ISSN NO. 2320-5407

Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/2347
DOI URL: http://dx.doi.org/10.21474/IJAR01/2347

RESEARCH ARTICLE

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

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Manuscript Info

Manuscript History

Received: 30 September 2016
Final Accepted: 30 October 2016
Published: November 2016

Key words:-
2-Aminothiazole, anticancer, HepG2, MCF7.

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 C_2 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 $\mathbf{5_a}$ and $\mathbf{5_b}$, 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 IC_{50} 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 IC_{50} values ranging from 4.95-10.8 µg/mL.

Introduction:-

Five-membered aromatic nitrogen and sulphur containing heterocyclesoccurring 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}.The ^{1}HNMR 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-d_6 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 (1_a):-
An equimolar mixture of phenacylbromide (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 allowed to attain room temperature, ethanol was evaporated and the precipitate formed is then filtered, dried and crystallized from ethanol to yield compound **1**.

White needle crystals, **yield**: 0.05 g (75 %), **m.p.**: 147-149^{o}C as reported [13]. **Anal. form**: $C_9H_8N_2S$ (176.24). **IR (KBr, cm^{-1})**: 3269, 3167 (NH_2); 2985, 2958 (CH-aromatic); 1627 (C=N); 1571 (C=C). **MS m/z (relative intensity %):** 178 (M^{+}+2, 5); 177 ($M^{+\cdot}$+1 , 12); 176 ($M^{+\cdot}$, 100).

Phenyl-3-(4-phenylthiazol-2-yl)thiourea (2):-
An equimolar mixture of 2-aminothiazole derivative $\mathbf{1_a}$ (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**.

Yellow powder, **yield:** 0.13 g (41 %), **m.p.**: 106-108^{o}C. **Anal. form**: $C_{16}H_{13}N_3S_2$ (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). **^{1}H-NMR (DMSO-d_6, δ ppm):** 5.73 (s, 1H, NH-thiazole, D_2O exchangeable); 6.95-6.98 (m, 1H, NH-C_6H_5-C_4-H); 7.22-7.28 (m, 2H, NH-C_6H_5-$C_{3,5}$-H); 7.32 (s, 1H, thiazole-C_5-H); 7.38-7.53 (m, 1H, thiazole-C_6H_5-C_4-H); 7.60 (t, 2H, J=7.8 Hz, thiazole-C_6H_5-$C_{3,5}$-H); 7.81 (d, 2H, J=7.5 Hz, NH-C_6H_5-$C_{2,6}$-H); 7.90 (d, 2H, J=7.8 Hz, thiazole-C_6H_5-$C_{2,6}$-H); 8.76 (s,1H, NH-C_6H_5, D_2O exchangeable). **MS m/z (relative intensity %):** 311 (M^{+}, 2).

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, phenacyl bromide (0.2 g, 1 mmol) was added. The reaction mixture was then heated under reflux in absolute ethanol (15 mL) for 22 hours. The reaction was allowed to cool, poured on to ice cold water and the precipitate formed was then filtered, dried and crystallized from ethanol to give compound **3**.

Brown powder, **yield**: 0.18 g (44 %), **m.p.**: 108-110^{o}C. **Anal.form**: $C_{24}H_{19}N_3S_2$ (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); 3055, 3032(CH-aromatic); 1610 (C=N); 1597 (C=C). **^{1}H NMR (DMSO-d_6, δ ppm):** 4.00 (s, 1H, thiazole-C_2-H); 6.48-6.60 (m, 1H, N-C_6H_5-C_4-H); 6.81-6.89 (m, 2H, N-C_6H_5-$C_{2,6}$-H); 6.93 (s, 1H, dihydrothiazole-C_5-H); 7.00-7.18 (m, 3H, N-C_6H_5-C_4-H); 7.24 (s, 1H, thiazole-C_5-H); 7.34-7.44 (m, 3H, thiazole NH-C_6H_5-$C_{3,5}$-H & thiazole-C_6H_5-C_4-H); 7.50-7.59 (m, 4H, dihydrothiazole-C_4-C_6H_5-$C_{2,6}$-H & thiazole-C_6H_5-$C_{3,5}$-H); 7.98 (d, 2H, J=8.1 Hz, thiazole-C_6H_5-$C_{2,6}$-H); 8.65 (s, 1H, NH, D_2O exchangeable). **MS m/z (relative intensity %):** 413 ($M^{+\cdot}$, 1.5); 412 ($M^{+\cdot}$-1, 3.6); 411 ($M^{+\cdot}$ -2, 11.5).

Synthesis of 4-Phenyl-N,N-di(prop-2-ynyl)thiazol-2-amine (4):-
To an equimolar mixture of 2-aminothiazole derivative $\mathbf{1_a}$ (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 heated under reflux for 20 hours, then allowed to cool and the precipitate formed was 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^{o}C. **Anal. Form**: $C_{15}H_{12}N_2S$ (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≡C); 1614 (C=N); 1600 (C=C). **^{1}H NMR (DMSO-d_6, δ ppm):** 4.15 (s, 4H, two CH_2); 6.99 (s, 1H, thiazole-C_5-H); 7.15-7.55 (m, 3H, C_6H_5-$C_{3,4}$-H); 7.78 (d, 2H, J=8.4Hz, C_6H_5-$C_{2,6}$-H); 8.5 (s, 2H, two -C≡CH). **MS m/z (relative intensity %):** 253 ($M^{+\cdot}$+1, 3); 252 ($M^{+\cdot}$, 1).

Synthesis of 2-((2-Imino-4-phenylthiazol-3(2H)-yl)methyl)succinic acid ($5_{a,b}$):-
An equimolar mixture of the selected 2-aminothiazole derivative $\mathbf{1}_{a,b}$(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 filtered, washed with ethanol, dried and crystallized from ethanol to yield compounds $\mathbf{5}_{a,b}$.

2-((2-Imino-4-phenylthiazol-3(2H)-yl)methyl)succinic acid (5_a):-
Pale yellow powder**, yield**: 0.14 g (45 %), **m.p.:** 100-102ºC. **Anal. form**: $C_{14}H_{14}N_2O_4S$ (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 ($M^{+\cdot}$, 15).

