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In vitro Anti Leukemia Cancer Activity of Some Novel Pyrazole Derivatives and Pyrazoles Containing Thiazole Moiety

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Abstract: The design and syntheses of several novel pyrazole derivatives (2, 5, 6 and 7) and pyrazole derivatives (3 and 4) containing thiazole moiety *via* by ethyl *β*-(*p*-chlorophenyl)–α-cyanoacrylate (1) and thiosemicarbazide as starting materials. Pyrazole derivatives (3 and 4) containing thiazole moiety were synthesized *via* cyclization of pyrazole derivative (2) with bromomethyl arylketones, to give compound 3, followed by acetylation. N- (3- methoxy-2-hydroxybenzal) -3- (*p*-chlorophenyl)-4- cyano-5-oxopyrazol-1-thiocarboxamide (6) was synthesized *via* reaction of compound 2 with 3-methoxy-2-hydroxybenzaldehyde. Structures of all compounds were confirmed by elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The cytotoxic activity of all the synthetic compounds were evaluated against Leukemia HL-60 compared with Doxorubicicn. The cytotoxic activity was checked *in vitro* for the recently prepared compounds by using the MTT assay. Compounds 4, 6 and 9 were the most active against Leukemia HL-60. The IC_{50} values of them were less than 5 µM in the range of 1.35-4.78 µM. In addition, compounds 3 and 5 showed less antiproliferative activity against Leukemia HL-60 cells with IC_{50} values in the range 5.39-8.82 µM. Compound 6 was the most potent cytotoxic activity. The studies biological activity includes cell cycle analysis, apoptosis detection assay and Topoisomerase II inhibition activity assay explained that compound 6 is a strong Topo II inhibitor.

Keywords: Pyrazole, Thiazole, Antiproliferative, Cell Cycle Analysis, Annexin-V, Topoisomerase II

1. Introduction

Nitrogen and sulphur heterocyclic compounds are extensively dispersed in natural world and are vital to life [1]. Among the wide range of heterocycles containing thiazole and pyrazolinone moeites investigated currently, heterocycles containing thiazole derivatives which have diverse biological activities [2-11]. Thiazole derivatives are remarkable in the biological activity of natural products and many potent biologically active molecules such as vitamine B1, sulphathiazole, abafungin and tiazofurin, epothilones, nizatidine and ritonavir [12-23].

Thiazoles are a familiar group of heterocyclic compounds possessing a wide variety of biological activities such as antimicrobial [24], antioxidant [25], antitubercular [26], anticonvulsant [27], anticancer [28–34], antiallergic [12] and anti-inflammatory [35, 36] agents.

Thiazole derivatives are recognized for their antihypertensive, antischizophrenia, hypnotics, anti-HIV, analgesic, fibrinogen receptor antagonist, bacterial DNA gyrase B inhibitor [12-23].

The presence of the pyrazole nucleus in different structures leads to diversified applications in different areas such as technology, medicine and agriculture. In particular, they are described as inhibitors of protein glycation, antimicrobial [37, 38], antidepressant [39, 40], anticonvulsant [41], antifungal [42, 43], and antitubercular [44, 45], anticonvulsant [46], DPPH radical Scavenging, anti-diabetic [47], antiamebic [48], as well as antiviral agents [49]. Pyrazole analogoues can selectively inhibit (cyclooxygenase enzyme) COX-2 [50] they are also expressing anti-inflammatory [51], analgesic [52], antihypertensive, antipyretic, sedatives, and antidiabetic activities [53-55].

Further, derivatives of pyrazoles have shown significant

pharmacological activities, such as antitumor [56-59] antileukemic [60-62] and antiproliferative [63, 64] agents. In addition to their ability to apply remarkable anticancer effects throughout the inhibition of different types of enzymes which play significant roles in cell division [65-67]. As an expansion of our preceding work [68-70], this research reported the syntheses of some novel heterocyclic compounds including thiazole moiety attached to pyrazolinone ring at N-1 using ethyl *β*- (p-chlorophenyl) –*α*-cyanoacrylate (1) as a key starting material.

These arrangements were suggested as a trial to increase the activity against leukemia cancer cells. Hoping to find out a new guide combination structure which that would have a considerable anti- leukemia potential with small concentrations.

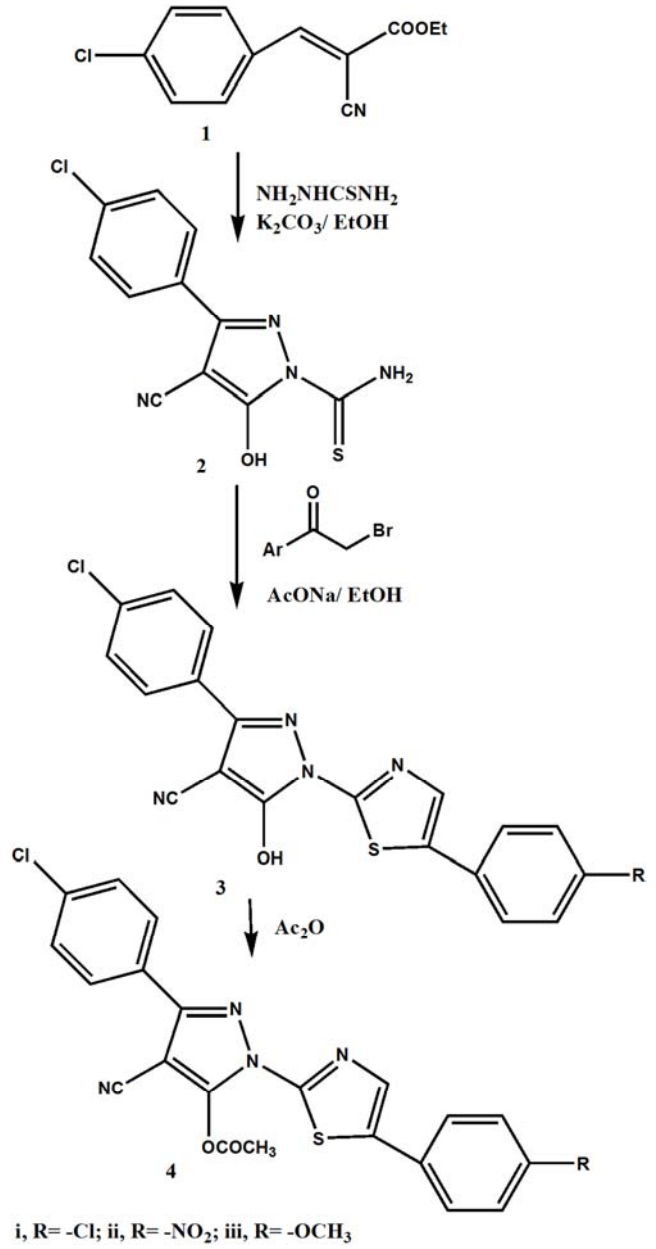

Figure 1. *Syntheses of pyrazole derivatives (3 and 4) containing thiazole moiety.*

2. Results and Discussion

2.1. Chemistry

The structural diversity and biological importance of pyrazole and thiazole derivatives have made them attractive targets for synthesis. The syntheses of the new heterocyclic compounds contain thiazole and pyrazole moieties (3 and 4) are described in "Figure 1".

The initial compound ethyl *β*- (*p*-chlorophenyl) - *α*- cyano-acrylate (1) was reacted with thiosemicarbazide and ethanol in the existence of anhydrous potassium carbonate to make the 3- (*p*-chlorophenyl)-4-cyano-5-hydroxypyrazol-1-thiocarboxamide (2).

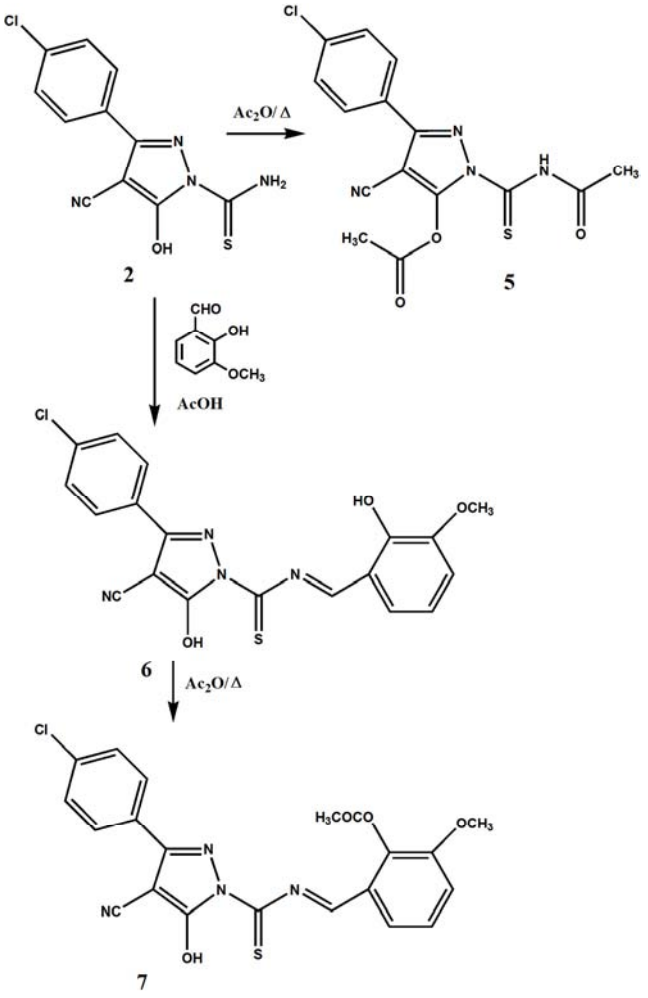

Figure 2. *Syntheses of some pyrazol-1-thiocarboxamide derivatives (5-7).*

Cyclocondensation of pyrazol- 1- thiocarboxamide derivative (2) with various aryl bromomethyl ketones (such as *p*- chlorophenacyl bromide, *p*- nitrophenacyl bromide and *p*-meth-oxyphenacylbromide) and ethanol in the existence of fused sodium acetate gave 5- hydroxyl-4-cyano-3-(chlorophenyl) -1- (5- arylthiazol -2-yl) – pyrazoles (3a-c).

