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Synthesis of new heterocycles via methylenebis(2-(2-methoxyphenyl)thiazolidin-4-one) as potential anticancer agents

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Abstract: NaOAc catalyzed condensation of bis(2-(2-methoxyphenyl)thiazolidin-4-one) with *p*-methoxybenzaldehyde in AcOH gave the corresponding chalcones. Cyclization of the synthesized chalcones with NH_2OH by refluxing in AcOH afforded the related isoxazoles fused with thiazoles. All the synthesized isoxazoles were evaluated against different tumor cell lines. Almost all compounds showed activity against prostate cancer cell lines.

Keywords: Bis thiazolo isoxazoles; cytotoxic activity; Knovenagal condensation; cyclisation.

1. Introduction

The isoxazole moiety is a crucial pharmacophore, biochemical synthon in medicinal chemistry. The dynamic potency of isoxazoles has sparked a lot of research, it's been extensively studied for use in antiepileptic[1], PPAR agonists[2], acetylcholine esterase inhibitor[3], anti-inflammatory[4], acrosin inhibitor[5], anti bacterial [6],A-Precursor protien[7] Protein tyrosine phosphate inhibitor[8,9]anti viral [10], anti convulsant[11], insecticidal[12], antitubercular[13], immuno modulatory[14] and hypolipomic[15] treatments.

Thiazolidinone derivatives also have wide biological activities and pharmacological properties[16].This interesting core has received considerable attention for its myriad biological activities,such as antibacterial[17],antidiabetic[18], antibiofilm[19], anticancer[20], antifungal[21,22], anti-inflammatory[23],tyrosinase inhibitory[24], cyclooxygenase-2 inhibitory[25], and anti-HIV [26,27] , nematicidal [28] properties. It has also been used to treat heart diseases. [29]

"Following the successful introduction of thiazolidinones, isoxazoles and continuation of our work on the development of novel heterocyclics[30, 31] we have synthesized some novel methylene bis *p*-methoxyphenyl thiazolo isoxazoles and evaluated their anticancer activity."

2. Experimental

2.1. Chemical Methods and Apparatus

"Reagents from the commercial range are employed in accordance with instructions. It is possible to dry purify solvents outside of the refining reagent range if required. On Merck silica F254 Silica plates that had been used to monitor the progress and purity of compounds, and composite

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compounds were either subjected to UV light or submerged in 1 percent $KMno_4$ solution.SGCcolumns (60-120) mesh are often used for separation. All melting points were processed and measured by Fischer –Johns. Data was captured on KBr discs using the Perkin –Elmer FLIR Spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra measured Varian –Gemini spectrometer (300MHz at ^{1}H and 75 MHz at ^{13}C) ppm changes and coupling constants (J) compared to TMS. The Mass spectra were captured using a VG micro mass 7070H spectrometer. Perkin Elmer 240 CHN analysis of the essential elements (C,H,N) was within 0.4 percent of theory."

2.2. Biological methods and Apparatus

SRB – assay kit ab235935 was purchased from Abcan, MCF cancer lines purchased from Sigma Aldrich, Prostate cancer cell lines were taken from patient suffering from Prostate cancer and HeLa cancer cell lines purchased from Thermo Fischer scientific. Olimpus Xi71 microscope was used to capture images of the strained cells using SA-gel levels. PI was taken by fluorescence activated cell sorting (FACS Calibur System; BD Bio- science, Erembodegem, Belgium) in a FL-2 fluorescence detector (10000 events were recorded for each condition) using FCS express 4 software (De Novo Software, Los Angeles, CA) flow cyto metry data was analyzed.

2.3. Chemistry

*Compounds are synthesized using a standard procedure **2(a-g)***: A mixture of **1** (5 mmol), p-methoxy Benzaldehyde (10 mmol) and anhydrous NaOAc (5 mmol) was refluxed for 3 hours in glacial AcOH (10 mL).After removing acetic acid under reduced pressure it was poured in ice cold water, the particles separated was filtered, washed with water and recrystallized it from glacial acetic acid to afford **2a-g**.

*(5Z, 5'Z)-2 ,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene)) bis(5-(4-methoxy benzylidi ne)-3-phenylthiazolidin-4-one)(**2a**)*:m.p:145-44^{o}C.IR(KBr):ν_{max}3026,2985,1721,1530,1472,666cm^{-1}.^{1}HNMR ($CDCl_3$, 300MHz):δ 3.83(s,6H,OCH_3),3.80(s,6H,OCH_3),3.50(s,2H, CH_2), 6.95 (d,*J* = 9.1 Hz, 2H, Ar-H), 7.15-7.06 (m, 8H, Ar-H),7.29(s,2H, CH=C), 7.33-7.30 (m, 8H, Ar-H + CH-S),7.50-7.43(m,8H, Ar-H).^{13}CNMR($CDCl_3$,75MHz):δ 42.3, 56.7, 56.0, 62.1, 114.3, 114.1, 123.5, 125.0, 125.4, 125.8, 126.2,128.8, 130.8, 132.0, 133.3, 135.5, 135.7, 154.6, 161.6,172.9. ESI-MS: *m/z* 819 $[M+1]^+$. Anal. Calcd. for $C_{49}H_{42}F_2N_2O_6S_2$: C, 71.86; H, 5.17; N, 3.42. Found: C, 71.67; H, 4.97; N, 3.35.

*(Z)-3-(4-chlorophenyl)-2-(2-methoxy-5-(4-methoxy-3-((Z)-5-(4-methoxybenzylidene)-4-oxothiazolidin-2-yl)benzyl)phenyl)-5-(4-methoxybenzylidene)thiazolidin-4-one(**2b**)*:m.p: 153-55 ^{o}C.IR (KBr): ν_{max} 3035, 2989, 1719, 1535, 1182,747, 686 cm^{-1}. ^{1}H NMR ($CDCl_3$,300 MHz): δ 3.67 (s, 2H, CH_2),3.82 (s, 6H, OCH_3), 3.83(s,6H,OCH_3), 6.92 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.03 (d, *J* = 9.1 Hz, 4H, Ar-H), 7.06 (s, 2H, CH-S),7.10 (d, *J* = 8.3 Hz, 2H, Ar-H) 7.25 (s,2H,Ar-H),7.38-7.30(m 10H, Ar-H+CH=C),7.53(d, *J* =8.8Hz,4H,Ar-H)..^{13}C NMR ($CDCl_3$, 75 MHz): δ 42.3, 56.0, 56.7, 62.1, 114.1, 114.3, 123.5, 125.8, 125.4, 132.0, 133.9, 135.5, 154.6, 161.6, 172.9. ESI-MS: *m/z* 886 (M^+). Anal. Calcd. fo $C_{49}H_{40}Cl_2N_2O_6S_2$: C, 66.28; H, 4.54; N, 3.16. Found: C, 66.17; H, 4.41; N, 3.09.