2-((4-(4-Bromophenyl)-2-iminothiazol-3(2H)-yl)methyl)succinic acid (5_b):-
Colourless crystals, **yield**: 0.23 g (60 %), **m.p.:** 142-144 ˚C. **Anal. form**: $C_{14}H_{13}BrN_2O_4S$ (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); 2933 (C-H aromatic); 2856 (C-H aliphatic); 1701 (C=O); 1627 (C=N); 1540 (C=C); 840 (p-Br-phenyl). **^{1}H NMR (DMSO-d_6,** δ **ppm):** 2.01 (s, 2H, N-CH_2); 5.70 (s, 2H, CH_2COOH); 6.12(s, 1H, CHCOOH); 6.94 (s, 1H, NH, D_2O exchangeable); 7.03 (s, 1H, thiazole-C_5-H); 7.53 (d, 2H, J=8.4Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 7.74 (d, 2H, J=8.4Hz, 4-Br- C_6H_4-$C_{3,5}$-H); 12.22 (s, 2H, two COOH, D_2O exchangeable). **MS m/z (intensity %):** 387 ($M^{+\cdot}$+2, 3); 384 ($M^{+\cdot}$-1, 4).

Synthesis of 4-Phenylthiazol-2-ylamino-N-(thioformyl)benzamide (6):-
A suspension of 2-aminothiazole derivative$\mathbf{1}_a$ (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 precipitate formed was filtered, washed with acetone, dried and crystallized from ethanol to yield compound **6**.

White powder, **yield:** 0.156 g (45%), **m.p**.: 80-82ºC. **Anal.form.**: $C_{17}H_{13}N_3OS_2$ (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). **^{1}H NMR (DMSO-d_6,** δ **ppm):** 5.45 (s, 1H, NH-thiazole, D_2O exchangeable); 7.42-7.49 (m, 2H, COC_6H_5-$C_{3,5}$-H); 7.55 (d, 2H, J=7.5Hz, thiazole-C_6H_5-$C_{3,5}$-H); 7.62-7.65 (m, 2H, thiazole-C_6H_5-$C_{2,6}$-H); 7.68 (s, 1/2H, OH-tatumer, D_2O exchangeable); 7.86-8.04 (m, 1H, CO-C_6H_5-C_4-H); 8.13 (d, 2H, J=8.1Hz, CO-C_6H_5-$C_{2,6}$-H); 12.81 (s, 1/2H, NH-CO, D_2O exchangeable). **MS m/z (relative intensity %):** 340 ($M^{+\cdot}$+1, 2); 339 ($M^{+\cdot}$, 6).

Synthesis of N-(6-Phenyl-2H-thiazolo[3,2-b][1,2,4]thiadiazol-2-ylidene) benzamide (7):-
Equimolar amounts ofcompound**6** (0.3 g, 1 mmol) and phosphorous oxychloride (0.14 g, 0.13 mL, 1 mmol) wereheated together at 250 ºC 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 ºC. **Anal. form**: $C_{17}H_{11}N_3OS_2$ (337.42). **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). **^{1}H NMR (DMSO-d_6,** δ **ppm):** 6.90-6.95 (m, 1H, C_6H_5-C_4-H); 7.02 (s, 1H, thiazole-C_5-H); 7.09-7.13 (m, 2H, C_6H_5-$C_{3,5}$-H); 7.40-7.46 (m, 2H, CO-C_6H_5-$C_{3,5}$-H); 7.52 (d, 2H, J = 6.9 Hz, C_6H_5-$C_{2,6}$-H); 7.59-7.61 (m, 1H, CO-C_6H_5-C_4-H); 7.71 (d, 2H, J = 6.6Hz, CO-C_6H_5-$C_{2,6}$-H).

Synthesis of N-(4-(4-Bromophenyl)thiazol-2-yl)-2-formylbenzamide (8):-
An equimolar mixture of 2-aminothiazole derivative $\mathbf{1}_b$ (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 ºC.**Anal. form**: $C_{17}H_{11}BrN_2O_2S$ (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).**^{1}H NMR (DMSO-**

***d_6*, δ ppm):** 6.80 (s, 1H, thiazole-C_5-H); 7.00-7.20 (m, 4H, 4-Br-C_6H_4); 7.41 (s, 1/2H, OH-tautomer, D_2O exchangeable); 7.45-7.60 (m, 2H, 2-CHO-C_6H_4-$C_{4,5}$-H); 7.61-7.96 (m, 2H, 2-CHO-C_6H_4-$C_{3,6}$-H); 9.08 (s, 1/2H, NH, D_2O exchangeable); 9.25 (s, 1H, CHO, D_2O exchangeable). **MS m/z (relative intensity %):** 389 ($M^{+\cdot}$+2, 5); 388 ($M^{+\cdot}$+1, 21); 387 ($M^{+\cdot}$, 11).

Synthesis of N-(4-Chlorobenzylidene)-4-(4-bromophenyl)thiazol-2-amine (9):-
To a suspension of 2-aminothiazole derivative **1_b** (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**.

Yellowe powder**, yield**: 0.23 g (62 %), **m.p.**:204-206°C. **Anal. form**: $C_{16}H_{10}BrClN_2S$ (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); 1616 (C=N); 1489 (C=C); 1068 (p-Cl-phenyl); 829 (C-Br). **^{1}H NMR (DMSO-*d_6*, δppm):** 6.68 (s, 1H, thiazole-C_5-H); 6.96 (s, 1H, N=CH); 7.00-7.8 (m, 6H, 4-Br-C_6H_4-$C_{3,5}$-H & 4-Cl-C_6H_4); 7.82-8.17 (m, 2H, 4-Br-C_6H_4-$C_{2,6}$-H). **MS m/z (relative intensity %):** 380 ($M^{+\cdot}$+2, 5); 378 ($M^{+\cdot}$, 10).

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 concentrated, allowed to cool to room temperature and the precipitate formed was filtered, washed with ethanol, dried and crystallized from ethanol to yield compound **10**.

White powder, **yield**: 0.17 g (37 %), **m.p.**: 398-400°C. **Anal. form**: $C_{18}H_{12}BrClN_2OS_2$ (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); 3032 (CH-aromatic); 2922 (CH-aliphatic); 1683 (C=O); 1640 (C=N); 1060 (p-Cl-phenyl); 800 (C-Br). **MS m/z (intensity %):** 454 ($M^{+\cdot}$+2, 4); 452 ($M^{+\cdot}$, 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** (0.38 g, 1 mmol) in dry benzene/ triethylaminemixture (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.**: 140-142ºC. **Anal.form**: $C_{18}H_{11}BrCl_2N_2OS$ (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); 1693 (C=O); 1531 (C=C aromatic). **^{1}H NMR (DMSO-*d_6*, δ ppm):** 2.30 (s, 1H, azetidinyl-C_4-H); 4.05-4.18 (m, 1H; azetidinyl-C_3-H); 6.98 (s, 1H, thiazole-C_5-H); 7.05-7.21 (m, 4H, 4-Cl-C_6H_4); 7.35-7.43 (m, 4H, 4-Br-C_6H_4). **MS m/z (intensity %):** 458 ($M^{+\cdot}$+4, 0.5); 454 ($M^{+\cdot}$, 6).

