INVESTIGATIONS SYNTHESIS, CHARACTERIZATION AND EVALUATION OF THE ANTICANCER ACTIVITY OF NOVEL 2-AMINOPHENYLTHIAZOLE DERIVED HETEROCYCLES.

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Abstract

Novel substituted 2-aminophenylthiazole derivatives were synthesized via various synthetic pathways. Among which were compounds bearing different side chains attached to the thiazole backbone through occupying C2 position 2-4, 6, 8-12 and 15-22. Also thiazole derivatives 23-25 bearing substituted pyrimidines were designed to be synthesized. Moreover, we have synthesized iminothiazolyl succinic acid derivatives 5a and 5b, fused thiazolothiadiazole analogue 7, as well as, the thiazolopyrimidine derivatives 13 and 14. The newly synthesized compounds were evaluated for their in vitro anticancer activity against human Liver cancer HepG2 and Breast cancer MCF7 cell lines compared to the reference drug Doxorubicin. Compounds 3, 12, 15, 17, 18, 21, 22 and 24 showed significant activity against HepG2 cell lines with IC50 values ranging from 4.78-11.4 µg/mL. However, compounds 3, 7, 15, 17, 18 and 22 exerted highly potent anticancer activity against MCF7 cell line with IC50 values ranging from 4.95-10.8 µg/mL.

Introduction:

Five-membered aromatic nitrogen and sulphur containing heterocycles occurring in a diversity of natural products and drugshave received great biological interest [1]. Among which are various thiazole derivatives having versatile biological activities embracing the antiviral [2], antibacterial [3], antifungal [1], anti-HIV [4], anticancer [1,4,5], anti-inflammatory[6], neuroprotective and antioxidant [4], anticonvulsant [4], antidiabetic[1] and antihypertensive [4] activities. Moreover, different fused thiazolothiadiazole derivatives were recognized as highly potent anticancer agents against liver cancer HepG2 and breast cancer MCF7 cell lines [7, 8]. In addition, various fused thiazolopyrimidine derivatives have attracted great attention in various pharmacological fields embracing their anticancer activity against various cancer cell lines [9-12].

Materials and Methods:

Melting points of all the synthesized compounds were determined in an open capillary method and were uncorrected. IR spectra were recorded using KBr disc technique on Nikolet IR 200 FT IR Spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Cairo University and values are represented in cm⁻¹. The¹HNMR Spectra were recorded on Varian Gemini EM-300 MHZ, NMR Spectrometer at laboratories of the nuclear magnetic resonance, Chemical Warfare Department, Ministry of Defense. DMSO-d6 was used as a solvent;

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chemical shifts were measured in δ ppm, relative to TMS as internal standard. Electron impact Mass Spectra were recorded at 70 ev on DI-50 unit of Schimadzu GC/MS-QP5050A Spectrometer at Regional center for Mycology and Biotechnology, Al-Azhar University. Microanalyses were carried out at Regional Center for Mycology and Biotechnology, Al-Azhar University.

**Synthesis of 2-Amino-4-phenylthiazole (1a):**
An equimolar mixture of phenacyl bromide (0.076 g, 1 mmol) and thiourea (0.2 g, 1 mmol) was heated under reflux in absolute ethanol (15 mL) for 20 hours. The reaction mixture was then concentrated, allowed to cool and the solid formed was filtered, washed with dioxan, dried and crystallized from ethanol to give compound 1a.

Yellow powder, yield: 0.18 g (44 %), m.p.: 108-110°C, Anal. form: C_{16}H_{10}N_{3}S_{2} (413.56), Calcd. (%) C, 69.70; H, 4.63; N, 10.16. Found (%) C, 70.00; H, 4.80; N, 10.35. IR (KBr, cm⁻¹): 3387 (NH); 3078, 3032 (CH-aromatic); 1610 (C=N); 1582 (C=C).

**Phenyl-3-(4-phenylthiazol-2-yl)thiourea (2):**
An equimolar mixture of 2-aminothiazole derivative 1a (0.18 g, 1 mmol) and phenyl isothiocyanate (0.14 g, 1 mmol) in dioxan (15 mL) was heated under reflux for 20 hours. The reaction mixture was then concentrated, allowed to cool and the solid formed was filtered, washed with dioxan, dried and crystallized from ethanol to yield the target compound 2.

Brown powder, yield: 0.13 g (41 %), m.p.: 106-108°C, Anal. form: C_{16}H_{12}N_{2}S (311.42), Calcd. (%) C, 61.71; H, 4.21; N, 13.49; S, 20.59. Found (%) C, 61.88; H, 4.23; N, 13.67; S, 20.69. IR (KBr, cm⁻¹): 3361, 3261 (NH); 3078, 3032 (CH-aromatic); 1627 (C=N); 1575 (C=C). MS m/z (relative intensity %): 236 (M^+2, 5); 177 (M^+1 , 12); 176 (M^+, 100).

**Synthesis of 2,3-Dihydro-N,4-diphenyl-3-(4-phenylthiazol-2-yl)thiazol-2-amine (3):**
To a solution of thiourea derivative 2 (0.31 g, 1 mmol) in absolute ethanol (15 mL) was heated under reflux for 22 hours. The reaction mixture was then allowed to attain room temperature, ethanol was evaporated and the precipitate formed is then filtered, dried and crystallized from ethanol to give compound 3.

Brown powder, yield: 0.18 g (44 %), m.p.: 108-110°C, Anal. form: C_{24}H_{19}N_{2}S (413.56), Calcd. (%) C, 69.70; H, 4.63; N, 10.16. Found (%) C, 70.00; H, 4.80; N, 10.35. IR (KBr, cm⁻¹): 3387 (NH); 3055, 3032(CH-aromatic); 1610 (C=N); 1597 (C=C). ^1H NMR (DMSO-d_6, δ ppm): 4.00 (s, 1H, thiazole-C_2-H); 6.84-6.88 (m, 2H, N-C\_6H\_5-C\_3-H); 7.34-7.44 (m, 3H, thiazole-NH-C\_6H\_5-C\_3-H & thiazole-C\_6H\_5-C\_3-H); 7.60-7.68 (m, 2H, J=8.1 Hz, thiazole-C\_6H\_5-C\_3-H & thiazole-C\_6H\_5-C\_3-H); 7.98 (d, 2H, J=8.1 Hz, thiazole-C\_6H\_5-C\_3-H & thiazole-C\_6H\_5-C\_3-H); 8.65 (s, 1H, NH, N-C\_6H\_5, D\_2O exchangeable). MS m/z (relative intensity %): 413 (M^+, 1.5); 412 (M^+-1, 3.6); 411 (M^+-2, 11.5).

**Synthesis of 4-Phenyl-N,N-di(prop-2-ynylationzol-2-yl)thiazol-2-amine (4):**
To an equimolar mixture of 2-aminothiazole derivative 1a (0.18 g, 1 mmol) and propargyl bromide (0.24 g, 2 mmol) in dry acetone (15 mL), catalytic amount of anhydrous potassium carbonate (0.01 mmol) and potassium iodide (0.01 mmol) were added. The reaction mixture was then heated under reflux for 20 hours, then allowed to cool and the precipitate formed was then filtered, washed with ethanol, dried and crystallized from ethanol to yield the target compound 4.