1-substituted-pyrazole derivatives (3) was heated with acetic anhydride under reflux to form 5- acetoxy-4-cyano-3-(*p*-chlorophenyl-1-(5-aryl-thiazole-2-yl)-pyrazoles (4a-c).

The synthetic strategies adopted to obtain N-substituted 5-

oxopyrazole-1-thiocarboxamide derivatives, is demonstrated in "Figure 1".

The conversion of compound 2 into the N- acetyl 5-acetoxy-4-cyano-(*p*-chlorophenyl)-pyrazol-1-thiocarboxamide (5) was achieved by heating the 5-hydroxy-4-cyano-3-(*p*-chlorophenyl)-pyrazol-1-thiocarboxamide (2) with acetic anhydride under reflux.

Condensation of pyrazole derivative (2) with 3-methoxy-2-hydroxybenzaldehyde in acetic acid yielded the target N-(*m*-methoxy-*o*-hydroxybenzal) -5-hydroxy-4-cyano-3-(*p*-chlorophenyl)-pyrazol-1-thiocarboxamide (6). Acetylation of the latter compound with acetic anhydride under boiling resulted in the corresponding N-(*m*-methoxy-*o*-acetoxy-benzal)-5-hydroxy-4-cyano-3-(*p*-chlorophenyl)-pyrazole-1-thiocarboxamide 7, " Figure 2".

2.2. NMR Spectra Investigation of Substituted Pyrazoles and Pyrazole Containing Thiazole Moiety

2.2.1. Substituted Pyrazoles (2 and 5)

FT-IR spectrum of compound 2 showed the predictable absorption bands at δ 3437 corresponding to hydroxyl group, and δ 3328, δ 3181 due to NH_2 group. Stretching vibration bands at δ 2235, 1632, 1605, 1585 cm^{-1} revealed the presence of CN, C=C and C=N, respectively.

In the ^{1}H-NMR spectrum of compound 2 "Figure 3" displayed two singlet signals at δ 11.43 and 7.46 ppm assignable to the protons of OH and NH_2 groups respectively, beside two doublet signals at δ7.84 & 8.02 ppm revealed to adjacent two protons of aromatic ring.

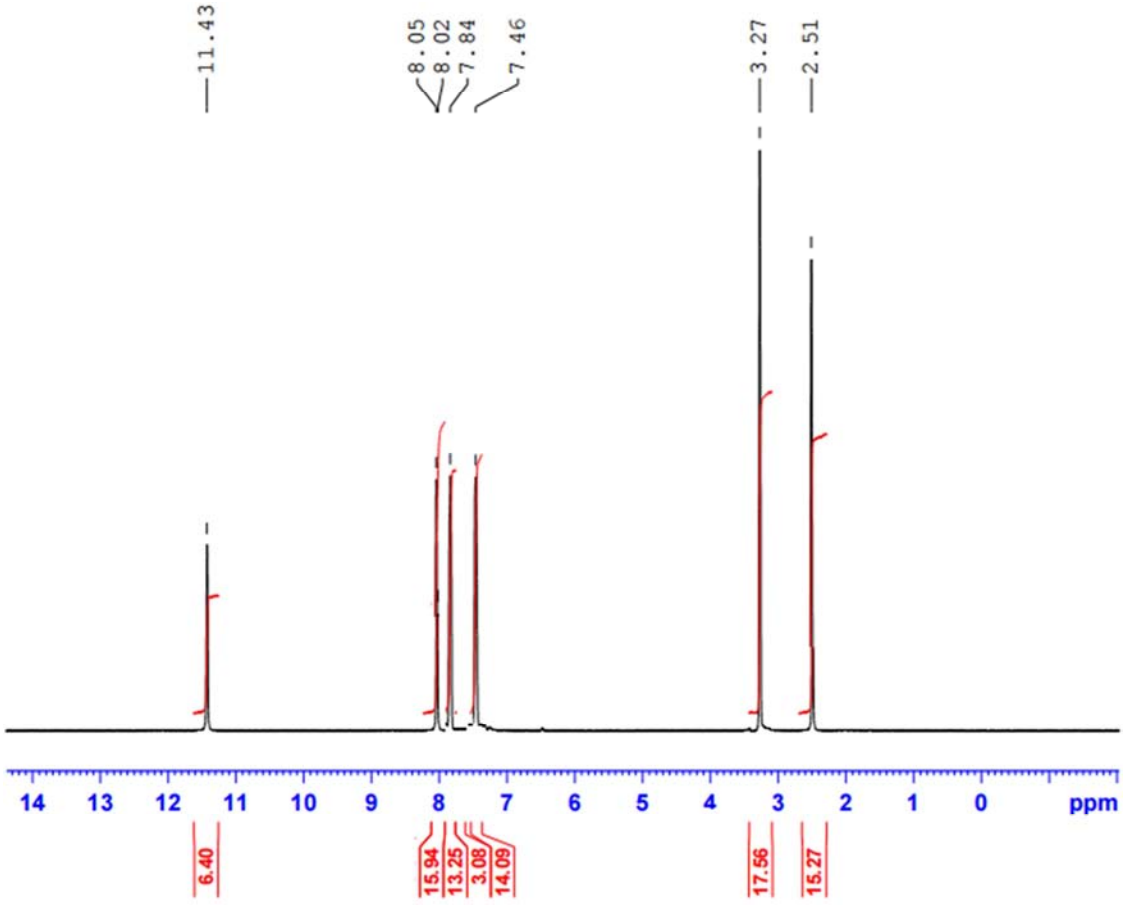

Figure 3. *^{1}H-NMR spectrum of compound 2.*

IR spectrum of compound 5 displayed the lack of the absorption bands of NH_2, but the absorption bands observed at 3216 cm^{-1} attributed to NH group. Bands at 1606, 1583 were attributed to C=C. Bands at 1715, 1695 were due to C=O group. Absorption band at 1625 cm^{-1} was due to C=N group, and band at 2252 cm^{-1} was due to CN group and bands 1098, 1071cm^{-1} revealed the presence of C-O group.

^{1}H-NMR spectrum of compound 5 "Figure 4" gave cut evidence singlet signal at δ 11.73 ppm attributed to the proton of NH group and two singlet signals at δ 2.21, 2.05 ppm due to two acetyl groups. The outstanding proton signals are appearing in the predictable area of basic nucleus. In ^{13}C-NMR spectrum of compound 5 "Figure 5" showed signal at δ 193.55 was due to C=S group. Two signals appeared in δ 169.90 and 167.94 were assignable to C=O group, in addition to two signals showed at 23 and 22.28 ppm characteristic of two methyl groups. Also, displayed signals in the range δ 154.25-115.83 ppm corresponding to carbons of aromatic, pyrazole and cyano groups.

