

Anticancer activity of some new series of 2-(substituted)amino-1,3-thiazole derivatives

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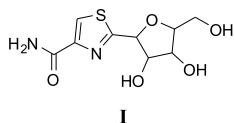
A series of thiazole derivatives were synthesized and structurally elucidated by IR, ^1H NMR, ^{13}C NMR, mass and elemental analyses. The prepared compounds were screened for their cytotoxic activity against Leukemia HL-60 cell line. Compound **4b** was considered as the most promising antitumor candidate among the tested compounds. Mechanism of action of compound **4b** evaluated by flow cytometric assay revealed cell cycle arrest at G2/M phase and pre-G1 apoptosis. The ratio of apoptosis was also determined. Moreover, compound **4b** increased the concentration of caspase 3 by 4 fold more than untreated control.

Keywords: Thiazole, Anticancer, Cell Cycle Analysis, Annexin V, FITC, Doxorubicin.

INTRODUCTION

Thiazole ring is a simple organic compound related to azoles. Thiazole molecules containing thiazole amine moiety exhibit interesting biological activities depending on the substitution pattern at the thiazole ring¹. The thiazole compound was first described by Hantzsch and Waber in 1887. The structure of thiazole compound was established by Popp in 1889². The thiazole ring is a crucial part of vitamin B-1 (thiamine) and epothilone, thiazole dyes are used for dyeing cotton.

The rapid spread of cancer has sparked an intense worldwide search for new structure lead which may be of use in designing novel antitumor drugs. In this context, thiazoles and thiazoles linked to various heterocyclic rings through different linkages have recently attracted great attention^{3–8}. The antitumor activities of the natural antineoplastic antibiotics bleomycin, netropsin and thiazole netropsin have been reported⁹. Most of the aforementioned drugs have the thiazole carboxamide moiety as a common feature. Among these, triazofurin **I** was reported to possess potential anticancer activity.



Keeping in view the importance of thiazole compounds and in continuation of our search on biologically active molecule^{10–12}, we report the synthesis of simple thiazole derivatives by using p-chlorophenacylbromide and thiourea as starting materials. The synthesized *N*-substituted thiazole derivatives were considered a scaffold for further optimization to obtain a promising anticancer agent.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategy adopted to obtain the target *N*-substituted-1,3-thiazole derivatives are depicted in schemes 1 and 2.

The starting 4-(p-chlorophenyl)-2-aminothiazole (**2**) was synthesized from p-chlorophenacyl bromide with thiourea in ethanol in the presence of fused sodium acetate according to published procedure¹³. The 2-amino-

thiazole derivatives **2** was confirmed *via* its acetylation with acetic anhydride under reflux to give 5-(p-chlorophenyl)-2-acetylaminothiazole (**3**). FT-IR spectrum of compound **3** showed the expected absorption band at 3275 (NH), 1687 (C=O) and 1632 (C=N) cm^{-1} . In the ^1H NMR spectrum of compound **3**, the acetyl amino protons (NHCOCH_3) resonated at a singlet signal at δ 2.17 (COCH_3) and δ 12.85 (NHCO) ppm. The protons of aromatic and H-5 of thiazole appeared as a doublet of doublet from aromatic protons at δ 7.49 and δ 7.91 ppm and singlet at δ 7.66 ppm for H-5-thiazole ring (Fig. 1a). ^{13}C NMR spectrum of compound **3** showed two signals at δ 169.16 and 22.96 ppm corresponding to carbon atoms of acetyl amino (NHCOCH_3). The signals due to the aromatic and thiazole carbons were observed within the expected chemical shift at δ 158.60, 147.93, 133.66, 132.65, 129.21 (two carbons), 127.82 (two carbons) and δ 109.06 ppm (Fig. 1b).

Ethyl β -aryl- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyanoacrylate **4a,b** were obtained from the condensation of 5-(p-chlorophenyl)-2-aminothiazole with α,β -unsaturated ester namely (Ethyl β -(p-hydroxyphenyl)- α -cyanoacrylate and Ethyl β -(p-methoxyphenyl)- α -cyanoacrylate) in ethanol in the presence of fused sodium acetate. The IR spectra of thiazole derivatives **4a, b** revealed the presence of stretching vibration for NH bands at 3285–3282 cm^{-1} , in addition to absorption bands in the region of 2214–2210 cm^{-1} corresponding to cyano group (CN), and bands in the region 1720–1714 and 1634–1630 cm^{-1} , which indicate the presence of C=O and C=N groups.

The ^1H NMR spectra of compounds **4 a,b** showed a sharp two signals as triplet and quartet at δ 1.27–1.31 and δ 4.26–4.30 ppm due to the pattern of ethoxy group ($\text{CH}_3\text{CH}_2\text{CO}-$) and sharp singlet signals at δ 8.24–8.28 ppm due to the NH protons. Protons of aromatic, olefinic and H-5 of thiazole were observed within the chemical shift in region at δ 6.95–8.08 ppm and exhibited the expected integral values. The protons of hydroxyl group (OH) in compound **4a** appeared as a broad singlet signal at δ 10.85 ppm, while the protons of methoxy group (OCH_3) in compound **4b** appeared as a sharp signal at δ 3.86 ppm (Fig 2).

