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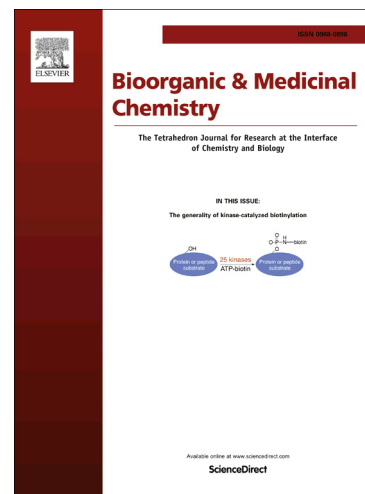
Design, Synthesis, and Biological Evaluation of 1,9-Diheteroarylnona-1,3,6,8-tetraen-5-ones as a New Class of Anti-Prostate Cancer Agents

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Abstract — In search of more effective chemotherapeutics for the treatment of castration-resistant prostate cancer and inspired by curcumin analogues, twenty five (1E,3E,6E,8E)-1,9-diarylnona-1,3,6,8-tetraen-5-ones bearing two identical terminal heteroaromatic rings have been successfully synthesized through Wittig reaction followed by Horner-Wadsworth-Emmons reaction. Twenty-three of them are new compounds. The WST-1 cell proliferation assay was employed to assess their anti-proliferative effects toward both androgen-sensitive and androgen-insensitive human prostate cancer cell lines. Eighteen out of twenty-five synthesized compounds possess significantly improved potency as compared with curcumin. The optimal compound, **78**, is 14- to 23-fold more potent than curcumin in inhibiting prostate cancer cell proliferation. It can be concluded from our data that 1,9-diarylnona-1,3,6,8-tetraen-5-one can serve as a new potential scaffold for the development of anti-prostate cancer agents and that pyridine-4-yls and quinolin-4-yl act as optimal heteroaromatic rings for the enhanced potency of this scaffold. Two of the most potent compounds, **68** and **75**, effectively suppress PC-3 cell proliferation by activating cell apoptosis and by arresting cell cycle in the G_0/G_1 phase.

1. Introduction:

The American Cancer Society recently estimates that about 180,890 American men will be diagnosed as new prostate cancer cases and that over 26,000 men will die of prostate cancer in 2016.[1] These data indicate that prostate cancer is still the most common cancer and the second leading cause of cancer-affiliated death among men in the United States. It is now well known that most prostate cancer patients die of castration-resistant prostate cancer due to the inevitable progression of resistance to first-line treatment with docetaxel.[2,3] There is thus an urgent need to search for new chemotherapeutics for this deadly disease. The dietary curcumin (**1**) (Figure 1) was chosen by us and others as lead compound for the development of new anti-prostate cancer agents based on: i) its well-documented safety profile in humans;[4] and ii) its well-evidenced therapeutic potential in treating prostate cancer, especially for castration-resistant prostate cancer, in several cell culture systems and human xenograft mouse models.[4-16] To overcome its disadvantage (moderate potency and poor bioavailability) as a drug candidate,[6,17,18] chemical manipulations of curcumin have been performed by us and many other research groups.[5,19-21] Consequently, curcumin-based compounds with various lengths of central linkers, including 11-atom linker, 7-atom linker, 5-carbon linker, and 3-carbon linker, have so far been reported to possess similar or increased cytotoxic and anti-proliferative potency against prostate cancer cell lines, as compared with curcumin.[5] However, there is no report available on the curcumin mimics containing a 9-atom linker as anti-prostate cancer agents. Our laboratory has recently discovered that basic *N*-containing heteroaromatic rings contribute significantly to the enhanced cytotoxic and anti-proliferative potency of curcumin-based compounds (e.g. **2**-**6**) with 5-carbon or 7-carbon linker toward prostate cancer cells.[5]

As part of our ongoing project in quest for more effective chemotherapeutics inspired by dietary natural products for the treatment of castration-resistant prostate cancer, this study aims to systematically investigate the effect of central linker length in curcumin mimics possessing terminal

heteroaromatic rings on *in vitro* cell proliferation in prostate cancer cell models. Consequently, twenty five (1*E*,3*E*,6*E*,8*E*)-1,9-diarylnona-1,3,6,8-tetraen-5-ones (**59**-**83**) bearing two identical terminal heteroaromatic rings were designed for the evaluation of their anti-prostate cancer potential in cell-based models. Among them, 1,9-di-3-pyridinyl-1,3,6,8-nonatetraen-5-one (**72**) and 1,9-di-4-pyridinyl--1,3,6,8-nonatetraen-5-one (**74**) are known compounds.[22,23] No synthesis has been reported so far. All other twenty-three (1*E*,3*E*,6*E*,8*E*)-1,9-diarylnona-1,3,6,8-tetraen-5-ones are new compounds.
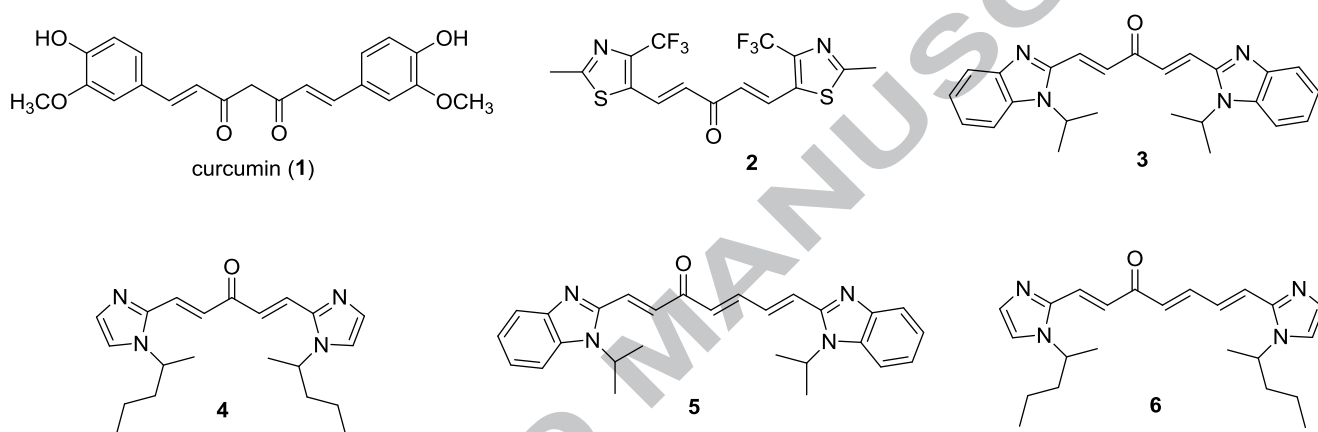
Figure 1. Structures of curcumin (**1**) and curcumin-based anticancer agents (**2**-**6**)

2. Results and Discussion:

2.1. Chemistry

Twenty three new and two known (1*E*,3*E*,6*E*,8*E*)-1,9-diarylnona-1,3,6,8-tetraen-5-ones (**59**-**83**) bearing two identical terminal heteroaromatic rings have been successfully synthesized as illustrated in Scheme 1. Specifically, these target compounds have been achieved by Horner-Wadsworth-Emmons reaction of one equivalent of 1,3-bis(diethylphosphonato)acetone (**58**) with 2.2 equivalents of the appropriate (*E*)-3-aryl-2-propenal (**33**-**57**), using potassium carbonate as base and employing water and ethanol (3:2, v/v) as a co-solvent. The (*E*)-3-aryl-2-propenals (**33**-**57**) have been prepared via Wittig

