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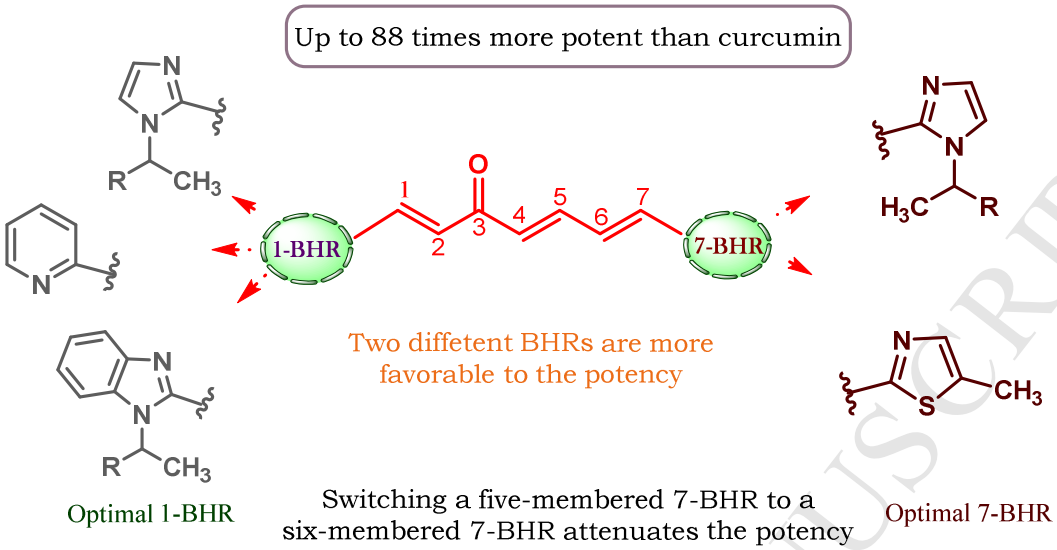
Graphical Abstract



Structure-Activity Relationship Studies of 1,7-Diheteroarylhepta-1,4,6-trien-3-ones with Two Different Terminal Rings in Prostate Epithelial Cell Models

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ABSTRACT: To systematically investigate the structure-activity relationships of 1,7-diheteroarylhepta-1,4,6-trien-3-ones in three human prostate cancer cell models and one human prostate non-neoplastic epithelial cell model, thirty five 1,7-diarylhepta-1,4,6-trien-3-ones with different terminal heteroaromatic rings have been designed for evaluation of their anti-proliferative potency *in vitro*. These target compounds have been successfully synthesized through two sequential Horner-Wadsworth-Emmons reactions starting from the appropriate aldehydes and tetraethyl (2-oxopropane-1,3-diyl)bis(phosphonate). Their anti-proliferative potency against PC-3, DU-145 and LNCaP human prostate cancer cell lines can be significantly enhanced by the manipulation of the terminal heteroaromatic rings, further demonstrating the utility of 1,7-diarylhepta-1,4,6-trien-3-one as a potential scaffold for the development of anti-prostate cancer agents. The optimal analog **40** is 82-, 67-, and 39-fold more potent than curcumin toward the three prostate cancer cell lines, respectively. The experimental data also reveal that the trienones with two different terminal aromatic rings possess greater potency toward three prostate cancer cell lines, but also have greater capability of suppressing the proliferation of PWR-1E benign human prostate epithelial cells, as compared to the corresponding counterparts with two identical terminal rings and curcumin. The terminal aromatic rings also affect the cell apoptosis perturbation.

Key words: 1,7-diheteroarylhepta-1,4,6-trien-3-one, antiproliferative activity, prostate cancer, structure-activity relationship, cell apoptosis

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1. Introduction

Curcumin (**1**) is the major chemical component of well-known turmeric (*Curcuma longa*) that has long been used as a yellow spicy curry ingredient and as a traditional Ayurvedic, Chinese, and Hindu medicine [1]. Curcumin is a well-established lead compound for the development of new anti-prostate cancer agents due to its safety profiles in human and its demonstrated potential in treating prostate cancer [1-4]. Several strategies have been applied to optimize curcumin in the hope of improving its potency and/or bioavailability. Chemical manipulations on curcumin have been established as a meaningful approach to the development of curcumin-based anti-prostate cancer agents with more drug-like properties [5]. 1,5-Diheteroarylpenta-1,4-dienones [6,7], 1,7-diheteroarylhepta-1,4,6-trienones [8], and 1,9-diheteroarylnona-1,4,6,8-tetraenones [9] have been identified by us as potential scaffolds for developing curcumin-based anti-prostate cancer agents because of their apparently improved potency as compared with curcumin. Among them, the 1,4,6-trienone motif was envisioned and illustrated as a good bioisostere of the keto-enol central spacer in curcumin due to the indistinguishable shape and size [8]. Our previous study demonstrates that 1-alkyl-1*H*-imidazol-2-yl in compounds **2**-**6** and 1-alkyl-1*H*-benzo[*d*]imidazole-2-yl in compounds **7**-**11** are significantly beneficial to the *in vitro* antiproliferative potency in three prostate cancer cell models [8]. All 1,7-diheteroaryl-1,4,6-trienones investigated in our previous study have two identical nitrogen-containing terminal aromatic rings.

To systematically investigate the structure-activity relationships of 1,7-diheteroaryl-1,4,6-heptatrien-3-ones in prostate epithelial cell models, this study aims to further explore the effect of two different terminal aromatic rings of the trienones on anti-proliferative potency in androgen-sensitive and androgen-insensitive prostate cancer cell models, as well as on PWR-1E non-neoplastic prostate epithelial cells. Consequently, thirty five new (1*E*,4*E*,6*E*)-heptatrien-3-ones (**12**-**46**, see Schemes 1 and 2, Table 1) that retain the 7-carbon central trienone linker but with two different nitrogen-containing heteroaromatic rings were designed and synthesized for the *in vitro* evaluation of their inhibitory ability towards cell proliferation of cancerous and noncancerous prostate epithelial cells.