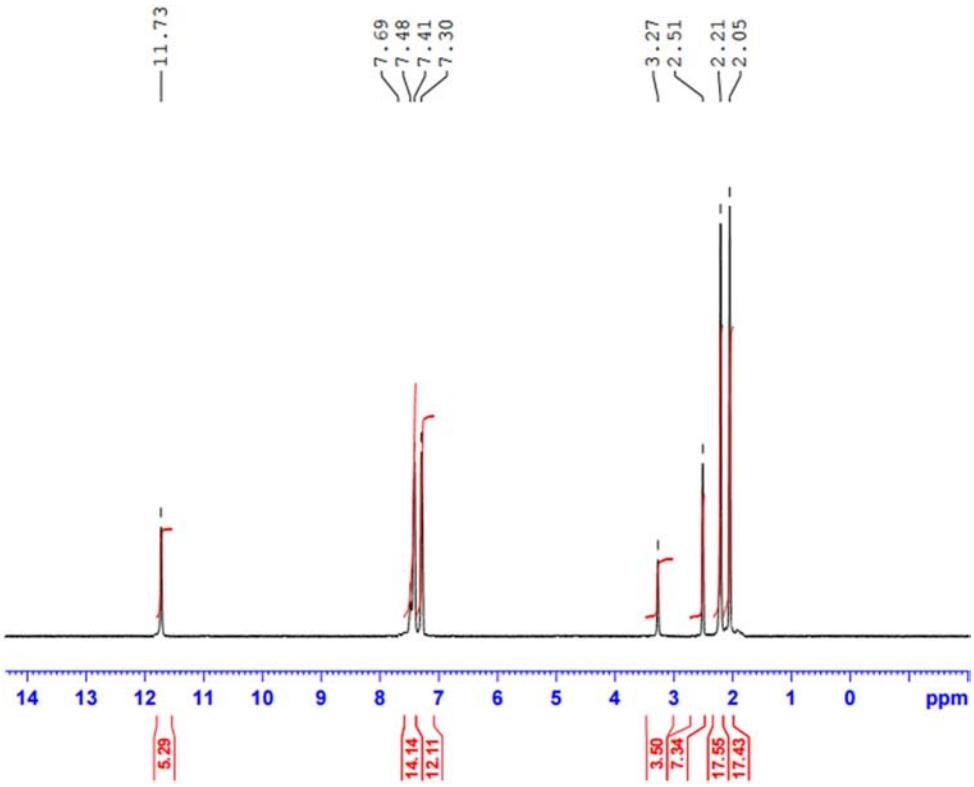

Figure 4. *[1]H-NMR spectrum of compound 5.*

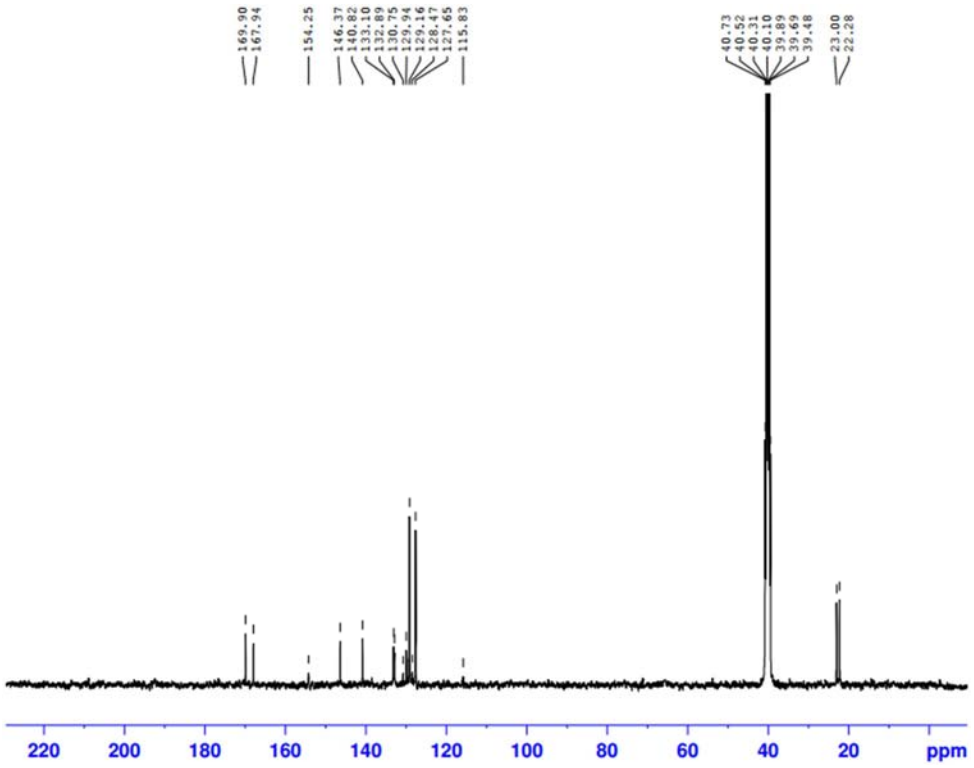

Figure 5. *[13]C-NMR spectrum of compound 5.*

2.2.2. 1-(Thiazolyl)-Pyrazole Derivatives (3 and 4)

The [1]H-NMR spectra of compound 3a "Figure 6" displayed a sharp singlet signal at δ 12.20 ppm due to the proton of OH group for the pyrazole ring at C-5. Protons of the aromatic and thiazole ring of compound 3a were showed at δ 7.39-8.05 ppm.

[13]C-NMR spectrum of compound 3a "Figure 7" showed carbons of aromatic and thiazole ring noticed within the chemical shift regions and demonstrated integral values.

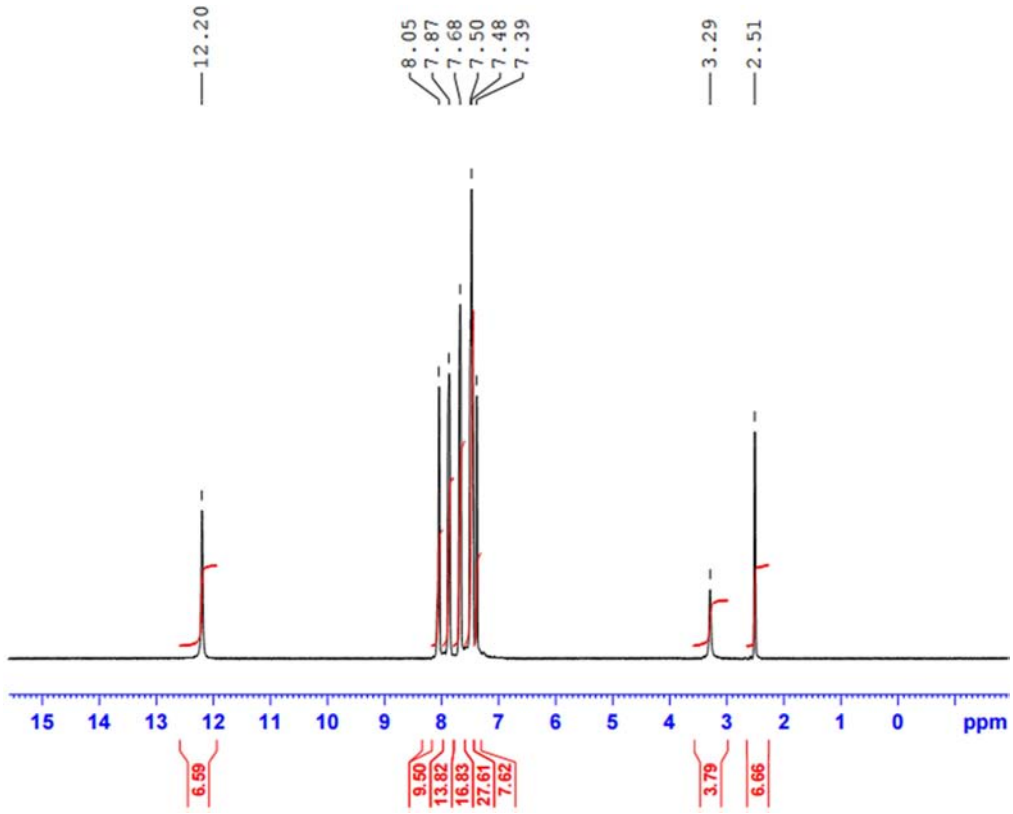

Figure 6. *[1]H-NMR spectrum of compound 3a.*

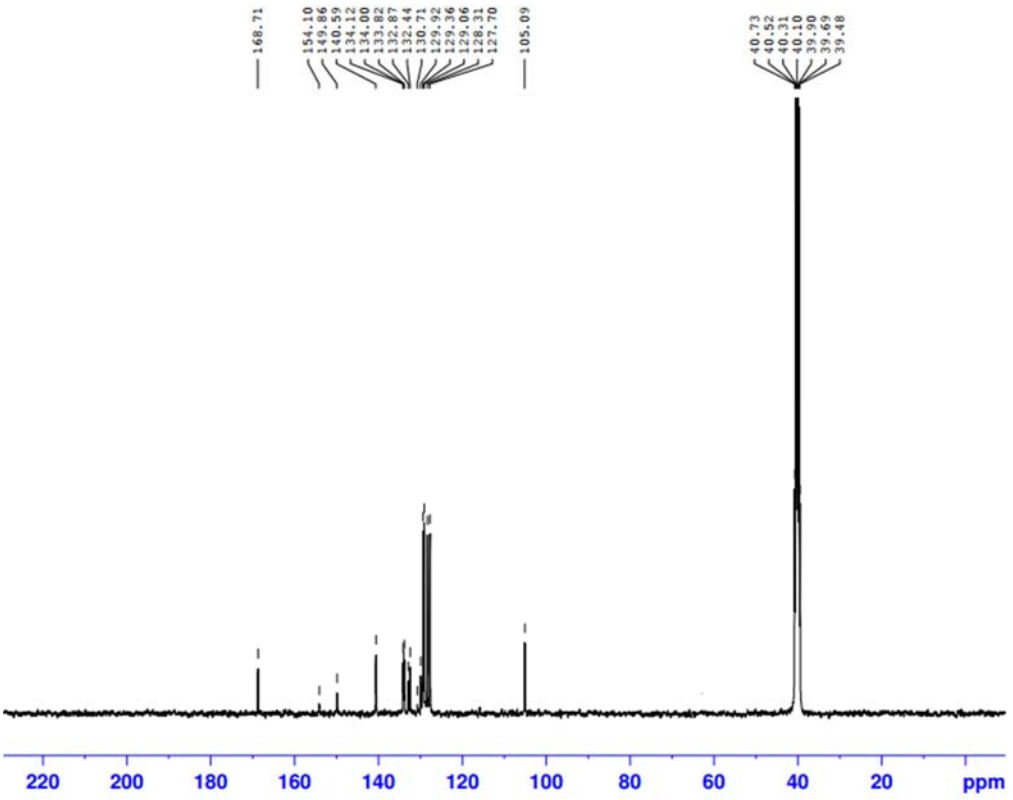

Figure 7. *[13]C-NMR spectrum of compound 3a.*

The [1]H-NMR spectra of compound 3b "Figure 8" showed a sharp singlet signal at δ 11.43 ppm characteristic to the proton of OH group for the pyrazole ring at C-5. Protons of the aromatic and thiazole ring of compound 3b were assignable at δ 7.45-8.33 ppm.