reaction of the appropriate carbaldedyde (**7**-**31**) with (triphenylphosphoranylidene)aldehyde (**32**) at room temperature in DMF for one to four days. TLC was used to detect the completion of the reaction. 1-Alkyl-1*H*-imidazole-2-carbaldehydes (**13**-**17**) were synthesized from 1*H*-imidazole-2-carbaldehydes mediated by potassium carbonate based on the procedure illustrated in the literature.[24,25] 1-Alkyl-1*H*-benzo[*d*]imidazole-2-carbaldehydes (**27**-**31**) were obtained from benzene-1,2-diamine and glycolic acid through a three-step transformation as previously reported by us.[19] Other starting carbaldehydes were purchased from Fisher Scientific. 1,3-Bis(diethylphosphonato)acetone (**58**) was readily synthesized from carbazic acid methyl ester, 1,3-dichloroacetone, and triethyl phosphite according to the reported procedure.[26] Curcumin, as the positive reference control, was prepared by Claisen-Schmidt condensation of vanillin with acetylacetone according to the procedure described in the literature.[27]
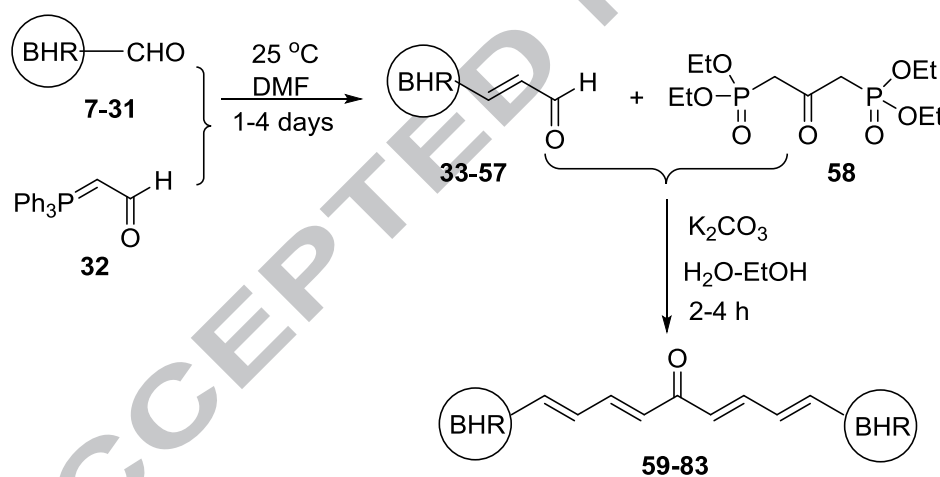
Scheme 1. Synthesis of 1,9-diarylnona-1,3,6,8-tetraen-5-ones (**59**-**83**)

Table 1. Structures of basic nitrogen-containing heteroaromatic rings (BHR) in 1,9-diarylnona-1,3,6,8-tetraen-5-ones (**59-83**)

BHR	(thiazol-2-yl)	(4-methylthiazol-2-yl)	(thiazol-5-yl)	(5-methylisoxazol-3-yl)	(4-bromo-1-methyl-pyrazol-3-yl)
compds	**59**	**60**	**61**	**62**	**63**
BHR	(5-chloro-1-methyl-3-(trifluoromethyl)pyrazol-4-yl)	(1-isopropylimidazol-2-yl)	(1-(sec-butyl)imidazol-2-yl)	(1-isobutylimidazol-2-yl)	(1-(sec-butyl)imidazol-2-yl)
compds	**64**	**65**	**66**	**67**	**68**
BHR	(1-(pentan-2-yl)imidazol-2-yl)	(pyridin-2-yl)	(6-methylpyridin-2-yl)	(pyridin-3-yl)	(6-(trifluoromethyl)pyridin-3-yl)
compds	**69**	**70**	**71**	**72**	**73**
BHR	(pyridin-4-yl)	(3-fluoropyridin-4-yl)	(quinolin-2-yl)	(2-chloroquinolin-3-yl)	(quinolin-4-yl)
compds	**74**	**75**	**76**	**77**	**78**
BHR	(1-methylbenzimidazol-2-yl)	(1-ethylbenzimidazol-2-yl)	(1-propylbenzimidazol-2-yl)	(1-isopropylbenzimidazol-2-yl)	(1-isobutylbenzimidazol-2-yl)
compds	**79**	**80**	**81**	**82**	**83**

2.2. Anti-proliferative effects toward three prostate cancer cell lines

To evaluate the *in vitro* anticancer potential of the synthesized 1,9-diarylnona-1,3,6,8-tetraen-5-ones (**59-83**), we first determined their antiproliferative activity toward both androgen-sensitive (LNCaP) and androgen-insensitive (PC-3 and DU145) human prostate cancer cell lines using the WST-1 cell proliferation assay. The IC_{50} values, acquired based on the inhibitory rates for at least 5 concentrations, are summarized in Table 2. Curcumin was employed as a positive control for comparison in the parallel experiment. Among the series of compounds tested, eighteen out of twenty-five 1,9-diarylnona-1,3,6,8-tetraen-5-ones (**59-62**, **65**, **66**, **68**, **69**, **71**, **72**, **74**, **75**, **78-83**) display 2-23 times

higher antiproliferative potency than curcumin in three prostate cancer cell models (Table 2), suggesting that 1,9-diarylnona-1,3,6,8-tetraen-5-one can serve as a new potential scaffold of anti-prostate cancer agents. The data in Table 2 suggest that *para* pyridyls in **74** and **75**, and quinolin-4-yl in **78** are favorable terminal heteroaromatic rings for the promising potency of these three compounds in three prostate cancer cell lines. This is different from another two promising scaffolds, (1*E*,4*E*)-1,5-diheteroarylpenta-1,4-dien-3-ones and (1*E*,4*E*,6*E*)-1,7-diaryl-1,4,6-heptatrien-3-ones that we previously reported, in which *ortho* pyridyl confers greater cytotoxic and antiproliferative potency than *para* pyridyl in various prostate cancer models.[5] Additionally, in our previous studies, 1-alkyl-1*H*-imidazol-2-yl and 1-alkyl-1*H*-benzo[*d*]imidazole-2-yl serve as optimal heteroaromatic rings for the antiproliferative potency of 1,5-diheteroarylpenta-1,4-dien-3-ones (e.g. **3** and **4**)[19] and 1,7-diheteroarylhepta-1,4,6-trien-3-ones (e.g. **5** and **6**).[20] However, the current study indicates that these two groups of heteroaromatic rings can only bestow moderate antiproliferative potency to 1,9-diarylnona-1,3,6,8-tetraen-5-ones (e.g. **82** and **69**). As illustrated in Table 3, the optimal 1,5-diheteroarylpenta-1,4-dien-3-one (**3**)[19] has much greater anti-proliferative potency than **5** and **78**, the optimal mimics from 1,7-diheteroarylhepta-1,4,6-trien-3-ones[20] and 1,9-diarylnona-1,3,6,8-tetraen-5-ones. This indicates that the length of the linker exhibits important impact on anti-proliferative potency of these curcumin mimics and that the scaffold with 5-carbon linker is the most promising one. On the other hand, the optimal mimic with 7-carbon linker (**5**) is slightly more potent than the optimal mimic with 9-carbon linker (**78**), suggesting no potency contribution from the linker length in mimics with 9-carbon linker. The above-mentioned data collectively indicate that i) the enhanced anti-proliferative potency for **75** and **78**, as compared with curcumin, may be fully due to the terminal heteroaromatic groups; and ii) 1,9-diarylnona-1,3,6,8-tetraen-5-ones with two identical terminal heteroaromatic groups can serve as a new scaffold for the development of curcumin-based anti-prostate cancer agents.

Table 2. Anti-Proliferative Activity of Diheteroarylnona-1,3,6,8-tetraen-5-ones

Compd.	IC$_{50}$ (μM)[a]			IC$_{50}$ (curcumin)/IC$_{50}$(dienone)		
	PC-3[b]	DU145[c]	LNCaP[d]	PC-3[b]	DU145[c]	LNCaP[d]
Curcumin	25.43 ± 2.15	26.23 ± 0.65	13.61 ± 2.69	1	1	1
59	6.21 ± 0.15	6.38 ± 0.65	6.85 ± 0.35	4	4	2
60	3.00 ± 1.18	7.37 ± 1.44	8.01 ± 2.87	9	4	2
61	10.47 ± 2.43	5.30 ± 2.36	8.09 ± 1.20	2	5	2
62	4.19 ± 0.24	4.19 ± 1.35	2.58 ± 1.04	6	6	5
63	24.77 ± 8.06	11.35 ± 2.96	>20	1	2	<1
64	>20	18.53 ± 10.59	>20	<1	1	<1
65	3.86 ± 0.23	2.45 ± 1.29	6.14 ± 2.68	7	11	5
66	7.43 ± 4.05	2.69 ± 0.99	4.94 ± 0.47	3	10	5
67	>15	>15	> 20	<1.7	<1.7	<1
68	2.36 ± 0.56	1.21 ± 0.43	2.43 ± 1.31	11	22	6
69	2.50 ± 1.06	1.75 ± 0.52	5.62 ± 3.57	10	15	2
70	45.69 ± 19.56	3.59 ± 2.63	17.53 ± 4.89	<1	7	<1
71	10.39 ± 4.71	8.29 ± 2.97	6.05 ± 4.93	3	3	2
72	5.22 ± 3.97	6.55 ± 3.26	4.94 ± 0.47	5	4	3
73	>15	>15	5.89 ± 2.64	<1.7	<1.7	2.3
74	1.79 ± 0.24	1.60 ± 0.08	1.63 ± 0.34	14	16	8
75	1.14 ± 0.12	1.78 ± 0.13	2.17 ± 0.2	22	15	6
76	11.74 ± 0.61	14.36 ± 0.34	13.62 ± 3.64	2	2	1
77	>15	>15	17.71 ± 4.88	<1.7	<1.7	<1