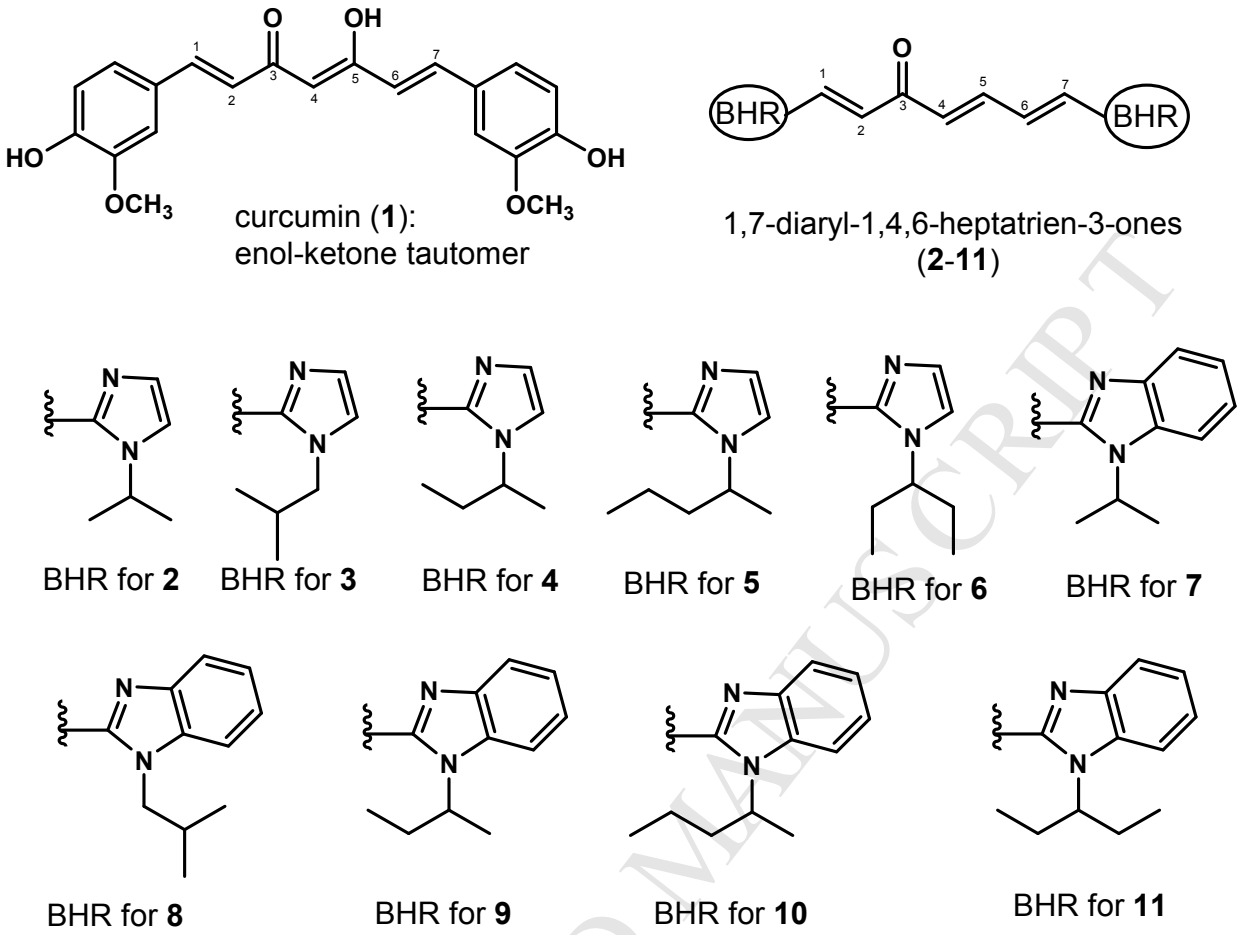


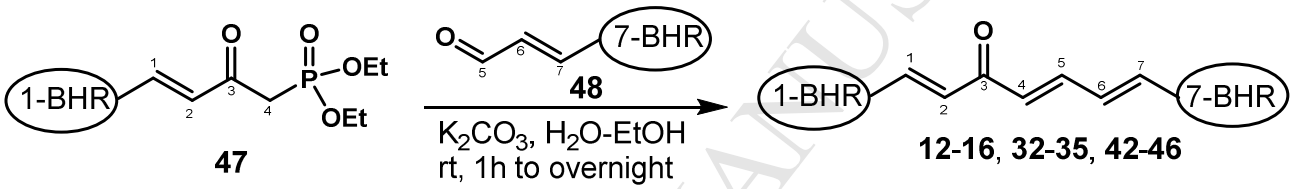
Fig. 1. Structures of curcumin and its analogues