Synthesis of Ethyl (4-(4-bromophenyl)thiazol-2-yl carbamoyl)formate (12):-
An equimolar mixture of 2-aminothiazole **1_b** (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**: $C_{13}H_{11}BrN_2O_3S$ (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). **^{1}H NMR (DMSO-*d_6*, δ ppm):** 3.98 (t, 3H, J=7 Hz, CH_2CH_3); 4.32 (q, 2H, J=7Hz, CH_2CH_3); 7.09 (s, 1H, thiazole-C_5-H); 7.59 (d, 2H, J=8.4Hz, 4-Br-C_6H_4-$C_{3,5}$-H); 7.85 (d, 2H, J=8.4Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 8.7 (s,

1/2H, OH-tautomer, D_2O exchangeable); 13.10 (s, 1/2H, NH, D_2O exchangeable). **MS m/z (relative intensity %):** 357 ($M^{+\cdot}$+2, 2); 356 ($M^{+\cdot}$+1, 12).

Synthesis of 3-(4-Bromophenyl)-7-(methylthio)-5-oxo-5H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (13):-
An equimolar mixture of **1_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^{o}C. **Anal.form**: $C_{14}H_8BrN_3OS_2$ (378.27). **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); 2240 (C≡N); 1660 (C=O); 1589 (C=N); 1525 (C=C); 829 (C-Br). **MS m/z (relative intensity %):** 380 ($M^{+\cdot}$+2, 1); 378 ($M^{+\cdot}$, 3).

Synthesis of Ethyl 3-(4-bromophenyl)-5-(4-chlorophenyl)-7-methyl-5H-thiazolo [3,2-a]pyrimidine-6-carboxylate (14):-
An equimolar mixture of compound **1_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^{o}Cfor 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 ^{o}C.**Anal. form**: $C_{22}H_{18}BrClN_2O_2S$ (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); 2880 (CH-aliphatic); 1716 (C=O); 1624 (C=N); 1525 (C=C); 1242, 1070 (C-O-C); 1070 (p-Cl-phenyl); 829 (C-Br). **^{1}H NMR (DMSO-d_6, δ ppm):** 0.80-1.30 (m, 3H, O-CH_2-CH_3); 1.30 (s, 3H, CH_3); 3.80-4.40 (m, 2H, O-CH_2-CH_3); 5.02 (s, 1H, thiazolopyrimidine-C_5-H); 6.15 (s, 1H, thiazolopyrimidine-C_2-H); 7.14-7.82 (m, 8H, 4-Cl-C_6H_4& 4-Br-C_6H_4).

Synthesis of 4-(4-Bromophenyl)-N,N-(diethyl formate)thiazol-2-amine (15):-
2-Aminothiazole derivative **1_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 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^{o}C. **Anal.form**: $C_{15}H_{15}BrN_2O_4S$ (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=N); 1577 (C=C); 1253, 1064 (C-O-C); 866 (C-Br). **MS m/z (relative intensity %):** 401 ($M^{+\cdot}$+2, 4); 400 ($M^{+\cdot}$+1, 2); 399 ($M^{+\cdot}$, 5); 398 ($M^{+\cdot}$-1, 3).

Synthesis of 4-(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^{o}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^{o}C. **Anal. form**: $C_{11}H_7BrN_4O_2S$ (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_6H_4-$C_{2,6}$-H); 7.60-7.66 (m, 2H, 4-Br-C_6H_4-$C_{3,5}$-H); 7.68 (s, 1H, NH, D_2O exchangeable); 7.71 (s, 1H, NH, D_2O exchangeable).

Synthesis of N-(4-(4-Bromophenyl)thiazol-2-yl)-2-chloroacetamide (17):-
To a well stirred solution of 2-aminothiazole derivative **1_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**: $C_{11}H_8BrClN_2OS$ (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). **^{1}H NMR (DMSO-*d*$_6$, δ ppm):** 4.17 (s, 2H, CH_2); 7.14 (s, 1/2H, OH tautomer, D_2O exchangeable); 7.62 (d, 2H, J=8.4Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 7.71 (s, 1H, thiazole-C_5-H); 7.84 (d, 2H, J=8.4 Hz, 4-Br-C_6H_4-$C_{3,5}$-H); 12.59 (s, 1/2H, NH, D_2O exchangeable). **MS m/z (relative intensity %):** 336 (M$^{+\cdot}$ +4, 0.2); 334 (M$^{+\cdot}$ +2, 5); 332 (M$^{+\cdot}$, 17).

Synthesis of 2-(4-(4-Bromophenyl)thiazol-2-ylamino)thiazol-4(5H)-one (18):-
An equimolar mixture of compound **17** (0.33 g, 1 mmol) and ammonium thiocyanate (0.076 g, 1 mmol) was heated under reflux in absolute ethanol (15 mL) for 1 hour. The reaction mixture was then allowed to cool, poured onto ice cold water and the solid formed was filtered, washed with ethanol, dried and crystallized from dioxan to afford compound **18**.

White crystalline powder, **yield**: 0.1 g (28 %), **m.p.**: 95-97°C. **Anal.form**: $C_{12}H_8BrN_3OS_2$ (354.25). **Calcd**. (%): C, 40.69; H, 2.28; N, 11.86; S, 18.10. **Found** (%): C, 40.72; H, 2.31; N, 11.95; S, 18.19. **IR (KBr, cm^{-1}):** 3444 (broad OH tautomer); 3267 (NH); 3089, 3064 (C-H aromatic); 2931 (C-H aliphatic); 1730 (C=O); 1639 (C=N); 1574 (C=C); 850 (C-Br). **^{1}H NMR (DMSO-*d*$_6$, δ ppm):** 4.03 (s, 1H, thiazole-CH_2-under DMSO); 7.07 (s, 1H, thiazole-C_5-H); 7.30 (s, 1H, OH tautomer, D_2O exchangeable); 7.46 (s, 1H, NH, D_2O exchangeable); 7.64 (d, 2H, J=8.7Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 7.73 (d, 2H, J=8.7Hz, 4-Br-C_6H_4-$C_{3,5}$-H).

1-(4-(4-Bromophenyl)thiazol-2-yl)hydrazine (19):-
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, 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**: $C_9H_8BrN_3S$ (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_2); 2926 (CH-aromatic); 1589 (C=N); 1487 (C=C); 827 (C-Br). **^{1}H NMR (DMSO-*d*$_6$, δ ppm):** 3.72 (s, 2H, NH_2, D_2O exchangeable); 4.38 (s, 1H, NH, D_2O exchangeable); 7.22 (s, 1H, thiazole-C_5-H); 7.78 (d, 2H, J=8.4 Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 8.20 (d, 2H, J=8.4 Hz, 4-Br-C_6H_4-$C_{3,5}$-H). **MS m/z (relative intensity):** 272 (M$^{+\cdot}$ +2, 4); 271 (M$^{+\cdot}$ +1, 8).