78	1.09 ± 0.17	1.20 ± 0.01	0.98 ± 0.19	23	22	14
79	2.63 ± 0.51	3.50 ± 0.17	0.97 ± 0.34	10	8	14
80	2.48 ± 0.41	2.70 ± 0.28	1.49 ± 2.24	10	10	9
81	7.24 ± 0.58	9.76 ± 0.66	3.65 ± 0.85	4	3	3.7
82	2.67 ± 0.26	3.36 ± 0.36	3.00 ± 0.77	10	8	4.5
83	2.82± 0.38	4.08 ± 0.36	4.31 ± 0.99	9	6	3.2

[a] IC_{50} is the drug concentration effective in inhibiting 50% of the cell viability measured
 by WST-1 cell proliferation assay (WST-1) or trypan blue exclusion assay (TB) after 3 days exposure.
[b] Human androgen-insensitive prostate cancer cell line
[c] Human androgen-insensitive prostate cancer cell line
[d] Human androgen-sensitive prostate cancer cell line

Table 3. The Effect of Linker Length of Curcumin Mimics on Anti-Proliferative Activity

Compd.	Linker	IC_{50}(curcumin)/IC_{50}(curcumin mimic)			Note
		PC-3	DU145	LNCaP	
3[a]	5-carbon	111	50	30	The optimal candidate for the series
4[a]	5-carbon	82	90	57	
5[b]	7-carbon	36	29	11	The optimal candidate for the series
6[b]	7-carbon	14	25	11	
82	9-carbon	10	8	4.5	
69	9-carbon	10	15	2	
78	9-carbon	23	22	14	The optimal candidate for the series

[a]Data from ref. 19; [b]Data from ref. 20.

2.3. Effects of 68 and 75 on PC-3 cell apoptosis and cell cycle progression

The anti-proliferative effect of curcumin against androgen-insensitive human prostate cancer cells is linked to its capability of promoting cancer cell apoptosis and cell cycle regulation.[28,29] To determine whether the suppression of PC-3 cell proliferation by the 1,9-diarylnona-1,3,6,8-tetraen-5-ones is

associated with activation of cell apoptosis and cell cycle arrest, two of the most potent compounds, **75** and **68**, were selected for further investigation. **74**, **75**, and **78** are the three most potent compounds that contain *para* pyridyls or quinolin-4-yl. Among them, **75** was chosen as the representative due to the presence of a fluorine atom in the aromatic ring which might bring in beneficial pharmacokinetic properties.[30,31]

PC-3 cells were treated with **75** and **68**, respectively, at 0-100 μM final concentration range for 16 h. The specific apoptotic inducer staurosporine was used as a positive control for early apoptosis in the experiments (data not shown). The early apoptotic PC-3 cells can be readily discriminated from the late apoptotic and necrotic cells by the double staining assay, consisting of the violet excitable dye F2N12S and dead cell stain SYTOX AADVanced, in a flow cytometer. Data summarized in Figures 2 and 3 demonstrate that a 16-hour treatment of both **68** and **75** can significantly promote androgen-insensitive PC-3 cell apoptosis in a dose-responsive manner. It is worth noting that **75** induced PC-3 cell apoptosis much more effectively than **68** even though they possess similar inhibitory effect on PC-3 cell proliferation, indicating that terminal *para* pyridyl is beneficial to cell apoptosis activation. For example, 10 μM of compound **68** induce only modest early phase of apoptosis (6%) in PC-3 cells as compared with control cells; whereas 10 μM of compound **75** activated substantial early apoptotic PC-3 cells (51%). Also, the early apoptotic cell population reached a maximum (47%) when PC-3 cancer cells were exposed to compound **68** at 30 μM concentration; in contrast, treatment of the cells with compound **75** led to a much higher maximum of early apoptosis (75%) that was reached at a lower dose (20 μM).

PC-3 cell cycle regulation mediated by compounds **68** and **75** was assessed by flow cytometric analysis with propidium iodide DNA staining. As illustrated in Figure 4, the PC-3 cell population in the G_0/G_1 phase increased by 21.3% and 29.7%, respectively, after treating with **68** and **75** for 16 h, as compared with the respective control cells. In contrast, **68** and **75** decreased the PC-3 cell population in

the G_2/M phase by 12.8% and 17.0%, respectively. Therefore, it can be concluded that both compounds can significantly arrest PC-3 cell cycle at the G_0/G_1 phase, with **75** as the slightly stronger promoter.
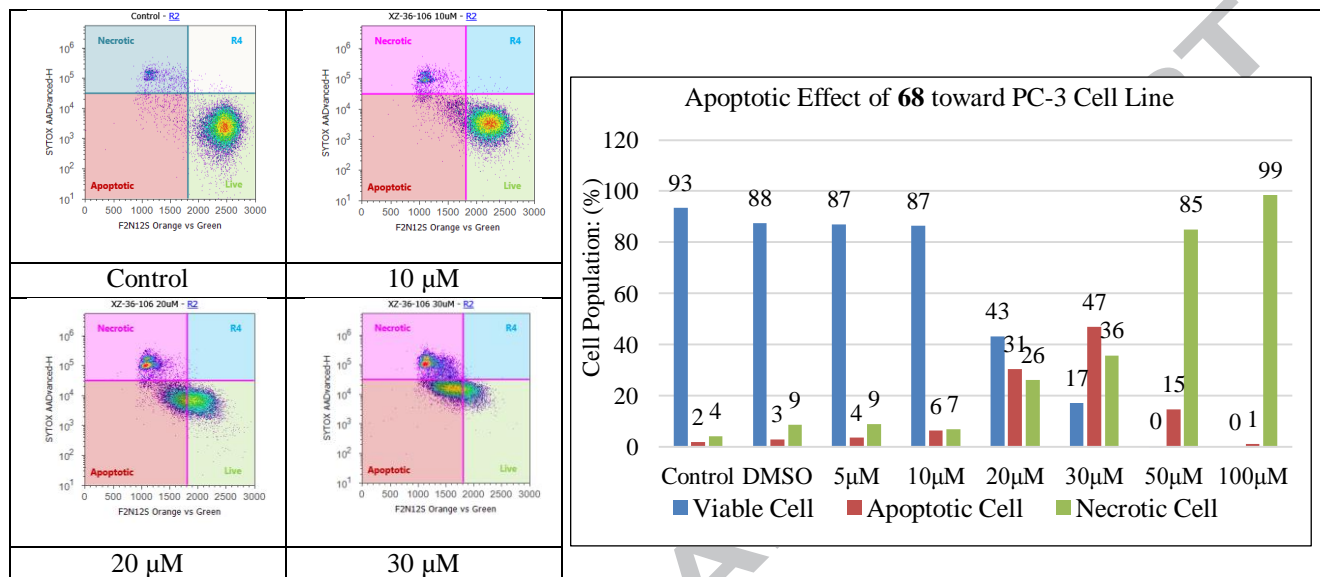