2. Results and Discussion

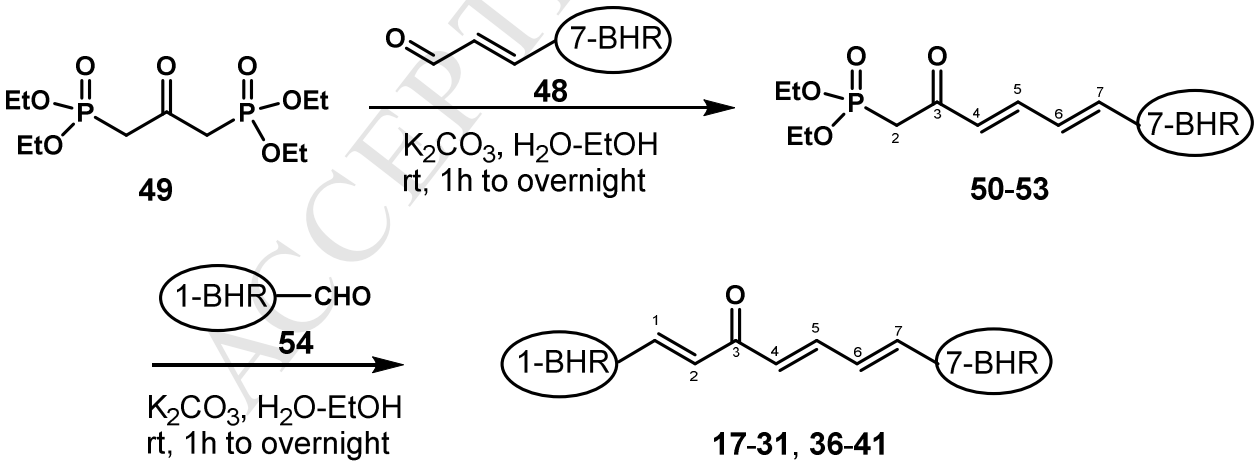
2.1 Chemistry

Initially, the protocol developed in our previous study was used to synthesize fourteen 1,7-diaryl-1,4,6-heptatrien-3-ones (**12**-**16**, **32**-**35**, **42**-**46**) with two different terminal heteroaromatic rings. Specifically, the syntheses were completed via the Horner-Wadsworth-Emmons reaction of (*E*)-diethyl(2-oxo-4-(1-BHR)-but-3-en-1-yl)phosphonates (**47**) with (2*E*)-3-(7-BHR)-2-propenals (**48**) using potassium carbonate as base (Scheme 1). Both phosphonates (**47**) and propenals (**48**) can be readily obtained using the procedure previously described by us [8]. Due to the unsatisfactory yields (7-39%) for the Horner-Wadsworth-Emmons reaction as illustrated in Scheme 1, the new reaction sequence with high efficiency was developed as shown in Scheme 2 and applied to the synthesis of the remaining twenty-one 1,7-diaryl-1,4,6-heptatrien-3-ones (**17**-**31**, **36**-**41**). This synthetic sequence started with the

preparation of four common ((3*E*,5*E*)-2-oxo-6-(aryl)hexa-3,5-dien-1-yl)phosphonates (**50**-**53**) by treating the appropriate (2*E*)-3-(7-BHR)-2-propenal (**48**) with 1 equivalent of 1,3-bis(diethylphosphonato)acetone (**49**). The 1,7-diaryl-1,4,6-heptatrien-3-ones (**17**-**31**, **36**-**41**) were obtained in 35-86% yields by the Horner-Wadsworth-Emmons reaction of ((3*E*,5*E*)-2-oxo-6-(aryl)hexa-3,5-dien-1-yl)phosphonates (**50**-**53**) with the appropriate heteroarylformaldehyde (**54**). It should be noted that we had already synthesized enough amount of compounds **12**-**16**, **32**-**35**, and **42**-**46** via Scheme 1 (Method A) for our structure-activity relationship studies when we figured out the more efficient synthetic method (Scheme 2, Method B). That is the reason why these compounds were not synthesized using Method B. According to the structural similarity, the method B described in Scheme 2 could be an efficient method for the synthesis of compounds **12**-**16**, **32**-**35**, and **42**-**46** as well.

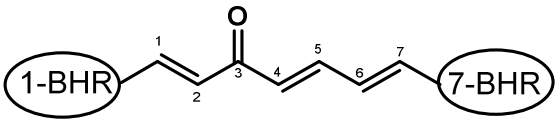


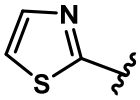
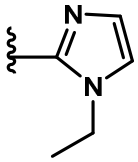
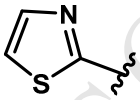
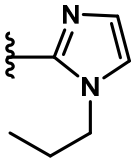
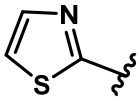
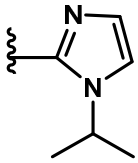
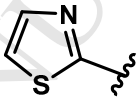
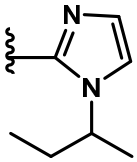
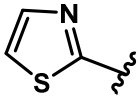
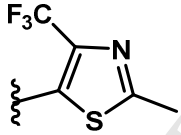
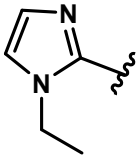
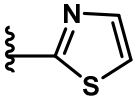
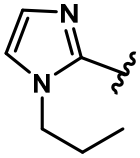
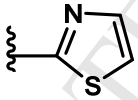
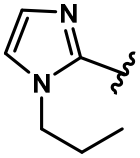
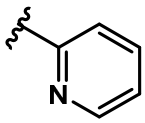
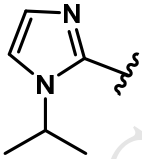
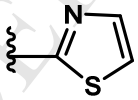
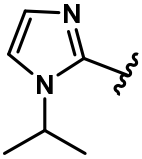
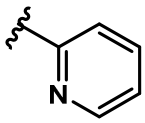
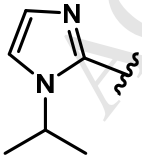
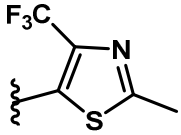
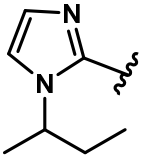
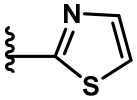
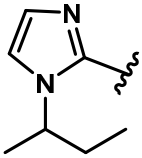
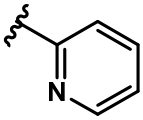
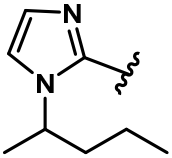
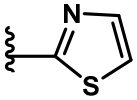
Scheme 1. Synthesis of 1,7-diheteroaryl-1,4,6-heptatrien-3-ones (**12**-**16**, **32**-**35**, and **42**-**46**) (Method A).



Scheme 2. Synthesis of 1,7-diheteroaryl-1,4,6-heptatrien-3-ones (**17**-**31** and **36**-**41**) (Method B).

Table 1. Structures of 1,7-diarylhepta-1,4,6-trien-3-ones with different nitrogen-containing heteroaromatic rings (**12-46**)



Compd	1-BHR	7-BHR	Compd	1-BHR	7-BHR
12			**13**		
14			**15**		
16			**17**		
18			**19**		
20			**21**		
22			**23**		
24			**25**		

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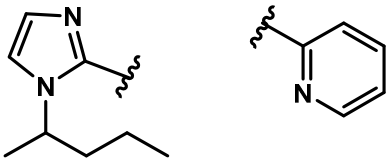

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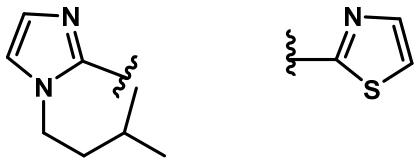

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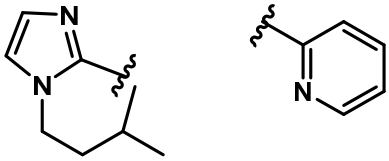

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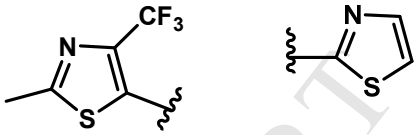

