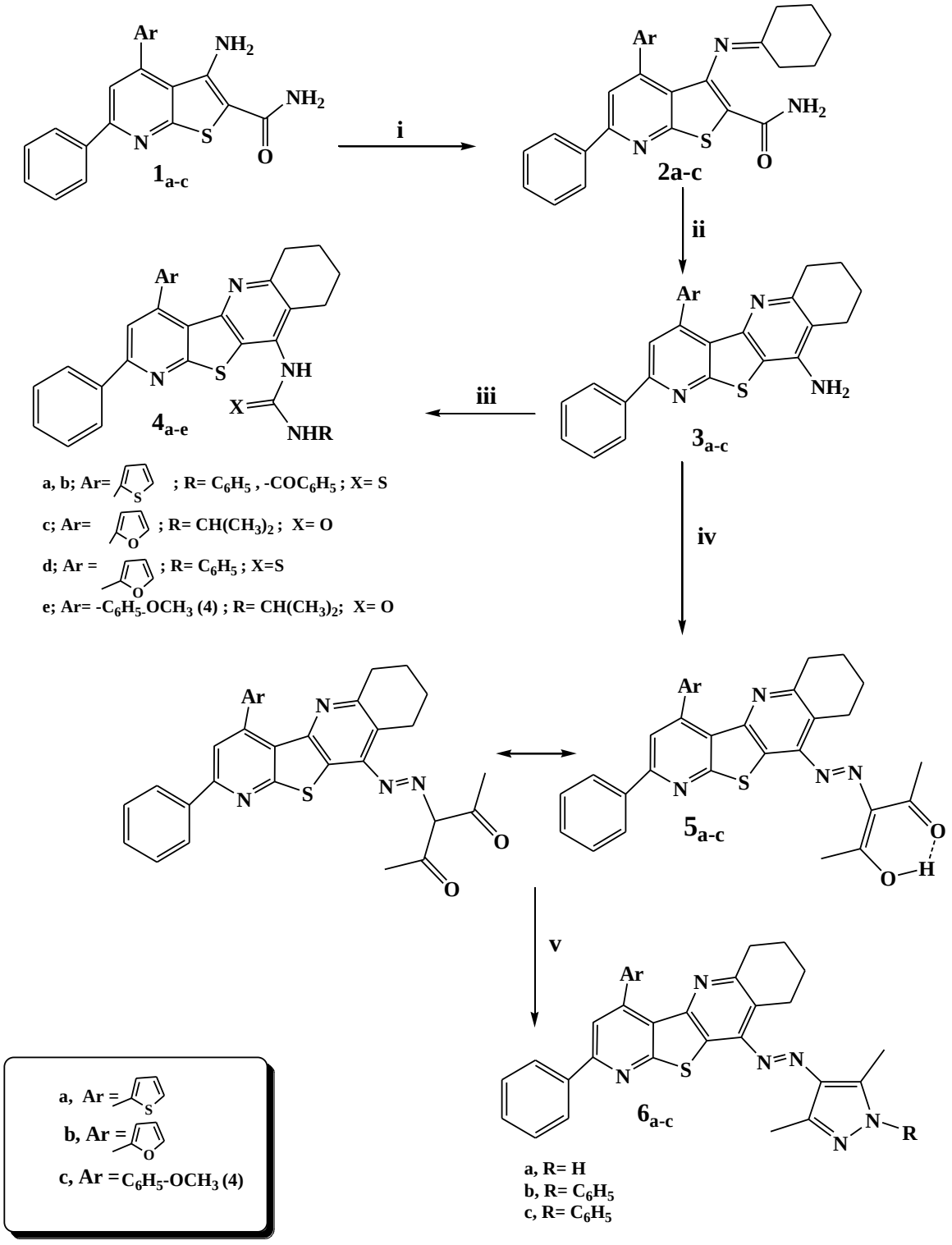

Scheme 1: Synthesis of the amino derivatives **3a-c**, urea (thiourea) derivatives **4a-e,** the azo derivatives **5a-c** and pyrazolo derivatives **6a-c**. i) cyclohexanone, gl. acetic acid, reflux for 3h, ii)$POCl_5$, refux for 4h, iii) different iso(thio)cyanates, DMF, reflux for 6h, iv) $NaNO_2$, HCl, stirr at 0 ^{o} C then acetylacetone, acetone, stirr at r.t., v) hydrazine derivatives, ethanol,reflux for 5h.