*(5Z,5'Z)-2,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-methoxybenzyliden e)-3-(4-nitrophenyl)thiazolidin-4-one)(**2c**)*:m.p:189-91^{o}C.IR(KBr):ν_{max}3036,2995,1720, 1542, 1535, 1340, 1187, 684 cm^{-1}.^{1}H NMR ($CDCl_3$,300 MHz): δ 3.71 (s, 2H, CH_2), 3.81 (s, 6H, OCH_3),3.85(s,6H,OCH_3), 6.96 (d, *J* = 9.0 Hz 2H, Ar-H), 7.03-7.0 (m,6H, Ar-H+CH-S), 7.18(d, *J* = 8.4 Hz, 2H, Ar-H), 7.29 (s, 2H, Ar-H), 7.31 (s, 2H, C=CH), 7.51(d, *J* = 8.7 Hz, 4H, Ar-H),7.71 (d, *J* = 9.7 Hz, 4H, Ar-H),8.27(d, *J* = 8.7 Hz, 4H, Ar-H).^{13}C NMR ($CDCl_3$,75 MHz): δ 42.38, 56.0, 56.7, 62.1, 114.3, 114.1, 121.6, 123.5, 125.8,125.4, 128.8, 130.8, 132. 0, 133.9, 135.5, 143.0, 144.3, 154.6, 161.6, 172.9. Mass: *m/z* 908 $[M]^+$. Anal. Calcd. for $C_{49}H_{40}N_4O_{10}S_2$: C, 64.74; H, 4.44; N, 6.16. Found: C, 64.63; H, 4.29; N, 6.07.

*(5Z,5'Z)-2,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-methoxybenzyli dene)- 3-p-tolylthiazolidin-4-one)(**2d**):* m.p:167-69 °C. IR (KBr): ν_{max} 3062,2990,1720,685 cm^{-1}. ^1H NMR ($CDCl_3$,300 MHz): δ 2.34 (s, 6H, CH_3), 3.76 (s, 6H, OCH_3), 3.79 (s, 2H,CH_2), 3.81 (s, 6H, OCH_3), 6.66 (s, 2H, CH-S), 6.94 (d,*J*= 9.3Hz, 2H, Ar-H), 7.03 (d,*J*= 8.6Hz, 4H, Ar-H), 7.15 (d, *J*= 8.6Hz, 2H, Ar-H), 7.23 (d, *J* = 8.3 Hz, 4H, Ar-H), 7.30 (s, 2H, CH=C),7.41-7.38(m,6H,Ar-H),7.49(d, *J*= 9.8Hz,4H,Ar-H). ^{13}C NMR ($CDCl_3$,75 MHz): δ 21.1, 42.3, 56.0, 56.7, 62.1, 114.1, 114.3, 122.5, 125.4, 125.8, 128.8, 130.6, 130.8, 132.0, 134.7,134.9, 135.9, 154.6, 161.6, 172.9. ESI-MS: *m/z* 846 $[M]^+$. Anal. Calcd. for $C_{51}H_{46}N_2O_6S_2$: C, 72.32; H, 5.47; N, 3.31. Found: C, 72.16; H, 5.32; N, 3.24.

*(5Z,5'Z)-2,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-hydroxyphenyl)-5-(4-methoxybenzylidene)thiazolidin-4-one)(**2e**)*: m.p: 171-73 °C. IR (KBr): ν_{max} 3410, 3035, 2962, 1722, 1530, 1271, 682 cm^{-1}.^1H NMR ($CDCl_3$,300 MHz): δ 3.68 (s, 2H, CH_2), 3.82 (s, 12H, OCH_3), 5.44 (s, 2H, OH), 6.87 (d, *J* = 9.1 Hz, 4H, Ar-H), 6.91 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.03 (d, *J* = 9.1 Hz, 4H, Ar-H), 7.10 (s, 2H, Ar-H), 7.11 (s, 2H, CH-S), 7.29-7.66 (m, 6H, Ar-H),7.34 (s,2H,CH=C),7.52 (d, *J* = 7.1 Hz, 4H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 42.3, 56.0, 56.7, 62.1, 114.1, 114.3, 117.2, 123.5, 125.4, 127.8, 128.8, 128.9, 132.0, 130.8, 133.9, 135.5, 154.6, 157.0, 161.6, 172.9. ESI-MS *m/z* 850 $[M]^+$. Anal. Calcd. for $C_{49}H_{42}N_2O_8S_2$: C, 69.16; H, 4.97; N, 3.29. Found: C, 69.74; H, 4.75; N, 3.14.

*(5Z,5'Z)-2,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-fluorophenyl)-5-(4-methoxybenzylidene)thiazolidin-4-one)(**2f**)*:m.p:181-83 °C. IR (KBr): ν_{max} 3065, 2992, 1720, 1530,1270,686 cm^{-1}. ^1H NMR ($CDCl_3$,300 MHz): δ 3.59 (s, 2H, CH_2), 3.82 (s, 6H, OCH_3), 3.85 (s, 6H, OCH_3), 6.93(d, *J* = 9.0 Hz, 2H, Ar-H), 7.03(d, *J* = 9.7 Hz, 4H, Ar-H),7.10 (d, *J* = 9.5 Hz, 4H, Ar-H), 7.16 (t, 4H, Ar-H), 7.36 (s, 2H, =CH), 7.41 (s, 2H, CH-S), 7.47 (t, 4H, Ar-H), 7.53 (d, *J*= 8.1Hz, 4H, Ar-H). ^{13}C NMR ($CDCl_3$,75 MHz): δ 42.3, 56.0, 56.7, 114.1, 114.3, 116.7, 123.5, 125.4, 126.1, 126.8, 128.8, 130.0, 132.0, 133.9, 135.5, 154.6, 159.1, 161.6, 172.9. ESI-MS: *m/z* 854 $[M]^+$. Anal. Calcd. for $C_{49}H_{40}F_2N_2O_6S_2$: C, 68.83; H, 4.72; N, 3.28. Found: C, 68.74; H, 4.60; N, 3.10.