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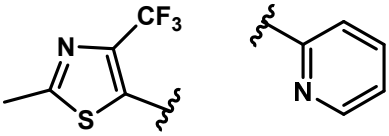

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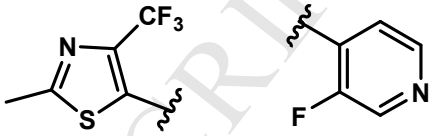

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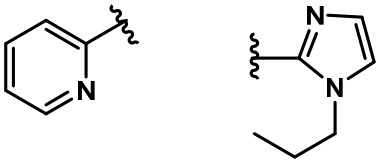

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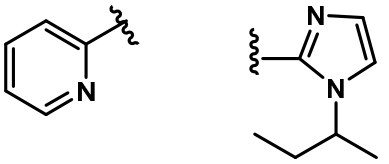

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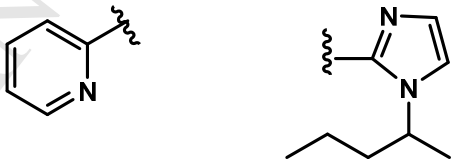

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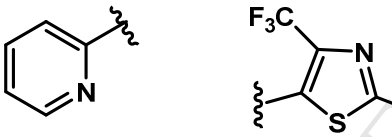

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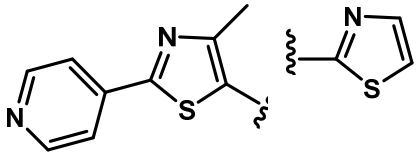

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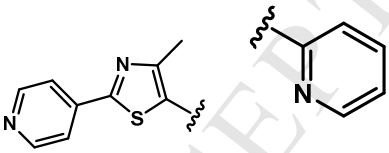

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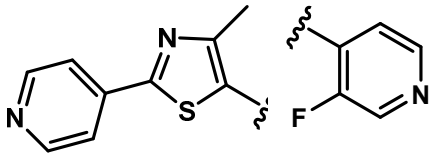

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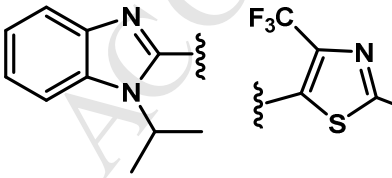

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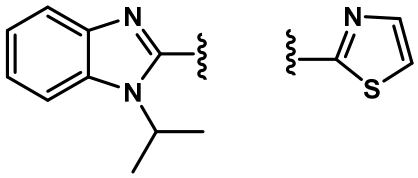

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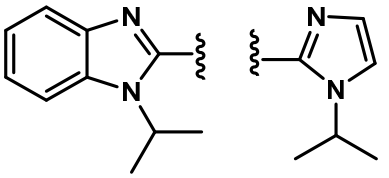

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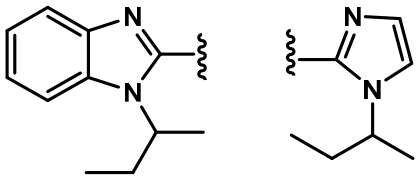
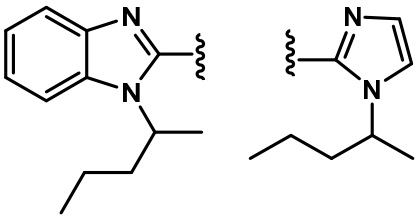
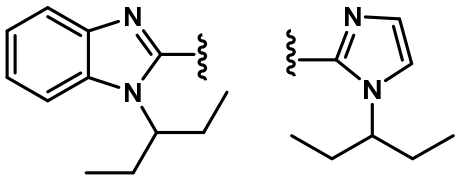
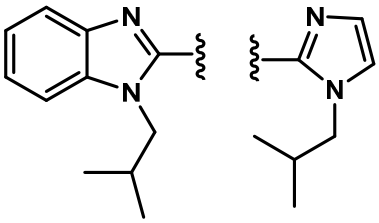


44 

45 

46 

2.2 Anti-proliferative activity towards prostate cancer cell lines and structure-activity relationships.

The *in vitro* anti-proliferative activity of these thirty-five new trienones was first evaluated on human prostate cancer cell models, employing both androgen-sensitive and androgen-insensitive prostate cancer cell lines (PC-3, DU145, and LNCaP). WST-1 cell proliferation assay was employed for the potency assessment, in which a water-soluble tetrazolium salt can be converted to formazan catalyzed by cell mitochondrial dehydrogenases. Consequently, the amount of produced formazan dye directly associates with live cell populations in the culture. Curcumin was used as a positive control for comparison in the parallel experiments and the anti-proliferative potency of each test trienone was represented as IC_{50} values. As illustrated in Table 2, all these trienones with two different aromatic rings have greater anti-proliferative potency than curcumin as indicated in their IC_{50} values. Their IC_{50} values against PC-3, DU-145 and LNCaP human prostate cancer cell lines are in the ranges of 0.29-14.02 μM, 0.33-8.68 μM, and 0.27-6.32 μM, respectively. They are up to 88-, 80-, 50-fold more potent than curcumin in the three prostate cancer cell models.

The structure-antiproliferative activity relationships of trienones on the basis of this study in combination with our previous study [8] can be summarized as below:

- It can be clearly concluded that 1,7-diaryl-1,4,6-heptatrien-3-ones with two different heteroaromatic rings are more potent towards both androgen-sensitive and androgen-insensitive prostate cancer cell lines than the corresponding ones with two identical aromatic rings. This conclusion is supported by the following data: i) the optimal trienone (**7**) with two identical

terminal heteroaromatic rings is 36-, 29-, and 11-fold more potent than curcumin toward PC-3, DU145, and LNCaP prostate cancer cell lines [8]; while the optimal trienones with two different terminal aromatic rings (**40** and **41**) are 82-88, 44-67, and 39-51 times more potent than curcumin; and ii) trienones (**42** and **43**) having two different terminal heteroaromatic rings are apparently more potent than their corresponding counterparts (**7** & **2**, and **9** & **4**) with two identical heteroaromatic rings (Table 3).