Synthesis of4-(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, 0.20 mL, 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**: $C_{16}H_{11}Br_2N_3OS$ (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$^{+\cdot}$+2, 2).

Synthesis of6-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**: $C_{16}H_9Br_2N_3S$ (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$^{+\cdot}$+2, 0.40); 435 (M$^{+\cdot}$, 0.3).

Synthesis of4-(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^{o}C. **Anal.form**: $C_{10}H_9BrN_4S$ (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, NH_2); 2966, 2927 (C-H aromatic); 1633 (C=N); 1533 (C=C aromatic); 850 (C-Br). **^{1}H-NMR (DMSO-*d*$_6$,** δ **ppm):** 6.99 (s, 2H, NH_2, D_2O exchangeable); 7.04 (s, 1H, thiazole-C_5-H); 7.53 (d, 2H, J= 8.7Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 7.76 (d, 2H, J=8.7 Hz, 4-Br-C_6H_4-$C_{3,5}$-H); 8.30 (s, 1H, NH, D_2O exchangeable); 12.20 (s, 1H, C=NH, D_2O exchangeable). **MS m/z (relative intensity %):** 299 ($M^{+\cdot}$+2, 1); 297 ($M^{+\cdot}$, 3).

Synthesis ofN-(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^{o}C. **Anal.form**: $C_{15}H_{13}BrN_4S$ (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); 2897, 2879, 2860 (C-H aliphatic); 1600 (C=N); 1556, 1539 (C=C); 827 (C-Br). **^{1}H NMR (DMSO-*d*$_6$,** δ **ppm):** 3.00 (s, 6H, two CH_3); 3.47 (s, 1H, NH, D_2O exchangeable); 7.36 (s, 1H, thiazole-C_5-H); 7.43-7.92 (m, 4H, 4-Br-C_6H_4); 8.15 (s, 1H, pyrimidine-C_5-H). **MS m/z (relative intensity %):** 363 ($M^{+\cdot}$+2, 2).

Synthesis of N-(4-(4-Bromophenyl)thiazol-2-yl)-4-methyl-6-phenyl-pyrimidin-2-amine (24):-
A mixture of compound **22** (0.29 g, 1 mmol) and benzoylacetone (0.32 g, 0.32 mL, 2 mmol) was heated in an oil bath at 250^{o}C for 2 hours. The reaction mixture was 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^{o}C. **Anal.form**: $C_{20}H_{15}BrN_4S$ (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). **^{1}H NMR (DMSO-*d*$_6$,** δ **ppm):** 2.44 (s, 3H, CH_3); 6.34 (s, 1H, thiazole-C_5-H); 7.48-7.86 (m, 3H, C_6H_5-$C_{3,4,5}$-H); 7.88-7.95 (m, 4H, C_6H_5-$C_{2,6}$-H & 4-Br-C_6H_4-$C_{2,6}$-H); 8.00 (d, 2H, J=8.4Hz, 4-Br-C_6H_4-$C_{3,5}$-H); 8.27 (s, 1H, pyrimidine-C_5-H); 10.53 (s, 1H, NH, D_2O 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^{o}C for 2 hours. The reaction mixture was then allowed to cool, triturated with ethanol/ether mixture (1:1, 4 mL) 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^{o}C. **Anal.form**: $C_{13}H_9BrN_4O_2S$ (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 (NH); 2981, 2958 (CH-aromatic); 1685 (C=O); 1590 (C=N); 1560, 1544 (C=C); 821 (C-Br).**^{1}H NMR (DMSO-*d*$_6$,** δ **ppm):** 7.60 (s, 1H, thiazole-C_5-H); 7.61 (d, 2H, J=6.6 Hz, 4-Br-C_6H_4-$C_{2,6}$-H); 7.71 (s, 1H, NH, D_2O exchangeable); 7.83 (d, 2H, J=6.6 Hz, 4-Br-C_6H_4-$C_{3,5}$-H); 7.90 (s, 1H, pyrimidine-C_5-H); 12.21 (s,1H, pyrimidine-NH, D_2O); 12.44 (s, 1H, OH, D_2O exchangeable).

Results and Discussion:-

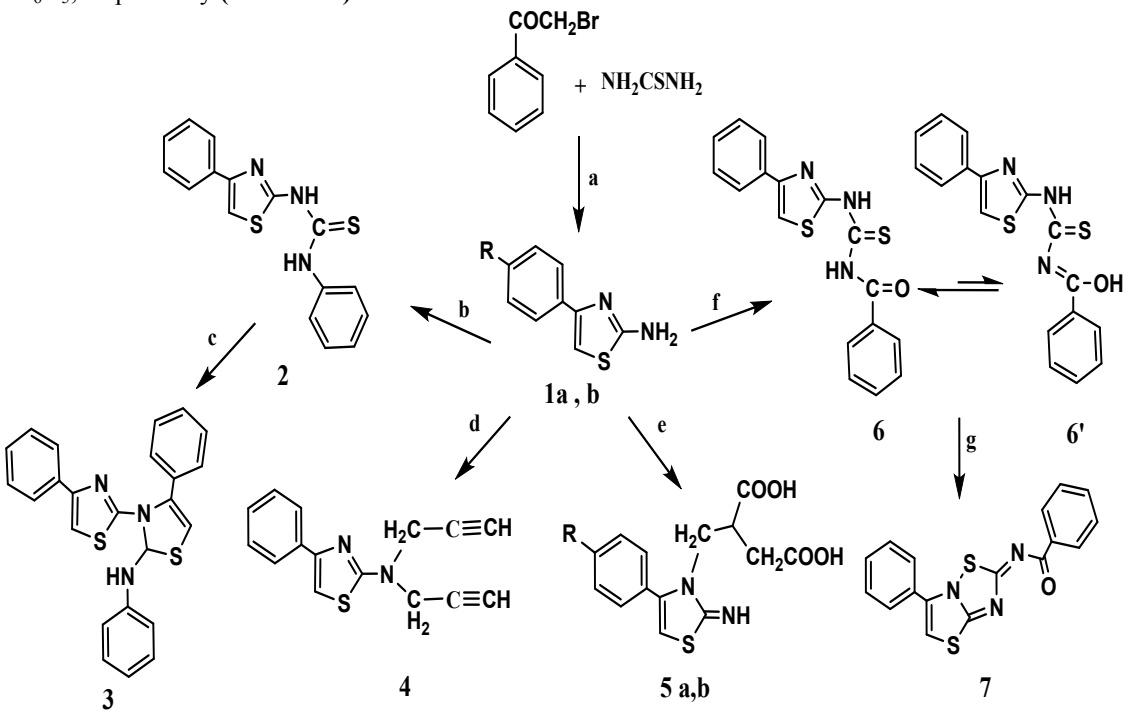
2-Amino-4-phenylthiazole derivative**1$_a$** was prepared according to the reported procedure [13] which was further utilized as a building block to synthesize various phenylthiazole derivatives substituted either at the thiazole amino function or fused to the thiazole back bone.However, treatment of compound **1$_a$**with phenylisothiocyanate furnished the corresponding thiourea derivative **2** that was further cyclized to its aminothiazoleanalogue **3**. The **^{1}H NMR** spectrum of compound **2** revealed a deuterium oxide exchangeable singlet signal at δ 8.76 ppm attributed to NH-C_6H_5 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 **^{1}H NMR** spectrum of compound **3**

revealed a deuterium oxide exchangeable singlet signal at δ 8.65 ppm attributed to NH. In addition,dihydrothiazole C_5-H was observed as a singlet signal at δ 6.93 ppm.