*(5Z,5'Z)-2,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-methoxybenzylide ne)-3-o-tolylthiazolidin-4-one)(**2g**)*: m.p:157-59 °C. IR (KBr): ν_{max} 3062, 2967, 1722, 1532, 1269, 685 cm^{-1}.^1H NMR ($CDCl_3$,300 MHz): δ 2.32 (s, 6H, CH_3), 3.68 (s, 2H, CH_2), 3.82 (s, 6H, OCH_3), 3.84 (s,6H,OCH_3), 6.92 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.03 (d, *J* = 8.5Hz, 4H, Ar-H),7.11(d, *J* = 9.5Hz, 4H, Ar-H),7.16 (s, 2H, Ar-H) 7.21 (d, *J* = 8.4 Hz, 4H, Ar-H), 7.26 (s, 2H, Ar-H),7.33 (s 2H, CH=C),7.41 (s, 2H, CH-S),7.54 (d, *J*=9.2 Hz, 4H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.2, 42.3, 56.0, 56.7, 63.4, 114.1, 114.3, 123.5, 125.4, 125.8, 126.1, 128.8, 130.8, 132.0, 133.9, 134.5, 135.5, 154.6, 161.6, 171.3.Mass**:** *m/z* 846 $[M]^+$. Anal. Calcd. for $C_{51}H_{46}F_2N_2O_6S_2$: C, 72.32; H, 5.47; N, 3.31. Found: C, 72.16; H, 5.35; N, 3.19.

*Compounds synthesized using a standard procedure **3(a-g)**:* A solution of glacial acetic acid, compounds **2(a-g)** (5mmol) and hydroxylamine hydrochloride (10 mmol) was refluxed for 8 hours. Pure components were obtained by filtering, washing and crystallisation of reaction mixture in water after it had been concentrated and cooled.

*(4-methoxy-3-(3-(4-methoxyphenyl)-6-phenyl-3,3a,5,6-tetrahydroisoxazolo[3,4-d]thiazo l-5-yl)phenyl)(4-methoxy-3-(3-(4-methoxyphenyl)-6-phenyl-3,3a,5,6-tetrahydrothiazolo [4,5-c]isoxazol-5-yl)phenyl)methane (**3a**):* m.p: 179-81 °C. IR (KBr): ν_{max} 3065, 2970, 1600, 1562, 1470, 1270, 1065, 820 cm^{-1}.^1H NMR ($CDCl_3$,300 MHz): δ 3.65 (s, 2H, CH_2), 3.78 (s, 6H, OCH_3), 3.81(s, 6H, OCH_3), 4.39 (d, *J* = 2.2 Hz, 2H, S-CH), 5.61(s, 2H, CH-N), 6.21 (d, *J*= 8.5 Hz, 2H, CH-O), 6.65 (d, *J* = 9.1 Hz, 4H, Ar-H), 6.81 (t, 2H, Ar-H), 6.91-6.90 (m, 6H, Ar-H), 7.24-7.21 (m, 12H, Ar-H).^{13}C NMR ($CDCl_3$,75 MHz): δ 42.3, 56.0,56.7,58.4, 69.2, 82.3, 113.9, 114.3, 124.6, 126.7, 128.8,129.3, 129.5, 132.3, 133.9, 137.3, 155.0, 158.8. ESI-MS: *m/z* 848 $[M+1]^+$. Anal. Calcd. for $C_{49}H_{44}F_2N_4O_6S_2$: C, 69.32; H, 5.22; N, 6.60. Found: C, 69.20; H, 5.08; N, 6.45.

*(3-(6-(4-chlorophenyl)-3-(4-methoxyphenyl)-3,3a,5,6-tetrahydroisoxazolo[3,4-d]thiazol-5-yl)-4-methoxyphenyl)(3-(6-(4-chlorophenyl)-3-(4-methoxyphenyl)-3,3a,5,6-tetrahydrot hiazolo[4,5-c] isoxazol-5-yl)-4-methoxyphenyl)methane (**3b**)*: m.p:167-69 ^{o}C.IR(KBr):ν_{max}30 70, 2962, 1624, 1590, 1496, 1003, 839, 673 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.61 (s, 2H, CH_2), 3.77 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 4.71 (d, *J* = 2.2 Hz, 2H, S-CH), 5.61 (s, 2H, CH-N), 6.48 (d, *J* = 9.1 Hz, 2H, CH-O), 6.57 (d, *J* = 8.5 Hz, 4H, Ar-H), 6.90 (m, 6H, Ar-H), 7.16 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.24-7.22 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 42.38, 56.0, 56.7, 58.4, 69.2, 82.3, 113.9, 114.3, 124.6, 127.6, 128.8, 129.3, 130.3, 131.6, 132.2, 133.9, 135.6, 155.0, 158.8. ESI-MS: *m/z* 894 $[M]^+$. Anal. Calcd. for $C_{49}H_{42}Cl_2N_4O_6S_2$: C, 64.12; H, 4.61; N, 6.10. Found: C, 64.06; H, 4.50; N, 6.01.