Dark brown powder, yield: 0.1 g (39 %), m.p.: 298-300°C, Anal. form: C_{16}H_{12}N_{2}S (252.33), Calcd. (%) C, 71.40; H, 4.79; N, 11.10. Found (%) C, 71.59; H, 4.85; N, 11.21. IR (KBr, cm⁻¹): 2953, 2918 (CH-aromatic); 2848 (CH-aliphatic); 2320 (C=O); 1614 (C=N); 1600 (C=C). ^1H NMR (DMSO-d_6, δ ppm): 6.15 (s, 1H, two CH, ppm): 6.99 (s, 1H, thiazole-C_3-H); 7.15-7.55 (m, 3H, C\_6H\_5-C\_3-H & thiazole-C\_6H\_5-C\_3-H); 7.78 (d, 2H, J=8.4Hz, C\_6H\_5-C\_3-H & thiazole-C\_6H\_5-C\_3-H); 8.5 (s, 2H, two -C=CH). MS m/z (relative intensity %): 253 (M^+1, 3); 252 (M^+, 1).
Synthesis of 2-((2-Imino-4-phenylthiazol-3(2H)-yl)methyl)succinic acid (5a,b):-
An equimolar mixture of the selected 2-aminothiazole derivative 1a,b (1 mmol) and itaconic acid (0.26 g, 1 mmol) in absolute ethanol (15 mL) was stirred at room temperature for 48 hours. The reaction mixture was then concentrated, and the precipitated solid was washed with ethanol, dried and crystallized from ethanol to yield compounds 5a,b.

2-((2-Imino-4-phenylthiazol-3(2H)-yl)methyl)succinic acid (5a):
Pale yellow powder, yield: 0.14 g (45 %), m.p.: 100-102°C. Anal. form: C_{14}H_{12}N_{2}O_{2}S (306.34). Calcd. (%): C, 54.89; H, 4.61; N, 9.14. Found (%): C, 55.13; H, 4.62; N, 9.26. IR (KBr, cm^{-1}): 3383 (OH); 3267, 3126 (NH); 2954, 2929 (CH-aromatic); 2879, 2858 (CH-aliphatic); 1701 (C=O); 1631 (C=N); 1598 (C=C). MS m/z (relative intensity %): 306 (M^{+}, 15).

2-(4-(4-Bromophenyl)-2-iminothiazol-3(2H)-yl)methyl)succinic acid (5b):
White powder, yield: 0.23 g (60 %), m.p.: 142-144 °C. Anal. form: C_{14}H_{12}BrN_{2}O_{2}S (385.23). Calcd. (%): C, 43.65; H, 3.40; N, 7.27. Found (%): C, 43.75; H, 3.42; N, 7.43. IR (KBr, cm^{-1}): 3429 (OH); 3184 (NH); 3033 (OH tautomer); 3059, 3028 (CH-aromatic); 1670 (C=O); 1631 (C=N); 1531 (C=C). MS m/z (relative intensity %): 387 (M^{+}+2, 3); 384 (M^{+}-1, 4).

Synthesis of 4-Phenylthiazol-2-ylamino-N-(thioformyl)benzamide (6):-
A suspension of 2-aminothiazole derivative 1a (0.18 g, 1 mmol) in acetone (15 mL) was added to benzoyl isothiocyanate (0.16 g, 1 mmol) [prepared by refluxing equimolar mixture of benzoyl chloride (0.14 g, 0.12 mL, 1 mmol) and ammonium thiocyanate (0.76 g, 1 mmol) in acetone (15 mL) for 2 hours]. The reaction mixture was then heated under reflux for 20 hours, allowed to cool and the precipitated solid was filtered, washed with acetone, dried and crystallized from ethanol to yield compound 6.

White powder, yield: 0.156 g (45%), m.p.: 80-82 °C. Anal. form: C_{14}H_{12}N_{2}O_{2}S (339.43). Calcd. (%): C, 60.15; H, 3.86; N, 12.38. Found (%): C, 60.29; H, 3.84; N, 12.54. IR (KBr, cm^{-1}): 3369 (OH tautomer); 3273, 3172 (NH); 3082, 3064, 3032 (C-H aromatic); 1660 (C=O); 1624 (C=N); 1577 (C=C). 1H NMR (DMSO-d_{6}, δ ppm): 7.42-7.47 (m, 1H, thiazole-C_{5}-H); 7.02 (s, 1H, CO-C_{6}-H); 6.94-6.95 (m, 1H, C_{4}-H); 7.40-7.46 (m, 2H, CO-C_{6}-H); 8.13 (d, 2H, J=8.4 Hz, CO-C_{6}-H); 12.81 (s, 1H, NH-CO, D_{2}O exchangeable). MS m/z (relative intensity %): 340 (M^{+}+1, 2); 339 (M^{+}, 6).

Synthesis of N-(6-Phenyl-2H-thiazolo[3,2-b][1,2,4]thiadiazol-2-ylidene) benzamide (7):-
Equimolar amounts of compound 6 (0.3 g, 1 mmol) and phosphorous oxychloride (0.14 g, 0.13 mL, 1 mmol) were heated together at 250 °C for 20 hours. The reaction mixture was then allowed to cool, poured onto crushed ice and was allowed to stand in the refrigerator for 48 hours. The obtained solid was then filtered, dried and washed with several solvents to yield the target compound 7.

Dark green powder, yield: 0.25 g (84 %), m.p.: 68-70 °C. Anal. form: C_{14}H_{12}N_{2}O_{2}S (373.42). Calcd. (%): C, 60.51; H, 3.29; N, 12.45. Found (%): C, 60.32; H, 3.92; N, 12.54. IR (KBr, cm^{-1}): 3059, 3028 (CH-aromatic); 1670 (C=O); 1598 (C=N); 1544 (C=C). 1H NMR (DMSO-d_{6}, δ ppm): 6.90-6.95 (m, 1H, thiazole-C_{5}-H); 7.09-7.14 (m, 2H, CO-C_{6}-H); 7.40-7.46 (m, 2H, CO-C_{6}-H); 7.52 (d, 2H, J=6.9 Hz, C_{6}-C_{5,6}-H); 7.59-7.61 (m, 1H, CO-C_{6}-C_{5}-H); 7.71 (d, 2H, J=6.6 Hz, CO-C_{6}-C_{5,6}-H).

Synthesis of N-(4-(4-Bromophenyl)thiazol-2-yl)-2-formylbenzamide (8):-
An equimolar mixture of 2-aminothiazole derivative 1a (0.25 g, 1 mmol) and 2-formylbenzoic acid (0.15 g, 1 mmol) in methanol (15 mL) was heated under reflux for 40 hours. The reaction mixture was then concentrated, allowed to cool and the residue was triturated with hot ethanol, left to cool to isolate a solid product that was filtered, washed with ethanol, dried and crystallized from ethanol to yield compound 8.

Pale yellow powder, yield: 0.12 g, (32 %), m.p.: 120-122 °C. Anal. form: C_{14}H_{12}BrN_{2}O_{2}S (387.25). Calcd. (%): C, 52.73; H, 2.86; N, 7.23. Found (%): C, 52.85; H, 2.89; N, 7.43. IR (KBr, cm^{-1}): 3367 (broad OH tautomer); 3284 (NH); 3086, 2964 (CH-aromatic); 1755, 1710 (two C=O); 1629 (C=N); 1570 (C=C); 746 (C-Br). 1H NMR (DMSO-d_{6}, δ ppm): 7.25-7.28 (m, 2H, 4-Br- C_{6}-H); 7.31-7.34 (m, 3H, 2-Br- C_{6}-H); 7.40-7.46 (m, 2H, CO-C_{6}-H); 7.54-7.60 (m, 2H, CO-C_{6}-H); 7.68-7.74 (m, 2H, CO-C_{6}-H); 8.13 (d, 2H, J=8.4 Hz, CO-C_{6}-H); 12.81 (s, 1H, NH-CO, D_{2}O exchangeable). MS m/z (relative intensity %): 340 (M^{+}+1, 2); 339 (M^{+}, 6).
Synthesis of N-(4-Chlorobenzylidene)-4-(4-bromophenyl)thiazol-2-amine (9):-

To a suspension of 2-aminothiazole derivative 1 (0.25 g, 1 mmol) in methanol (15 mL) containing glacial acetic acid (4 mL), 4-chlorobenzaldehyde (0.14 g, 1 mmol) was added. The reaction mixture was then heated under reflux for 9 hours, concentrated, allowed to cool to yield an oily residue that was treated with boiling ethanol. The mixture was then allowed to cool, triturated with water and the formed precipitate was filtered, washed with water, dried and crystallized from ethanol to afford compound 9.