Figure 2. Evolution of viable, apoptotic, and necrotic PC-3 cells populations in response to increasing dosages of compound **68**.
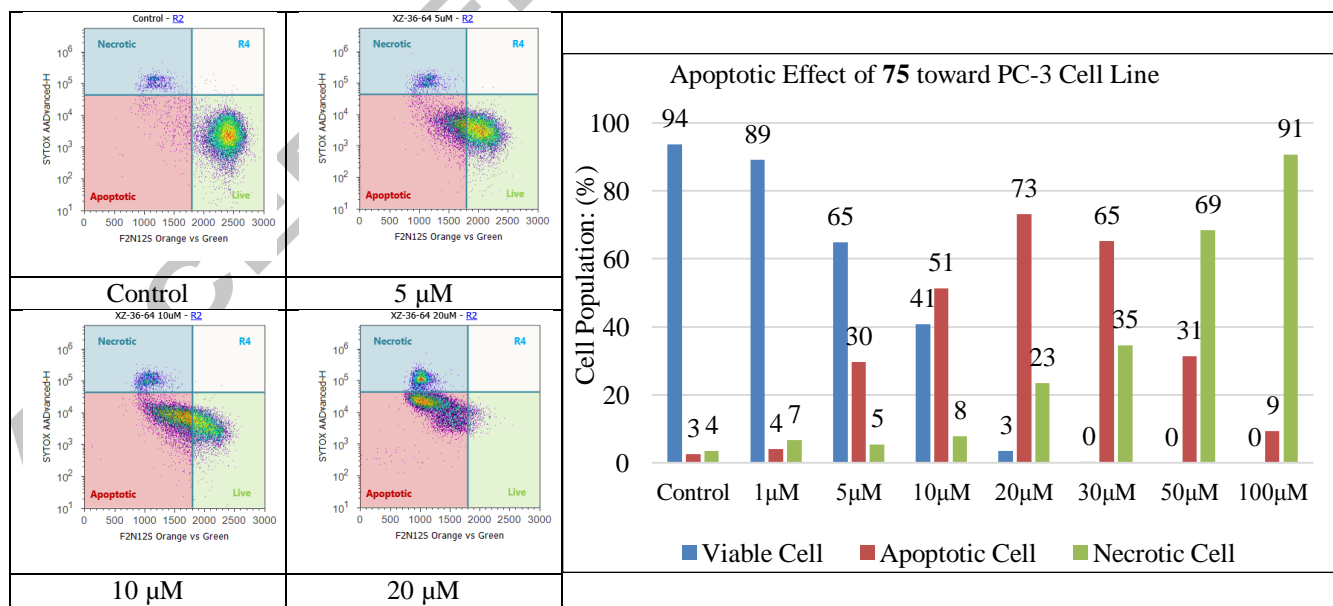

Figure 3. Evolution of viable, apoptotic, and necrotic PC-3 cells populations in response to increasing dosages of compound **75**.

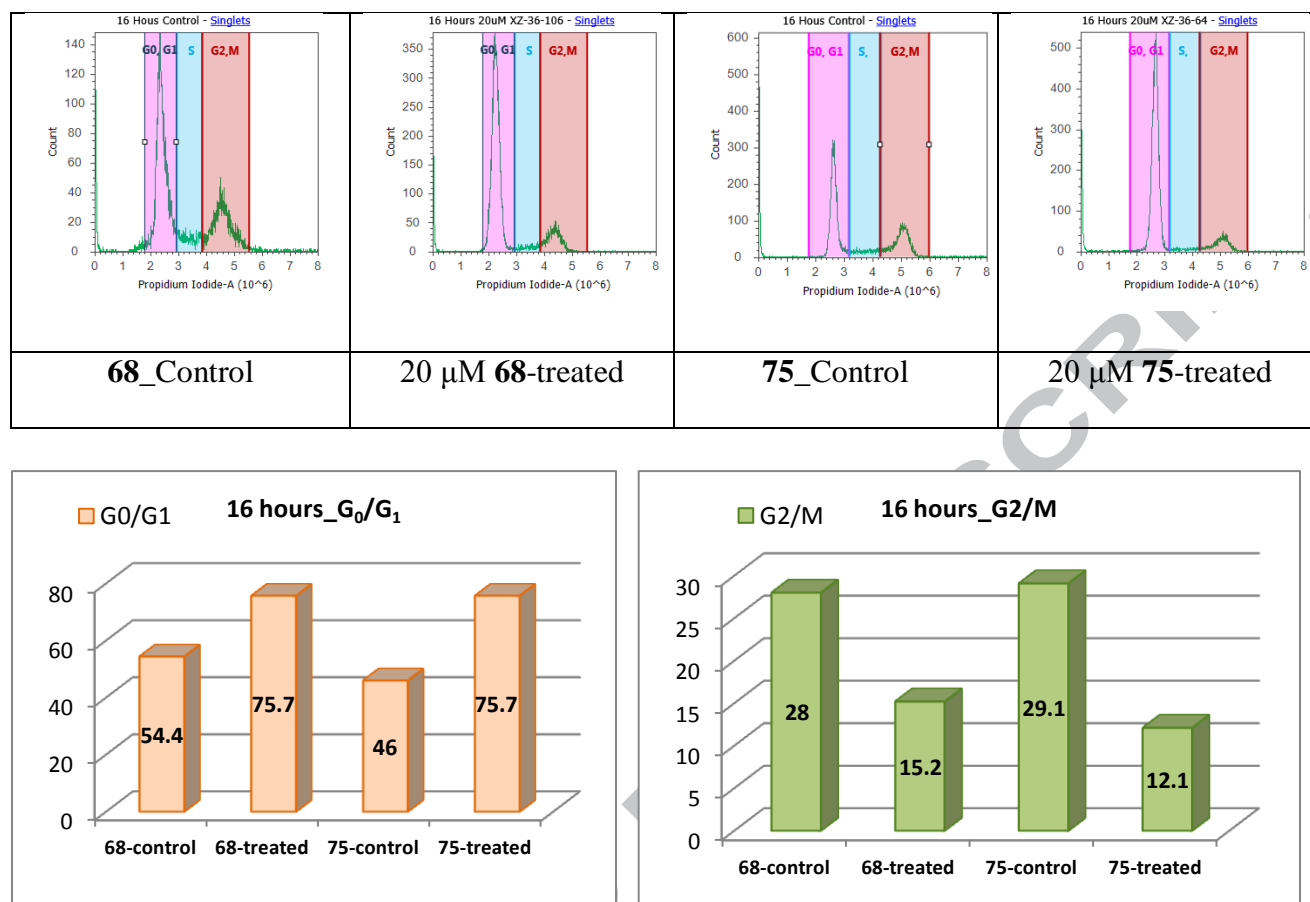| **68**_Control | 20 μM **68**-treated | **75**_Control | 20 μM **75**-treated |

Figure 4. Cell cycle analysis of PC-3 prostate cancer cells. PC-3 cancer cells were untreated or treated with **68** and **75**, respectively, at 20 μM. Cells were harvested after 16 hours, fixed, stained, and analyzed for DNA content.

3. Conclusion

In summary, twenty-five symmetric 1,9-diarylnona-1,3,6,8-tetraen-5-ones with two identical nitrogen-containing terminal heteroaromatic rings have been synthesized. Eighteen 1,9-diarylnona-1,3,6,8-tetraen-5-ones exhibit significantly improved antiproliferative potency as compared with curcumin. This indicates that (1*E*,3*E*,6*E*,8*E*)-1,9-diarylnona-1,3,6,8-tetraen-5-one can serve as a potential scaffold for further exploration of the therapeutic potential for the treatment of prostate cancer. Our findings

also suggest that *para* pyridyls and quinolin-4-yl serve as favorable heteroaromatic rings for the enhanced potency of this new scaffold. Two of the most potent compounds, **75** and **68**, effectively inhibit PC-3 cell proliferation by inducing cell apoptosis and by arresting cell cycle regulation in the G_0/G_1 phase.

4. Experiments

4.1. General synthetic procedures: NMR spectra were obtained on a Bruker Fourier 300 spectrometer in CDCl$_3$, or DMSO-d$_6$. The chemical shifts are given in ppm referenced to the respective solvent peak, and coupling constants are reported in Hz. Anhydrous THF and dichloromethane were purified by PureSolv MD 7 Solvent Purification System from Innovative Technologies (MB-SPS-800). All other reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using silica gel (32-63 μm). Preparative thin-layer chromatography (PTLC) separations were carried out on thin layer chromatography plates preloaded with silica gel GF254.

4.2. General procedure for the synthesis of (*E*)-3-aryl-2-propenals (33-57).[20,32]

The reaction mixture of aldehyde (0.5 mmol) and 2-(triphenylphosphoranylidene)acetaldehyde (0.51 mmol) in DMF (0.5 mL) was stirred for 1-4 days and detected with TLC. The reaction mixture was extracted with dichloromethane (10 mL × 3). The combined organic extracts were rinsed with brine (5 mL × 3) and dried over anhydrous magnesium sulfate, and the volatile components were evaporated under vacuum to give the crude product, which was subjected to the PTLC purification using hexanes/ethyl acetate (1/1, v/v) as eluent to give the respective product. All intermediates were directly used for the next step reaction after confirming with their ^{1}H NMR data. The ^{1}H NMR data for the

sixteen known (*E*)-3-aryl-2-propenals (**33**, **34**, **36**, **37**, **39-46**, **48**, **49**, **56**, **57**) are in consistent with those reported in the literature.[20] The yields and ^{1}H NMR spectral data for the other nine intermediates are listed below.