- 1-(*α*-Branched alkyl)-1*H*-imidazol-2-yl in trienones **22**-**26**, 1-(*α*-branched alkyl)-1*H*-benzo[d]imidazole-2-yl in compounds **40**-**45**, and pyridin-2-yl in trienones **32**-**35** serve as the optimal heteroaromatic rings at the C-1 terminal (1-BHR) for enhanced *in vitro* potency of this scaffold.

- 2-Methyl-4-trifluoromethyl-thiazole-5-yl in trienones **16**, **22**, **36**, and **40** and 1-(*α*-branched alkyl)-1*H*-imidazol-2-yl in trienones **14**, **15**, **32**, **33**, **34**, and **35** are the optimal heteroaromatic rings at the C-7 terminal for great potency.

- If both 1-BHR and 7-BHR are five-membered aromatic rings, swapping the location of two terminal rings has no effect on their potency (Table 4). For example, **12** and **17**, **14** and **20**, **13** and **18**, **15** and **23** exhibited approximately similar potency based on the ratios of IC_{50}(curcumin)/IC_{50}(trienone). The only exception is compound **16** with the 2-methyl-4-trifluoromethyl-thiazole-5-yl moiety on C-7, which is two-fold more potent than its counterpart **29** possessing thiazol-2-yl unit on C-7.

- If one side terminal is a five-membered aromatic ring and the other side is a six-membered ring, location of the five-membered ring to C-7 (as 7-BHR) significantly contributes to greater anti-proliferative potency toward three prostate cancer cell lines as described in Table 4. For example, compounds **32**, **33**, **34**, **35**, and **36** having a five-membered ring on C-7 exhibited greater potency than their respective counterparts (**19**, **21**, **24**, **26**, or **30**) with a six-membered ring on C-7.

- A generalized pharmarcophore for the 1,7-diheteroarylhepta-1,4,6-trien-3-ones was proposed in Fig. 2.

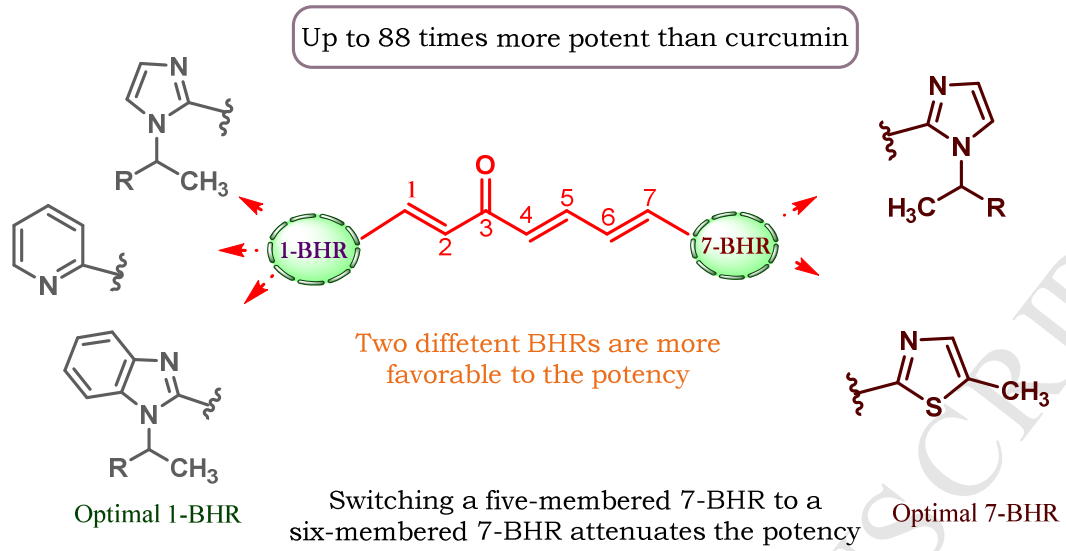


Fig. 2. Proposed pharmarcophore for the 1,7-diheteroarylhepta-1,4,6-trien-3-ones

Table 2. Anti-proliferative activity of 1,7-diheteroarylhepta-1,4,6-trien-3-ones

Compd	IC_{50} (μM)[a]			IC_{50} (curcumin)/IC_{50}(trienone)		
	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
12	1.52 ± 0.47	1.59 ± 0.42	0.75 ± 0.07	17	16	18
13	1.13 ± 0.10	1.53 ± 0.29	0.58 ± 0.08	23	17	24
14	1.37 ± 0.18	1.14 ± 0.28	0.73 ± 0.03	19	23	19
15	1.23 ± 0.04	0.81 ± 0.11	0.54 ± 0.09	21	32	25
16	1.28 ± 0.20	1.79 ± 0.27	1.10 ± 0.24	20	15	12
17	1.09 ± 0.17	1.06 ± 0.37	0.42 ± 0.04	23	25	32
18	1.65 ± 0.22	1.40 ± 0.25	0.67 ± 0.06	15	19	20
19	1.91 ± 0.07	1.82 ± 0.14	1.07 ± 0.21	13	14	13
20	1.21 ± 0.14	1.27 ± 0.04	1.15 ± 0.06	21	21	12
21	1.18 ± 0.25	1.12 ± 0.24	0.66 ± 0.16	22	23	21