Yellow powder, yield: 0.23 g (62 %), m.p.:204-206°C. Anal. form: C_{16}H_{10}BrClN_{2}S (377.69). Calcd. (%): C, 50.88; H, 2.67; N, 7.42. Found (%): C, 51.08; H, 2.68; N, 7.51. IR (KBr, cm⁻¹): 3064, 2954 (CH-aromatic); 2854 (CH-aliphatic); 1683 (C=O); 1640 (C=N); 1060 (p-Cl-phenyl); 800 (C-Br). MS m/z (relative intensity %): 389 (M⁺+2, 5); 388 (M⁺+1, 21); 378 (M⁺, 11).

Synthesis of 3-(4-(4-Bromophenyl)thiazol-2-yl)-2-(4-chlorophenyl) thiazolidin-4-one (10):-

An equimolar mixture of  compound 9 (0.38 g, 1 mmol) and thioglycolic acid (0.05 g, 0.05 mL, 1 mmol) in tetrahydrofuran (30 mL ) containing a catalytic amount of anhydrous zinc chloride (0.01 g, 0.01 mmol) was heated on a water bath under reflux for 16 hours. The reaction mixture was then left to be stirred at 50 °C for 2 hours then heated under reflux for 20 hours. The reaction mixture was filtered while hot and the filtrate was triturated with ethanol and poured onto crushed ice with constant stirring. The obtained precipitate was filtered, washed with ethanol and dried to yield compound 10.

White powder, yield: 0.17 g (37 %), m.p.: 398-400°C. Anal. form: C_{18}H_{12}BrClN_{2}O_{2}S (451.79). Calcd. (%): C, 47.85; H, 2.68; N, 6.20; S, 14.19. Found (%): C, 47.98; H, 2.77; N, 6.38; S, 14.27. IR(KBr, cm⁻¹): 3421 (broad OH tautomer); 2922 (CH-aliphatic); 1683 (C=O); 1640 (C=N); 1060 (p-Cl phenyl); 800 (C-Br). MS m/z (intensity %): 454 (M⁺+2, 4); 452 (M⁺, 14).

Synthesis of 1-(4-(4-Bromophenyl)thiazol-2-yl)-3-chloro-4-(4-chlorophenyl) azetidin-2-one (11):-

To a well stirred solution of compound 9 (0.25 g, 1 mmol) in dry benzene/ triethylamine mixture (10 mL, 9:1), chloroacetyl chloride (0.17 g, 0.16 mL, 1 mmol) was added dropwise while stirring at room temperature. The reaction mixture was left to be stirred at 50 °C for 2 hours then heated under reflux for 20 hours. The reaction mixture was filtered while hot and the filtrate was triturated with ethanol and poured onto crushed ice with constant stirring. The obtained precipitate was filtered, washed with ethanol/water mixture, dried and crystallized from methanol to yield compound 11.

Buff powder, yield: 0.17 g (37 %), m.p.: 140-142°C. Anal. form: C_{18}H_{12}BrCl_{2}N_{2}O_{2}S (454.17). Calcd. (%): C, 47.60; H, 2.44; N, 6.17. Found (%): C, 47.73; H, 2.51; N, 6.34. IR(KBr, cm⁻¹): 3363 (OH); 3030-3053 (C-H aromatic); 2987, 2954 (CH-aromatic); 2854 (CH-aliphatic); 1616 (C=N); 1068 (p-Cl-phenyl); 829 (C-Br). ¹H NMR (DMSO-d₆, δ ppm): 6.68 (s, 1H, thiazole-C₆-H); 6.96 (s, 1H, N=CH); 7.00-7.8 (m, 6H, 4-Br-C₆-H); 7.82-7.17 (m, 2H, 4-Br-C₆H₄C₅,H₂& &-H). MS m/z (relative intensity %): 380 (M⁺+2, 5); 378 (M⁺, 10).

Synthesis of Ethyl (4-(4-bromophenyl)thiazol-2-yl carbamoyl)formate (12):-

An equimolar mixture of 2-aminothiazole 1b (0.25 g, 1 mmol) and diethyl oxalate (0.15 mL, 0.14 g, 1 mmol) in ethanol (15 mL) containing piperidine (0.5 mL) was heated under reflux for 40 hours. The reaction mixture was allowed to attain room temperature and then triturated with 10 % hydrochloric acid to yield crystals that were filtered, washed with ethanol, dried and recrystallized from ethanol to afford compound 12.

White crystals, yield: 0.13 g (37 %), m.p.:146-148°C. Anal. form: C_{13}H_{14}BrN_{2}O_{2}S (355.21). Calcd. (%):C, 43.96; H, 3.12; N, 7.89. Found (%): C, 44.08; H, 3.15; N, 7.96. IR (KBr, cm⁻¹): 3446 (OH-tautomer); 3346 (NH); 2987, 2953 (CH-aromatic); 2854 (CH-aliphatic); 1741, 1685 (two C=O); 1637 (C=N); 1571 (C=C); 1274, 1074 (C-O-C); 750 (C-Br). ¹H NMR (DMSO-d₆, δ ppm): 3.98 (t, 3H, J=7 Hz, CH₃CH₂); 4.32 (q, 2H, J=7 Hz, CH₃CH₂); 7.09 (s, 1H, thiazole-C₆-H); 7.59 (d, 2H, J=8.4Hz, 4-Br-C₆H₄C₅,H₂& &-H); 7.85 (d, 2H, J=8.4Hz, 4-Br-C₆H₄C₅,H₂& &-H). 8.7 (s,
1/2H, OH-tautomer, D$_2$O exchangeable); 13.10 (s, 1/2H, NH, D$_2$O exchangeable). **MS m/z (relative intensity %):**
357 (M$^+$+2, 2); 356 (M$^+$+1, 12).

**Synthesis of 3-(4-Bromophenyl)-7-(methylthio)-5-oxo-5H-thiazo[3,2-a]pyrimidine-6-carbonitrile (13):**
An equimolar mixture of I$_b$ (0.25 g, 1 mmol), ethyl 2-cyano-3,3-bis(methylthio)acrylate (0.22 g, 1 mmol) in presence of a catalytic amount of anhydrous potassium carbonate (0.014 g, 0.01 mmol) was heated under reflux in dimethylformamide (15 mL) for 20 hours. The reaction mixture was allowed to cool to yield a precipitate that was filtered, dried and washed with several solvents to give compound 13.

Dark brown powder, **yield:** 0.09 g (24 %), **m.p.:** 70-72°C. **Anal. form:** C$_{12}$H$_9$BrN$_2$O$_2$S (389.81). Calcd. (%): C, 44.45; H, 2.13; N, 11.11; S, 16.95. **Found (%):** C, 44.59; H, 2.14; N, 11.23; S, 17.02. **IR (KBr, cm$^{-1}$):** 2978, 2962 (C-H aromatic); 2854 (CH-aliphatic); 1720, 1697 (two C=O); 1620, 1585 (C=N); 1560 (C=C); 840 (C-Br). **MS m/z (relative intensity %):** 380 (M$^+$+2, 1); 378 (M$^+$, 3).

**Synthesis of Ethyl 3-(4-bromophenyl)-N,N-(diethyl formate)thiazol-2-amine (15):**
An equimolar mixture of compound I$_b$ (0.25 g, 1 mmol), 4-chlorobenzaldehyde (0.14 g, 1 mmol) and ethyl acetoacetate (0.13 g, 0.12 mL, 1 mmol) was heated in an oil bath at 250°C for 48 hours. The reaction mixture was allowed to cool, triturated with ethanol/water mixture then, the formed precipitate was filtered, washed with ethanol, dried and crystallized from dimethylformamide/water mixture to yield compound 14.