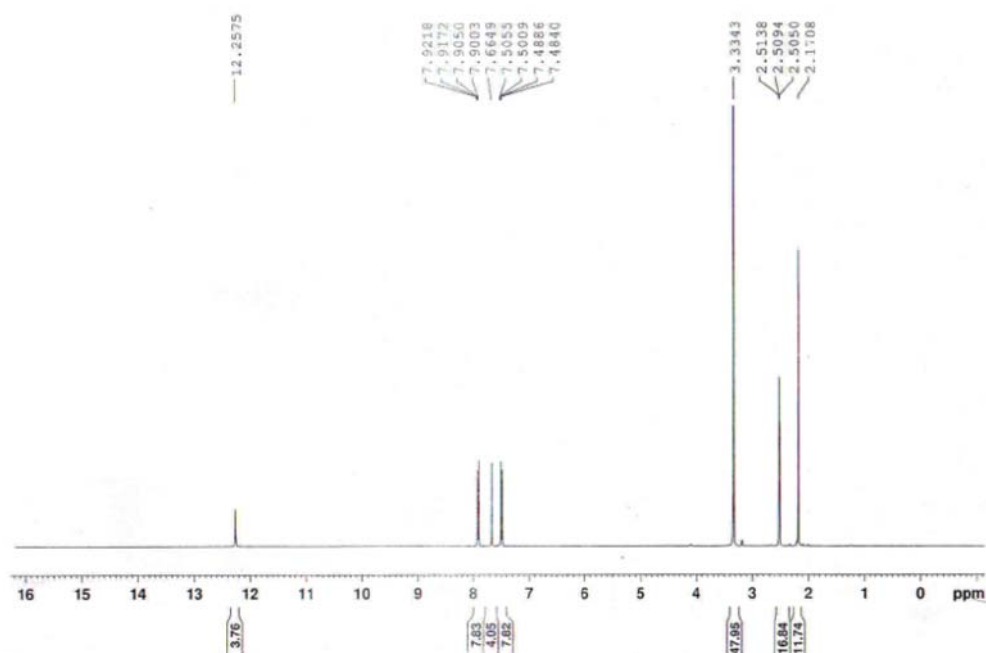


Figure 1a. ^1H NMR Spectrum of compound 3

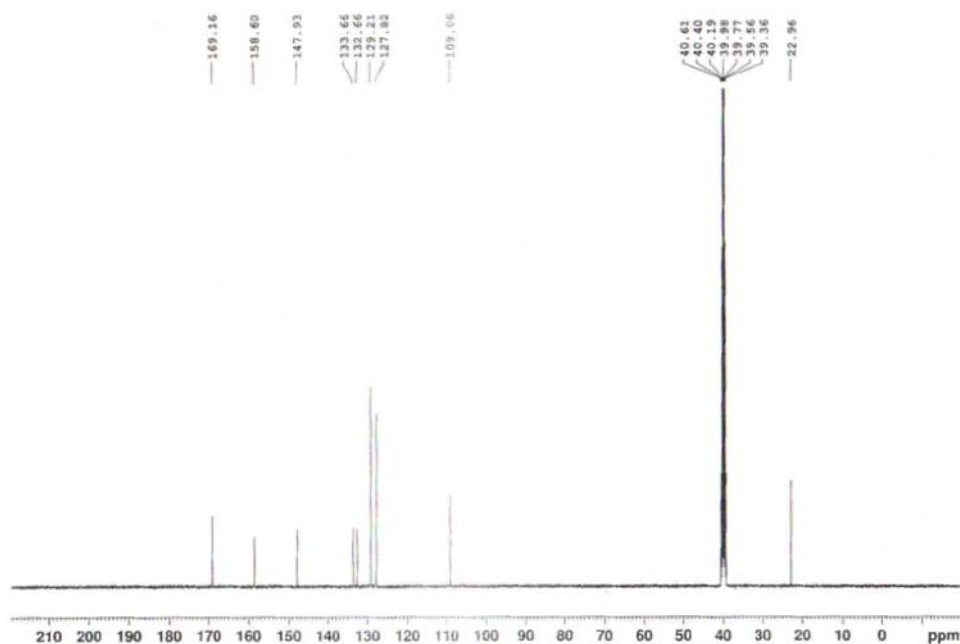


Figure 1b. ^{13}C NMR Spectrum of compound 3

Structure of ethyl β -(p-hydroxyphenyl)- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyano acrylate (**4a**) was confirmed *via* its transformation into ethyl β -(p-acetoxyphenyl)- β -[4-(p-chlorophenyl)-thiazole-2-ylacetyl-amino]- α -cyano acrylate (**5**) by refluxing with acetic anhydride.

Reaction of 4-(p-chlorophenyl)-2-aminothiazole with cinnamic acid in pyridine under reflux was expected to give β -phenyl- β -[4-(p-chlorophenyl)thiazole-2-ylamino] acylic acid (**6**), but only *N*-[4-(p-chlorophenyl)thiazol-1-yl] cinnamide (**7**) was yielded. FT-IR spectrum of compound **7** showed absorption bands at 3283, 1682 and 1630 cm^{-1} for NH, C=O and C=N groups, respectively. In the ^1H NMR spectrum of compound **7**, the amide proton (NHCO) resonated as broad singlet signal at δ 12.40 ppm. The proton signals of aromatic, olefinic and H-5 of thiazole ring are observed in the expected region at δ 6.51–7.82 ppm (Fig. 3).