4.2.1. (*E*)-3-(Thiazol-5-yl)acrylaldehyde (35): 43% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.64 (1H, d, *J* = 7.5 Hz), 8.89 (1H, s), 8.10 (1H, s), 7.65 (1H, d, *J* = 15.6 Hz), 6.49 (1H, dd, *J* = 15.6, 7.5 Hz).

4.2.2. (*E*)-3-(5-Chloro-1-methyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)acrylaldehyde (38): 53% yield, ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.64 (1H, d, *J* = 7.5 Hz), 7.33 (1H, d, *J* = 16.2 Hz), 6.84 (1H, dd, *J* = 16.2, 7.5 Hz), 3.96 (3H, s).

4.2.3. (*E*)-3-(6-(Trifluoromethyl)pyridin-3-yl)acrylaldehyde (47): 85% yield, ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.79 (1H, d, *J* = 7.5 Hz), 8.89 (1H, s), 8.08 (1H, d, *J* = 8.4 Hz), 7.78 (1H, d, *J* = 8.1 Hz), 7.55 (1H, d, *J* = 16.2 Hz), 6.85 (1H, dd, *J* = 16.2, 7.5 Hz).

4.2.4. (*E*)-3-(Quinolin-2-yl)acrylaldehyde (50): 45% yield, ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.86 (1H, d, *J* = 7.8 Hz), 8.22 (1H, d, *J* = 8.4 Hz), 8.13 (1H, d, *J* = 8.4 Hz), 7.84 (1H, d, *J* = 8.1 Hz), 7.77 (1H, dt, *J* = 6.9, 1.2 Hz), 7.73 (1H, d, *J* = 15.9 Hz), 7.69 (1H, d, *J* = 8.4 Hz), 7.60 (1H, dt, *J* = 8.1, 0.9 Hz), 7.13 (1H, dd, *J* = 16.2, 7.8 Hz).

4.2.5. (*E*)-3-(2-Chloroquinolin-3-yl)acrylaldehyde (51): 70% yield, ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.83 (1H, d, *J* = 7.5 Hz), 8.43 (1H, s), 8.02 (1H, d, *J* = 9.6 Hz), 7.98 (1H, d, *J* = 16.5 Hz), 7.89 (1H, d, *J* = 8.1 Hz), 7.81 (1H, d, *J* = 7.8 Hz), 7.62 (1H, d, *J* = 7.8 Hz), 6.84 (1H, dd, *J* = 15.9, 7.5 Hz).

4.2.6. (*E*)-3-(Quinolin-4-yl)acrylaldehyde (52): 72% yield, ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.89 (d, *J* = 7.5 Hz, 1H), 8.96 (d, *J* = 4.5 Hz, 1H), 8.23 (d, *J* = 16.2 Hz, 1H), 8.20-8.11 (overlapped, 2H), 7.78 (t, *J* = 7.2 Hz, 1H), 7.65 (t, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 4.5 Hz, 1H), 6.89 (dd, *J* = 15.9, 7.5 Hz, 1H).

4.2.7. (*E*)-3-(1-Methyl-1*H*-benzo[*d*]imidazol-2-yl)acrylaldehyde (53): 46% yield, ^{1}H NMR (300 MHz, CDCl₃) δ 9.82 (1H, d, J = 7.5 Hz), 7.84 (1H, dd, J = 6.3, 3.6 Hz), 7.54 (1H, d, J = 15.6 Hz), 7.42-7.34 (4H, overlapped), 3.95 (3H, s).

4.2.8. (*E*)-3-(1-Ethyl-1*H*-benzo[d]imidazol-2-yl)acrylaldehyde (54): 54% yield, ^{1}H NMR (300 MHz, CDCl₃) δ 9.80 (1H, d, J = 7.2 Hz), 7.82 (1H, dd, J = 6.3, 3.3 Hz), 7.49 (1H, d, J = 15.6 Hz), 7.43-7.30 (4H, overlapped), 4.37 (2H, q, J = 7.5 Hz), 1.49 (3H, t, J = 7.5 Hz).

4.2.9. (*E*)-3-(1-Propyl-1*H*-benzo[*d*]imidazol-2-yl)acrylaldehyde (55): 72% yield, ^{1}H NMR (300 MHz, CDCl₃) δ 9.79 (1H, d, J = 6.9 Hz), 7.80 (1H, dd, J = 6.6, 5.7 Hz), 7.47 (1H, d, J = 15.6 Hz), 7.41-7.29 (4H, overlapped), 4.26 (2H, t, J = 7.2 Hz), 1.89 (2H, Hex, J = 7.5 HZ), 0.97 (3H, t, J = 7.2 Hz).

4.3. General procedure for the synthesis of 1,9-diheteroarylnona-1,3,6,8-tetra-en-5-ones (59-83):
To the solution of tetraethyl (2-oxopropane-1,3-diyl)bis(phosphonate) (1 mmol) in 4 mL ethanol, potassium carbonate (5 mmol) in 6 mL water was added dropwise. After 30 min at room temperature, the appropriate (*E*)-3-aryl-2-propenal (2.2 mmol) in 0.5 mL ethanol was added to the solution dropwise. The inorganic solids were removed by filtration after 2-4 h when no starting material observed by TLC, and the filtrate was diluted with with 1 M HCl (10 mL) and extracted with dichloromethane (10 mL × 3). The collected aqueous layer was neutralized by saturated aqueous NaHCO₃ and then extracted with dichloromethane (10 mL × 3). The combined organic extracts were dried over anhydrous magnesium sulfate, and the volatile components were evaporated under vacuum to give the crude product, which was subjected to purification using dichloromethane/methanol (100/3, v/v) as eluent to give the respective product. Some physical and spectral data for the title compounds are listed below.

4.3.1. (1*E*,3*E*,6*E*,8*E*)-1,9-Di(thiazol-2-yl)nona-1,3,6,8-tetraen-5-one (59): brown solid, mp 131-132 $^{\text{o}}$C, 60% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.87 (2H, d, *J* = 3.2 Hz), 7.44 (4H, dd, *J* = 15.1, 10.0 Hz), 7.36 (2H, d, *J* = 3.2 Hz), 7.27–7.12 (2H, overlapped), 6.66 (2H, d, *J* = 15.1 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 188.4, 165.4, 144.5, 141.6, 132.6, 131.9, 131.5, 120.3. IR (film) v_{max}: 1656, 1606, 1401, 1328, 1063 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{15}$H$_{12}$N$_2$OS$_2$ [M+H]$^+$: 301.0469; found: 301.0466.

4.3.2. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(4-methylthiazol-2-yl)nona-1,3,6,8-tetraen-5-one (60): yellow solid, mp 138-139 $^{\text{o}}$C, 54 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.44 (2H, dd, *J* = 15.0, 10.5 Hz), 7.22 (2H, dd, *J* = 15.3, 10.8 Hz), 7.10 (2H, d, *J* = 15.3 Hz), 6.94 (2H, s), 6.66 (2H, d, *J* = 13.8 15.3 Hz), 2.50 (6H, s). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.0, 165.1, 155.3, 142.2, 133.2, 131.9, 131.8, 115.7, 17.8. IR (film) v_{max}: 3104, 2921, 1644, 1598, 1578, 1509, 1439 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{17}$H$_{17}$N$_2$OS$_2$ [M+H]: 329.0782; found 329.0778.

4.3.3. (1*E*,3*E*,6*E*,8*E*)-1,9-Di(thiazol-5-yl)nona-1,3,6,8-tetraen-5-one (61): yellow solid, mp 165-166 $^{\text{o}}$C, 50 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.75 (2H, s), 7.91 (2H, s), 7.40 (2H, dd, *J* = 15.0, 11.1 Hz), 7.13 (2H, d, *J* = 15.3 Hz), 6.73 (2H, dd, *J* = 15.3, 11.1 Hz), 6.54 (2H, d, *J* = 15.0 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.0, 153.9, 144.7, 142.6, 137.4, 130.69, 130.66, 130.36. IR (film) v_{max}: 3071, 1640, 1603, 1575, 1493, 1387 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{15}$H$_{13}$N$_2$OS$_2$ [M+H]: 301.0469; found 301.0465.

4.3.4. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(5-methylisoxazol-3-yl)nona-1,3,6,8-tetraen-5-one (62): yellow solid, mp 183-184 $^{\text{o}}$C, 62% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.41 (dd, *J* = 15.2, 9.0 Hz, 2H), 7.00–6.84 (m, 4H), 6.60 (d, *J* = 15.3 Hz, 2H), 6.17 (s, 2H), 2.44 (s, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 188.6, 170.0, 161.0, 141.8, 133.2, 131.1, 128.4, 99.2, 12.4. IR (film) v_{max}: 1658, 1610, 1445, 904, 725 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{17}$H$_{16}$N$_2$O$_3$ [M+H]$^+$: 297.1239; found: 297.1237.