*(4-methoxy-3-(3-(4-methoxyphenyl)-6-(4-nitrophenyl)-3,3a,5,6-tetrahydroisoxazolo[3,4-d]thiazol-5-yl)phenyl)(4-methoxy-3-(3-(4-methoxyphenyl)-6-(4-nitrophenyl)-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazol-5-yl)phenyl)methane(**3c**)*: m.p: 210-11 ^{o}C. IR (KBr): ν_{max} 3065, 2990, 1570, 1600, 1472, 1340, 1271, 820 cm^{-1}.^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.77 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 5.07 (d, *J* = 2.2 Hz, 2H, CH-S), 5.61 (s, 2H, CH-N), 6.33 (d, *J* = 9.2 Hz, 2H, CH-O), 6.91-6.81 (m,8H, Ar-H), 6.97 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.21 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.31-7.27 (m, 8H, Ar-H), 8.09 (d, *J* = 8.6 Hz, 4H, Ar-H).^{13}C NMR ($CDCl_3$,75 MHz): δ 42.3,56.0, 56.7, 58.4, 69.2, 82.3, 113.9, 114.3, 124.6, 126.2, 126.8, 128.8, 129.3, 130.3, 132.3,133.9, 143.9, 146.5, 155.0, 158.8. ESI-MS: *m/z* 939 $[M+1]^+$. Anal. Calcd. for $C_{49}H_{42}F_2N_6O_{10}S_2$: C, 62.67; H, 4.51; N, 8.95. Found: C, 62.64; H, 4.42; N, 8.72.

*(4-methoxy-3-(3-(4-methoxyphenyl)-6-p-tolyl-3,3a,5,6-tetrahydroisoxazolo[3,4-d]thiazol-5-yl)phenyl)(4-methoxy-3-(3-(4-methoxyphenyl)-6-p-tolyl-3,3a,5,6-tetrahydrothiazolo [4,5-c] isoxazol-5-yl)phenyl)methane(**3d**)*: m.p: 152-54 ^{o}C. IR (KBr): ν_{max} 3062, 2991, 1600, 1472, 1570, 1271, 821 cm^{-1}.^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.34 (s, 6H, CH_3), 3.66(s, 2H, CH_2), 3.79 (s,6H, OCH_3), 3.81 (s, 6H, OCH_3), 4.39 (d, *J* = 2.3 Hz, 2H, CH-S), 5.61 (s, 2H, CH-N), 6.16 (d, *J* = 9.5 Hz, 2H, CH-O), 6.60 (d, *J* = 8.6 Hz, 4H, Ar-H), 6.92-6.90 (m, 6H, Ar-H), 7.07 (d, *J* = 8.6 Hz, 4H, Ar-H), 7.17 (d, *J* = 8.9 Hz, 4H, Ar-H). 7.26-7.23 (m, 6H, Ar-H). ^{13}C NMR (DMSO-d_6 75 MHz): δ 21.1, 42.3, 56.0, 56.7, 58.4, 69.2, 82.3, 113.9, 114.3, 124.6, 127.1, 128.8, 129.3, 130.3, 132.3, 133.9, 136.5, 137.0, 155.0, 158.8.1. ESI-MS: *m/z* 876 $[M]^+$. Anal. Calcd. for $C_{51}H_{48}F_2N_4O_6S_2$: C,69.84; H,5.52;N,6.39. Found: C, 69.74; H, 5.46; N, 6.11.

*4,4'-(5,5'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-methoxyphenyl)-3,3a-dihydrothiazolo[4,5-c]isoxazole-6,5(5H)-diyl))diphenol(**3e**):* m.p:164-66^{o}C. IR (KBr):ν_{max} 3345,3062, 2970, 1624, 1496, 1257, 839 cm^{-1}.^{1}H NMR ($CDCl_3$,300 MHz): δ 3.63(s, 2H, CH_2), 3.77 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 3.87 (s, 2H, OH), 4.51 (d, *J* = 2.6 Hz, 2H, CH-S), 5.61 (s, 2H, CH-N), 6.32 (d, *J* = 9.4 Hz, 2H, CH-O), 6.47 (d, *J* = 9.1 Hz, 4H, Ar-H), 6.69 (d, *J* = 9.1 Hz, 4H, Ar-H),6.91-6.90 (d, *J* = 8.6 Hz, 6H, Ar-H), 7.16 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.25- 7.23 (m, 6H, Ar-H). **^{13}C** NMR ($CDCl_3$, 75 MHz): δ 42.3, 56.0, 56.7, 58.4, 69.2, 82.3, 113.9, 114.3, 116.5, 124.6, 127.8. 128.8, 129.3, 132.0, 132.3, 133.9, 154.0, 155.0, 158.1, 158.8. ESI-MS: *m/z* 882 $[M+2]^+$. Anal. Calcd. for $C_{49}H_{44}F_2N_4O_8S_2$: C, 66.80; H, 5.03; N, 6.36. Found: C, 66.70; H, 4.92; N, 6.20.

*(3-(6-(4-fluorophenyl)-3-(4-methoxyphenyl)-3,3a,5,6-tetrahydroisoxazolo[3,4-d]thiazol-5-yl)-4-methoxyphenyl)(3-(6-(4-fluorophenyl)-3-(4-methoxyphenyl)-3,3a,5,6-tetrahydro thiazo lo [4,5-c]isoxazol-5-yl)-4-methoxyphenyl)methane(**3f**)*: m.p: 176-78 ^{o}C. IR (KBr): ν_{max} 3064, 2970, 1600, 1570, 1470, 1269, 1046, 821 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.63 (s, 2H, CH_2), 3.78 (s, 6H, OCH_3),3.81 (s, 6H, OCH_3), 4.57 (d, *J* = 2.2 Hz, 2H, CH-S), 5.61 (s, 2H, CH-N), 6.42 (d, *J* = 9.3 Hz, 2H, CH-O), 6.62 (m, 4H, Ar-H), 6.95-6.90 (m, 10H, Ar-H), 7.16 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.26-7.24 (d, *J* = 8.1 Hz, 6H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 42.3, 56.0, 56.7, 58.4, 69.2, 82.3, 113.9, 114.3, 117.6, 124.6, 128.8, 129.3, 132.3, 133.9, 135.9, 155.0, 158.8, 162.6. ESI-MS: *m/z* 860 $[M+1]^+$.*Anal. Calcd. for* $C_{49}H_{42}F_2N_4O_6S_2$: C, 66.50; H, 4.78; N, 6.33. Found: C, 66.40; H, 4.57; N, 6.25.