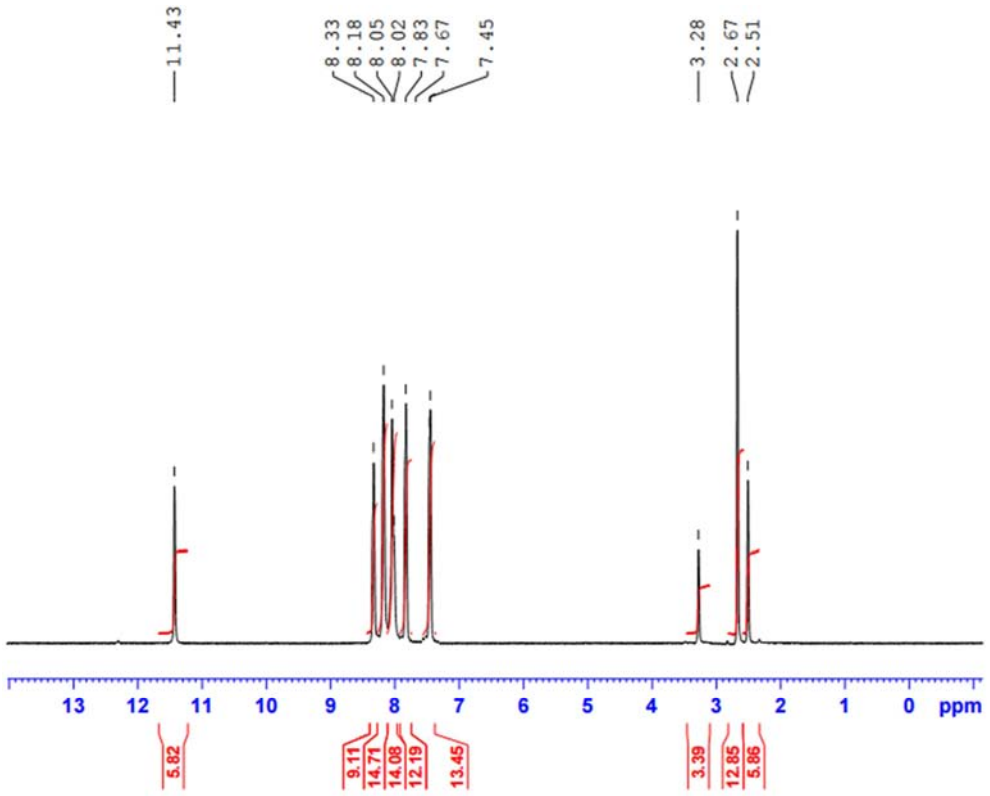

Figure 8. *[1]H-NMR spectrum of compound 3b.*

The [1]H-NMR spectra of compound 3c "Figure 9" displayed a sharp singlet signal at 12.16 ppm due to the proton of OH group for the pyrazole rings at C-5. Protons of the aromatic and thiazole ring of compound 3c were due to δ 6.97-8.04 ppm.

[1]H-NMR spectrum of compound 3c explained a sharp singlet signal at δ 3.79 ppm due to the protons of methoxy group (OCH_3).

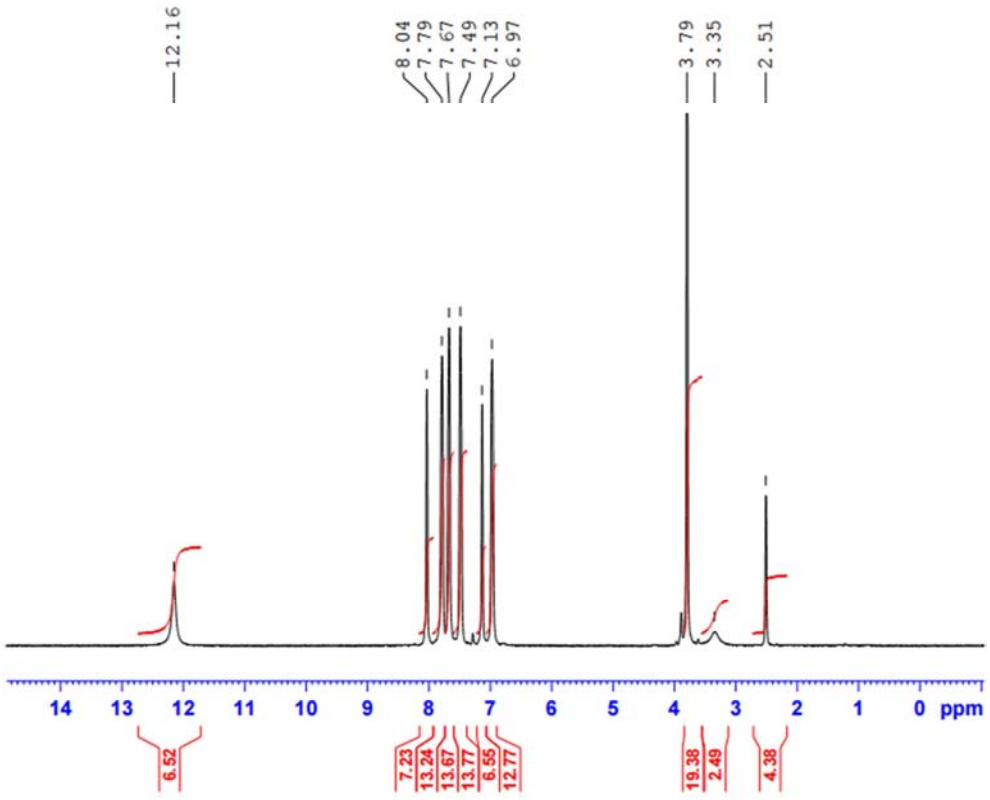

Figure 9. *[1]H-NMR spectrum of compound 3c.*

In the ^{1}H-NMR spectra of compounds 4a-c showed the absence of singlet signals in the region at δ 12.33-11.91 ppm represented to the protons of hydroxyl groups (OH), while the ^{1}H-NMR spectra of compounds 4a-c showed the new singlet signals in the region at δ 2.32-2.50 ppm assignment to acetyl groups (CH_3CO). The ^{1}H-NMR spectrum of compound 4a "Figure 10" as example displayed two singlet signals at δ 8.80 and 2.42 ppm due to the protons of H-thiazole and methyl group. In addition, viewing multiplet signals in the region at δ 7.50- 8.15 ppm corresponding to the protons of aromatic rings.

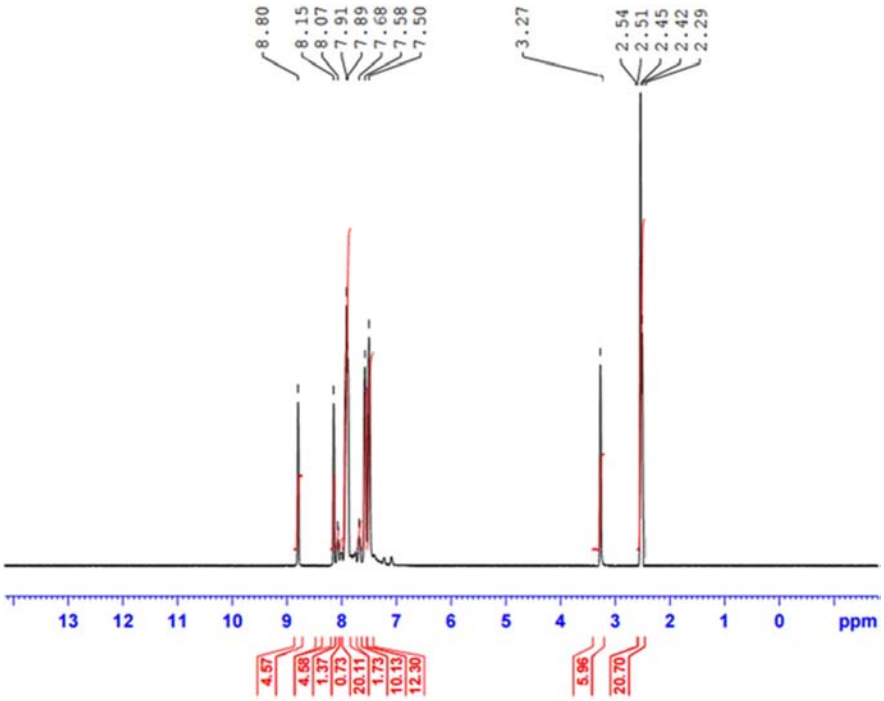

Figure 10. *^{1}H-NMR spectrum of compound 4a.*

In the ^{13}C-NMR spectrum of compound 4a "Figure 11" revealed two new signals at δ 171.99 & 22.85 ppm due to the two carbon atoms of acetyl group (CH_3CO). The signals due to the carbons of aromatic, thiazole, pyrazole rings and cyano group were noticed in the region at δ156.43-114.20 ppm.