Condensation of thiazole derivative **7** with 2-hydroxy benzaldehyde in the presence of piperidine as catalyst under fusion led to the formation of *N*-(o-hydroxyphenyl, hydroxy) methyl, *N*-(4-(p-chlorophenyl)thiazol-2-yl) cinnamide (**8**). The structure of **8** was established *via* its acetylation with acetic anhydride to give *N*-(o-acetoxyphe-nyl, acetoxy) methyl-*N*-(4-(p-chlorophenyl)thiazol-2-yl) cinnamide (**9**). FT-IR spectrum of compound **9** showed the expected absorption bands at 1766, 1692 and 1632 cm^{-1} for carbonyl and C=N groups, respectively. The absence of absorption band corresponding to hydroxyl group stretching frequency. In the ^1H NMR spectrum of compound **9** showed three singlet signals at δ 2.14, 2.17 and 5.91 ppm due to the protons of two methyl for the acetyl groups and O-CH-N group. The protons of aromatic, olefinic and H-5 of thiazole ring appeared in the region at δ 7.15–7.92 ppm as multiplet signals (Fig. 4).

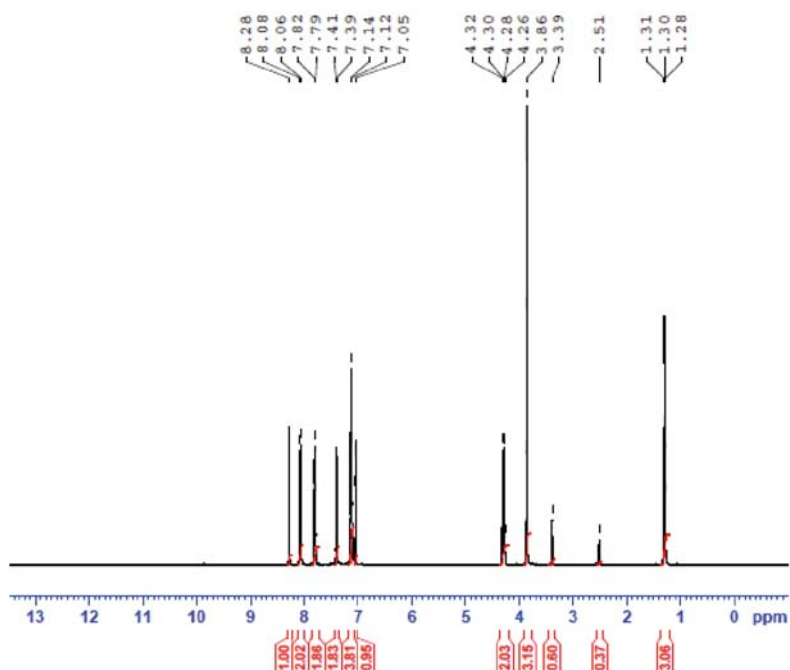


Figure 2. ^1H NMR Spectrum of compound **4b**

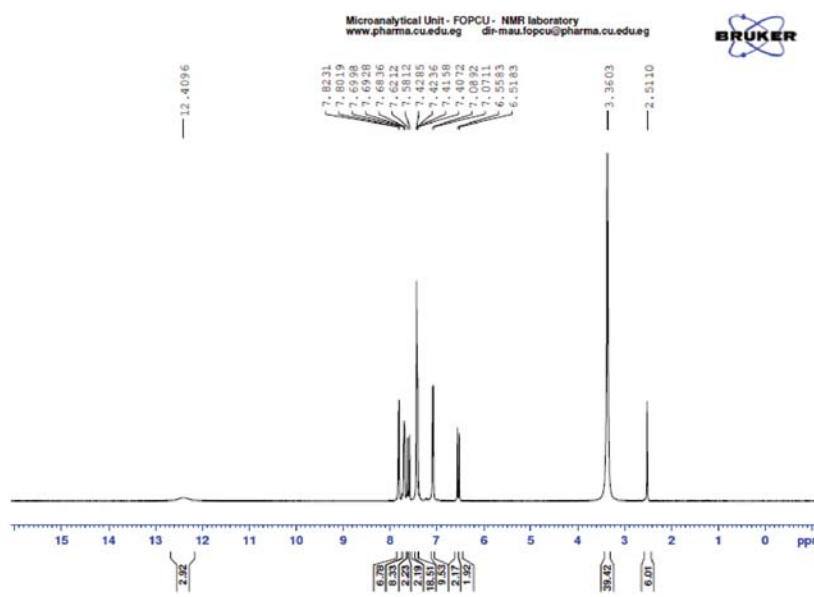


Figure 3. ^1H NMR Spectrum of compound **7**

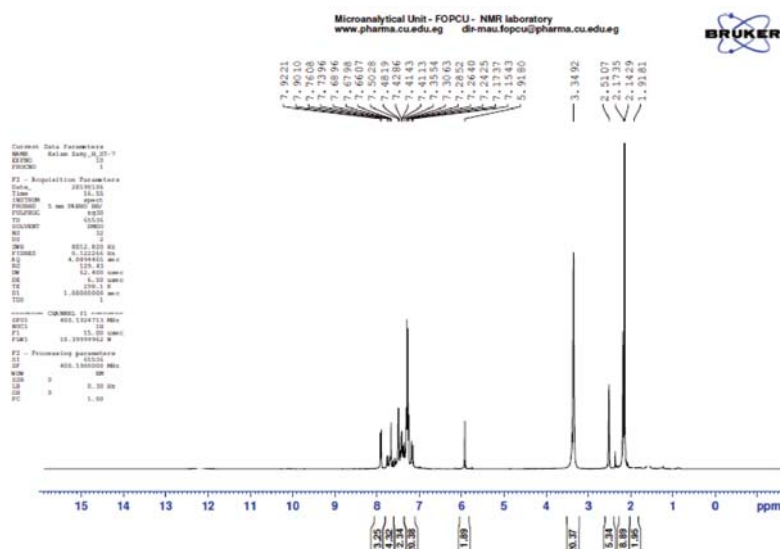
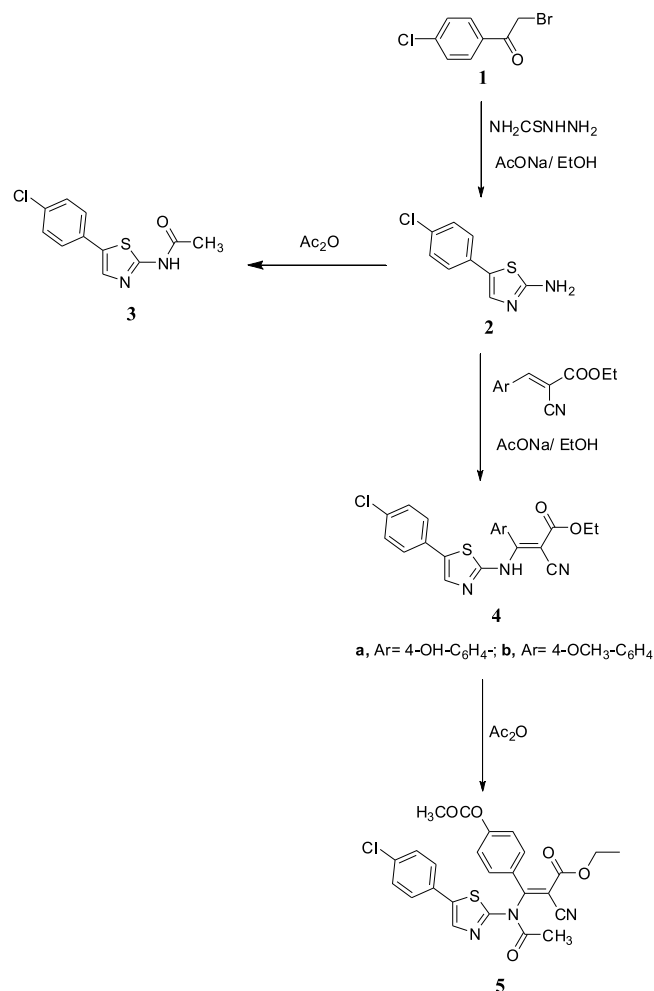
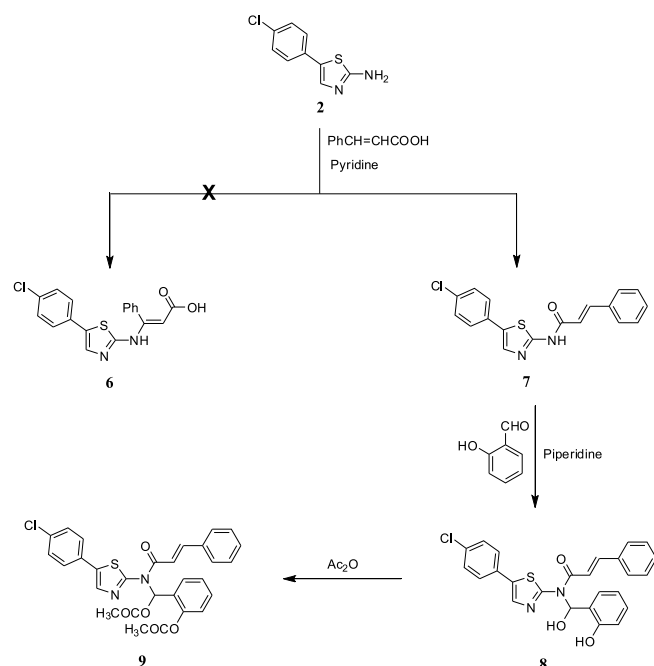


Figure 4. ^1H NMR Spectrum of compound **9**



Scheme 1. Synthesis of 2-*N*-(substituted) amino thiazole derivatives (3–5).



Scheme 2. Synthesis of 1,3-thiazole derivatives (7–9).

Evaluation of biological activity

In vitro cytotoxic activity against Leukemia HL-60 cell line

In order to examine the anticancer activity of the prepared thiazole derivatives, the effect of the synthesized compounds on the viability of Leukemia HL-60 cell line

was measured using colorimetric MTT assay after 48 h of incubation. The percentage of cell viability of thiazole derivatives and reference compound are presented in **Table 1** and represented graphically in **Fig. 5**. The reference compound in our assay is Doxorubicin (Dox). As shown in **Table 1** and **Fig. 5**, compounds **4a**, **4b** and **7** showed significant anti-proliferative activity compared to the reference compound Dox against HL-60 cell line. Overall, derivatives of Ethyl β -aryl- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyano acrylate **4a,b** were found to have superior anti-cancer activity compared to the other thiazole derivatives. Among the thiazole derivatives compound Ethyl β -(4-methoxyphenyl)- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyano acrylate (**4b**) was found to be superior to the other thiazole derivatives in terms of anti-cancer activity with IC_{50} value of $1.3 \pm 0.29 \mu\text{M}$.