(4-methoxy-3-(3-(4-methoxyphenyl)-6-o-tolyl-3,3a,5,6-tetrahydroisoxazolo[3,4-d]thiazol-5-yl)phenyl)(4-methoxy-3-(3-(4-methoxyphenyl)-6-o-tolyl-3,3a,5,6-tetrahydrothiazolo[4, 5-c]isoxazol-

5-yl)phenyl)methane (**3g**): m.p: 156-58 °C.IR (KBr): ν_{max} 3062, 2990, 1600, 1570, 1470, 1271, 1040, 822 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.18 (s, 6H, CH_3), 3.65 (s, 2H, CH_2), 3.77 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 4.80 (d, *J* = 2.2 Hz, 2H, S-CH), 5.61 (s, 2H, CH-N), 6.44 (d, *J* = 9.4 Hz, 2H, CH-O), 6.58 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.67 (t, 2H, Ar-H), 6.80-6.91 (m, 6H, Ar-H), 7.10-7.06 (m, 6H, Ar-H), 7.24 (d, *J* =8.9 Hz, 6H, Ar-H). ^{13}C NMR ($CDCl_3$, 75MHz): δ 17.2, 42.3, 56.0, 56.7, 59.2, 69.2, 82.3, 113.9, 114.3, 124.6, 126.4, 127.0, 128.4, 129.3, 130.3, 132.3, 133.9, 135.4, 137.6, 155.0, 158.8. ESI-MS: *m/z* 854 $[M]^+$. Anal. Calcd. for $C_{51}H_{48}F_2N_4O_6S_2$: C, 69.84; H, 5.52; N, 6.39. Found: C, 6.75; H, 5.41; N, 6.29.

2.4. Biological Assay

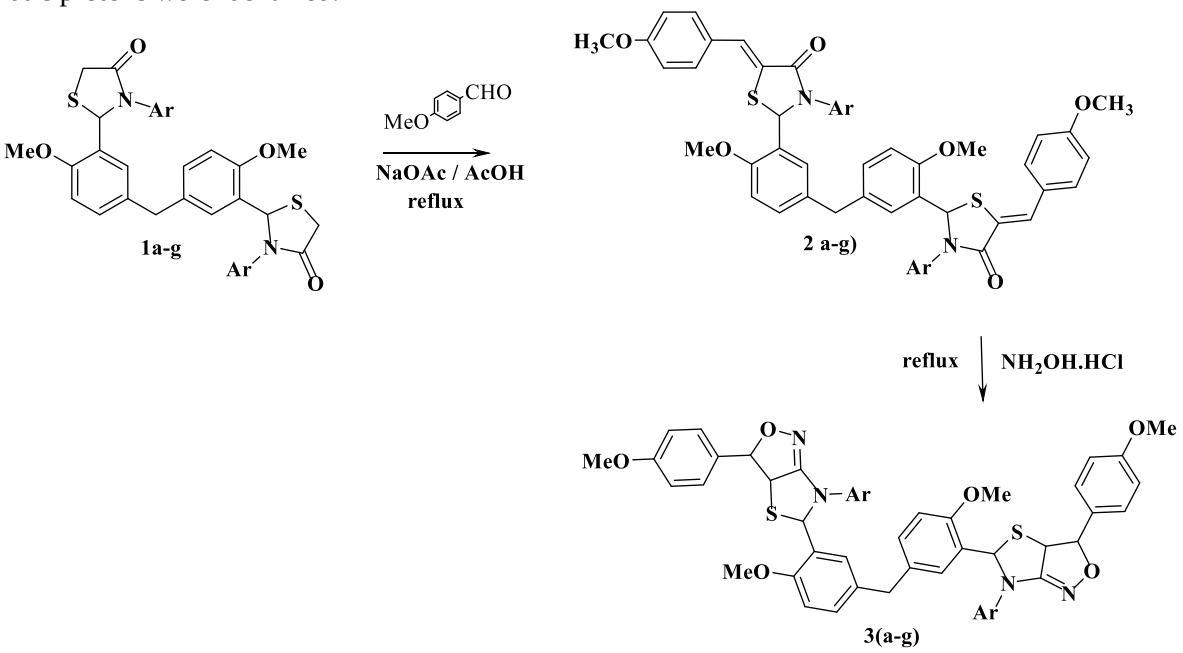
2.4.1. Cytotoxicity Evaluation

All the newly synthesized compounds were evaluated their cytotoxic activity against different cancer cell lines such as MCF-7, Prostate cancer (DU145) and HEL cell lines with Sulforamide B assay, based on the IC_{50} value compound **3b** picked up and evaluated its activity against prostate cancer cell lines by taking in different concentrations in different methods[32,33].

3. Results and Discussion

3.1. Chemistry

Its necessary to use compounds **1 (a-g)** [30] in the manufacture of target molecules. In the presence of NaOAc anhydrous to glacial acetic acid, compounds **1 (a-g)** were employed to extract methylene –bis –thiazolidinones **2 (a-g)** (Scheme 1). Cyclo-condensation of **2 (a-g)** in NaOAc water soluble acetic acid at reflux heat yielded **3 (a-g)** with optional to outstanding yields of **3** (**a-g**) (Scheme1).The results are summarized in Table 1. The composition was determined through spectroscopic analysis. It was confirmed that the carbonyl group had been cyclized when the amide carbonyl (C=O) absorbtion band at 1700cm^{-1} was absent in the compounds **3(a-g)** but was present in compounds **2** (**a-g**). Bands C=N, N-O were found in compounds **3 (a-g)** at 1601 and 1472 cm^{-1} respectively. ^1H-NMR of **3 (a-g)** in $CDCl_3$ showed methylene bridge protons at 3.61–3.71 as a singlet, S-CH fused proton at 4.39-5.07 as a doublet ,and CH-O proton of isoxazole ring at 6.16-6.44 as a doublet .These signals indicate that the cyclisation step has been completed. The expected sites for all of **3 (a-g)** additional aromatic and aliphatic protons were identified.



Scheme 1. Synthetic Pathway for compound **3a-g**

The ^{13}C NMR spectra of compounds **3 (a-g)**, recorded in $CDCl_3$, show substantial signals corresponding to the carbons of the thiazolo-isoxazole ring at δ 58.2, 69.2, and 129.7. This is more evidence confirming their structures. There were $[M^+]/[M+1]^+$ peaks in the mass spectra of all the synthesized compounds, and those peaks corresponded to their chemical formulae. The compounds were also subjected to anticancer testing.