We carried out the synthesis of the target *N,N*-dipropynylthiazole-2-amine derivative **4** by refluxing one equivalent of 2-amino-4-phenyl-thiazole $\mathbf{1_a}$ with two equivalents of propargyl bromide.The **^{1}H NMR** spectrum of compound **4** revealed a singlet signal at δ 4.15 ppm attributed to two CH_2 protons. In addition to a singlet signal at δ 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 $\mathbf{5_{a,b}}$ were synthesized by stirring the appropriate 2-amino-4-phenylthiazole derivatives$\mathbf{1_{a,b}}$with an equimolar amount of itaconic acid at room temperature. The **IR** spectra of compounds $\mathbf{5_{a,b}}$ 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 of2-amino-4-phenylthiazole $\mathbf{1_a}$ with benzoyl isothiocyanatewhich was further cyclized to its correspondingthiadiazolederivative**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 δ 5.45 and δ 7.68 ppm attributed to NH and OH tautomer; respectively. However, the **^{1}H NMR** spectrum of compound **7** revealed two multiplet signals at δ 7.40-7.46 ppm and δ 7.59-7.61ppm attributed to $C_{3,5}$ and C_4 protons of CO-C_6H_5; respectively.**(Scheme 1)**



a: R=H; **b**: R=Br

Reagents and conditions: (a) Absolute ethanol/ reflux; **(b)** Phenyl isothiocyanate/ dioxan/ reflux;
(c) Phenacyl bromide/ absolute ethanol/ reflux; **(d)** Popargyl bromide/ anhydrous K_2CO_3/ KI/ absolute Ethanol/ reflux;
(e) Itaconic acid/ absolute ethanol/ stirring/ r. t; **(f)** Aroyl isothiocyanate/acetone/ reflux; **(g)** $POCl_3$/ fusion.

Scheme 1

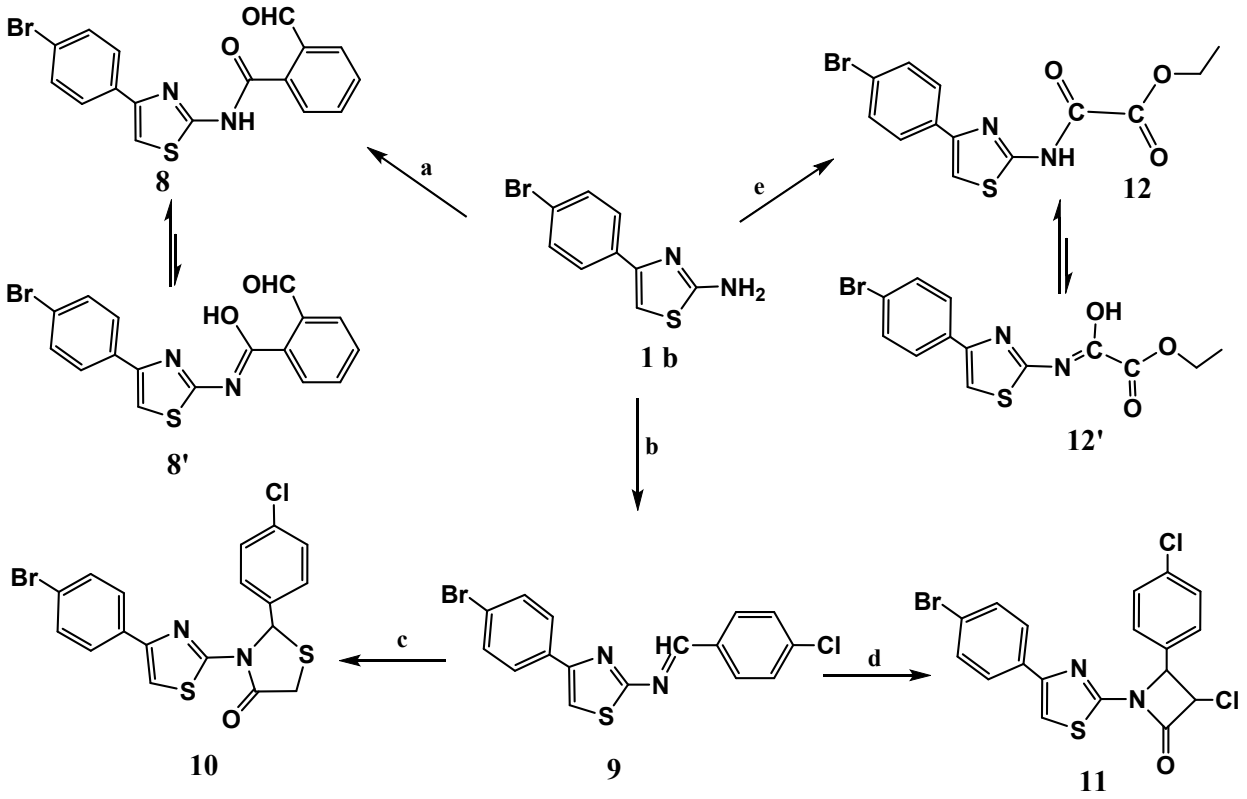
In our recent study, the benzamide derivative **8** was prepared via the reaction of equimolar amounts of 2-formylbenzoic acid and 2-aminothiazole derivative $\mathbf{1_b}$ in methanol under reflux conditions. The **IR** spectrum of compound **8** showed an absorption band at 3367 cm^{-1} corresponding 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 δ 7.41 and 9.08 ppm attributed to OH tautomer andthiazole 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 abroad 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 δ 2.30 ppm attributed to azetidinyl-C_4-H proton. In addition to a multiplet signal at δ 4.05-4.18 ppm due to azetidinyl-C_3-H proton.