22	0.63 ± 0.04	0.40 ± 0.09	0.41 ± 0.07	41	66	33
23	1.16 ± 0.03	1.17 ± 0.21	0.74 ± 0.06	22	22	18
24	1.47 ± 0.32	1.12 ± 0.01	1.29 ± 0.14	17	23	11
25	0.99 ± 0.09	0.74 ± 0.08	0.69 ± 0.05	26	35	20
26	1.03 ± 0.25	1.00 ± 0.22	0.55 ± 0.18	25	26	25
27	3.19 ± 1.09	5.74 ± 0.12	4.33 ± 0.89	8	5	3
28	1.93 ± 0.07	1.75 ± 0.24	1.42 ± 0.35	13	15	10
29	3.63 ± 0.98	3.61 ± 0.87	2.10 ± 0.31	7	7	6
30	4.73 ± 0.31	6.14 ± 0.42	4.99 ± 0.67	5	4	3
31	2.69 ± 0.02	3.16 ± 0.32	1.28 ± 0.34	9	8	11
32	1.02 ± 0.03	0.71 ± 0.07	0.37 ± 0.06	25	37	37
33	0.86 ± 0.17	0.75 ± 0.15	0.38 ± 0.01	30	35	36
34	0.70 ± 0.07	0.89 ± 0.27	0.28 ± 0.02	36	30	49
35	0.64 ± 0.06	0.66 ± 0.05	0.38 ± 0.03	39	40	36
36	1.81 ± 0.08	1.72 ± 0.29	1.16 ± 0.23	14	15	12
37	14.02 ± 3.98	8.68 ± 0.67	6.32 ± 0.27	2	3	2
38	1.79 ± 0.07	2.07 ± 0.10	1.58 ± 0.50	14	13	9
39	1.67 ± 0.27	2.00 ± 0.26	2.09 ± 0.34	15	13	7
40	0.31 ± 0.01	0.39 ± 0.08	0.35 ± 0.03	82	67	39
41	0.29 ± 0.06	0.60 ± 0.04	0.27 ± 0.01	88	44	50
42	0.53 ± 0.06	0.33 ± 0.08	0.60 ± 0.16	48	80	23
43	0.42 ± 0.13	0.64 ± 0.37	0.30 ± 0.06	60	41	45
44	0.54 ± 0.07	0.79 ± 0.15	0.55 ± 0.05	47	33	25
45	0.51 ± 0.04	0.61 ± 0.06	0.72 ± 0.10	50	43	19

46	2.74 ± 0.28	2.40 ± 0.36	1.88 ± 0.34	9	11	7

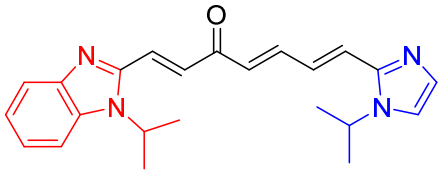
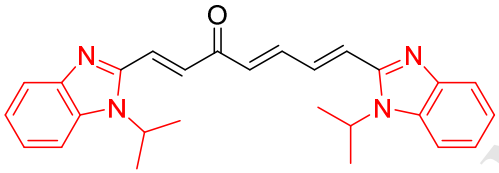
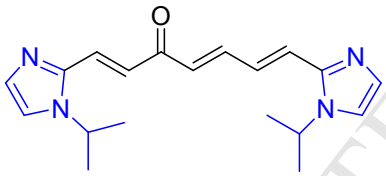
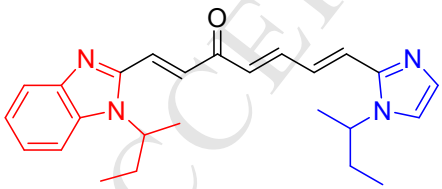
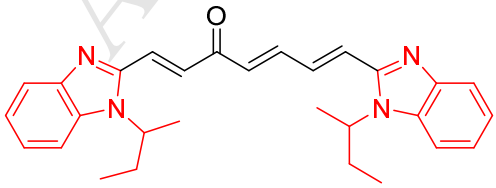
[a] IC_{50} is the drug concentration effective in inhibiting 50% of the cell viability measured by WST-1 cell proliferation assay after 3 days exposure. The data were presented as the mean ± standard deviation of the mean.

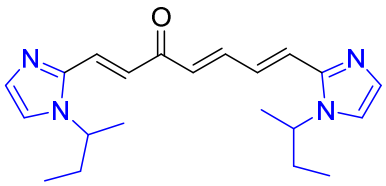
[b] Human androgen-insensitive prostate cancer cell line

[c] Human androgen-insensitive prostate cancer cell line

[d] Human androgen-sensitive prostate cancer cell line

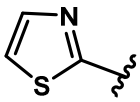
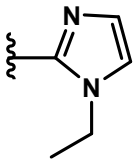
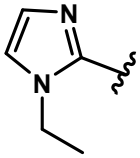
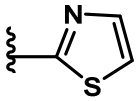
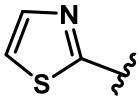
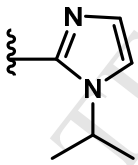
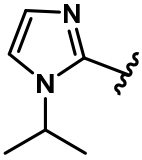
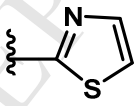
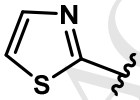
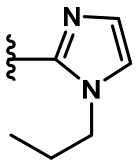
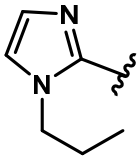
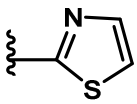
Table 3. Comparison of potency for trienones with identical and different aromatic rings

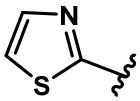
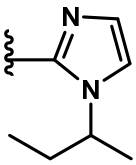
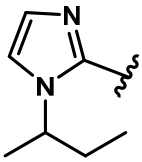
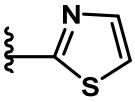
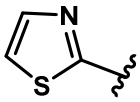
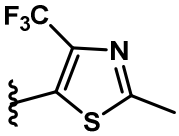
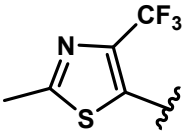
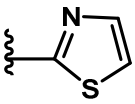
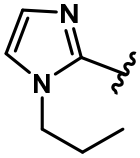
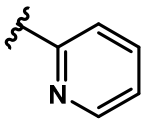
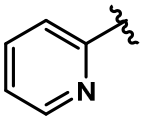
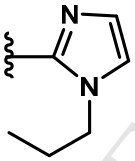
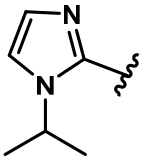
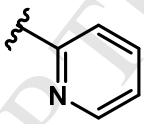
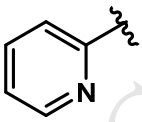
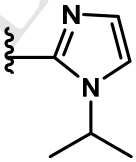
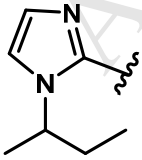
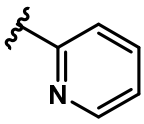
Compd.	Structure	IC_{50}(curcumin)/IC_{50}(trienone)			
		PC-3	DU145	LNCaP	PWR-1E
42		48	79	23	7
7[a]		36	29	11	10
2[a]		10	16	9	0.9-1.3
43		60	87	45	28
9[a]		25	26	11	24