Table 1. Synthesis of compounds **2(a-g)** and **(3a-g)**

Compound	Ar	Yield (%)
2a	C_6H_5-	71
2b	*p*-Cl-C_6H_4-	69
2c	*p*-NO_2-C_6H_4-	77
2d	*p*-Me-C_6H_4-	65
2e	*p*-OH-C_6H_4-	70
2f	*p*-F-C_6H_4-	69
2g	*o*-Me-C_6H_4-	66
3a	C_6H_5-	67
3b	*p*-Cl-C_6H_4-	63
3c	*p*-NO_2-C_6H_4-	70
3d	*p*-Me-C_6H_4-	68
3e	*p*-OH-C_6H_4-	71
3f	*p*-F-C_6H_4-	67
3g	*o*-Me-C_6H_4-	65

3.2. In Vitro Cytotoxic Activity

3.2.1. Cytotoxicity Evaluation Against Different Cancer Cell Lines

By performing a Sulforhodamine B assay (SRB) on different representative cell lines, the cytotoxic effect of the compounds was tested. At first in flat bottom 96-well plate(5000 cells/100µL) in a medium containing 10% serum was seeded in the cell of interest, was followed by incubation for 18-20 hrs in an incubator which was continuously supplied with 5% CO_2 to make sure of proper adherence of the cells to the surface bottom of the wells. The cells were treated with the compound after 18 hrs. Concentration of the compounds were prepared by diluting the known concentration. The final concentration of compound 0 to 100 µm was made by adding 2µL aliquot. Every compound was tested in triplicate. The cytotoxicity was determined as the average of that triplicate standard control of anti cancer drug like DMSO and doxorubicin were taken as vehicle and positive controls. After that in an incubator maintaining at 37 ^oC with a constant supply of 5% CO_2 the plates were incubated for 48 hrs, the cells were fixed using 10% TCA solution. It was incubated for 1hour at 4 ^oC. Then the plate was rinsed carefully with MQ water and air dried, 0.057 SRB solution was added for 30 min approximately before rinsing off using 1% acetic acid. The plates were air dried absorbed and measured using Perkin Elmer Multimode reader at 510 nm.To solubilize the SRB and to measure absorbance,100 mL of 10mµ Tris base was added .To calculate the IC_{50} values the measure of absorbance is directly proportional to cell growth. In this study four types of cancer cell lines namely (MCF-7), Prostate cancer (DU145) and HEL are used initial screening. For the cytotoxic effect of the series of compounds a cell lines were tested. Based on the IC_{50} values obtained, compound **3b** picked to study on prostate cell line(DU145).

3.2.2. Morphological Change

The effect on the morphology of the cells was to find out the cytotoxic ability of the compound. The objectivity and the procedure of this is to keep a 24 well plate seeded with cells in a

manner previously described and incubated for 18-20 hrs.with an increasing concentration of **3b** the cells were treated. After that the experiment was terminated and the cells were observed under the microscope &images were captured using Olympus Xi71 microscope (Figure 1). This is done only after 48 hours of incubation.

3.2.3. Assay for Colony Establishment

The following experiment was tested in order to know the long term effect of the **3b** on the anchorage independent nature of cancer cells. With minor modifications as stated earlier the experiment was conducted by a soft agar assay. In order to achieve final concentration of 0.35% of agar in IX growth medium with 10% serum concentration base agar was prepared by mixing 1% of agarose (Bacto agar:Becton,Dickison,Sparks,MD) with 2x DMEM along with 20% FBS &2x antibiotics in 6 well plated experiment. To obtain of a final concentration of 0.35% agar, solidification of the base agar 2.5x 10^4 cells were mixed with cultivation medium containing compound at various concentrations along with agar solution. This was spread on top of the base a gas previously solidified for a period of 9 days plate was incubated with periodic refilled every 3 days with medium compound .Plates were monitored regularly for appearance of colonies turns purple in color after 9 days. The extra strained was washed off using MQ water. The colonies then photographed and counted using a microscope (Figure 2).

3.2.4. Caspases-3 and -9 Activity Determination

Caspase 9 and caspase 3 activities were analyzed in the cell lysates obtained from DU 145 cells previously treated with compound **3b**. Ac-DEVD-AMC and Ac-LEHD-AFC for caspase 3 and caspase 9 respectively was observed using fluorogenic substrates. After 48 h treatment of cells with compound **7b**, harvested cells (50 mμ HEPES,5mμCHAPS,5mμ DIT ,Ph 7.5) were lysed directly in Caspase lysis buffer with the respective substrate (AC-DEVD-AFC/AC-LEHD-AMC) in 20mμ HEPES (pH 7.5) ,0.1% CHAPS ,2mM EDTA and 5 Mm DTT at 37 ºC for 2h.The lysates were incubated using an excitation and emission wave length of 400/505 nm (for AFC) and 380/460nm (for AMC) which is directly proportional to Caspase-9 &3 activity respectively the release of AFC and AMC was analyzed by a fluorimeter. With total protein concentration estimation estimated by broad ford method and the relative capsase activities were calculated as the ratio of values between mock treated (DMSO) & treated cells the observed fluorescence values were normalized (Figure 3).

3.2.5. Assay of Senescence

By limiting their proliferation compounds with anti cancer potential may have the potential to induce senescence in cells. By measuring senescence associated beta-galactosidase (SA-βgal) activity (pH 6.0) in DU145 cells exposed to compound **3b** (Figure 4) the ability to induce cellular senescence. As previously described, A 24 well plate was seeded with DU145 cells. The media was aspirated after 48h the cells were washed with PBS (2x1min) after incubation for 5 min at room temperature. The resultant fixed cells were then stained with freshly prepared staining solution (40 mM citric acid /Na phosphate buffer, 5 mM K_4 [Fe (CN) $_6$] $3H_2O$, 5 mM K_3 [Fe (CN) $_6$], 150 mM sodium chloride, 2 mM magnesium chloride and 1 mg/mL X-gal in distilled water) overnight. By repeated washings with PBS & then plate was allowed for drying at room temperature the excess stain was removed. Under an Olympus Xi71 microscope the cells were stained with SA-β gal levels were observed and photographed.