4.3.5. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(4-bromo-1-methyl-1*H*-pyrazol-3-yl)nona-1,3,6,8-tetraen-5-one (63):

yellow oil, 46 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.45 (2H, dd, J = 15.0, 11.4 Hz), 7.41 (2H, s),

7.27 (2H, dd, J = 15.6, 10.8 Hz), 6.89 (2H, d, J = 15.6 Hz), 6.59 (2H, d, J = 15.0 Hz). ^{13}C NMR (75

MHz, CDCl$_3$) δ 189.7, 147.0, 143.5, 132.2, 130.33, 130.23, 129.3, 95.2, 40.5. IR (film) v_{max}: 3127,

2926, 1698, 1620, 1588, 1575, 1304, 1163, 1087, 1004 cm^{-1}. HR-MS (ESI) *m/z*: calcd for

C$_{17}$H$_{17}$N$_4$OBr$_2$ [M+H]: 450.9769; found 450.9762; 452.9748, found 452.9741; 454.9728, found

454.9720.

4.3.6. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(5-chloro-1-methyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)nona-1,3,6,8-

tetraen-5-one (64): yellow solid, mp 188-189 ^{o}C, 54 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.41 (2H,

dd, J = 15.0, 10.8 Hz), 7.03 (2H, dd, J = 15.9, 10.8 Hz), 6.82 (2H, d, J = 15.6 Hz), 6.58 (2H, d, J =

15.3 Hz), 3.94 (6H, s). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.3, 143.6, 139.7 (q, J_{CF} = 37.5 Hz), 132.6,

130.1, 128.7, 127.1, 125.2 (q, J_{CF} = 267.8 Hz), 114.5, 37.8. IR (film) v_{max}: 3052, 1657, 1601, 1523,

1485, 1410, 1358, 1243, 1169 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{19}$H$_{15}$N$_4$OCl$_2$F$_6$ [M+H]: 499.0528;

found 499.0540; 501.0498; found 501.0509; 503.0468; found 503.0475.

4.3.7. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(1-isopropyl-1*H*-imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (65): deep

brown solid, mp 145-146 ^{o}C, 59 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.51-7.38 (4H, overlapped),

7.17 (2H, s), 7.06 (2H, s), 6.83 (2H, dt, J = 13.8, 6.3 Hz), 6.62 (2H, dt, J = 14.1, 7.2 Hz), 4.54 (2H,

hept, J = 6.6 Hz), 1.49 (12H, d, J = 6.9 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.6, 154.8, 150.5, 142.8,

140.4, 137.5, 131.7, 131.6, 124.0, 123.9. IR (film) v_{max}: 2977, 2928, 1642, 1599, 1463, 1417 cm^{-1}.

HR-MS (ESI) *m/z*: calcd for C$_{21}$H$_{27}$N$_4$O [M+H]: 351.2185; found 351.2181.

4.3.8. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(1-(*sec*-butyl)-1*H*-imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (66): deep

brown solid, mp 148-150 ^{o}C, 52 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48-7.43 (4H, overlapped),

7.20 (2H, s), 7.02 (2H, s), 6.82 (2H, d, J = 13.5 Hz), 6.64 (2H, d, J = 13.8 Hz), 4.27 (2H, Hex, J = 6.9

Hz), 1.68-1.73 (4H, m), 1.48 (6H, d, J = 6.9 Hz), 0.86 (6H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, CDCl$_3$)

δ 189.4, 144.7, 142.9, 130.93, 130.87, 130.86, 125.4, 118.1, 54.0, 31.5, 22.3, 11.3. IR (film) v_{max}:

3028, 2964, 1642, 1459, 1418, 1400 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{23}$H$_{31}$N$_4$O [M+H]: 379.2498;

found 379.2493.

4.3.9. (1E,3E,6E,8E)-1,9-Bis(1-isobutyl-1H-imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (67): deep

brown oil, 42 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.46 (4H, dd, J = 14.4, 7.8 Hz), 7.18 (2H, s),

6.95 (2H, s), 6.77 (2H, d, J = 14.1 Hz), 6.65 (2H, d, J = 13.8 Hz), 3.81 (4H, d, J = 7.2 Hz), 2.12-2.00

(2H, m), 0.96 (12H, d, J = 6.9 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.4, 144.8, 142.8, 131.2, 130.0,

125.0, 122.8, 54.3, 31.2, 20.6. IR (film) v_{max}: 3109, 2960, 2926, 1642, 1599, 1468, 1444, 1278, 1245

cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{23}$H$_{31}$N$_4$O [M+H]: 379.2498; found 379.2494.

4.3.10. (1E,3E,6E,8E)-1,9-Bis(1-(pentan-3-yl)-1H-imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (68):

brown oil, 72 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.46-7.41 (4H, overlapped), 7.20 (2H, s), 6.95

(2H, s), 6.80 (2H, d, J = 13.5 Hz), 6.61 (2H, d, J = 13.8 Hz), 3.98-3.93 (2H, m), 1.89-1.66 (8H, m),

0.79 (12H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.4, 145.6, 143.0, 131.2, 130.8, 130.7,

125.6, 118.1, 60.3, 29.7, 11.2. IR (film) v_{max}: 3071, 2967, 2934, 1640, 1597, 1507, 1458, 1420, 1275

cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{25}$H$_{35}$N$_4$O [M+H]: 407.2811; found 407.2806.

4.3.11. (1E,3E,6E,8E)-1,9-Bis(1-(pentan-2-yl)-1H-imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (69):

brown oil, 61 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.50-7.37 (4H, overlapped), 7.18 (2H, s), 7.01

(2H, s), 6.81 (2H, d, J = 13.8 Hz), 6.62 (2H, d, J = 13.8 Hz), 4.32 (2H, hex, J = 6.9 Hz), 1.74 (4H, q, J

= 7.5 Hz), 1.46 (6H, d, J = 6.6 Hz), 1.30-1.10 (4H, m), 0.9 (6H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz,

CDCl$_3$) δ 189.4, 144.6, 142.9, 131.0, 130.9, 130.7, 125.5, 118.1, 52.2, 40.5, 22.7, 20.0, 14.3. IR (film)

v_{max}: 3104, 2959, 2932, 1641, 1597, 1506, 1457, 1418, 1402 cm^{-1}. HR-MS (ESI) m/z: calcd for

C$_{25}$H$_{35}$N$_4$O [M+H]: 407.2811; found 407.2807.

4.3.12. (1*E*,3*E*,6*E*,8*E*)-1,9-Di(pyridin-2-yl)nona-1,3,6,8-tetraen-5-one (70): deep brown solid, mp 128-129 ^{o}C, 39% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.63 (2H, d, *J* = 4.2 Hz), 7.70 (2H, t, *J* = 7.5 Hz), 7.55-7.43 (4H, overlapped), 7.22 (2H, dd, *J* = 6.6, 4.8 Hz), 7.04 (2H, dd, *J* = 14.1, 6.6 Hz), 6.70 (2H, d, *J* = 13.8 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.6, 154.8, 150.5, 142.8, 140.4, 137.5, 131.7, 131.6, 124.0, 123.9. IR (film) v_{max}: 1646, 1600, 1469, 1431, 1250, 1002 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{19}$H$_{17}$N$_2$O [M+H]: 289.1341; found 289.1338.

4.3.13. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(6-methylpyridin-2-yl)nona-1,3,6,8-tetraen-5-one (71): deep brown solid, mp 85-86 ^{o}C, 46 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.57 (2H, t, *J* = 7.5 Hz), 7.49 (2H, dd, *J* = 15.9, 11.1 Hz), 7.44 (2H, dd, *J* = 15.9, 11.4 Hz), 7.19 (2H, d, *J* = 7.8 Hz), 7.05 (2H, d, *J* = 6.3 Hz), 7.00 (2H, dt, *J* = 13.8, 4.5 Hz), 6.67 (2H, dt, *J* = 13.8, 4.5 Hz), 2.59 (s, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.0, 158.7, 153.6, 142.4, 140.4, 136.8, 130.7, 130.5, 123.0, 120.4, 24.6. IR (film) v_{max}: 3106, 2923, 1646, 1617, 1600, 1574, 1507, 1451 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{21}$H$_{21}$N$_2$O [M+H]: 317.1654; found 317.1651.