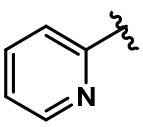
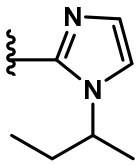
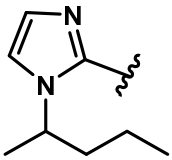
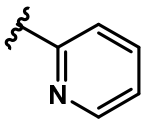
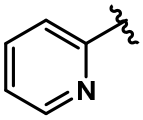
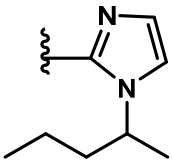
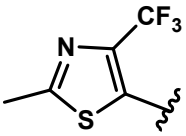
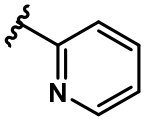
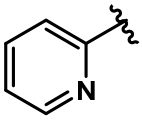
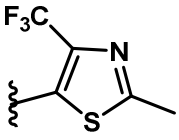
4[a]		14	25	11	2.5-5.6

[a]The IC_{50}(curcumin)/IC_{50}(trienone) ratios for compounds **7**, **2**, **9**, and **4** have been reported in our previous paper [8]

Table 4. Effects of the location of terminal aromatic rings on the potency

Compd	1-BHR	7-BHR	IC_{50}(curcumin)/IC_{50}(trienone)		
			PC-3	DU145	LNCaP
12			17	16	18
17			23	25	32
14			19	23	19
20			21	21	12
13			23	17	24
18			15	19	20

15			21	32	25
23			22	22	18
16			20	15	12
29			7	7	7
19			13	14	13
32			25	37	37
21			22	23	21
33			30	35	36
24			17	23	11

34			36	30	49
26			25	26	25
35			39	40	36
30			5	4	3
36			14	15	12

2.3 Anti-proliferative activity towards PWR-1E non-neoplastic human prostate epithelial cell line.

PWR-1E human prostatic epithelial cells were initially originated from a non-malignant prostate with mild hyperplasia and immortalized by a hybrid virus of adenovirus 12 and Simian 40. This cell line expresses markers of normal prostatic epithelial cells, including prostate specific antigen and androgen receptor, and mimics normal growth and differentiation responses to androgen [10]. Curcumin was used as a positive control, which has generally been considered to possess human safety profile as validated by human clinical trials [3,11] and animal studies [12]. No anti-proliferative potency of curcumin against non-cancerous PWR-1E cells has so far been reported. However, it has been revealed that the apoptotic cell death pathway could be triggered by androgen deprivation in both normal human primary prostate epithelial cells and androgen-sensitive human prostate cancer cells [13]. In this study, we observed the IC_{50} values for curcumin fall into the range of 8.14-9.54 μM, suggesting no differential responses of curcumin to LNCaP prostate cancer epithelial cell lines and PWR-1E benign human prostate epithelial cells. This is reasonable given that curcumin can downregulate the expression

and activity of androgen receptor and suppress the gene expression of prostate-specific antigen in LNCaP prostate cancer cells [14] and that both LNCaP prostate cancer cells and PWR-1E non-neoplastic prostate epithelial cells express androgen receptor and androgen specific antigen. As shown in Table 5, the 1,7-diaryl-1,4,6-heptatrien-3-ones (compounds **2-6**) with two identical 1-alkyl-1*H*-imidazol-2-yl as terminal aromatic rings have equivalent or slightly greater potency (within 3 folds) in inhibiting PWR-1E cell proliferation, as compared with curcumin. In contrast, those 1,7-diaryl-1,4,6-heptatrien-3-ones with two identical 1-alkyl-1*H*-benzo[d]imidazole-2-yl as terminal aromatic rings (compounds **7-11**) and those with two different aromatic rings (compounds **13**, **15**, **17**, **22**, **23**, **25**, **26**, **32-35**, and **40-43**) possess significantly stronger ability in inhibiting PWR-1E cell proliferation. Considering both potency towards prostate cancer cells and selectivity between prostate cancer cells and non-neoplastic cells, compounds **2-6** and **42** are the optimal 1,7-diaryl-1,4,6-heptatrien-3-ones for further investigation as anti-prostate cancer agents.

Table 5. Anti-proliferative activity of 1,7-diheteroarylhepta-1,4,6-trien-3-ones with two identical terminal aromatic rings (**2-11**) and those with two different aromatic rings (**13**, **15**, **17**, **22**, **23**, **25**, **26**, **32-35**, and **40-43**) toward PWR-1E non-neoplastic prostate epithelial cells

Compd	IC_{50} (μM)[a]	IC_{50} (curcumin) / IC_{50} (trienone)	Compd	IC_{50} (μM)[a]	IC_{50} (curcumin) / IC_{50} (trienone)
Curcumin	8.14-9.54	1	**13**	0.49	18
2	6.39-10.16	0.9-1.3	**15**	0.44	20
3	4.85-5.80	1.7	**17**	0.62	14
4	1.45-3.54	2.5-5.6	**22**	0.39	23
5	2.72-4.75	2.0-3.0	**23**	0.57	16
6	4.99	1.9	**25**	0.50	18
7	0.80	10	**26**	0.31	29
8	0.23	36	**32**	0.54	16
9	0.33	24	**33**	0.40	22
10	<1	>8	**34**	0.41	21
11	0.24	34	**35**	0.038	239
			40	0.048	186
			41	0.195	46

42	1.34	7
43	0.32	28

[a] IC_{50} is the drug concentration effective in inhibiting 50% of the normal cell viability measured by WST-1 cell proliferation assay after 3 days exposure.
[b] Human prostatic epithelial cells derived from prostate with mild hyperplasia.

2.3 Effects of trienone 40 on PC-3 cell cycle progression and apoptosis

Curcumin has been demonstrated to block PC-3 prostate cancer cell cycle progression by arresting the G_1/S phase [15]. Our previous study revealed that treatment of PC-3 cells with compound **7**, the most potent 1,7-diheteroarylhepta-1,4,6-trien-3-one with two identical aromatic rings, led to appreciable cell cycle arrest at the G_1/G_0 phase [8]. To evaluate the effect of terminal aromatic rings of the trienone scaffold on the PC-3 prostate cancer cell cycle regulation, compound **40**, as one of the most potent trienones with two different aromatic rings, was selected for further assessment using flow cytometry analysis with propidium iodide DNA staining. The similar cell cycle regulation has been identified in this study when exposing PC-3 cells to trienone **40** at 5 μM concentration (Fig. 3). Specifically, trienone **40** was found to cause accumulation of PC-3 cells in the G_0/G_1 phase from 49% and 61% for control cells at 16 hours and 24 hours, respectively, to 65% and 64% for the compound-treated cells. The cell population in the G_2 phase decreased from 33% in control cells to 23% at 16 hours, and from 26% in control cells to 23% at 24 hours.