Table 1. *In vitro* antitumor activity of 1,3-thiazole derivatives 3–9 over Leukemia HL-60 cell line. Data are expressed as the mean \pm three experiments

Compound No.	IC ₅₀ values [μM]
	HL-60
3	42.1 ± 1.08
4a	8.9 ± 0.48
4b	1.3 ± 0.29
5	58.3 ± 0.93
7	7.3 ± 0.35
8	13.6 ± 0.86
9	82.4 ± 1.62
Dox	3.01 ± 0.07

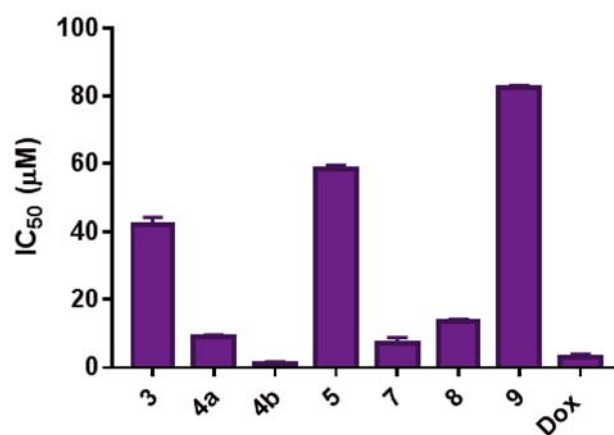


Figure 5. IC_{50} values (μM) noted for 1,3-thiazole derivatives and Doxorubicin against leukemia HL-60 cell line

Cell cycle analysis

Due to the importance of the cell cycle in the process of tumor cell proliferation, HL-60 cell growth inhibition due to cell cycle arrest using DNA flow cytometric assay for 48 h was evaluated³. In this assay, HL-60 cells were treated with compound **4b** at its IC_{50} concentration value for 48 h. It is evident from **Fig. 6**, compound **4b** showed G2/M cell cycle arrest compared with untreated control. Compound **4b** showed 37.08% of cells in G2/M phase in comparison with the untreated cells which had 7.13%. Moreover, compound **4b** revealed G0 phase arrest marked by appearance of peak at G0 phase of the cell cycle distribution profile which indicate HL-60 cell apoptosis. In conclusion, compound **4b** showed cell cycle

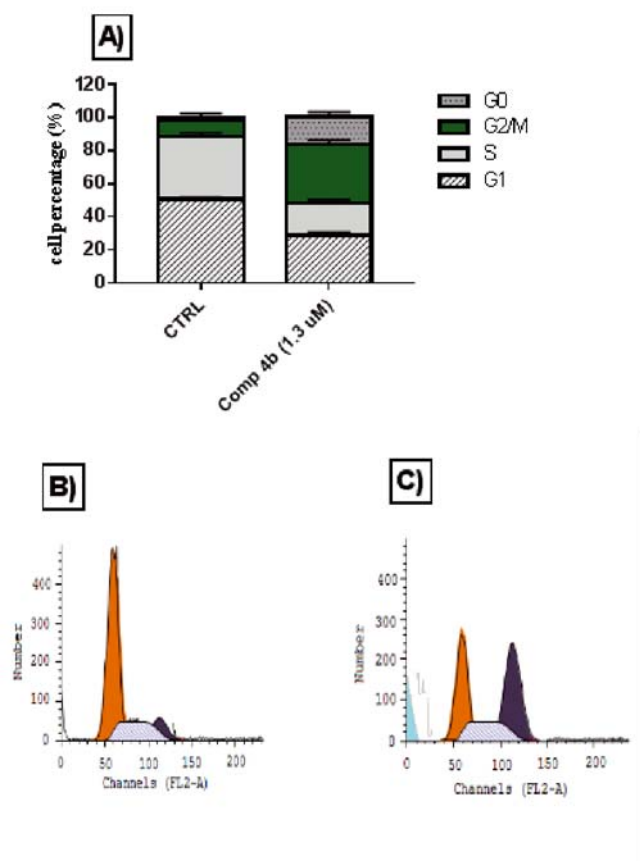


Figure 6. Effect of compound **4b** on the HL-60 cell line determined by flow cytometry analysis after 48 h of incubation. **A)** Graphical presentation, **B)** Cell cycle analysis of control group, **C)** Cell cycle analysis of compound **4b**

arrest at G2/M phase with apoptosis inducing activity in HL-60 treated cells.

Annexin V-FITC Staining assay

Several thiazole derivatives have been reported in the literature as apoptosis inducer in several tumor cell line¹⁴. Therefore apoptosis ratio was determined in HL-60 cells for 48 h. In this study, HL-60 cells were incubated with compound **4b** for 48 h and then stained with Annexin V-FITC followed by flow cytometry assay. The results in this assay (**Fig. 7**) showed that, compound **4b** could enhance the early apoptosis stage by 4.88% compared with the untreated cells. Moreover, compound **4b** could increase the late apoptosis stage with 7.7% in comparison with the control group. These data indicated the involvement of apoptosis in inhibition of HL-60 cells induced by compound **4b**.