4.3.14. (1*E*,3*E*,6*E*,8*E*)-1,9-Di(pyridin-3-yl)nona-1,3,6,8-tetraen-5-one (72): yellow solid, mp 205 ^{o}C (decomposed), 10 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.73 (2H, d, *J* = 1.5 Hz), 8.56 (2H, d, *J* = 8.1 Hz), 7.82 (2H, d, *J* = 8.1 Hz), 7.49 (2H, dd, *J* = 15.6, 9.9 Hz), 7.33 (2H, dd, *J* = 8.1, 4.8 Hz), 7.05 (1H, d, *J* = 15.6 Hz), 7.00 (2H, s), 6.97 (1H, d, *J* = 15.6 Hz), 6.63 (2H, d, *J* = 8.1 Hz). ^{13}C NMR (75 MHz, CDCl$_3$ + CD$_3$OD) δ 189.6, 150.1, 149.2, 143.1, 138.0, 134.4, 132.7, 130.6, 129.6, 124.5. IR (film) v_{max}: 3028, 2923, 1642, 1620, 1580, 1479, 1415 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{19}$H$_{17}$N$_2$O [M+H]: 289.1341; found 289.1337.

4.3.15. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(6-(trifluoromethyl)pyridin-3-yl)nona-1,3,6,8-tetraen-5-one (73): yellow solid, mp 168-169 ^{o}C, 22 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.81 (2H, s), 7.96 (2H, d, *J* = 8.1 Hz), 7.69 (2H, d, *J* = 8.1 Hz), 7.48 (2H, dd, *J* = 15.0, 10.2 Hz), 7.10-6.98 (4H, overlapped), 6.68

(2H, d, J = 15.3 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ188.9, 149.4, 148.3 (q, J_{CF} = 34.5 Hz), 142.4, 136.3, 135.3, 135.2, 131.7, 122.0 (q, J_{CF} = 273.3 Hz), 121.22, 121.19. IR (film) v_{max}: 3037, 1667, 1605, 1395 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{21}$H$_{15}$N$_2$OF$_6$ [M+H]: 425.1088; found 425.1083.

4.3.16. (1E,3E,6E,8E)-1,9-Di(pyridin-4-yl)nona-1,3,6,8-tetraen-5-one (74): brown solid, mp 133-134 ^{o}C, 16% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.63 (4H, d, J = 6.0 Hz), 7.48 (2H, dd, J = 15.0, 10.8 Hz), 7.35 (4H, d, J = 6.0 Hz), 7.13 (2H, dd, J = 15.6, 11.1 Hz), 6.92 (2H, d, J = 15.6 Hz), 6.66 (2H, d, J = 15.3 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.0, 150.9, 144.0, 142.5, 139.0, 131.8, 131.7, 121.8. IR (film) v_{max}: 1646, 1601, 1415, 1357, 1249, 1073 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{19}$H$_{17}$N$_2$O [M+H]: 289.1341; found 289.1336.

4.3.17. (1E,3E,6E,8E)-1,9-Bis(3-fluoropyridin-4-yl)nona-1,3,6,8-tetraen-5-one (75): brown solid, mp 142-143 ^{o}C, 44 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.50 (2H, d, J = 1.2 Hz), 8.42 (2H, d, J = 5.1 Hz), 7.47 (2H, dd, J = 15.3, 10.8 Hz), 7.40 (2H, t, J = 5.7 Hz), 7.20 (2H, dd, J = 15.9, 10.8 Hz), 7.04 (2H, d, J = 15.6 Hz), 6.68 (2H, d, J = 15.3 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.0, 157.5 (d, J_{CF} = 262.5 Hz), 146.6 (d, J_{CF} = 5.3 Hz), 142.7, 139.7 (d, J_{CF} = 24.8 Hz), 134.2 (d, J_{CF} = 6.8 Hz), 131.8 (d, J_{CF} = 46.5 Hz), 131.5, 131.4, 121.7. IR (film) v_{max}: 3041, 1648, 1605, 1491, 1417, 1250 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{19}$H$_{15}$N$_2$OF$_2$ [M+H]: 325.1152; found 325.1148.

4.3.18. (1E,3E,6E,8E)-1,9-Di(quinolin-2-yl)nona-1,3,6,8-tetraen-5-one (76): brown solid, mp 172-173 ^{o}C, 38 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.14 (4H, dd, J = 12.3, 8.7 Hz), 7.80 (2H, d, J = 8.1 Hz), 7.74 (2H, dt, J = 7.2, 1.2 Hz), 7.61-7.52 (8H, overlapped), 7.31-7.21 (2H, overlapped), 6.76 (2H, dt, J = 11.4, 3.0 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.4, 155.1, 148.7, 142.9, 141.3, 137.4, 133.0, 132.1, 130.8, 130.0, 128.4, 128.2, 127.6, 120.7. IR (film) v_{max}: 3053, 1654, 1619, 1609, 1569, 1504, 1429 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{27}$H$_{21}$N$_2$O [M+H]: 389.1654; found 389.1650.

4.3.19. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(2-chloroquinolin-3-yl)nona-1,3,6,8-tetraen-5-one (77): yellow solid, mp 141-142 °C, 5 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.41 (2H, s), 8.02 (2H, d, *J* = 8.4 Hz), 7.87 (2H, d, *J* = 8.1 Hz), 7.77 (2H, dt, *J* = 6.9, 1.2 Hz), 7.62 (2H, d, *J* = 9.0 Hz), 7.59 (2H, dd, *J* = 15.6, 10.8 Hz), 7.48 (2H, d, *J* = 15.6 Hz), 7.11 (2H, dd, *J* = 15.6, 11.1 Hz), 6.72 (2H, d, *J* = 15.0 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.3, 150.6, 148.0, 143.0, 136.3, 135.3, 131.7, 131.5, 131.1, 129.5, 129.1, 128.5, 128.3, 127.9. IR (film) v_{max}: 3071, 1640, 1603, 1575, 1493, 1387 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{27}$H$_{19}$N$_2$OCl$_2$ [M+H]: 457.0874; found 457.0868; 459.0845; found 459.0837; 461.0815; found 461.0808.

4.3.20. (1*E*,3*E*,6*E*,8*E*)-1,9-Di(quinolin-4-yl)nona-1,3,6,8-tetraen-5-one (78): Yellow solid, mp 189-190 °C, 16% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.93 (d, *J* = 4.5 Hz, 2H), 8.15 (dd, *J* = 8.3, 4.0 Hz, 4H), 7.76 (dd, *J* = 14.9, 5.1 Hz, 2H), 7.75 (d, *J* = 14.9 Hz, 2H), 7.69–7.56 (m, 6H), 7.20 (dd, *J* = 15.4, 11.1 Hz, 2H), 6.74 (d, *J* = 15.2 Hz, 2H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 188.7, 150.3, 149.0, 142.41 141.3, 135.5, 132.9, 131.2, 130.5, 129.8, 127.3, 126.0, 123.2, 117.5. IR (film) v_{max}: 1644, 1610, 1596, 1577, 1505, 1263, 1073 cm^{-1}; HRMS (ESI): *m/z* calcd for C$_{27}$H$_{20}$N$_2$O [M+H]$^+$: 389.1654; found: 389.1652.

4.3.21. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (79): brown solid, mp 198 °C (decomposed), 28 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.86-7.77 (4H, overlapped), 7.54 (2H, dd, *J* = 15.0, 11.4 Hz), 7.39-7.32 (6H, overlapped), 7.03 (2H, d, *J* = 15.0 Hz), 6.77 (2H, d, *J* = 15.0 Hz), 3.87 (6H, s). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.6, 149.8, 142.2, 136.5, 136.1, 133.2, 124.8, 124.7, 124.5, 124.2, 119.6, 110.4, 30.3. IR (film) v_{max}: 3058, 2925, 1644, 1600, 1460, 1322 cm^{-1}. HR-MS (ESI) *m/z*: calcd for C$_{25}$H$_{23}$N$_4$O [M+H]: 395.1872; found 395.1872.

4.3.22. (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(1-ethyl-1*H*-benzo[*d*]imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (80): yellow solid, mp 139-141 °C, 52 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.81-7.72 (4H, overlapped),

7.52 (2H, dd, J = 15.0, 11.7 Hz), 7.36-7.26 (6H, overlapped), 6.96 (2H, d, J = 14.4 Hz), 6.73 (2H, d, J = 15.0 Hz), 4.29 (4H, q, J = 7.2 Hz), 1.46 (6H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.2, 149.4, 144.0, 142.2, 135.7, 135.1, 132.5, 125.3, 124.1, 123.8, 120.5, 110.2, 39.0, 16.3. IR (film) v_{max}: 3059, 2977, 2928, 1643, 1600, 1580, 1470, 1447, 1409, 1382 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{27}$H$_{27}$N$_4$O [M+H]: 423.2185; found 423.2181.