Furthermore,2-aminothiazole derivative $\mathbf{1_b}$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 δ 3.98 ppm attributed to CH_3 protons and a quartet signal at δ 4.32 ppm attributed to CH_2 protons. In addition to, two deuterium oxide exchangeable signals at δ 8.7 ppm and δ 13.10 ppm due to OH and NH of the tautomer respectively.**(Scheme 2)**

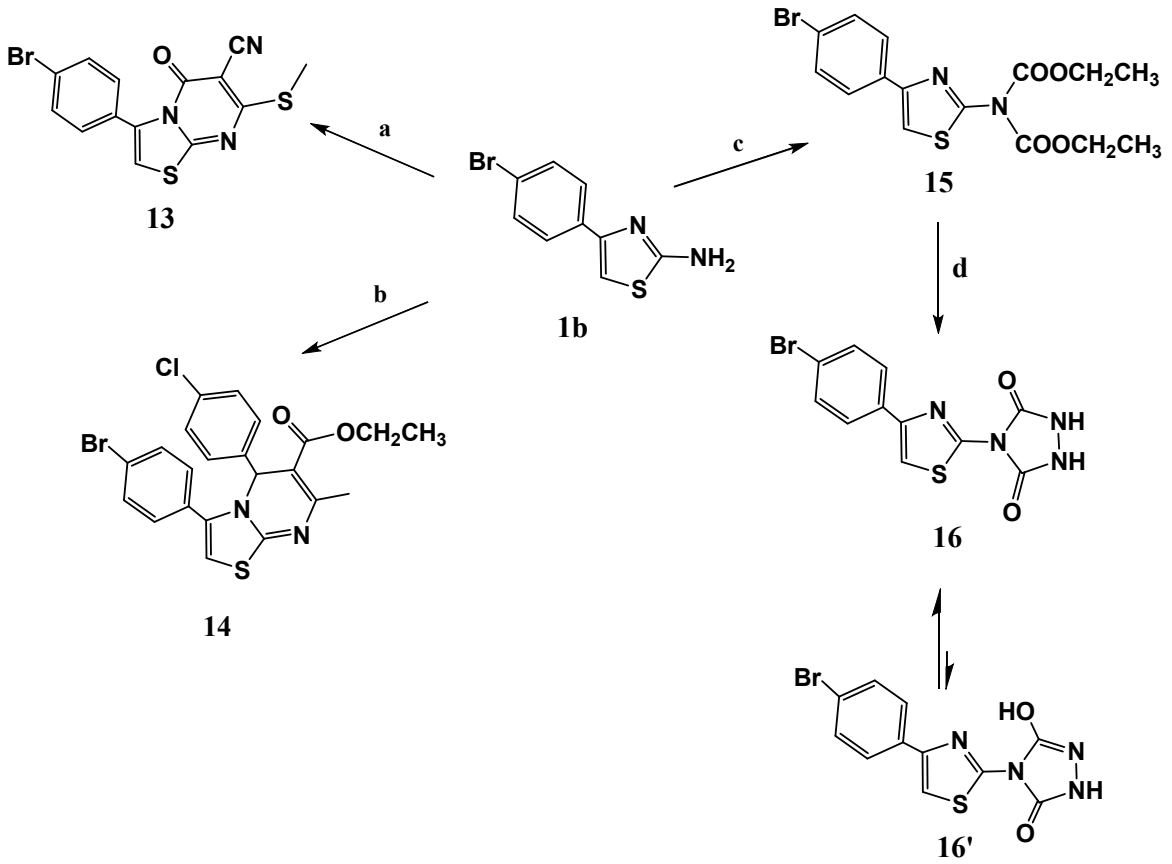
Compound **13** was prepared via refluxing equimolar amounts of 2-aminothiazole derivative $\mathbf{1_b}$and 2-ethoxy-3,3-bis(methylthio)acrylonitrilein dimethylformamide containing anhydrous potassium carbonate as a base. The **IR**spectrum of Compound **13** showed two characteristic absorption bands at 2240 and 1660 cm^{-1} corresponding to C≡N and C=O functions; respectively.



Reagents and conditions: **(a)** 2-Formylbenzoic acid/ methanol/ reflux; **(b)** 4-Chlorobenzaldehyde/ absolute ethanol/ gl.AcOH/ reflux;**(c)** Thioglycolic acid/ anhydrous $ZnCl_2$/ THF/ W.B; **(d)** Chloroacetyl chloride/ TEA/ benzene/ reflux; **(e)** Diethyl oxalate/ piperidine/absolute ethanol/ reflux.

Scheme 2

Thethiazolopyrimidine derivative **14** was obtained via heating of the multicomponent reaction mixture of 2-aminothiazole derivative $\mathbf{1_b}$, 4-chlorobenzaldehyde and ethyl acetoacetate. The **IR** spectrum of compound **14** lacked absorption bands due to NH_2 function and showed an absorption band at 1716 cm^{-1} corresponding to ester carbonyl function. While, the **^{1}H 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-CH_2-CH_3 and O-CH_2-CH_3 protons; respectively.



Reagents and conditions: (a) 2-cyano-3,3-bis(methylthio)acrylate / K_2CO_3/ DMF/ reflux;
(b) 4-Chlorobenzaldehyde / EAA/ fusion; **(c)** Ethyl chloroformate/ fusion; **(d)** Hydrazine hydrate/ fusion.

Scheme 3

In our recent study, $\mathbf{1_b}$ upon treatment with excess of ethyl chloroformate yieldedthetarget *N,N*-diethylformate thiazole-2-amine **15**.The **IR** spectrum of compound **15** lacked absorption bands corresponding to NH_2 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 **^{1}H 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 $\mathbf{1_b}$ 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 **^{1}H 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 CH_2 protons.