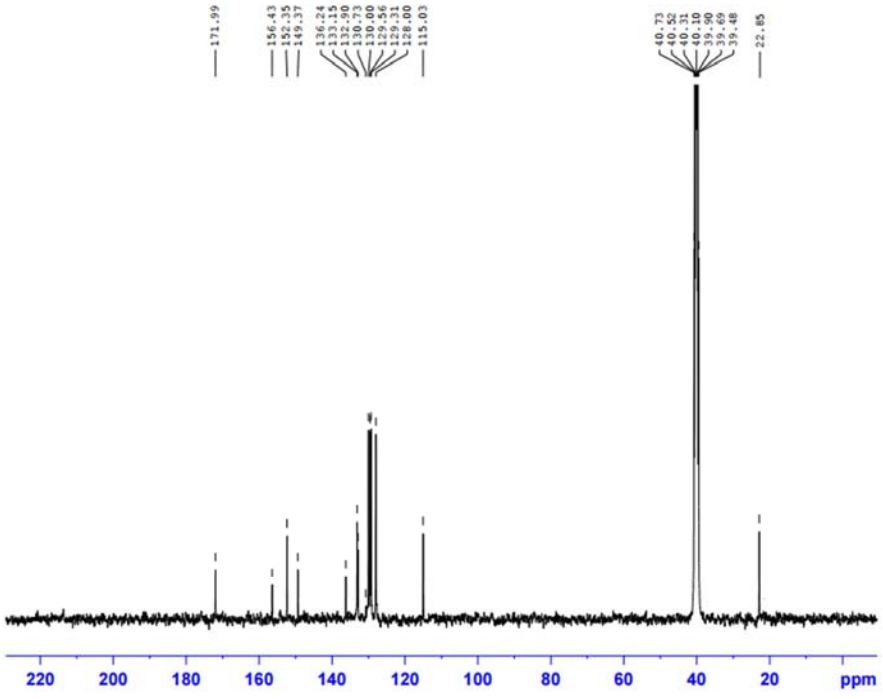

Figure 11. *^{13}C-NMR spectrum of compound 4a.*

^{1}H-NMR spectrum of compound 6 "Figure 12" revealed the presence of a singlet signal at δ 8.42 ppm demonstrated the existence of CH=N group. Aromatic protons resonate as multiplets at around δ 7.44-8.18 ppm, singlet signal showed at δ 3.88 ppm due to –OCH_3 and signal showed at δ 11.35 indicating the presence of OH group.