2.2.4. Caspase 3 assay

To ensure that, compound **4b** induce apoptosis, compound **4b** was screened for its ability to activate caspase 3. Leukemia HL-60 cell line was treated with compound **4b** at its IC_{50} concentration dose value and caspase 3 concentration was measured using ELISA assay. From the obtained data in **Fig. 8** it was observed that compound **4b** could enhance the concentration of caspase 3 as concluded from the optical density values by 4.55- fold compared with the untreated control. In conclusion, compound **4b** may induce apoptosis through activation of caspase 3.

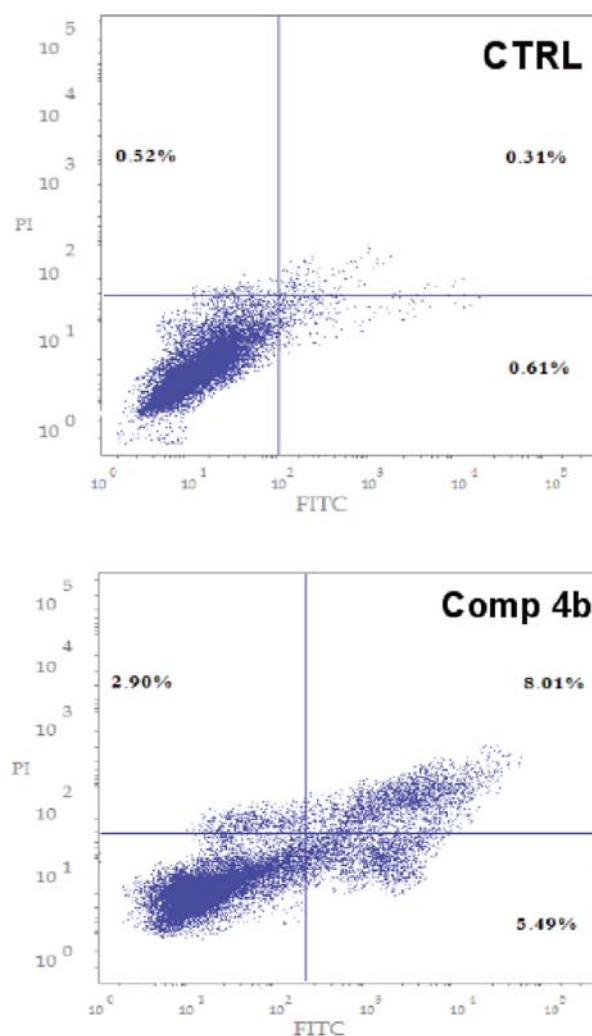


Figure 7. Annexin V-FITC of compound **4b** at its IC_{50} concentration dose value (μ M) compared to untreated control cells

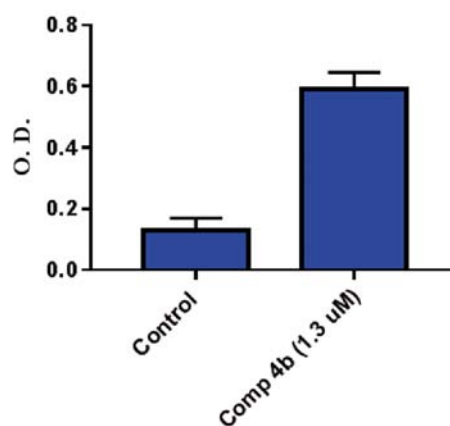


Figure 8. Effect of compound **4b** on the caspase 3 compared to untreated control

CONCLUSION

In the present synthesis of 4-(p-chlorophenyl)-2-amino-1,3-thiazole (**2**) by the reaction of p-chlorophenacyl bromide with thiourea. Treatment of 2-amino thiazole with α,β -unsaturated ester and acid in different condition led to the formation of 2-(substituted)amino thiazole derivatives **3**, **4** and **7**. Condensation of compound **7** with 2-hydroxy benzaldehyde yielded the corresponding *N*-(o-hydroxyphenyl, hydroxy) methyl-*N*-(4-p-chlorophenyl

thiazole-2-yl)-cinnamide (**8**). Acetylation of compound **4** and **8** with acetic anhydride give diacetyl derivatives **5** and **9**. The structure of the synthesized derivatives **2-9** were confirmed by IR, ^1H NMR, ^{13}C NMR, mass and elemental analyses. All the synthesized thiazole derivatives were screened for their cytotoxic activity against Leukemia HL-60 cell line. Compound **4b** was considered as the most promising antitumor compound among the tested compounds. Mechanism of action of compound **4b** evaluated by flow cytometric assay revealed cell cycle arrest at G2/M phase and pre-G1 apoptosis. The ratio of apoptosis was also determined. Moreover, compound **4b** increased the concentration of caspase 3 by more than 4 fold compared with untreated control. The synthesized *N*-substituted thiazole derivatives were considered a scaffold for further optimization to obtain a promising anticancer agents.

EXPERIMENTAL

Chemistry

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were run with a Bruker 400 DRX-Avance NMR spectrometer using DMSO- d_6 as solvent- chemical shifts are reported on ppm downfield from internal tetramethylsilane and are given in the scale. The IR data were obtained with a Shimadzu 470 spectrometer. Melting point were measured using open capillary tubes on a melt-temp melting point apparatus and are uncorrected. Mass spectral data were obtained with Probe Agilent MSD-5979 mass spectrometer. The elemental analysis was carried out on a Perkin-Elmer 2400 CHN analyzer. All chemicals were purchased from *Aldrich* chemical company, *Merk* and *Fluka*.