Grey powder, **yield:** 0.2 g (42 %), **m.p.:** 94-96°C. **Anal. form:** C$_{13}$H$_{19}$BrClN$_2$O$_2$S (489.81). Calcd. (%): C, 53.95; H, 3.70; N, 5.72. **Found (%):** C, 53.99; H, 3.77; N, 5.80. **IR (KBr, cm$^{-1}$):** 2980, 2960 (C-H aromatic); 1716 (C=O); 1624 (C=N); 1525 (C=C); 1242, 1070 (C-O-C); 1070 (p-Cl-phenyl); 829 (C-Br). **MS m/z (relative intensity %):** 401 (M$^+$+1, 2); 399 (M$^+$, 5); 398 (M$^+$-1, 3).

**Synthesis of 4-(4-Bromophenyl)-N,N-(diethyl formate)thiazol-2-amine (15):**
2-Aminothiazole derivative I$_b$ (0.25 g, 1 mmol) was heated under reflux in excess ethylchloroformate for 44 hours. The reaction mixture was then allowed to cool, triturated with ethanol/water mixture and the obtained precipitate was filtered, washed with ethanol, dried and recrystallized from ethanol to yield compound 15.

White needle crystals, **yield:** 0.18 g (46 %), **m.p.:** 50-52°C. **Anal. form:** C$_{13}$H$_{18}$BrN$_2$O$_2$S (399.26). Calcd. (%): C, 45.12; H, 3.79; N, 7.02. **Found (%)**: C, 45.26; H, 3.85; N, 7.11. **IR (KBr, cm$^{-1}$):** 2978 (C-H aromatic); 2858 (CH-aliphatic); 1789, 1720 (two C=O); 1639 (C=O); 1577 (C=C); 1253, 1064 (C-O-C); 866 (C-Br). **MS m/z (relative intensity %):** 401 (M$^+$+1, 2); 399 (M$^+$, 5); 398 (M$^+$-1, 3).

**Synthesis of 4-(4-Bromophenyl)thiazol-2-yl)-1,2,4-triazolidine-3,5-dione (16):**
An equimolar mixture of compound 15 (0.39 g, 1 mmol) and hydrazine hydrate 99% (0.05 g, 0.05 mL, 1 mmol) was heated in an oil bath at 250°C for 48 hours. The reaction mixture was then allowed to cool, triturated with ethanol and treated with 10 % hydrochloric acid (0.5 mL) to afford a precipitate that was filtered, washed with ethanol, dried and crystallized from ethanol to yield compound 16.

Red powder, **yield:** 0.2 g (60 %), **m.p.:** 58-60°C. **Anal. form:** C$_{11}$H$_9$BrN$_3$O$_2$S (339.17). Calcd. (%): C, 38.95; H, 2.08; N, 16.52. **Found (%)**: C, 38.99; H, 2.11; N, 16.60. **IR (KBr, cm$^{-1}$):** 3431 (broad OH tautomer); 3292, 3248 (NH); 2983, 2960 (CH-aromatic); 1720, 1697 (two C=O); 1620, 1585 (C=N); 1560 (C=C); 840 (C-Br). **$^1$H NMR (DMSO-d$_6$, δ ppm):** 7.21 (s, 1H, thiazole-C$_5$-H); 7.31 (d, 2H, J=6.9 Hz, 4-Br-C$_4$H$_4$-C$_2$H$_5$-H); 7.68 (s, 1H, NH, D$_2$O exchangeable); 7.71 (s, 1H, NH, D$_2$O exchangeable). **MS m/z (relative intensity %):** 7.60-7.66 (m, 2H, 4-Br-C$_4$H$_4$& 4-Br-C$_6$H$_4$).

**Synthesis of 4-(4-Bromophenyl)-N,N-(diethyl formate)thiazol-2-yl)-2-chloroacetamide (17):**
To a well stirred solution of 2-aminothiazole derivative I$_b$ (0.25 g, 1 mmol) in dimethylformamide (15 mL), chloroacetyl chloride (0.11 g, 0.1 mL, 1 mmol) was added dropwise while stirring. The reaction mixture was then left to be stirred for 24 hours at room temperature, then poured onto crushed ice to afford a solid precipitate that was filtered off, washed with ethanol, dried and recrystallized from ethanol to yield compound 17.
White needle crystals, yield: 0.18 g (55 %). m.p.: 170-172°C. Anal. Form: C_{11}H_{13}BrCIN_{2}OS (331.62). Calcd. (%): C, 39.84; H, 2.43; N, 8.45. Found (%): C, 39.95; H, 2.46; N, 8.51. IR (KBr, cm^{-1}): 3446 (OH tautomer); 3182 (NH); 3074, 2993 (CH-aromatic); 2927, 2854 (CH-aliphatic); 1654 (C=O); 1637 (C=N); 1558 (C=C); 831 (C-Br).

1H NMR (DMSO-d_{6}, δ ppm): 1.47 (s, 2H, CH_{2}); 7.14 (s, 1/2H, OH tautomer, D_{2}O exchangeable); 7.62 (d, 2H, J=4.3 Hz, 4-Br-C_{6}H_{4}-C_{2,5,6}-H); 7.71 (d, 1H, thiazole-C_{3}-H); 7.84 (d, 2H, J=8.4 Hz, 4-Br-C_{6}H_{4}-C_{3,5,6}-H); 12.59 (s, 1/2H, NH, D_{2}O exchangeable). MS m/z (relative intensity %): 336 (M^{+} + 4, 0.2); 334 (M^{+} + 2, 5); 332 (M^{+}, 17).

Synthesis of 2-(4-(4-Bromophenyl)thiazol-2-ylamino)-N-(5-bromo-2-(iminomethyl)phenol (20):-

To a suspension of 2-aminothiazole derivative 1_{b} (0.25 g, 1 mmol) in ethylene glycol (10 mL), hydrazine hydrate (0.05 g, 0.05 mL, 1 mmol) was added. The reaction mixture was then heated under reflux for 40 hours, then was allowed to cool, triturated with cold water to precipitate a solid that was filtered, washed with methanol, and dried and crystallized from ethanol to yield the target compound 19.

Dark brown powder, yield: 0.07 g (26 %). m.p.: 90-92°C. Anal. Form: C_{6}H_{6}BrN_{3}S (270.15). Calcd. (%): C, 40.01; H, 2.98; N, 15.55. Found (%): C, 40.13; H, 2.98; N, 15.70. IR (KBr, cm^{-1}): 3385, 3332, 3205 (NH, NH exchangeable); 3107 (C=O); 1610 (C=N); 1574 (C=C); 831 (C-Br). 1H NMR (DMSO-d_{6}, δ ppm): 3.72 (s, 2H, NH); 4.03 (s, 1H, thiazole-CH); 7.20 (s, 1H, NH, D_{2}O exchangeable); 7.46 (s, 1H, NH, D_{2}O exchangeable); 7.64 (d, 2H, J=8.4 Hz, 4-Br-C_{6}H_{4}-C_{2,5,6}-H); 7.73 (d, 2H, J=8.4 Hz, 4-Br-C_{6}H_{4}-C_{3,5,6}-H). MS m/z (relative intensity): 272 (M^{+} + 2, 4); 271 (M^{+}, +1, 8).

Synthesis of 24-(4-Bromophenyl)thiazol-2-ylamino)-N-(5-bromo-2-(iminomethyl)phenol (20):-

An equimolar mixture of compound 19 (0.27 g, 1 mmol) and 5-bromo salicylaldehyde (0.20 g, 1 mmol) in methanol (15 mL) was heated under reflux for 9 hours. The reaction mixture was then concentrated, allowed to cool and the formed precipitate was filtered, washed with methanol, dried and crystallized from ethanol to give compound 20.

Yellow powder, yield: 0.2 g (44 %). m.p.: 178-180°C. Anal. Form: C_{16}H_{11}Br_{2}N_{2}OS (453.15). Calcd. (%): C, 42.41; H, 2.45; N, 9.27. Found (%): C, 42.52; H, 2.44; N, 9.32. IR (KBr, cm^{-1}): 3406 (broad OH); 3228 (NH); 3041, 2958, 2926 (CH-aromatic); 2926, 2872 (CH-aliphatic); 1610 (C=N); 1560 (C=C); 829 (C-Br). MS m/z (relative intensity %): 455 (M^{+} + 2, 2).