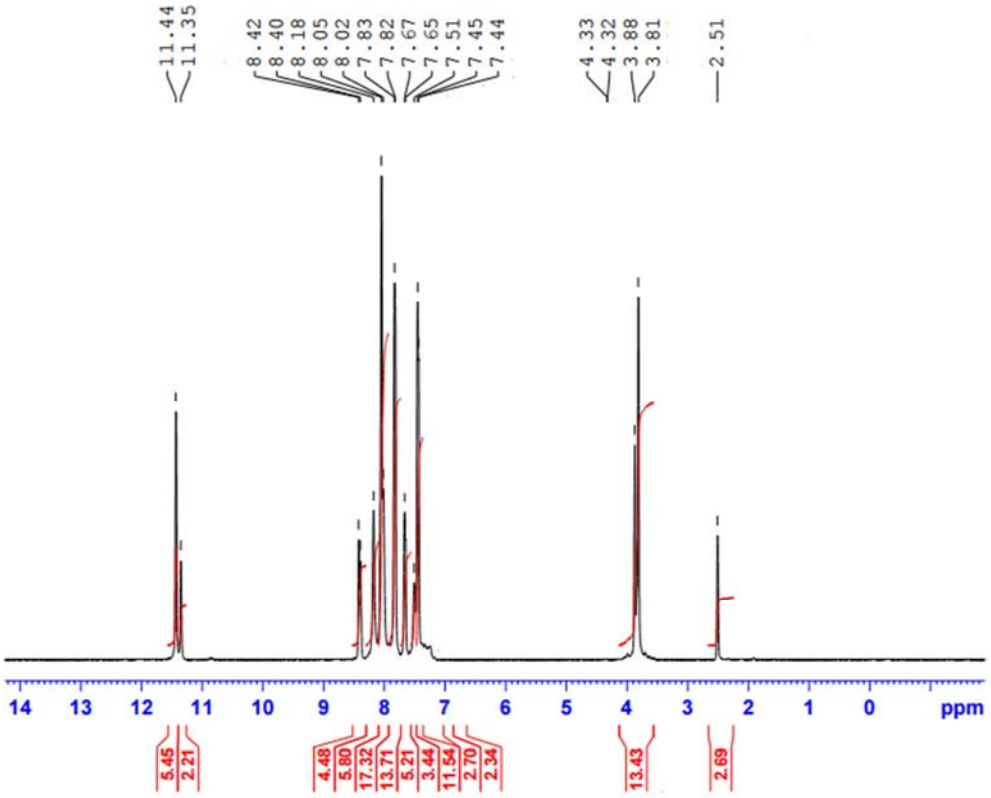

Figure 12. *^{1}H-NMR spectrum of compound 6.*

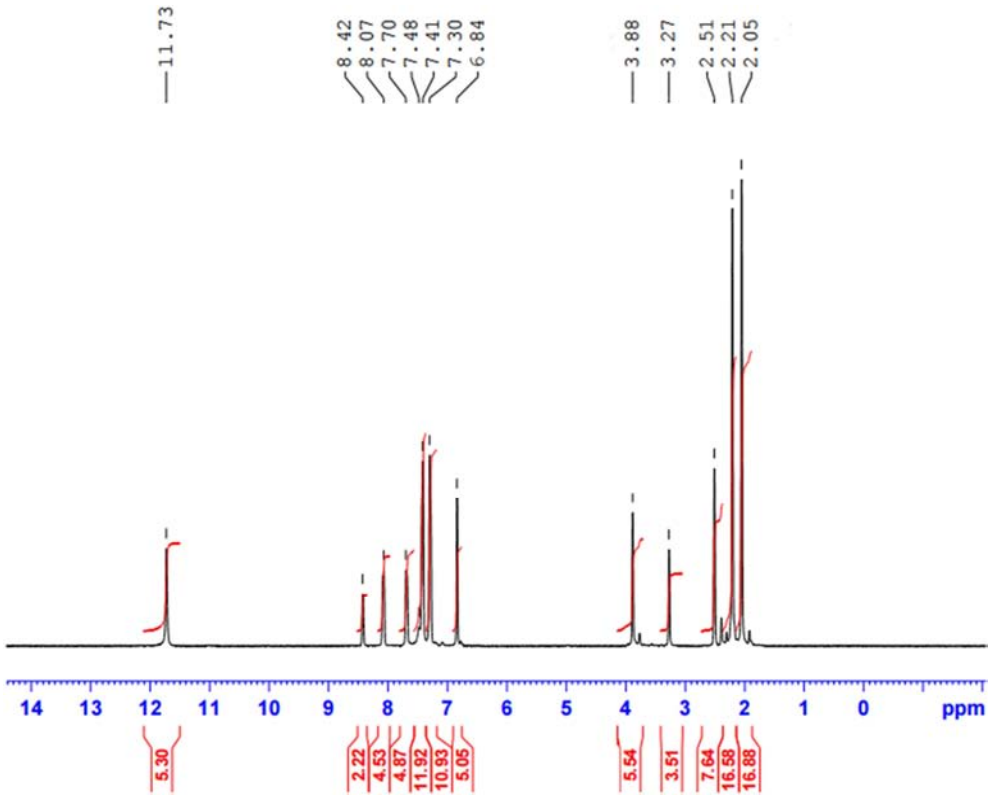

Figure 13. *^{1}H-NMR spectrum of compound 7.*

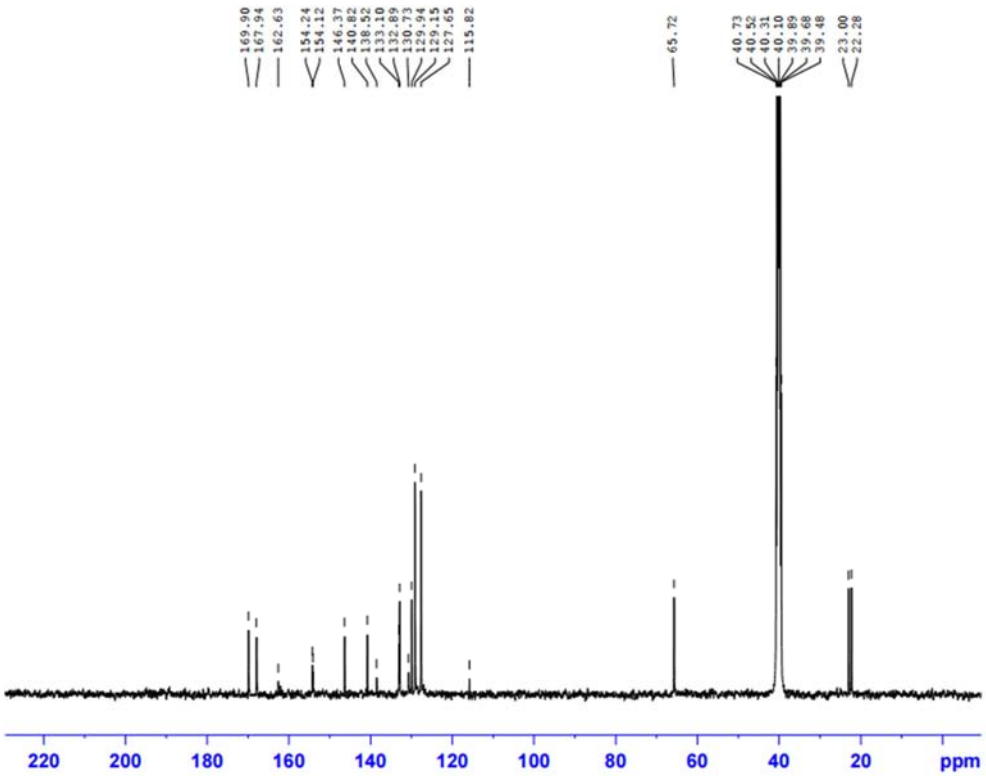

Figure 14. *^{13}C-NMR spectrum of compound 7.*

On the other hand, ^{1}H-NMR spectrum of compound 7 "Figure 13" exhibited signal at δ 2.21ppm due to CH_3CO group, a singlet signal showed at δ 3.88 ppm represented –OCH_3 group, in addition the aromatic multiplets in the region δ 6.84-8.07 ppm, signal showed at δ 11.73 due to OH group. ^{13}C-NMR spectrum of compound 7 "Figure 14" displayed signal at δ 167.94 ppm represented to C=O group, signals showed around δ 154.24-127.65 ppm corresponding to aromatic and pyrazole ring, signal resonating at δ 65.72 due to –OCH_3 and signal showed at δ 23 ppm stand for CH_3 group.

2.3. Biological Assay

2.3.1. In vitro Anti-leukemia Activity

Compounds 3-9 were checked for their anti-proliferative activity against leukemia HL-60 human cancer cell line by means of MTT assay method. Doxorubicin (Dox) was employed as positive reference. The IC_{50} values (μM) of the tested compounds and reference compound are mentioned in "Table 1" and represented graphically in "Figure 15". Compounds 4, 6 and 9 (4c) were the most active against Leukemia HL-60. The IC_{50} values of them were less than 5 μM in the range of 1.35-4.78 μM. In addition, compounds 3 and 5 showed less antiproliferative activity against Leukemia HL-60 cells with IC_{50} values in the range 5.39-8.82 μM. Structurally, pyrazole derivatives were the most active with increasing activity observed more than pyrazole containing thiazole moiety. Moreover, compound *N*- (3- methoxy-2-hydroxybenzal) -3- (*p*-chlorophenyl)-4- cyano-5-oxopyrazol-1-thiocarboxamide (6) possessed the highest cytotoxic activity with IC_{50} value of 1.35 μM compared with Dox IC_{50} value of 2.02 μM. Additionally, it was observed that, a decrease in the cytotoxic activity was shown as acetylation to the pyrazole ring, regardless of the substitution. In conclusion, it is clear that compounds 4, 6 and 9 (4c) were the most potent active compounds. Compound 6 gave the highest cytotoxicty (IC_{50} value is 1.35 μM) compared with the value of Dox (IC_{50} value is 2.02 μM).

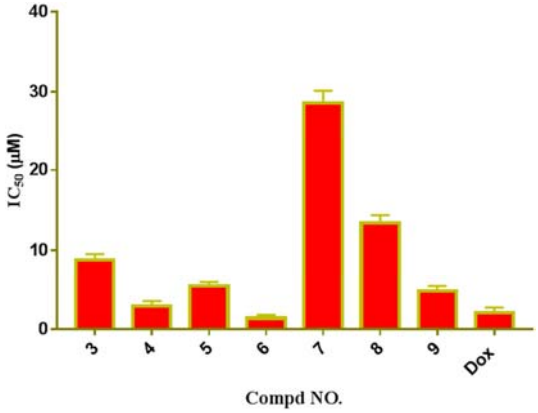

Figure 15. *Graphical presentation of the antitumor activity of the prepared compounds 3-9 over Leukemia HL-60 cell line.*

Table 1. *In vitro antitumor activity of pyrazole derivatives 3-9 over Leukemia HL-60 cell line. Records are represented as the mean ± three experiments.*

Compound No.	IC_{50} values (μM)
	HL-60
3	8.82±0.72
4	2.91±0.13
5	5.39±0.24
6	1.35±0.14

Compound No.	IC_{50} values (μM)
	HL-60
7	28.54±1.56
8	13.4±1.04
9	4.78 ±0.24
Dox	2.02±0.08

2.3.2. Analysis of Cell Cycle

Compound 6 had the highest promising cytotoxicty against Leukemia HL-60 cell line. So it was selected for extra investigation concerning its mechanism of cell proliferation inhibition mechanism. To discover the correlation between the cell cycle progression and anticancer effect, DNA flow cytometric assay by means of propidium iodode (PI) in Leukemia HL-60 cells after handling with compound 6 on its IC_{50} concentration dose for 24 hrs. "Figure 16". It is observed that compound 6 increased the percentage of G2/M phase from 11.05% to 39.22% compared with the untreated control. This increase was accompanied by a decrease in the other states of the cell cycle profile in Leukemia HL-60. This information recommended that, compound 6 evidently increased the percentage of G2/M phase. This leads to cell growth arrest in leukemia HL-60 cells.

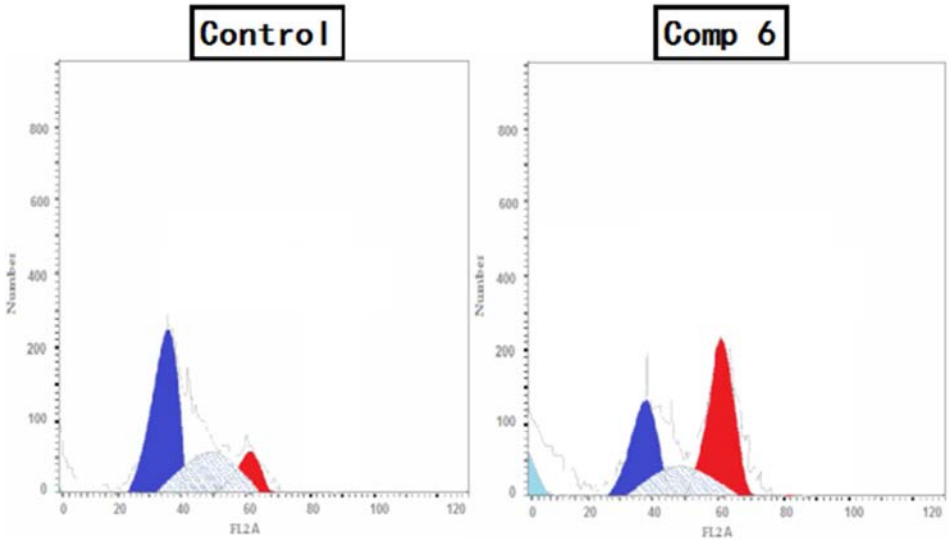

Figure 16. *Cell cycle distribution of human leukemia HL-60 cell line induced by compound 6. Cells were rigid and detected with PI staining to evaluate DNA content by flow cytometry.*

2.3.3. Apoptosis Detection Assay

According to cell death caused by compound 6, a biparamertric cytofluorimetric was analyzed using PI and Annexin-V-FITC which was in Leukemia HL-60 cells subsequent to treatment with compound 6 at its IC_{50} value for 24 hrs "Figure 17". It's revealed that compound 6 increases the value of total apoptosis compared with unprocessed control group. The value of early apoptosis was increased from 1.11% to 6.74% compared with the non-treated control group. Additionally, compound 6 can increase the percentage of late apoptosis from 0.31% to 4.31% compared with untreated control. This result recommended the interest of compound 6 in the apoptosis of Leukemia HL-60 cells-induced cell death.

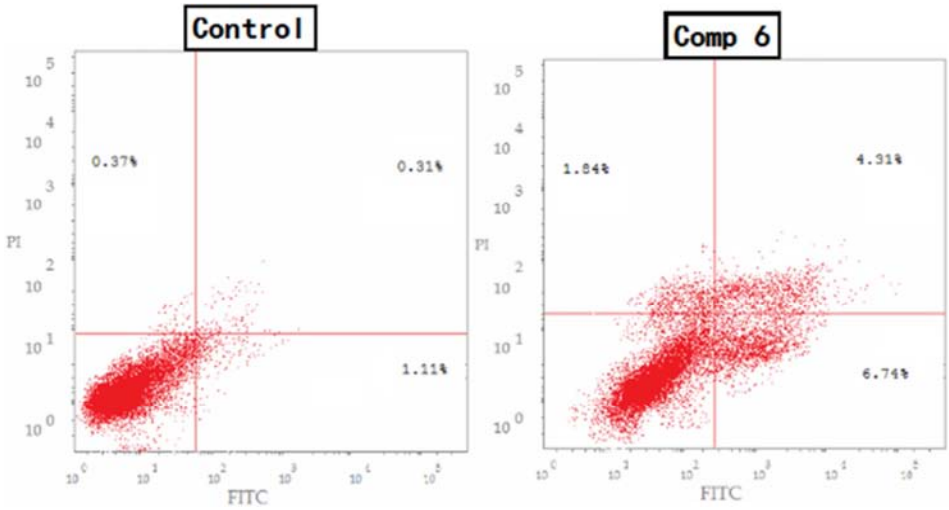

Figure 17. *Apoptosis effect of compound 6 on human leukemia HL-60 cell line. Apoptotic cells were checked with Annexin-V-FITC staining after incubating with compound 6 for 24 hrs.*

2.3.4. Assay of Topoisomerase II

Topoisomerase II (Topo II) is an enzyme which affects on the topology of DNA and acting a vital during cell division involving DNA replication, transcription, segregation and recombination. In this study, compound 6 was evaluated for Topo II inhibition activity IC_{50} value to evaluate whether the most active molecule was strong Topo II inhibitor. Five dose Topo II inhibition attempt was achieved by human DNA Topoisomerase II (Topo II) ELISA screening kit. Etoposide served as positive control in this study. As illustrated in "Figure 18", the Topo II inhibition activity assay revealed that compound 6 was efficient in inhibiting the Topo II activity with IC_{50} value 56.04 nM compared with etoposide which had IC_{50} value 41.11 nM. This result indicated that, compound 6 cytotoxic activity is mainly due to its potent DNA Topo II inhibition activity.