Synthesis of 4-(p-chlorophenyl)-2-aminothiazole (**2**)

An equimolar mixture of p-chlorophenacyl bromide (**1**, 0.01 mol), thiourea (0.01 mol) and fused sodium acetate (0.03 mol) in absolute ethanol (50 mL) was heated under reflux for 2 h. The reaction mixture was cooled and poured into water, then the precipitate formed was filtered off, washed with water, dried. Finally, the product was crystallized from ethanol to give **2**.

Yellow crystals, yield 83%, m.p 178–180°C. IR (KBr): 3335–3282 (NH_2), 1635 ($\text{C}=\text{N}$) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 4.09 (br. s, 2H, NH_2), 7.20 (s, 1h, H-5 of thiazole ring), 7.47 (d, 2H, Ar-H), 7.75 (d, 2H, Ar-H) ppm. MS: m/z (%) = 212 ($\text{M}^+ + 2$, 33.86), 210 (M^+ , 100) Anal. Calcd. For $\text{C}_9\text{H}_7\text{N}_2\text{ClS}$ (210): C, 51.31; H, 3.35; N, 13.30. Found: C, 51.23; H, 3.13; N, 13.19.

Synthesis of Ethyl β -aryl- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyano acrylate **4a,b**

A mixture of 4-(p-chloro phenyl)-2-aminothiazole (**2**, 0.01 mol), α,β -unsaturated ester (0.01 mol) and fused sodium acetate (0.03 mol) in absolute ethanol (50 ml) was heated under reflux for 4 h the reaction mixture was cooled and poured into water. The product formed was collected by filtration, washed with water, dried and purified by crystallization from ethanol to give **4**.

Ethyl β -(4-hydroxyphenyl)- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyano acrylate (**4a**)

Yellow crystals, yield 78%, m.p 125–127°C. IR (KBr): 3437 (OH), 3286 (NH), 2232 (CN), 1718 ($\text{C}=\text{O}$), 1633 ($\text{C}=\text{N}$), 1174, 1089 ($\text{C}-\text{O}$) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 1.29 (t, 3H, CH_3), 4.29 (q, 2H, OCH_2), 6.95–8.01 (m, 9H, Ar-H and H-5 of thiazole ring), 8.24 (s, 1H, NH), 10.85 (br. s, 1H, OH) ppm. MS: m/z (%) = 427 ($\text{M}^+ + 2$, 1.32), 425 (M^+ , 5.20) Anal. Calcd. For $\text{C}_{21}\text{H}_{16}\text{N}_3\text{ClO}_3\text{S}$ (425): C, 59.22; H, 3.79; N, 9.87. Found: C, 59.09; H, 3.48; N, 9.59.

Ethyl β -(4-methoxyphenyl)- β -[4-(p-chlorophenyl)-thiazole-2-ylamino]- α -cyano acrylate (**4b**)

Yellow crystals, yield 76%, m.p 118–120°C. IR (KBr): 3283 (NH), 2214 (CN), 1715 ($\text{C}=\text{O}$), 1633 ($\text{C}=\text{N}$), 1605, 1585 ($\text{C}=\text{C}$), 1126, 1015 ($\text{C}-\text{O}$) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 1.30 (t, 3H, CH_3), 3.86 (s, 3H, CH_3), 4.30 (q, 2H, OCH_2), 7.05–8.08 (m, 9H, Ar-H and H-5 of thiazole ring), 8.28 (s, 1H, NH) ppm. MS: m/z (%) = 441 ($\text{M}^+ + 2$, 1.10), 439 (M^+ , 4.33) Anal. Calcd. For $\text{C}_{22}\text{H}_{18}\text{N}_3\text{ClO}_3\text{S}$ (439): C, 60.07; H, 4.12; N, 9.55. Found: C, 60.02; H, 3.83; N, 9.38.

Synthesis of *N*-[4-(p-chlorophenyl)thiazol-1-yl] cinnamide (**7**)

An equimolar mixture of 4-(p-chloro phenyl)-2-aminothiazole (**2**, 0.01 mol), and cinnamic acid (0.01 mol) in pyridine (30 mL) was heated under reflux for 6 h. the reaction mixture was cooled and poured into water, and neutralized with dilute hydrochloric acid (2%). The product formed was collected by filtration, washed with water, dried and purified by crystallization with ethanol to give compound **7**.

Yellow crystals, yield 72%, m.p 110–112°C. IR (KBr): 3283 (NH), 2214 (CN), 1682 ($\text{C}=\text{O}$), 1630 ($\text{C}=\text{N}$), 1601, 1534 ($\text{C}=\text{C}$) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 6.51–7.82 (m, 12H, Ar-H, H olefinic and H-5 of thiazole ring), 12.40 (br. s, 1H, NHCO) ppm. MS: m/z (%) = 342 ($\text{M}^+ + 2$, 2.30), 340 (M^+ , 7.31) Anal. Calcd. For $\text{C}_{18}\text{H}_{13}\text{N}_2\text{ClOS}$ (439): C, 63.43; H, 3.84; N, 8.22. Found: C, 63.32; H, 3.56; N, 8.07.