Synthesis of 6-Bromo-1-(4-(4-bromophenyl)thiazol-2-yl)-1H-indazole (21):-

Compound 20 (0.45 g, 1 mmol) was heated under reflux in methanol (15 mL) containing glacial acetic acid (4 mL) for 9 hours. The reaction mixture was concentrated, triturated with ethanol and allowed to cool. The obtained solid was filtered, washed with ethanol, dried and crystallized from ethanol to yield compound 21.

Yellow powder, yield: 0.2 g (47 %). m.p.: 238-240°C. Anal. Form: C_{10}H_{10}Br_{2}N_{2}S (435.14). Calcd. (%): C, 44.16; H, 2.08; N, 9.66. Found (%): C, 44.24; H, 2.04; N, 9.76. IR (KBr, cm^{-1}): 3066, 2997 (C-H aromatic); 1625 (C=N); 1562 (C=C); 820 (C-Br). MS m/z (relative intensity %): 437 (M^{+} + 2, 0.40); 435 (M^{+}, 0.3).

Synthesis of 4-(Bromophenyl)thiazol-2-yl guanidine (22):-

A mixture of 1_{b} (0.25 g, 1 mmol) and cyanamide (0.84 gm, 2 mmol) in absolute ethanol (15 mL) containing concentrated hydrochloric acid (3 drops) for 48 hours. The reaction mixture was then concentrated, allowed to cool...
and the precipitated solid was filtered, washed with ethanol, dried and crystallized from ethanol to yield compound 22.

White powder, yield: 0.24 g, (83 %), m.p.: 142-144°C. Anal. form: C_{10}H_{8}BrN_{4}S (297.17). Calcd. (%): C, 40.42; H, 3.05; N, 18.85. Found (%): C, 40.53; H, 3.09; N, 18.97. IR (KBr, cm⁻¹): 3282, 3111 (NH, NH₂); 2966, 2927 (C-H aromatic); 1633 (C=C); 1533 (C=C aromatic); 850 (C-Br). ¹H NMR (DMSO-d₆, δ ppm): 6.99 (s, 2H, NH₂, D₂O exchangeable); 7.04 (s, 1H, thiazone-C₂-H); 7.53 (d, 2H, J=8.7 Hz, 4-Br-C₆H₄-C₂=O-H); 7.76 (d, 2H, J=8.7 Hz, 4-Br-C₆H₄-C₂=O-H); 8.30 (s, 1H, NH, D₂O exchangeable); 12.20 (s, 1H, C=NH, D₂O exchangeable). MS m/z (relative intensity %): 299 (Mⁿ⁺+2, 1); 297 (Mⁿ⁺, 3).

Synthesis of N-(4-(4-Bromophenyl)thiazol-2-yl)-4,6-dimethylpyrimidin-2-amine (23):-
To a solution of compound 22 (0.29 g, 1 mmol) in dimethylformamide (15 mL), acetylacetone (0.2 g, 0.20 mL, 2 mmol) was added. The reaction mixture was then heated under reflux for 48 hours. The reaction mixture was allowed to cool, poured onto ice cold water and was left to stand overnight. The formed precipitate was filtered, washed with water, dried and crystallized from dimethylformamide/water mixture to yield compound 23.

Brown powder, yield: 0.1 g (28 %), m.p.: 148-150°C. Anal. form: C_{13}H_{13}BrN_{4}S (361.26). Calcd. (%): C, 49.87; H, 3.63; N, 15.51. Found (%): C, 49.97; H, 3.67; N, 15.73. IR (KBr, cm⁻¹): 3309 (NH); 3053, 3034 (C-H aromatic); 2797, 2797, 2860 (C-H aliphatic); 1600 (C≡N); 1556, 1539 (C≡C); 827 (C-Br). ¹H NMR (DMSO-d₆, δ ppm): 3.00 (s, 6H, two CH₃); 3.47 (s, 1H, NH, D₂O exchangeable); 7.36 (s, 1H, thiazone-C₅-H); 7.43-7.92 (m, 4H, 4-Br-C₆H₄); 8.15 (s, 1H, pyrimidine-C₃-H). MS m/z (relative intensity %): 363 (Mⁿ⁺+2, 2).

Synthesis of N-(4-(4-Bromophenyl)thiazol-2-yl)-4-methyl-6-phenylpyrimidin-2-amine (24):-
A mixture of compound 22 (0.29 g, 1 mmol) and benzoylaceton (0.32 g, 0.32 mL, 2 mmol) was heated in an oil bath at 250°C for 2 hours. The reaction mixture was then allowed to cool, triturated with ethanol/diethylether mixture (1:1, 4 mL) and the obtained solid was filtered, washed with ethanol, dried and crystallized from ethanol to yield compound 24.

Dark brown powder, yield: 0.2 g (48 %), m.p.: 102-104°C. Anal. form: C_{20}H_{11}BrN_{4}S (423.33). Calcd. (%): C, 56.74; H, 3.57; N, 13.23. Found (%): C, 56.89; H, 3.62; N, 13.41. IR (KBr, cm⁻¹): 3115 (NH); 3040, 2970, 2924 (CH-aromatic); 2880, (CH-aliphatic); 1602 (C≡N); 1570 (C≡C); 856 (C-Br). ¹H NMR (DMSO-d₆, δ ppm): 2.44 (s, 3H, CH₃); 6.34 (s, 1H, thiazone-C₃-H); 7.48-7.86 (m, 3H, C₆H₅-C₆H₄-C₂=O-H & 4-Br-C₆H₄-C₂=O-H); 8.00 (d, 2H, J=8.4 Hz, 4-Br-C₆H₄-C₃=O-H); 8.27 (s, 1H, pyrimidine-C₃-H); 10.53 (s, 1H, NH, D₂O exchangeable).

Synthesis of 2-(4-(4-Bromophenyl)thiazol-2-ylamino)-6-hydroxypyrimidin-4(3H)-one (25):-
A mixture of compound 22 (0.29 g, 1 mmol) and diethyl malonate (0.32 g, 0.30 mL, 2 mmol) was heated in an oil bath at 250°C for 2 hours. The reaction mixture was then allowed to cool, triturated with ethanol/ether mixture (1:1, 4 mL) and the obtained product was filtered off, washed with ethanol, dried and recrystallized from ethanol to afford compound 25.

White crystals, yield: 0.2 g (56 %), m.p.: 128-130°C. Anal. form: C_{13}H_{8}BrN_{4}O₂S (365.21). Calcd. (%): C, 42.75; H, 2.48; N, 15.34. Found (%): C, 42.87; H, 2.53; N, 15.58. IR (KBr, cm⁻¹): 3431 (broad OH tautomer); 3155, 3101 (CH-aromatic); 1685 (C≡O); 1590 (C≡N); 1560, 1544 (C=C); 821 (C-Br). ¹H NMR (DMSO-d₆, δ ppm): 7.60 (s, 1H, thiazone-C₃-H); 7.61 (d, 2H, J=6.6 Hz, 4-Br-C₆H₄-C₂=O-H); 7.71 (s, 1H, NH, D₂O exchangeable); 7.83 (d, 2H, J=6.6 Hz, 4-Br-C₆H₄-C₃=O-H); 7.90 (s, 1H, pyrimidine-C₃-H); 12.21 (s, 1H, pyrimidine-NH, D₂O); 12.44 (s, 1H, OH, D₂O exchangeable).