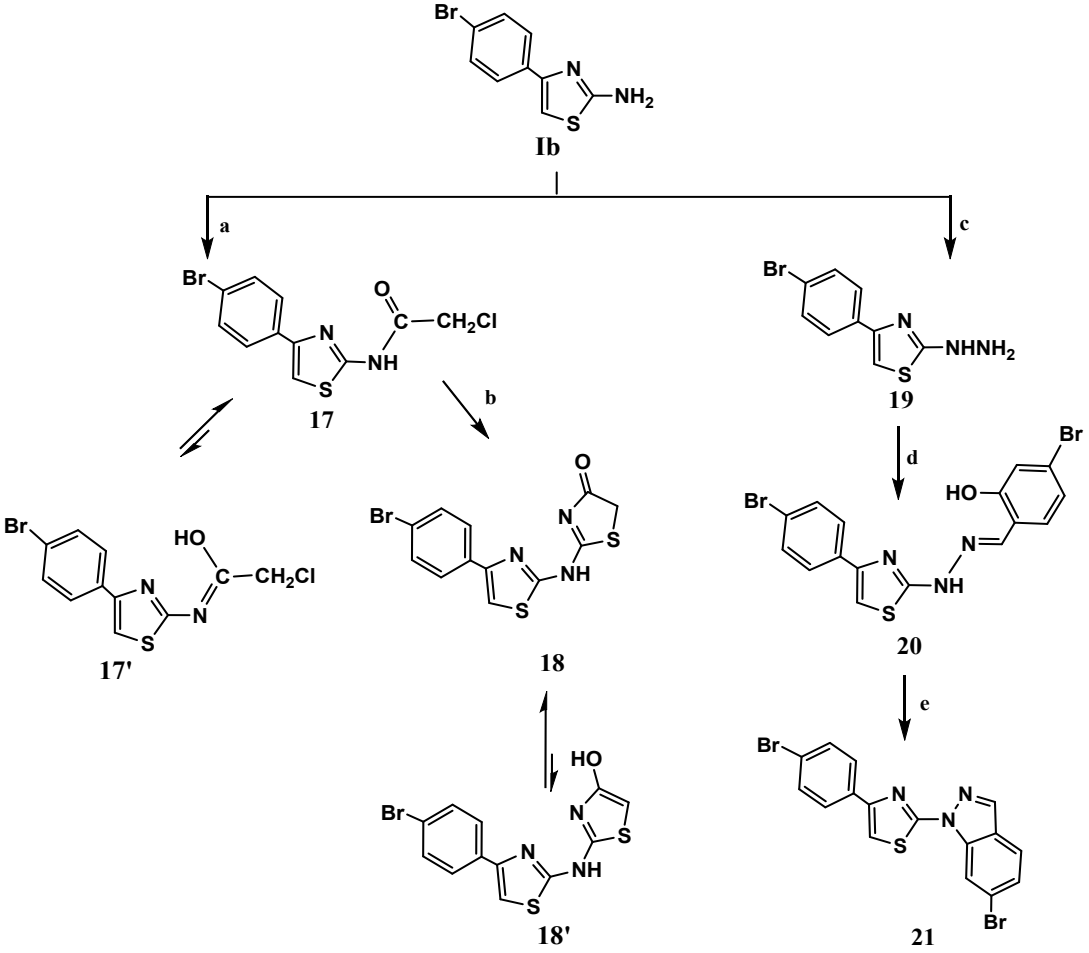
In addition, thecyclization of**17** to the target thiazolylthiazolone derivative **18**was achieved via heating equimolar mixture of **17** and ammonium thiocyanate in ethanol under reflux. The **IR** spectrum of compound **18** showed a broad absorption band at 3444 cm^{-1} corresponding to OH of the tautomer. The **^{1}H 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 of2-aminothiazole derivative $\mathbf{1_b}$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 δ 3.72 and 4.38 ppm attributed to NH and NH_2.

However, the thiazolyl hydrazine derivative **19** furnished the correspondingthiazolylamino-N-iminomethylphenol derivative **20**upon heating with oneequivalent of5-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** tothiazolylindazole 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 $\mathbf{1_b}$ to obtain the thiazolylguanidine intermediate **22**. The **^{1}H NMR** spectrum of compound **22** revealed three deuterium oxide exchangeable singlet signals at δ 6.99 ppm, δ 8.30 ppm and δ 12.20 ppm attributed to NH_2, NH and C=NH protons; respectively.



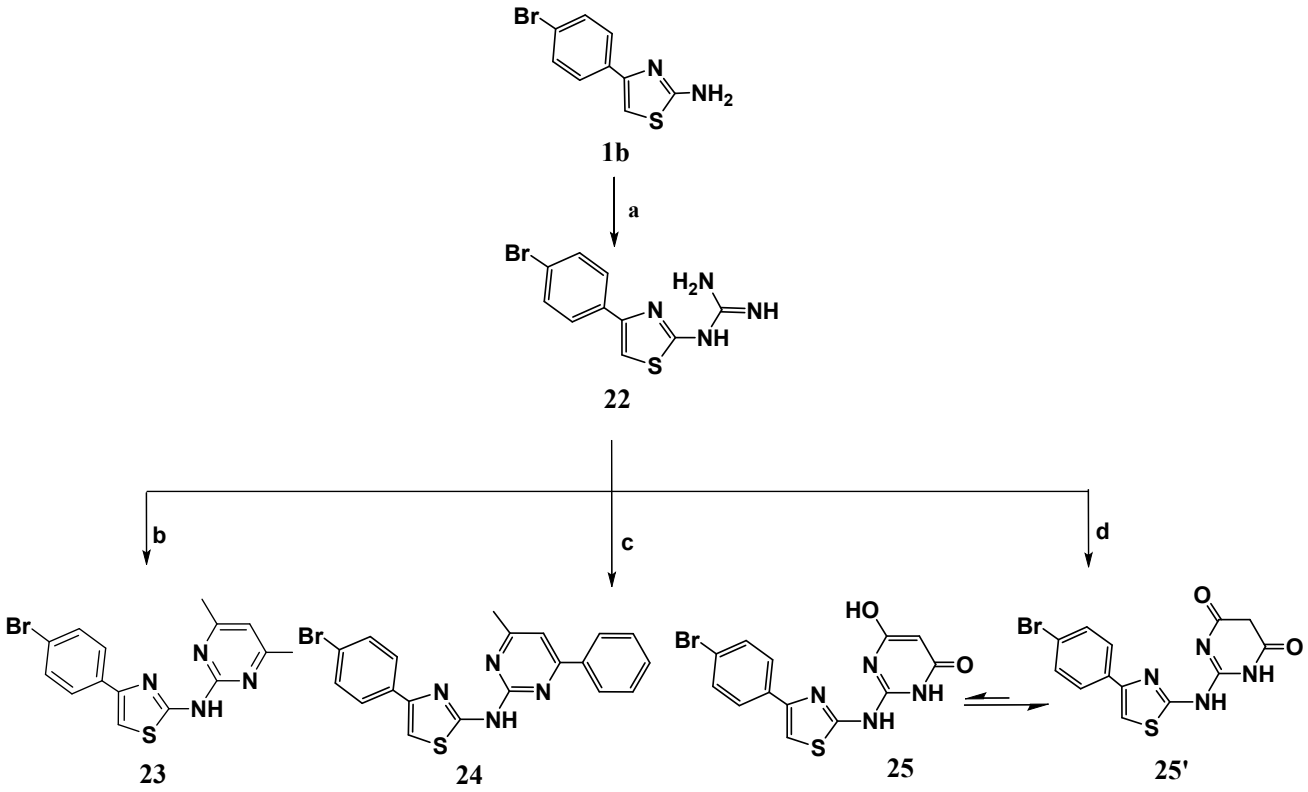
Reagents and conditions: (a) Chloroacetylchloride/ DMF/ stirring; **(b)** Ammonium thiocyanate/ absolute ethanol/ reflux; **(c)** Hydrazine hydrate/ ethylene glycol/ reflux; **(d)** 4-Bromosalicylaldehyde/ methanol/ reflux; **(e)** gl.AcOH/ methanol/ reflux.