3.2.6. PI Use for Cell Death Analysis

Cell death which is brought by compound **3b** as a measure of PI uptake was determined after treating with compound fixed in 70% ethanol and at desired concentration, the cells were harvested. In the form of pellet the cells were collected. All cells were resuspended in PI solution (RNase -0.1 mg/mL, Triton X-100-0.05%, PI-50 μg/mL) in the form of pellet and then incubated for 1h in dark at room temperature. By conducting repeated washings with PBS buffer the excess PI solution was

washed away.Analyzation of the resultant PI was taken by fluorescence activated cell sorting (FACS Calibur System; BD Bio- science, Erembodegem, Belgium) in a FL-2 fluorescence detector (10000 events were recorded for each condition) using FCS express 4 software (De Novo Software, Los Angeles, CA) flow cytometry data was analyzed.

Table 2. Cytotoxic activity of compounds **3a-3g**

		DU145		A549		HELA		MCF 7	
S.No	Sample Codes	IC_{50}	Std. Dev	IC_{50}	Std. Dev	IC_{50}	Std. Dev	IC_{50}	Std. Dev
1	3a	16.99	3.07	30.15	12.78	>100	-	40.47	21.16
2	3b	14.98	3.30	35.29	0.00	>100	-	55.16	5.03
3	3c	12.15	0.95	14.98	1.16	57.24	15.29	62.60	28.26
4	3d	36.71	17.26	32.64	6.44	>100	-	>100	-
5	3e	>100	-	28.81	0.52	>100	-	65.30	8.94
6	3f	15.91	2.47	21.64	4.58	66.07	41.33	19.73	2.21
7	3g	7.76	0.66	11.70	1.01	23.29	1.08	18.51	0.38
	Doxorubicin	6.70	0.10	8.49	0.13	10.89	0.09	8.62	1.52

Table 3. G0/G1 phase cell cycle arrest by Compound **3b** induced in DU145 cells

	G0/G1	S	G2/M
DMSO	70.36	5.22	22.64
5 µM	63.72	8.16	21.96
10 µM	68.15	5.64	24.55
15 µM	72.96	3.82	23.51
20 µM	73.11	3.69	21.05
25 µM	78.48	2.89	17.76

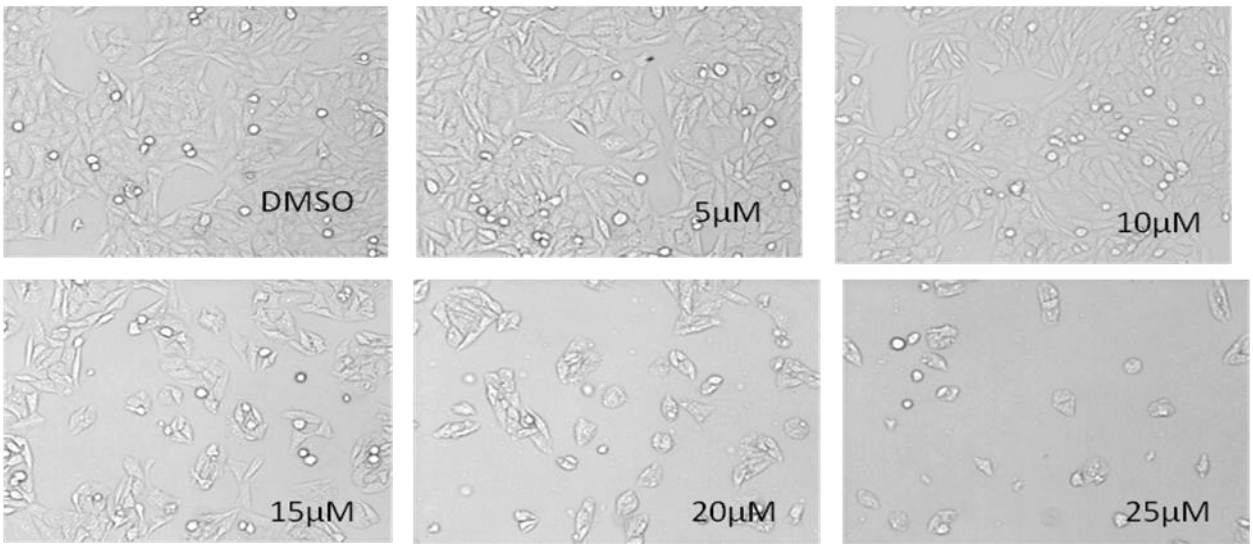


Figure 1. Exposure of DU145 cells to compound **3b**

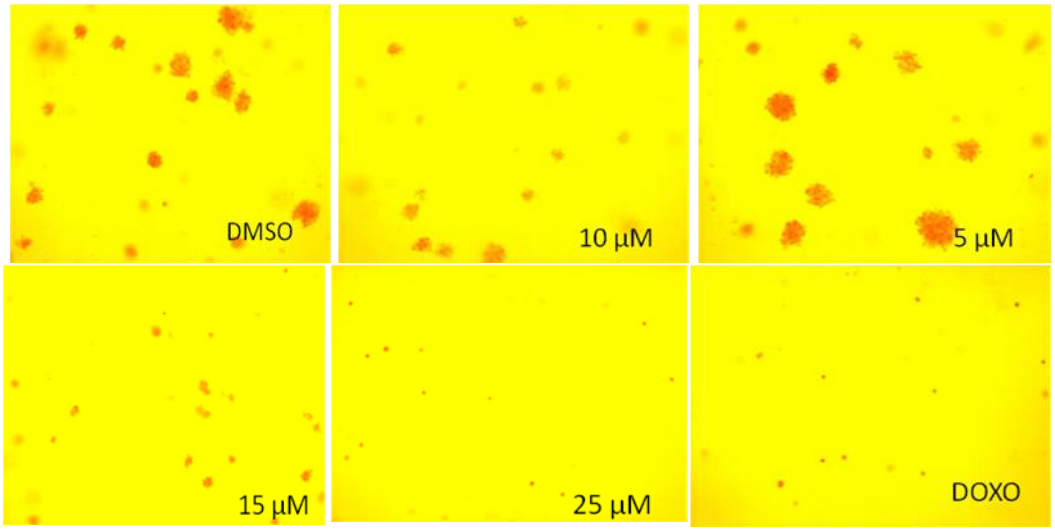


Figure 2. Long term effect of compound **3b** on the number of colony-forming DU145 cells