Results and Discussion:-
2-Amino-4-phenylthiazole derivative 1ₐ was prepared according to the reported procedure [13] which was further utilized as a building block to synthesize various phenylthiazole derivatives substituted either at the thiazole amino function or fused to the thiazole back bone. However, treatment of compound 1ₐ with phenylisothiocyanate furnished the corresponding thiourea derivative 2 which was further cyclized to its aminothiazole analogue 3. The ¹H NMR spectrum of compound 2 revealed a deuterium oxide exchangeable singlet signal at δ 8.76 ppm attributed to NH-C₆H₄ while, the IR spectrum of compound 3 showed NH function absorption band at 3387 cm⁻¹. In addition to, absorption bands at 1240, 1090 cm⁻¹ corresponding to C=S-C function. The ¹H NMR spectrum of compound 3...
revealed a deuterium oxide exchangeable singlet signal at $\delta$ 8.65 ppm attributed to NH. In addition, dihydrothiazole C$_3$-H was observed as a singlet signal at $\delta$ 6.93 ppm.

We carried out the synthesis of the target $N,N$-dipropynlythiazole-2-amine derivative 4 by refluxing one equivalent of 2-amino-4-phenyl-thiazole 1$_a$ with two equivalents of propargyl bromide. The $^1$H NMR spectrum of compound 4 revealed a singlet signal at $\delta$ 4.15 ppm attributed to two CH protons. In addition to a singlet signal at $\delta$ 8.5 ppm due to two acetylenic protons.

Previously iminosuccinic acid derivatives were obtained via treatment of different substituted aromatic amines with itaconic acid [14]. However, the reaction was postulated to proceed through addition of the ring nitrogen to the double bond of the methyl succinic acid (itaconic acid). The iminosuccinic acid derivatives 5$_{a,b}$ were synthesized by stirring the appropriate 2-amino-4-phenylthiazole derivatives 1$_{a,b}$ with an equimolar amount of itaconic acid at room temperature. The IR spectra of compounds 5$_{a,b}$ showed absorption bands at 3429-3383 cm$^{-1}$ corresponding to OH function and absorption bands at 3267-3116 cm$^{-1}$ due to NH function. In addition to absorption bands at 1701 cm$^{-1}$ corresponding to C=O function.

Furthermore, the thioformylbenzamide derivative 6 was prepared via the reaction of 2-amino-4-phenylthiazole 1$_a$ with benzoyl isothiocyanate which was further cyclized to its corresponding thiadiazole derivative 7 upon reflux with phosphorous oxychloride. The IR spectrum of compound 6 showed absorption band at 3369 cm$^{-1}$ corresponding to OH tautomer. The $^1$H NMR spectrum of compound 6 revealed two deuterium oxide exchangeable singlet signals at $\delta$ 5.45 and $\delta$ 7.68 ppm attributed to NH and OH protons, respectively. However, the $^1$H NMR spectrum of compound 7 revealed two multiplet signals at $\delta$ 7.40-7.46 ppm and $\delta$ 7.59-7.61 ppm attributed to C$_{3,5}$ and C$_4$ protons of CO-C$_6$H$_5$; respectively.

(Scheme 1)

Reagents and conditions: (a) Absolute ethanol/ reflux; (b) Phenyl isothiocyanate/ dioxan/ reflux; (c) Phenacyl bromide/ absolute ethanol/ reflux; (d) Propargyl bromide/ anhydrous K$_2$CO$_3$/ KI/ absolute Ethanol/ reflux; (e) Itaconic acid/ absolute ethanol/ stirring/ r.t; (f) Aroyl isothiocyanate/acetone/ reflux; (g) POCl$_3$/ fusion.

Scheme 1
In our recent study, the benzamide derivative 8 was prepared via the reaction of equimolar amounts of 2-formylbenzoic acid and 2-aminothiazole derivative 1b in methanol under reflux conditions. The IR spectrum of compound 8 showed an absorption band at 3367 cm\(^{-1}\) corresponding to OH tautomer. In addition to, two absorption bands at 1755, 1710 cm\(^{-1}\) corresponding to two C=O functions. The \(^1\)H NMR spectrum of compound 8 revealed two deuterium oxide exchangeable singlet signals at \(\delta\) 7.41 and 9.08 ppm attributed to OH tautomer and thiazole NH, respectively.

It is well established in the literature that, thioglycolic acid was reported to be utilized in various cyclocondensation reactions with different Schiff’s bases leading to their cyclized thiazolidinone analogues [8, 15]. Therefore, schiff’s base 9 was treated with thioglycolic acid in refluxing tetrahydrofuran containing zinc chloride as a catalyst to yield the target thiazolidinone derivative 10. The IR spectrum of compound 9 lacked absorption bands corresponding to NH\(_2\) function while, the IR spectrum of compound 10 showed a broad absorption band at 3421 cm\(^{-1}\) corresponding to OH tautomer. In addition to, an absorption band at 1683 cm\(^{-1}\) corresponding to C=O function.

Chloroacetyl chloride has been widely reported to be involved in the preparation of various azetidinone derivatives through the reaction with different Schiff’s bases [8, 16]. Therefore, thiazolylazetidin-2-one derivative 11 was prepared via stirring equimolar amounts of Schiff’s base derivative 9 and chloroacetyl chloride in dry benzene containing triethylamine. The \(^1\)H NMR spectrum of compound 11 revealed a singlet signal at \(\delta\) 2.30 ppm attributed to azetidinyl-C\(_4\)-H proton. In addition to a multiplet signal at \(\delta\) 4.05-4.18 ppm due to azetidinyl-C\(_3\)-H proton.

Furthermore, 2-aminothiazole derivative 1b upon heating under reflux in ethanol containing piperidine with equivalent amount of diethyl oxalate yielded the corresponding carbamoyl formate derivative 12. The IR spectrum of compound 12 showed an absorption band at 3446 cm\(^{-1}\) corresponding to OH tautomer and two absorption bands at 1741, 1685 cm\(^{-1}\) corresponding to two C=O functions. The \(^1\)H NMR spectrum of compound 12 revealed a triplet signal at \(\delta\) 3.98 ppm attributed to CH\(_3\) protons and a quartet signal at \(\delta\) 4.32 ppm attributed to CH\(_2\) protons. In addition to, two deuterium oxide exchangeable signals at \(\delta\) 8.7 ppm and \(\delta\) 13.10 ppm due to OH and NH of the tautomer respectively.\(\text{(Scheme 2)}\)

Compound 13 was prepared via refluxing equimolar amounts of 2-aminothiazole derivative 1b and 2-ethoxy-3,3-bis(methylthio)acrylonitrile in dimethylformamide containing anhydrous potassium carbonate as a base. The IR spectrum of Compound 13 showed two characteristic absorption bands at 2240 and 1660 cm\(^{-1}\) corresponding to C≡N and C=O functions, respectively.
Thethiazolopyrimidine derivative 14 was obtained via heating of the multicomponent reaction mixture of 2-aminothiazole derivative 1b, 4-chlorobenzaldehyde and ethyl acetoacetate. The IR spectrum of compound 14 lacked absorption bands due to NH₂ function and showed an absorption band at 1716 cm⁻¹ corresponding to ester carbonyl function. While, the ¹H NMR spectrum of compound 14 showed two multiplet signals at δ 0.80-1.30 ppm and 3.80-4.40 ppm attributed to O-CH₂-CH₃ and O-CH₂-CH₃ protons; respectively.

**Scheme 2**

Reagents and conditions: (a) 2-Formylbenzoic acid/ methanol/ reflux; (b) 4-Chlorobenzaldehyde/ absolute ethanol/ gl. AcOH/ reflux; (c) Thiglycolic acid/ anhydrous ZnCl₂/ THF/ W.B; (d) Chloroacetyl chloride/ TEA/ benzene/ reflux; (e) Diethyl oxalate/ piperidine/ absolute ethanol/ reflux.
Reagents and conditions: (a) 2-cyano-3,3-bis(methylthio)acrylate / K₂CO₃/ DMF/ reflux; (b) 4-Chlorobenzaldehyde / EAA/ fusion; (c) Ethyl chloroformate/ fusion; (d) Hydrazine hydrate/ fusion.