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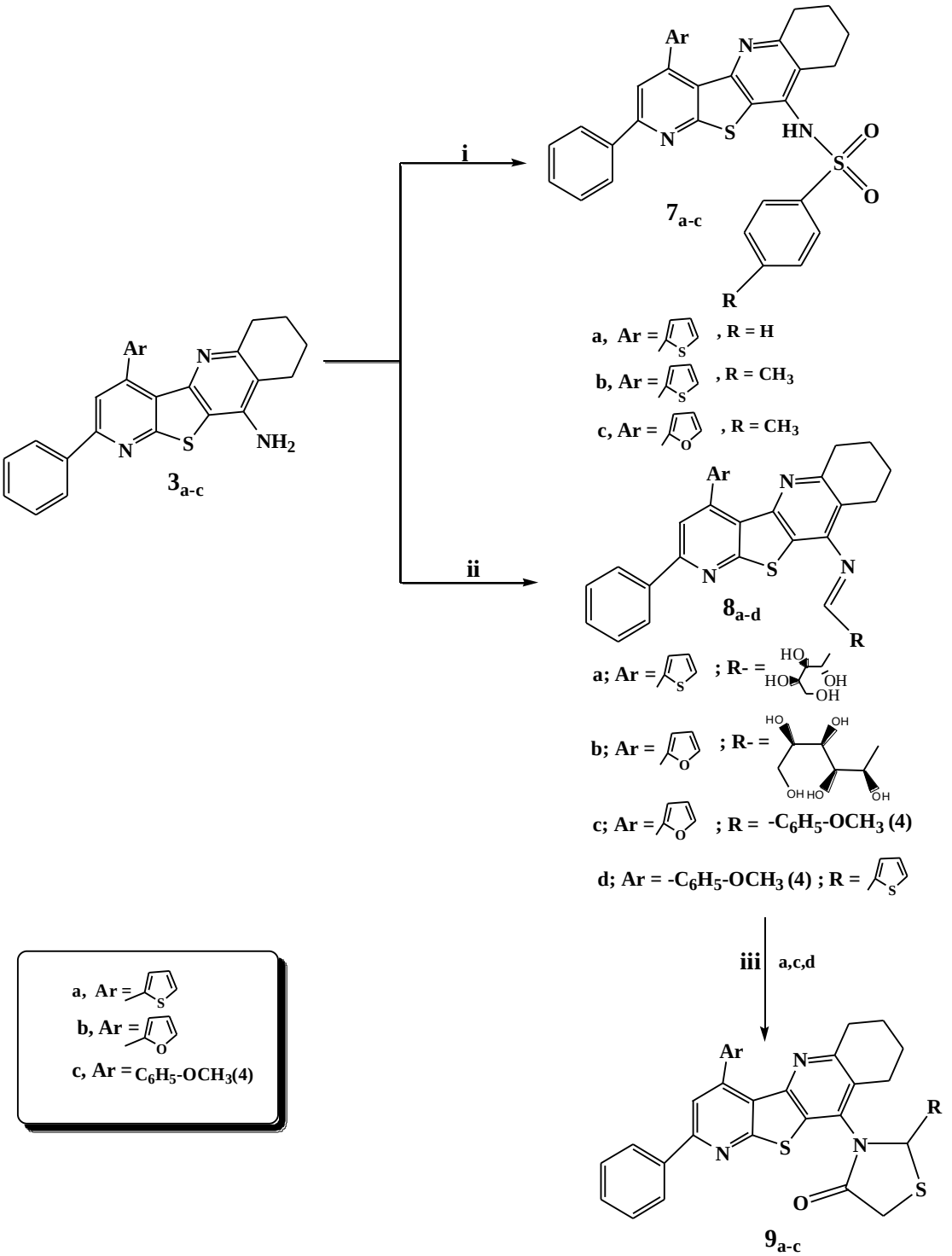

Scheme 2: Synthesis of sulfonamide derivatves **7a-c,** Schiff bases **8a-d**, thiazolidinone derivatives **9a-c**.
i) dry acetone, TEA, reflux for 4h, ii) different aldehydes and sugars, gl. acetic acid, reflux for 6-8h, iii) thioglycolic acid, dry benzene, reflux for 5h.

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CONCLUSION

The scope of this study was the synthesis of new derivatives bearing thienopyridine heterocyclic ring system fused with tetrahydroquinoline ring system aiming to gain new cytotoxic agents against breast carcinoma cell lines of higher activity than that obtained by the known marketed anticancer drugs such as Doxorubicin. Thus, by different routes various 6,7,8,9-tetrahydro-2-phenyl-4-substituted-2-yl-pyrido[3',2':4,5]thieno[3,2-b] quinolone analogues were synthesized incorporated with different heterocyclic/aromatic rings such as thiophene, furan and p-methoxyphenyl rings at position-4. Also, different substituents were conjugated with the parent nucleus at position-10 such as: free amino group, substituted (thio)urea side chains, different substituted pyrazole ring systems, different aryl sulfonamides, Schiff base side chains and their cyclized thiazolidinone ring systems. Cytotoxic evaluation of some selected derivatives exhibited that fusion of thienopyridine with tetrahydroquinoline ring systems produced new tetraheterocyclic compounds of growth inhibitory potency against breast carcinoma cell lines higher than that of the standard drug Doxorubicin as compounds **3a, 6a, 7a, 7b, 8a, 9a**, while the potency appeared to be slightly less than that of Doxorubicin by the derivatives 5a and 8c. These results can qualify these new derivatives as new candidates in the field of anticancer drug discovery.

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