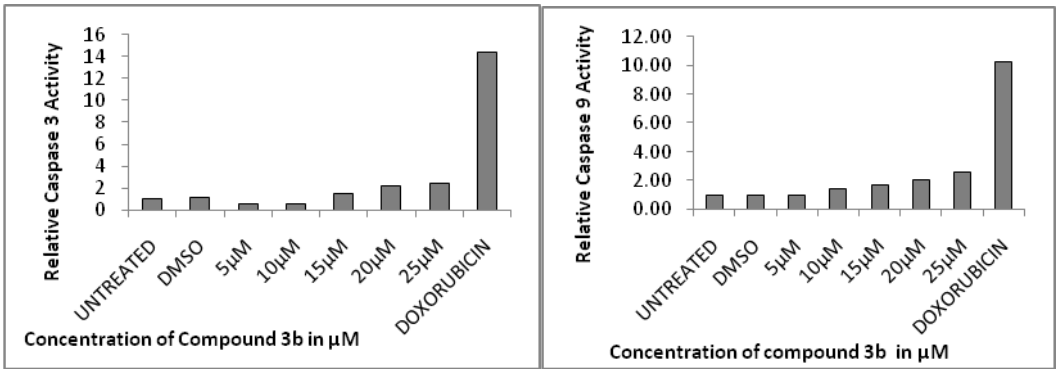


Figure 3. Caspases activation in DU145 cells

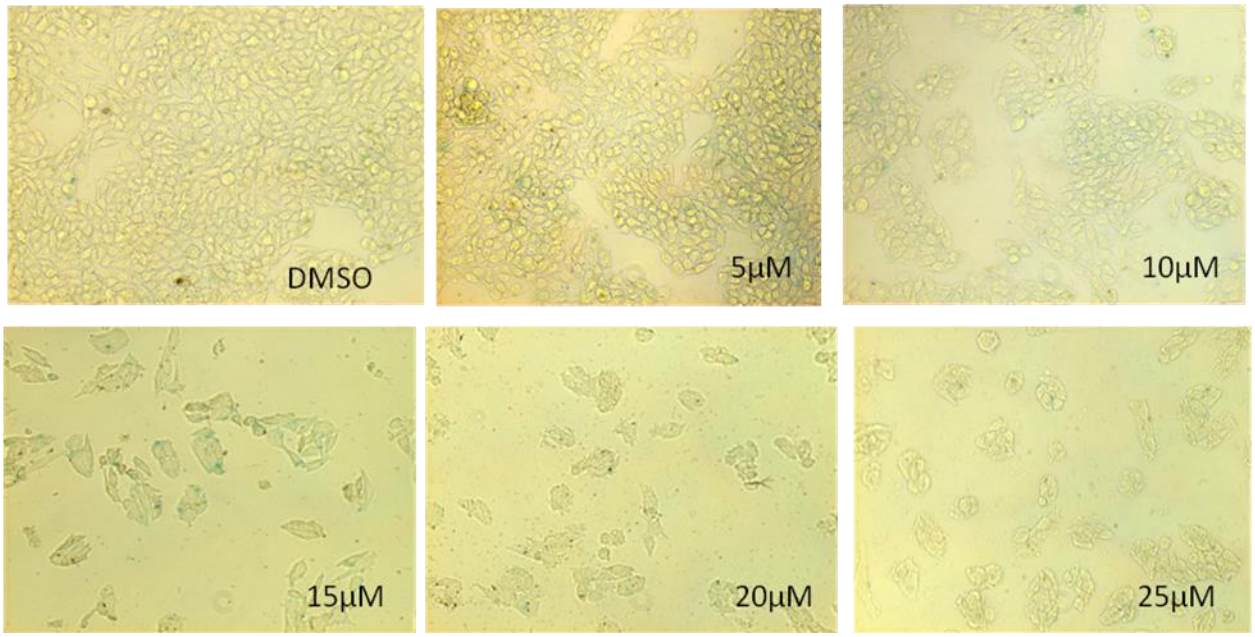


Figure 4. Senescence induced by compound **3b**

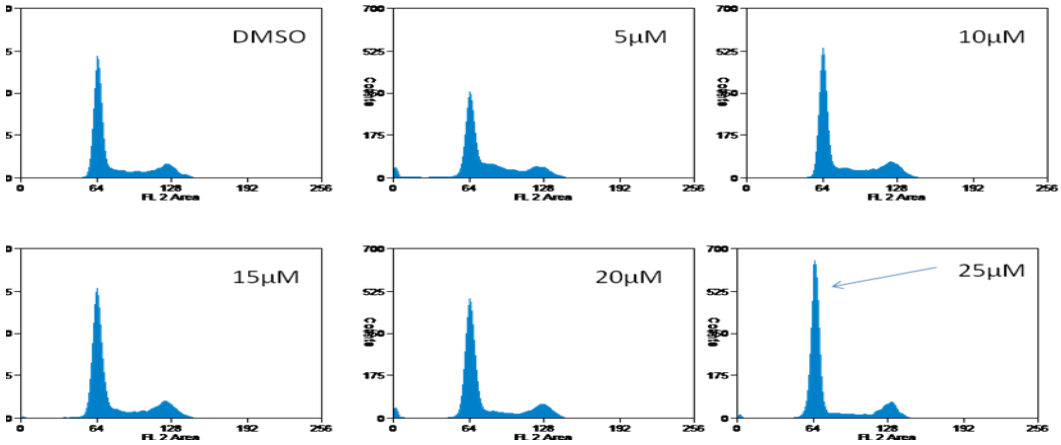


Figure 5. Cell cycle analysis of DU145 cells treated with compound **3b**

In conclusion, the cytotoxic activities of **3(a-g)** compounds were evaluated against various cell lines. Among the synthesized compounds, the compounds **3b** showed good cytotoxic activity against prostate cancer cell lines and emerged as potential molecules for further development.

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