Scheme 3

In our recent study, 1b upon treatment with excess of ethyl chloroformate yielded the target N,N-diethylformate thiazole-2-amine 15. The IR spectrum of compound 15 lacked absorption bands corresponding to NH₂ function, besides to, two absorption bands at 1789, 1720 cm⁻¹ due to the two ester carbonyl functions. In the present investigation, the target 1, 2, 4 thiazolidine-3, 5-dione derivative 16 was obtained by fusion of equimolar amounts of compound 15 and hydrazine hydrate. The ¹H NMR spectrum of compound 16 revealed two deuterium oxide exchangeable singlet signals at δ 7.68 ppm and δ 7.71 ppm attributed to two NH protons. (Scheme 3)

Furthermore, 2-aminothiazole derivative 1b was stirred with equimolar amounts of chloroacetyl chloride in dimethylformamide at room temperature to furnish the target chloroacetamide derivative 17. The IR spectrum of compound 17 showed an absorption band at 3446 cm⁻¹ corresponding to tautomeric OH function and an absorption band at 3182 cm⁻¹ due to NH function. In addition to, an absorption band at 1654 cm⁻¹ due to C=O function. The ¹H NMR spectrum of compound 17 revealed two deuterium oxide exchangeable singlet signals at δ 7.14 ppm and δ 12.59 ppm attributed to OH and NH of the tautomers; respectively. In addition to, a singlet signal at δ 4.17 ppm due to CH₂ protons.

In addition, the cyclization of 17 to the target thiazolylthiazolone derivative 18 was achieved via heating equimolar mixture of 17 and ammonium thiocyanate in ethanol under reflux. The IR spectrum of compound 18 showed a broad absorption band at 3444 cm⁻¹ corresponding to OH of the tautomer. The ¹H NMR spectrum of compound 18 revealed two deuterium oxide exchangeable singlet signals at δ 7.30 ppm and δ 7.46 ppm attributed to OH and NH protons.
The thiazolyl hydrazine derivative 19 was prepared by treatment of 2-aminothiazole derivative 1b with an equimolar amount of hydrazine hydrate in ethylene glycol. The $^1$H NMR spectrum of compound 19 revealed two deuterium oxide exchangeable singlet signals at $\delta$ 3.72 and 4.38 ppm attributed to NH and NH$_2$.

However, the thiazolyl hydrazine derivative 19 furnished the corresponding thiazolylamino-N-iminomethylphenol derivative 20 upon heating with one equivalent of 5-bromosalicylaldehyde in methanol under reflux. The IR spectrum of compound 20 showed a broad absorption band at 3406 cm$^{-1}$ corresponding to OH function.

Our aim was extended to study the effect of cyclization of the hydrazone derivative 20 to thiazolylindazole derivative 21 in refluxing glacial acetic acid/methanol mixture on the anticancer activity study. The IR spectrum of compound 21 lacked the absorption band due to OH and NH functions of its precursor. (Scheme 4).

It is well established in the literature that, various guanidine derivatives could be achieved via treatment different of amines with guanidinylation reagents [17, 18]. Therefore, we utilized cyanamide in the reaction with 2-aminothiazole derivative 1b to obtain the thiazolylguanidine intermediate 22. The $^1$H NMR spectrum of compound 22 revealed three deuterium oxide exchangeable singlet signals at $\delta$ 6.99 ppm, $\delta$ 8.30 ppm and $\delta$ 12.20 ppm attributed to NH$_2$, NH and C=NH protons, respectively.

Reagents and conditions: (a) Chloroacetyl chloride/DMF/stirring; (b) Ammonium thiocyanate/absolute ethanol/reflux; (c) Hydrazine hydrate/ethylene glycol/reflux; (d) 4-Bromosalicylaldehyde/methanol/reflux; (e) g.l./AcOH/methanol/reflux.

Scheme 4
Our efforts was directed to study the anticancer activity of various substituted pyrimidine heterocycles attached to the thiazole backbone through its amino function, a step that was achieved via cyclization of the side chain of the intermediate 22 by the aid of various active methylene bearing carbonyl compounds.

The thiazolylguanidine 22 upon treatment with either with acetyl acetone or benzoyl acetone furnished the target thiazolylmethylpyrimidine derivatives 23 and 24; respectively. The $^1$H NMR spectra of compounds 23 and 24 revealed singlet signals at $\delta$ 3.00 ppm due to two CH$_3$ protons of 23 and a singlet signals at $\delta$ 2.44 ppm attributed to CH$_3$ protons of 24 besides to, the NH proton deuterium oxide exchangeable singlet signal in each at $\delta$ 3.47 ppm and $\delta$ 10.53 ppm for compounds 23 and 24; respectively. In addition, the $^1$H NMR spectra of both 23 and 24 revealed the pyrimidine C$_5$-H singlet signal at $\delta$ 8.15 ppm and $\delta$ 8.27 ppm; respectively.

Finally, utilizing diethylmalonate in the cyclization of the guanidine derivative 22 yielded the target thiazolylaminopyrimidindione derivative 25. The IR spectrum of compound 25 showed a broad absorption band at 3431 cm$^{-1}$ corresponding to OH function. In addition to, absorption band at 1685 cm$^{-1}$ corresponding to C=O function. However, the $^1$H NMR spectrum of compound 25 displayed three deuterium oxide exchangeable singlet signals at $\delta$ 7.71, 12.21 and 12.44 ppm attributed to NH, pyrimidine N-H and OH tautomeric protons; respectively.

(Scheme 5)

Reagents and conditions: (a) Cyanamide/ absolute ethanol/ conc.HCl/ reflux; (b) Acetylacetone/ DMF/ reflux; (c) Benzoylacetone/ fusion; (d) Diethyl malonate / fusion.

**Scheme 5**

**Biological Activity:**

**Anticancer screening studies:**

Twenty four of the synthesized compounds (2-25) were screened for their in vitro cytotoxic activity against human hepatocellular liver carcinoma (HepG2) and human breast cancer (MCF7) cell lines in the regional center for mycology and biotechnology, at Al-Azhar University. Doxorubicin was used as the reference drug in this study. It is
well documented that doxorubicin induces its antitumor activity through several mechanisms including inhibition of topoisomerase II, DNA intercalation, generation of reactive oxygen species and DNA single and double strand breaks.

Cytotoxicity evaluation using viability assay:-
For cytotoxicity assay, the cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50μg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well micro titer plate incubated for 24h at 37°C in a humidified incubator with 5% CO2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 μl from different dilutions of tested sample in fresh maintenance medium and incubated at 37°C. A control of untreated cells was made in the absence of tested sample. A positive control containing Doxorubicin drug was also tested as reference drug for comparison. Six wells were used for each concentration of the test sample. Every 24h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader (SunRise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots [19, 20].

The six dose growth inhibition percent and the IC50 values of the tested compounds (2-25) against liver HepG2 and breast MCF7 cell lines are represented in tables (1-2) & (3-4); respectively. Growth inhibition curves of the most active compounds against both HepG2 and MCF7 cell lines are represented in figures 1 & 2; respectively.