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 variousactive 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 δ 3.00 ppm due to two CH_3 protons of **23** and a singlet signals at δ 2.44 ppm attributed to CH_3 protons of **24** besides to, the NH proton deuterium oxide exchangeable singlet signal in each at δ 3.47 ppm and δ 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 δ 8.15 ppm and δ 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 abroad 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 δ 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 fourof 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. Doxorubicinwas 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 37ºC in a humidified incubator with 5% CO_2. 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 37ºC. 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 (IC_{50}), 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 IC_{50} 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 IC_{50} values of the tested compounds of schemes 1, 2 &3 against HepG2 cell line

Sample conc. (µg /mL) / **Compound**	**Growth inhibition %**						**IC_{50} (µg/mL)**
	100	**50**	**25**	**12.5**	**6.25**	**3.125**	
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
Doxorubicin	90.76	88.45	84.26	77.78	70.82	55.16	4.65

Table 2:- Six dose growth inhibition percent and IC_{50} values of the tested compounds of schemes 4&5 against HepG2 cell line

Sample conc. (µg /mL) Compound	Growth inhibition %						IC_{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_{50} values of the tested compounds of schemes 1, 2&3 against MCF7 cell line

Sample conc. (µg /mL) Compound	Growth inhibition %						IC_{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_{50} values of the tested compounds of schemes 4&5 against MCF7 cell line

Sample conc. (µg /mL) Compound	Growth inhibition %						IC_{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

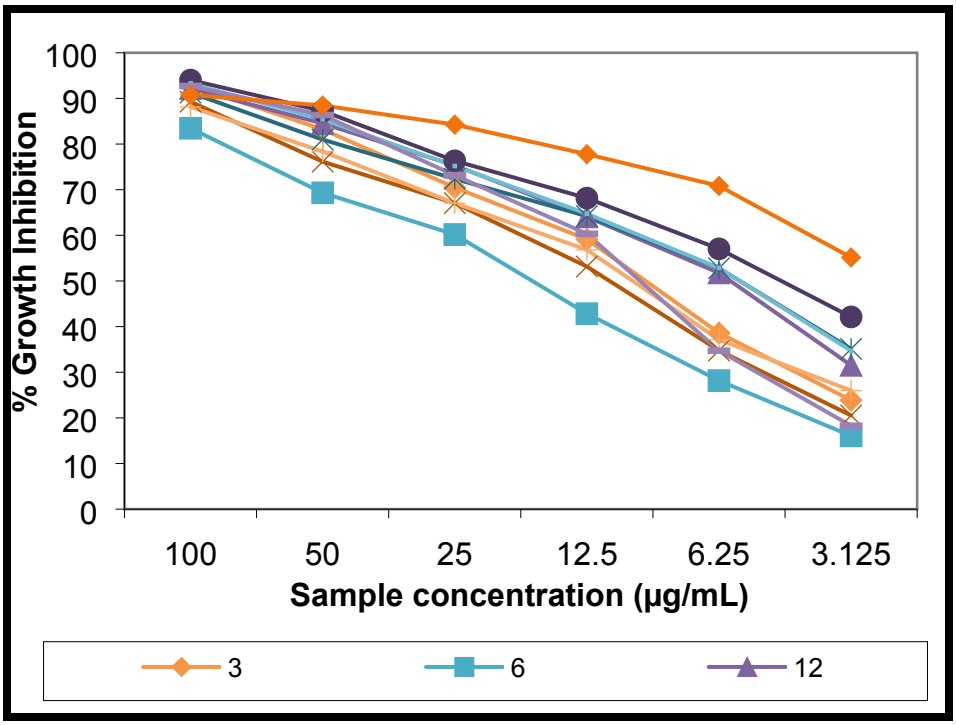


Figure 1:- Growth inhibition curves of the most active compounds against HepG2 cell line.

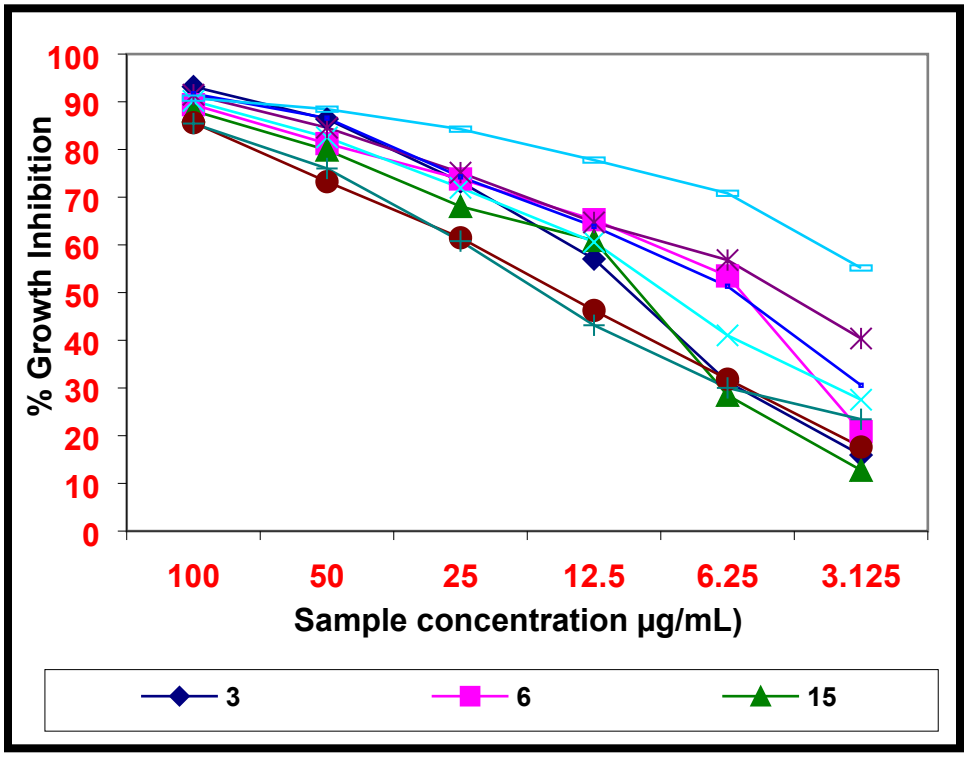


Figure 2:- Growth inhibition curves of the most active compounds against MCF7 cell line

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 IC_{50} 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 IC_{50} 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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