4.3.23. (1E,3E,6E,8E)-1,9-Bis(1-propyl-1H-benzo[d]imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (81): yellow solid, mp 205 ^{o}C (decomposed), 44 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.81 (2H, dd, J = 12.7, 4.5 Hz), 7.77 (2H, d, J = 12.8 Hz), 7.53 (2H, dd, J = 15.0, 11.4 Hz), 7.36-7.28 (6H, overlapped), 6.98 (2H, d, J = 15.0 Hz), 6.74 (2H, d, J = 15.3 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.1, 149.9, 143.9, 142.3, 136.2, 135.0, 132.4, 125.5, 124.1, 123.7, 120.4, 110.4, 45.7, 24.4, 12.1. IR (film) v_{max}: 3054, 2965, 2934, 1643, 1600, 1461, 1449, 1408 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{29}$H$_{31}$N$_4$O [M+H]: 451.2498; found 451.2492.

4.3.24.   (1E,3E,6E,8E)-1,9-Bis(1-isopropyl-1H-benzo[d]imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (82): deep brown solid, mp 113-115 ^{o}C, 68 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.88-7.79 (4H, overlapped), 7.58-7.49 (4H, overlapped), 7.34-7.29 (4H, overlapped), 7.08 (2H, d, J = 14.7 Hz), 6.77 (2H, d, J = 15.0 Hz), 4.90 (2H, hept, J = 6.9 Hz), 1.72 (12H, d, J = 6.9 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.2, 149.3, 143.9, 142.2, 135.8, 134.6, 132.6, 125.8, 123.9, 123.6, 120.6, 112.4, 48.6, 22.5. IR (film) v_{max}: 3055, 2978, 2934, 1643, 1598, 1489, 1446, 1408 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{29}$H$_{31}$N$_4$O [M+H]: 451.2498; found 451.2493.

4.3.25.   (1E,3E,6E,8E)-1,9-Bis(1-isobutyl-1H-benzo[d]imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (83): deep brown solid, mp 104-105 ^{o}C, 81 % yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.43 (2H, dd, J = 7.8, 3.6 Hz), 7.75 (2H, d, J = 14.4 Hz), 7.52 (2H, dd, J = 15.0, 11.7 Hz), 7.35-7.25 (6H, overlapped), 6.95 (2H, d, J = 14.7 Hz), 6.73 (2H, d, J = 15.0 Hz), 4.00 (4H, d, J = 7.5 Hz), 2.26-2.10 (2H, m), 0.97

(12H, d, J = 6.6 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.2, 150.1, 143.8, 142.3, 136.5, 135.0, 132.4, 125.7, 124.1, 123.7, 120.4, 110.7, 51.6, 30.6, 20.9. IR (film) ν_{max}: 3060, 2960, 2929, 1646, 1600, 1467, 1324 cm^{-1}. HR-MS (ESI) m/z: calcd for C$_{31}$H$_{35}$N$_4$O [M+H]: 479.2811; found 479.2805.

4.4. Cell culture. All cell lines were initially purchased from American Type Culture Collection (ATCC^{TM}). The PC-3 and LNCaP prostate cancer cell lines were routinely cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. Cultures were maintained in a high humidity environment supplemented with 5% carbon dioxide at a temperature of 37°C. The DU-145 prostate cancer cells were routinely cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS and 1% penicillin/ streptomycin.

4.5. WST-1 cell proliferation assay. PC-3, DU-145, or LNCaP cells were plated in 96-well plates at a density of 3,200 each well in 200 μL of culture medium. The cells were then treated with curcumin, or synthesized molecules separately at different doses for 3 days, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in a CO$_2$ incubator at 37 ^{o}C for three days. 10 μL of the premixed WST-1 cell proliferation reagent (Clontech) was added to each well. After mixing gently for one minute on an orbital shaker, the cells were incubated for additional 3 hours at 37 ^{o}C. To ensure homogeneous distribution of color, it is important to mix gently on an orbital shaker for one minute. The absorbance of each well was measured using a microplate-reader (Synergy HT, BioTek) at a wavelength of 430 nm. The IC$_{50}$ value is the concentration of each compound that inhibits cell proliferation by 50% under the experimental conditions and is the average from at least triplicate determinations that were reproducible and statistically significant. For calculating the IC$_{50}$ values, a linear proliferative inhibition was made based on at least five dosages for each compound.

4.6. Cell cycle analysis

PC-3 cells were plated in 24-well plates at a density of 200,000 each well in 400 µL of culture medium. After 3 h of cell attachment, the cells were then treated with compound **75** and **68** at 20 µM, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in CO_2 incubator at 37 ^{o}C for 16 h. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf 450 g for 5 min. After discarding the supernatant, the collected cells were re-suspended with 500 µL 80% cold ethanol to fix for 30 min in 4 ^{o}C. The fixed cells could be stored at -20 ^{o}C for one week. After fixation, the ethanol was removed after centrifuging and the cells were washed with PBS. The cells were then re-suspended with 100 µL of 100 mg/mL ribonuclease and were cultured at 37 ^{o}C for 30 min to degrade all RNA. The cells were stained with 200 µL of 50 µg/mL propidium iodide (PI) stock solution for 30 min at -20 ^{o}C, and then the fluorescence intensity of PI was detected in individual PC-3 cells using an Attune flow cytometer (Life Technologies) within 0.5-1 h after staining.

4.7. F2H12S and CYTOX AADvanced double staining assay

PC-3 cells were plated in 24-well plates at a density of 200,000 each well in 400 µL of culture medium. After 3 h of cell attachment, the cells were then treated with each test compound at different concentration for 15 h, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in CO_2 incubator at 37 ^{o}C for 16 h. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf value of 450 g for 5 min. The collected cells were re-suspended with 500 µL HBSS to remove proteins which may affect flow signal and centrifuged again. After discarding the supernatant, the collected cells were re-suspended with 300 µL HBSS and stained with 0.3 µL of F2N12S for 3-5 min followed by 0.3 µL SYTOX AADvanced for an additional 5 min. The fluorescence intensity of the two probes was further measured in individual PC-3 cells using an Attune flow cytometer (Life Technologies) within 0.5-1 h after staining.

4.8. Statistical analysis

All data are represented as the mean ± standard deviation (S.D.) for the number of experiments indicated. Other differences between treated and control groups were analyzed using the Student's t-test. A p-value < 0.05 was considered statistically significant.

Acknowledgments

Supplementary data

Supplementary data (comprised of copies of ^{1}H and ^{13}C NMR spectra for the final products) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.

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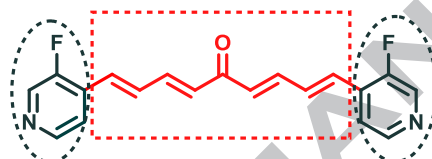
GRAPHICAL ABSTRACT

Design, Synthesis, and Biological Evaluation of 1,9-Diheteroarylnona-1,3,6,8-tetraen-5-ones as a New Class of Anti-Prostate Cancer Agents

Xiaojie Zhang, Rubing Wang, German Ruiz Perez, Guanglin Chen, Qiang Zhang, Shilong Zheng, Guangdi Wang, and Qiao-Hong Chen*
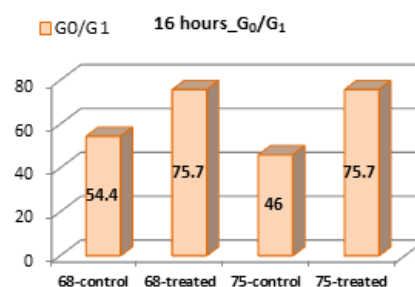
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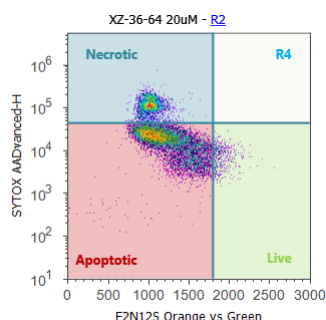
Cell Apoptosis Activation

IC_{50} = 1.14 μM (PC-3)
IC_{50} = 1.78 μM (DU145)
IC_{50} = 2.17 μM (LNCaP)

Cell Cycle Arrest at G0/G1 Phase

Key Words: Curcumin; Prostate cancer; Heteroaromatic ring, Cell proliferation, Cell Apoptosis; Cell Cycle Regulation.

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