Table 1:- Six dose growth inhibition percent and IC50 values of the tested compounds of schemes 1, 2 &3 against HepG2 cell line

| Sample conc. (μg/mL) | Compound | 100  | 50   | 25   | 12.5 | 6.25  | 3.125 | IC50 (μg/mL) |
|----------------------|----------|------|------|------|------|-------|-------|-------------|
| 2                    |          | 73.17| 60.32| 47.63| 31.41| 18.76 | 7.94  | 29.7        |
| 3                    |          | 92.94| 83.22| 70.48| 59.14| 38.61 | 23.88 | 9.72        |
| 4                    |          | 67.11| 38.27| 21.38| 10.85| 3.73  | 0.94  | 70.3        |
| 5a                   |          | 82.51| 70.15| 56.44| 32.72| 21.31 | 10.84 | 21.6        |
| 5b                   |          | 72.15| 58.04| 39.17| 21.55| 9.32  | 3.77  | 39.3        |
| 6                    |          | 83.46| 69.31| 60.18| 42.84| 28.17 | 16.06 | 17.7        |
| 7                    |          | 71.22| 33.75| 21.86| 10.37| 3.79  | 0.54  | 71.7        |
| 8                    |          | 79.22| 56.39| 32.76| 18.07| 9.32  | 2.17  | 43.2        |
| 9                    |          | 63.28| 48.11| 25.77| 12.49| 5.33  | 1.26  | 56.2        |
| 10                   |          | 65.13| 46.71| 25.85| 12.37| 5.74  | 2.58  | 58.9        |
| 11                   |          | 76.85| 63.22| 50.11| 26.87| 12.46 | 6.31  | 24.9        |
| 12                   |          | 92.17| 84.51| 75.32| 64.03| 51.76 | 31.51 | 5.98        |
| 13                   |          | 81.68| 69.33| 57.15| 41.84| 26.96 | 12.49 | 19.2        |
| 14                   |          | 83.18| 72.82| 60.57| 34.76| 20.28 | 9.33  | 19.9        |
| 15                   |          | 89.28| 76.14| 67.05| 53.16| 34.77 | 20.54 | 11.4        |
| 16                   |          | 73.24| 56.72| 38.11| 20.82| 5.77  | 1.46  | 41.0        |
| Doxorubicin          |          | 90.76| 88.45| 84.26| 77.78| 70.82 | 55.16 | 4.65        |
### Table 2: Six dose growth inhibition percent and IC\textsubscript{50} values of the tested compounds of schemes 4&5 against HepG2 cell line

| Sample conc. (µg/mL) | Compound | Growth inhibition % | IC\textsubscript{50} (µg/mL) |
|----------------------|----------|---------------------|-----------------------------|
|                      |          | 100                 | 50  | 25  | 12.5 | 6.25 | 3.125 |          |
| 27                   |          | 91.26               | 80.98 | 72.37 | 64.22 | 52.79 | 35.11 | 5.76   |
| 18                   |          | 94.02               | 87.26 | 76.33 | 68.15 | 57.04 | 42.12 | 4.78   |
| 19                   |          | 79.47               | 63.26 | 42.84 | 32.19 | 16.05 | 9.27  | 33.8   |
| 20                   |          | 80.41               | 69.82 | 58.44 | 35.11 | 20.34 | 8.25  | 20.5   |
| 21                   |          | 88.13               | 78.37 | 67.06 | 56.83 | 37.16 | 25.98 | 10.3   |
| 22                   |          | 93.27               | 85.41 | 75.19 | 64.73 | 52.87 | 34.71 | 5.76   |
| 23                   |          | 25.94               | 16.41 | 5.79  | 0.93  | 0.0   | 0.0   | >100   |
| 24                   |          | 92.71               | 86.35 | 73.16 | 60.33 | 34.72 | 18.28 | 9.38   |
| 25                   |          | 74.07               | 58.13 | 34.68 | 21.84 | 8.57  | 4.62  | 41.3   |
| Doxorubicin          |          | 90.76               | 88.45 | 84.26 | 77.78 | 70.82 | 55.16 | 4.65   |

### Table 3: Six dose growth inhibition percent and IC\textsubscript{50} values of the tested compounds of schemes 1, 2&3 against MCF7 cell line

| Sample conc. (µg/mL) | Compound | Growth inhibition % | IC\textsubscript{50} (µg/mL) |
|----------------------|----------|---------------------|-----------------------------|
|                      |          | 100                 | 50  | 25  | 12.5 | 6.25 | 3.125 |          |
| 2                    |          | 68.06               | 56.74 | 45.19 | 34.76 | 21.37 | 10.28 | 35.4   |
| 3                    |          | 93.16               | 86.38 | 73.24 | 57.05 | 31.27 | 15.94 | 10.8   |
| 4                    |          | 62.58               | 46.39 | 28.48 | 12.86 | 6.13  | 1.15  | 61.1   |
| 5a                   |          | 78.14               | 63.28 | 48.33 | 27.47 | 10.94 | 3.46  | 27.8   |
| 5b                   |          | 63.22               | 56.74 | 45.09 | 31.33 | 19.51 | 8.44  | 35.5   |
| 6                    |          | 79.38               | 68.44 | 56.82 | 37.15 | 25.41 | 12.79 | 20.7   |
| 7                    |          | 67.33               | 30.51 | 10.39 | 4.76  | 1.28  | 0.0   | 76.5   |
| 8                    |          | 76.14               | 57.26 | 30.7  | 15.33 | 8.11  | 4.24  | 43.2   |
| 9                    |          | 58.15               | 37.66 | 20.18 | 8.53  | 1.75  | 0.0   | 80.1   |
| 10                   |          | 62.46               | 31.83 | 15.94 | 8.57  | 1.28  | 0.0   | 79.7   |
| 11                   |          | 63.48               | 57.03 | 39.24 | 18.06 | 6.75  | 2.87  | 40.1   |
| 12                   |          | 89.39               | 81.24 | 73.82 | 65.28 | 53.49 | 20.86 | 5.92   |
| 13                   |          | 76.83               | 65.71 | 51.49 | 30.54 | 15.81 | 4.73  | 24.1   |
| 14                   |          | 80.33               | 65.48 | 53.32 | 27.49 | 15.13 | 7.62  | 23.4   |
| 15                   |          | 88.11               | 79.88 | 68.06 | 60.94 | 28.48 | 12.76 | 10.4   |
| 16                   |          | 75.82               | 63.54 | 26.36 | 9.15  | 3.66  | 1.28  | 40.9   |
| Doxorubicin          |          | 90.76               | 88.45 | 84.26 | 77.78 | 70.82 | 55.16 | 4.84   |

### Table 4: Six dose growth inhibition percent and IC\textsubscript{50} values of the tested compounds of schemes 4&5 against MCF7 cell line

| Sample conc. (µg/mL) | Compound | Growth inhibition % | IC\textsubscript{50} (µg/mL) |
|----------------------|----------|---------------------|-----------------------------|
|                      |          | 100                 | 50  | 25  | 12.5 | 6.25 | 3.125 |          |
| 17                   |          | 90.18               | 82.54 | 71.96 | 60.59 | 41.03 | 27.54 | 9.12   |
| 18                   |          | 91.48               | 84.57 | 75.15 | 64.71 | 56.82 | 40.37 | 4.95   |
| 19                   |          | 71.24               | 59.05 | 46.78 | 31.57 | 19.33 | 8.52  | 31.6   |
| 20                   |          | 85.62               | 73.25 | 61.49 | 46.26 | 31.85 | 17.63 | 15.8   |
| 21                   |          | 85.44               | 76.02 | 60.79 | 43.15 | 30.02 | 23.44 | 17.4   |
| 22                   |          | 91.69               | 86.58 | 74.26 | 63.88 | 51.33 | 30.57 | 6.05   |
| 23                   |          | 35.33               | 28.52 | 21.31 | 15.97 | 3.13  | 0.0   | >100   |
| 24                   |          | 27.82               | 10.33 | 3.57  | 0.83  | 0.0   | 0.0   | >100   |
| 25                   |          | 61.44               | 50.88 | 27.33 | 18.51 | 9.35  | 2.54  | 49.1   |
| Doxorubicin          |          | 90.76               | 88.45 | 84.26 | 77.78 | 70.82 | 55.16 | 4.84   |
Conclusion:-
Twenty five different aminothiazole derivatives were synthesized via various synthetic pathways. The newly synthesized compounds were evaluated for their cytotoxic activity against two human cancer cell lines, HepG2 and MCF7 cell lines. Among the tested compounds, 3, 7, 15, 17, 18, 21, 22 and 24 exhibited promising anticancer activity.
against HepG2 cell line with IC₅₀ values ranging from 4.78-11.4 µg/mL. While compounds 3, 7, 15, 17, 18 and 22 exerted highly potent anticancer activity against MCF7 cell line with IC₅₀ values ranging from 4.95-10.8 µg/mL. Among which compounds 3, 7, 15, 17, 18 and 22 showed dual activity against both cancer cell lines.

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