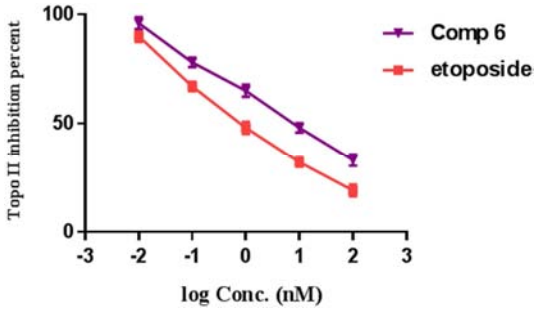

Figure 18. *In vitro DNA Topo II inhibition activity of compound 6 and etoposide.*

3. Experimental Section

3.1. Chemistry

Melting points were measured in open capillary tubes by Electrothermal digital melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR (400 MHz) spectra were recorded in DMSO-d6 on a Bruker 400 DRX-Avance NMR spectrometer using of TMS as internal standard and chemical shifts are expressed in δ (ppm) values. The IR spectra were recorded as KBr pellets on a Bruker (Tensor 37) FT-IR Spectrophotometer. Mass spectra were recorded on LCMS-QQQ instruments. Elemental analyses were achieved on Carlo Erba 1108 Elemental analyzer. All compounds were within ± 0.35% of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) on silica gel F254 aluminum sheets (Merck), and spots were visualized by UV lamp at 254–365 nm.

Syntheses of 3-(*p*-chlorophenyl) -4-cyano-5-hydroxypyrazol-1-thiocarboxamide (2)

A mixture of equimolar quantity of ethyl *β*-(*p*-chlorophenyl)-*α*- cyanoacrylate (0.01 mol) and thiosemicaebazide (0.01mol) in 70 mL ethanol and catalytic amount of anhydrous potassium carbmate (0.03 mol) was heated under reflux for 3-4 hr. The development of the reaction was monitor by TLC. After finishing of the reaction, the solid product was filtered off and washed with water. The solid was then dried, and recrystallized from ethanol to give 2 as yellow crystals, m.p. 200°C, yield 82%, IR (KBr) υ_{max}= 3437, 3281, 3165 (OH and NH_2), 1601, 1526 (C=C), 1490 (C=S), 1705 (C-O), 2256 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 7.46 (NH_2), 7.84-8.05 *dd*, 4H, Ar H), 11.43 (OH) ppm. MS: m/z (%) = 280 (M^++Z, 7.21), 278 (M^+, 21.30). Anal. Calcd. for $C_{11}H_7N_4ClOS$ (278): C, 47.48; H, 2.52; N, 20.14. Found: C, 47.24; H, 2.25; N, 20.02.

Syntheses of 5- hydroxyl -4- cyano- 3- (*p*-chlorophenyl)-1-(5-arylthiazol- 2-yl) – pyrazoles (3a-c)

A mixture of equimolar quantity of substituted pyrazole -1- thiocarboxamide (2, 0.01 mol) and aryl bromomethyl ketones (namely, *p*- chlorophenacyl bromide, *p*-nitrophenacyl bromide and *p*- methoxyphenacyl bromide, 0.01 mol) in 70 mL ethanol in the existence of fused sodium acetate (0.03 mol) was heated under reflux for 4hr. The reaction mixture was cooled and poured into water and neutralized with dilute hydrochloric acid (1%). The resulting solid was filtered off, washed with water, dried and re-crystallized from ethanol to give compound 3.

5- Hydroxyl -4- cyano- 3- (*p*-chlorophenyl)-1- (5-*p*-chlorophenyl-thiazol- 2-yl) – pyrazole (3a) as yellow crystals, yield 72%, m.p. 172°C. IR (KBr) υ_{max}= 3331-3010 (br-OH), 2227 (CN), 1092, 1024 (C-O), 1605, 1563 (C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 7.39-8.05 (*m*, 9H, ArH and H-thiazole), 12.20 (*s*, 1H, OH) ppm. ^{13}C NMR (DMSO-d_6) δ: 168.71, 154.10, 149.86, 140.59, 134.12, 134.00, 133.82, 132.87, 130.71, 129.92, 129.36, 129.06, 128.31, 127.70 (carbons of aromatic, thiazole and pyrazole rings), 105 (CN) ppm. MS: m/z (%) = 415 (M^+ +2, 3.20), 413 (M^+, 9.60), Anal. Calcd for $C_{19}H_{10}N_4Cl_2OS$ (413): C, 55.21; H, 2.42; N, 13.56. Found: C, 55.05; H, 2.22; N, 13.28.

5- Hydroxyl -4- cyano- 3- (*p*-chlorophenyl)-1- (5-*p*-nitrophenyl-thiazol- 2-yl) – pyrazole (3b) as yellow crystals, yield 68%, m.p. 160°C. IR (KBr) υ_{max}= 3315-3100 (*br*. OH), 2025 (CN), 1625 (C=N), 1605, 1585 (C=C), 1103, 1092 (C-O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 7.45-8.33 (*m*, 9H, Ar-H and H-thiazole), 11.43 (*s*, 1H, OH) ppm. Anal. Calcd for $C_{19}H_{10}N_5ClO_3S$ (423): C, 53.90; H, 2.36; N, 16.55. Found: C, 53.71; H, 2.18; N, 16.33.

5- Hydroxyl -4- cyano- 3- (*p*-chlorophenyl)-1- (6-*p*-methoxyphenyl-thiazol- 2-yl) – pyrazole (3c) as yellow crystals, yield 69%, m.p. 145°C. IR (KBr) υ_{max}= 3315-3151 (*br*-OH), 2258 (CN), 1623 (C=N), 1605, 1585 (C=C), 1175, 1083 (C-O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 3.79 (s, 3H, OCH_3), 6.97-8.04 (m, 9H, Ar-H and H-thiazole), 12.16 (br.0s, 1H, OH) ppm. MS: m/z (%) = 410 (M^++2, 4.23), 408 (M^+, 12.30). Anal. Calcd for $C_{20}H_{13}N_4ClO_2S$ (408): C, 58.82; H, 3.19; N, 13.73. Found: C, 58.58; H, 3.02; N, 13.47.

Syntheses of N- (3- methoxy-2-hydroxybenzal) -3- (*p*-chlorophenyl)-4- cyano-5-oxopyrazol-1-thiocarboxamide (6)

A mixture of equimolar quantity of compound 2 (0.01mol) and 3-methoxy-2-hydroxybenzaldehyde (0.01mol) in 30mL acetic acid was heated under reflux for 2hrs. The reaction mixture was cooled and added to water and neutralized with dilute sodium bicarbonate (2%). The product formed was

filtered off, washed with water, dried and purified with recystallization from ethanol to give 6 as orange crystals, yield 71%, m.p. 225°C. IR (KBr) υ_{max}= 3437 (OH), 3280 (NH), 2225 (CN), 1723 (C=O), 1208, 1089 (C-O), 1600, 1528 (C=C), 1442 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.88 (*s*, 3H, OCH_3), 7.44-8.18 (*m*, 7H, Ar-H), 8.42 (*s*, 1H, CH=N), 11.35 (OH) and 11.44 (NH) ppm. MS: m/z (%) = 414 (M^++2, 3.20), 412 (M^+, 10.20). Anal. Calcd for $C_{19}H_{13}N_4ClO_3S$ (412): C, 55.34; H, 3.16; N, 13.59. Found: C, 55.12; H, 3.02; N, 13.39.

General Procedure for acetylation reactions

A solution of compounds 2, 3, and 6 (0.01mol) in 30mL acetic anhydride was heated under reflux for 2 hrs, after that cooled & poured into ice-water. The reaction mixture was kept for 24hrs, and the producing solid was filtered, washed by water, dried and finally crystallized from ethanol to give 4, 5 and 7.