Synthesis of *N*-(o-hydroxyphenyl, hydroxy) methyl, *N*-(4-(p-chlorophenyl)thiazol-2-yl) cinnamide (**8**).

A mixture of **7** (0.01 mol), 2-hydroxybenzaldehyde (0.01 mol) and piperidine (2 mL) was fused on hot plate for 10–15 min, then added 50 mL ethanol. The reaction mixture was heated under reflux for 4 h, then cooled, poured into water and neutralized with dilute hydrochloric acid (2%). The separated solid product was filtered off, washed with water, dried and crystallized from ethanol to give **8**.

Orange crystals, yield 63%, m.p 135–137°C. IR (KBr): 3450–3300 (br. OH), 1685 ($\text{C}=\text{O}$), 1632 ($\text{C}=\text{N}$), 1605, 1585 ($\text{C}=\text{C}$), 1178, 1038 ($\text{C}-\text{O}$) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 6.09–8.02 (m, 17h, Ar-H, olefinic and H-5 of thiazole ring), 9.72 (s, 1H, OH), 11.57 (br. s, 1H, OH) ppm. MS: m/z (%) = 462 (M^+ , 100) Anal. Calcd. For $\text{C}_{25}\text{H}_{19}\text{N}_2\text{ClO}_3\text{S}$ (462): C, 64.86; H, 4.14; N, 6.05. Found: C, 64.74; H, 4.02; N, 5.83.

General procedure for acetylation reaction

A solution of **2**, **4** and **8** (0.01 mol) in acetic anhydride (15 mL) was heated under reflux for 2h, the reaction mixture was allowed to cool to room temperature, poured into water and left for 24 h. the solid formed was filtered off, washed with water and dried. Finally the product was crystallized from ethanol to give **3**, **5** and **9**.

5-(p-chlorophenyl)-2-acetyl aminothiazole (**3**)

Pale yellow crystals, yield 74%, m.p 205–207°C. IR (KBr): 3285 (NH), 1692 (C=O), 1630 (C=N), 1603, 1585 (C=C) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 2.17 (s, 3H, CH_3), 7.49 (d, 2h, Ar-H), 7.66 (s, 1H, H-5 of thiazole ring), 7.91 (d, 2h, Ar-H), 12.85 (s, 1h, NHCO) ppm. ^{13}C NMR (DMSO- d_6) δ : 169.16, 22.96 (CH_3CO), 158.60, 147.39, 133.66, 132.66, 129.21, 127.82, 109.06, (C of thiazole and aromatic ring) ppm. MS: m/z (%) = 254 ($\text{M}^+ + 2$, 33.20), 252 (M^+ , 100) Anal. Calcd. For $\text{C}_{11}\text{H}_9\text{N}_2\text{ClOS}$ (252): C, 52.28; H, 3.59; N, 11.08. Found: C, 52.11; H, 3.33; N, 11.01.

Ethyl β -(p-acetoxyphenyl)- β -[4-(p-chlorophenyl)-thiazole-2-ylacetyl amino]- α -cyano acrylate(**5**)

Yellow crystals, yield 63%, m.p 105–107°C. IR (KBr): 2224 (CN), 1762, 1724 (C=O), 1643 (C=N), 1605, 1578 (C=C), 1092, 1012 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 1.30 (t, 3H, CH_3), 2.10 (s, 3H, COCH_3), 2.26 (s, 3H, COCH_3), 4.29 (q, 2H, OCH_2), 6.95–8.11 (m, 9h, H-thiazole and Ar-H) ppm. ^{13}C NMR (DMSO- d_6) δ : 169.23, 162.24, 22.93, 21.14 ($2\times\text{COCH}_3$), 169.16, 62.84, 14.42 ($\text{COOCH}_2\text{CH}_3$), 158.62, 154.48, 154.43, 147.93, 133.64, 132.87, 132.66, 129.39, 129.18, 128.84, 127.79, 123.32, 109.01, 102.77 (C of thiazole and aromatic ring), 116 (CN) ppm. MS: m/z (%) = 511 ($\text{M}^+ + 2$, unstable), 509 (M^+ , unstable). Anal. Calcd. For $\text{C}_{25}\text{H}_{20}\text{N}_3\text{ClO}_5\text{S}$ (509): C, 58.88; H, 3.95; N, 8.24. Found: C, 58.63; H, 3.71; N, 8.03.

N-(o-acetoxyphenyl, acetoxy) methyl-N-(4-(p-chlorophenyl)thiazol-2-yl) cinnamide (**9**).

Pale yellow crystals, yield 71%, m.p 160–162°C. IR (KBr): 1766 (C=O), 1692 (C=O of amide), 1625 (C=N), 1603, 1545 (C=C), 1197, 1091 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 2.14 (s, 3H, COCH_3), 2.17 (s, 3H, COCH_3) 5.91 (s, 1H, OCH-N), 7.15–7.92 (m, 16h, H-thiazole and Ar-H) ppm. MS: m/z (%) = 549 ($\text{M}^+ + 2$, unstable), 547 (M^+ , unstable). Anal. Calcd. For $\text{C}_{29}\text{H}_{23}\text{N}_2\text{ClO}_5\text{S}$ (547): C, 63.67; H, 4.24; N, 5.12. Found: C, 63.36; H, 4.03; N, 5.07.

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