5-Acetoxy- 4-cyano-3- (*p*-chlorophenyl) -1- (5-*p*-chlorophenyl thiazol -2-yl) –pyrazole (4a) as pale yellow crystals, yield 67%, m.p. 220°C. IR (KBr) υ_{max}= 1735 (C=O), 1091, 1012 (C-O), 1625 (C=N), 1601, 1585 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.42 (*s*, 3H, $COCH_3$), 8.80 (*s*, 1H, H-thiazole) 7.50-8.15 (*m*, 8H, Ar-H) ppm. ^{13}C NMR (DMSO-d_6) δ: 171.99 (C=O), 156.43, 152.35, 149.37, 136.24, 133.15, 132.90, 130.73, 130.00, 129.56, 129.31, 128.00 (C of aromatic, thiazole and pyrazole rings), 115 (CN) and 22.85 (CH_3) ppm. MS: m/z (%) = 457 (M^++2, 3.25), 455 (M^+, 11.20). Anal. Calcd for $C_{21}H_{12}N_4Cl_2O_2S$ (455): C, 55.38; H, 2.64; N, 12.31. Found: C, 55.09; H, 2.42; N, 12.03.

5-Acetoxy- 4-cyano-3- (*p*-chlorophenyl) -1- (5-*p*-nitrophenyl thiazol -2-yl) –pyrazole (4b) as pale yellow crystals, yield 63%, m.p. 230°C. IR (KBr) υ_{max}= 2228 (CN), 1741 (C=O), 1083, 1041 (C-O), 1605, 1575 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.45 (*s*, 3H, $COCH_3$), 7.41-8.32 (*m*, 8H, Ar-H), 8.62 (*s*, 1H, H-thiazole) ppm. Anal. Calcd for $C_4H_{12}N_5ClO_4S$ (465): C, 54.19; H, 2.58; N, 15.05. Found: C, 54.01; H, 2.35; N, 14.89.

5-Acetoxy- 4-cyano-3- (*p*-chlorophenyl) -1- (5-*p*-methoxyphenyl thiazol -2-yl) –pyrazole (4c) as pale yellow crystals, yield 71%, m.p. 220°C. IR (KBr) υ_{max}= 2251 (CN), 1741 (C=O), 1125, 1087 (C-O), 1625 (C=N), 1605, 1583 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.82 (*s*, 3H, OCH_3), 6.98-8.01 (*m*, 8H, Ar-H), 8.35 (*s*, H, H-thiazole), 2.43(*s*, 3H, $COCH_3$) ppm. MS: m/z (%) = 452 (M^++2, 2.30), 450 (M^+, 7.50). Anal. Calcd for $C_{22}H_{15}N_4ClO_3S$ (450): C, 58.67; H, 3.33; N, 12.44. Found: C, 58.44; H, 3.11; N, 12.16.

N-Acetyl 5-Acetoxy- 4-cyano-3- (*p*-chlorophenyl) –pyrazole -1- thiocarboxamide (5) as pale yellow crystals, yield 68%, m.p. 226°C. IR (KBr) υ_{max}= 3216 (NH), 2252 (CN), 1715, 1695 (C=O), 1098, 1071 (C-O), 1625 (C=N), 1606, 1583 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.05 (*s*, 3H, $COCH_3$), 2.21 (*s*, 3H, $COCH_3$), 7.30-7.69 (*m*, 4H, Ar-H), 11.73 (*s*, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6) δ: 193.52 (C=S), 169.90, 167.94 (C=O), 154.25, 146.37, 140.82, 133.10, 132.89, 129.54, 128.16, 128.47, 127.65 (C of aromatic and pyrazole rings), 115.83 (CN), 23.00, 22.28 (CH_3). MS: m/z (%) = 364 (M^++1, 0.51), 362 (M^+, 1.70), 320 (100%). Anal. Calcd for $C_{15}H_{11}N_4ClO_3S$ (362): C, 49.72; H, 3.04; N, 15.47. Found: C, 49.59; H, 2.87; N, 15.35.

N-(*m*-Methoxy-*O*-acetoxy-benzal) 5- hydroxyl- 4- cyano-3-(*l*-chlorophenyl)-pyrazol-1-thiocarboxamide (7) as pale orange crystals, yield 71%, m.p. 180°C. IR (KBr) υ_{max}= 3432 (*br*-OH), 2256 (CN), 1721 (C=O), 1088, 1011 (C-O), 1628 (C=N), 1610, 1582 (C=C), 1446 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.21 (*s*, 3H, $COCH_3$), 3.88 (*s*, 3H, OCH_3), 6.84-8.07 (*m*, 7H, Ar-H), 8.42 (*s*, 1H, CH=N), 11.73 (*br.s*, 1H, OH) ppm. ^{13}C NMR (DMSO-d_6) δ: 169.90 (C=S), 167.94 (C=O), 154.24, 146.37, 140.83, 138.52, 133.10, 132.89, 129.94, 129.15, 127.65 (C-aromatic and pyrazole ring), 115.83 (CN), 65.72 (OCH_3), 23.00 (CH_3) ppm. MS: m/z (%) = 456 (M^++2, 0.7), 454 (M^+, 3.01). Anal. Calcd for $C_{21}H_{15}N_4ClO_4S$ (454): C, 55.51; H, 3.30; N, 12.33. Found: C, 55.28; H, 3.09; N, 12.13.

3.2. Biological Studies

3.2.1. Anti-tumor Activity Against Leukemia Cancer Cell Line (Leukemia HL-60)

The cytotoxic activity was checked *in vitro* for the recently prepared compounds by using the MTT assay. Cells were plated in 96- multiwall plate (10^5 cells/well) for 24 hrs before handling with the compounds. Examined compounds were dissolved in dimethyl sulfoxide. Several concentrations of the compound under assessment (10, 25, 50, and 100 µM) were added to the cell's monolayer. Triplicate wells were arranged for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 hrs at 37°C and in atmosphere of 5% CO_2. After 48 hrs, cells were fixed, washed and stained with 40µL of MTT solution (5 mg/mL of MTT in 0.9% NaCl) and was added in each well then incubated for extra 4 hrs. MTT crystals were dissolved in 180 µL of acidified isopropanol/well. The plate was shaking at room temperature, and then determining the absorbance at 570 nm by ELISA reader. The molar concentration requisite to inhibit 50% of cell capability (IC_{50}) was calculated, and compared with the reference drug doxorubicin. The surviving fractions were represented as means ± S.E.M.

3.2.2. Analysis of Cell Cycle for Compound 6

Leukemia HL-60 cells, ($3.0x10^5$ cells/well) was incubated at 37°C for 12 hrs. Compound 6 was added to target cells at its IC_{50} concentration and left for 24 hrs. After treatment, cells were collected and fixed with 75% ethanol at 20°C overnight. The cells were washed with PBS followed by centrifugation and then incubated with (10 mg/mL) Rnase (Sigma, USA) and (5 mg/mL) propidium iodide (PI, Sigma) before flow cytometry analysis (FACSCalibur cytometer using Cellquest software, BD Bioscience, USA).

3.2.3. Annexin V-FITC/PI and Detection of Apoptosis of Compound 6

Leukemia HL-60 cells ($1.5x10^5$ cells/well), incubated for 12 hrs. Cells were treated with compound 6 at its IC_{50} value for 24 hrs. Apoptosis was determined by staining cells with FITC-conjugated Annexin-V and Propidium Iodide, and then

analyzed by a *FACSCalibur* cytometer by using Cellquest software (BD Bioscience).

3.2.4. In vitro Topoisomerase II Enzyme Inhibition Assay

Compound 6 was selected to be examined against topo II using human DNA Topoisomerase II (Topo II) ELISA screening kit according to manufacturer's instructions. 100 mL of standard and sample was added per well and incubate for 2 hrs at 37°C. Take out the liquid of each well. Add 120 mL of biotin-antibody to each well then incubate for 1 hr at 37°C. Aspirate each well and wash three times. Add 100 mL of horseradish Peroxidase (HRP-avidin) to each well and then incubate for 1 h at 37°C. Repeat the aspiration/wash process for five times. Add 90 mL of 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate to each well and incubate for 15-30 min at 37°C, save from light. Add 50 mL of stop solution to each well and find out the optical density of each well within 5 min, using a ROBONEK P2000 ELISA reader to 450 nm. The values of % activity versus a series of compound log concentrations (-2nM, -1nM, 0nM, 1nM, 2nM) were then plotted using non-linear regression analysis of sigmoidal dose-response curve. The IC_{50} values for compounds 6 against topo II was estimated by the concentration causing a half-maximal percent activity and the records were compared with etoposide as standard. All experiments were made in triplicates.

4. Conclusion

The purpose of the current work was to syntheses and study *In vitro* antileukemia cancer activity of some novel pyrazole derivatives (2 and 5) and pyrazoles carrying the biologically active thiazole moieties at 1-position. All the synthesized compounds were established by using IR, MS, ^{1}H, ^{13}C NMR and elemental analyses. All of the synthesized compounds were monitored for their cytotoxic activity against Leukemia HL-60 cell line by using Doxorubicin as positive control. The results demonstrated that compound 6 was the most potent cytotoxic activity with IC_{50} value 1.35 μM. Exhaustive mechanism studies for cell cycle analysis of compound 6 were exposed cell cycle detain at G2/M phase and pre-G1 apoptosis. Topoisomerase II inhibition activity assay confirmed that compound 6 is a potent DNA Topoisomerase II inhibitor.

Declaration of Interest Section

Author has no declarations of interest to report

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