The growth suppression of PC-3 prostate cancer cells by curcumin and trienone **7** has been demonstrated to be, at least in part, associated with its capability of inducing PC-3 prostate cancer cell apoptosis [2, 16]. Consequently, F2N12S and CYTOX AADvanced double staining flow cytometry-based assay was chosen to discriminate PC-3 cells dying from apoptosis from those dying from necrosis in response to increasing concentrations of trienone **40**. Straurosporine was used as specific apoptotic inducer and positive apoptotic control in all these experiments (data were shown in Fig. 4). As illustrated in Fig. 5, trienone **40** induced appreciably higher levels of late apoptotic/necrotic cell death rather than apoptotic cell death in the androgen-insensitive PC-3 prostate cancer cell line after a 16-hour treatment. Specifically, 3-5 μM of trienone **40** could induce 43% late apoptotic/necrotic cells but merely 13-15% early apoptotic cells as compared with control cells; treatment with 10 μM of trienone **40** led to 55% late apoptotic/necrotic cells but only 27% early apoptotic cells; 20 μM of trienone **40** activated necrosis as well, with 63% late apoptotic/necrotic cells and 34% early apoptotic cells. Both apoptotic and necrotic cell population increased in response to increasing concentration of

trienone **40** (0-30 μM final concentration range). The apoptotic cell population reached maximum when treating PC-3 cancer cells with trienone **40** at 20 μM concentration. Trienone **40** is more potent than trienone **7** in inhibiting PC-3 cell proliferation but has less inductive effect on PC-3 cell apoptosis, indicating the cell death path way induced by the 1,7-diheteroarylhepta-1,4,6-trien-3-ones varies with different terminal heteroaromatic rings.

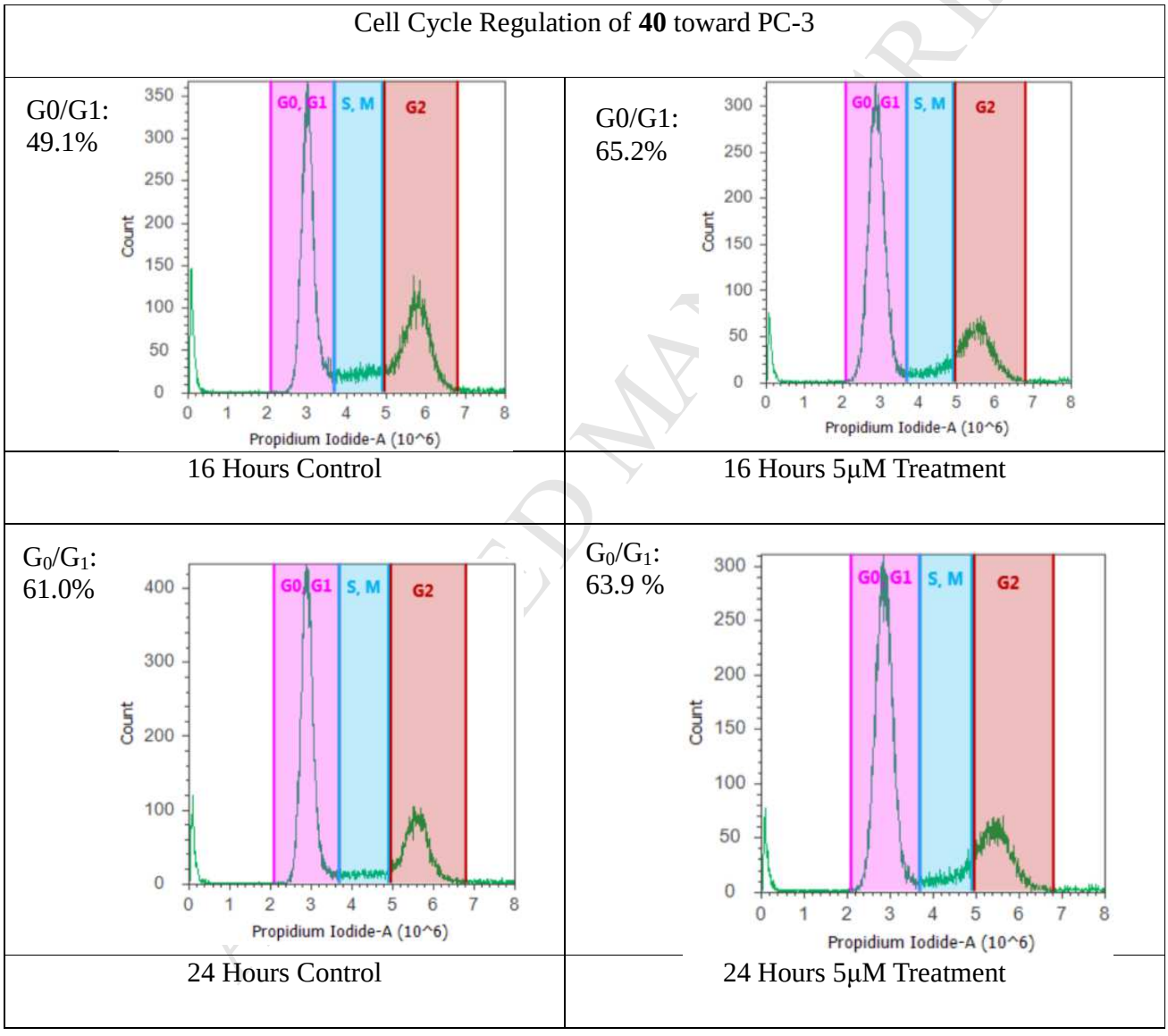


Fig. 3. Cell cycle analysis of PC-3 cells. PC-3 cancer cells were untreated or treated with trienone **40**. Cells were harvested after 16 and 24 hours then they were fixed, stained, and analyzed for DNA content. The distribution and percentage of cells in G_1/G_0, and G_2 phase of the cell cycle are indicated.

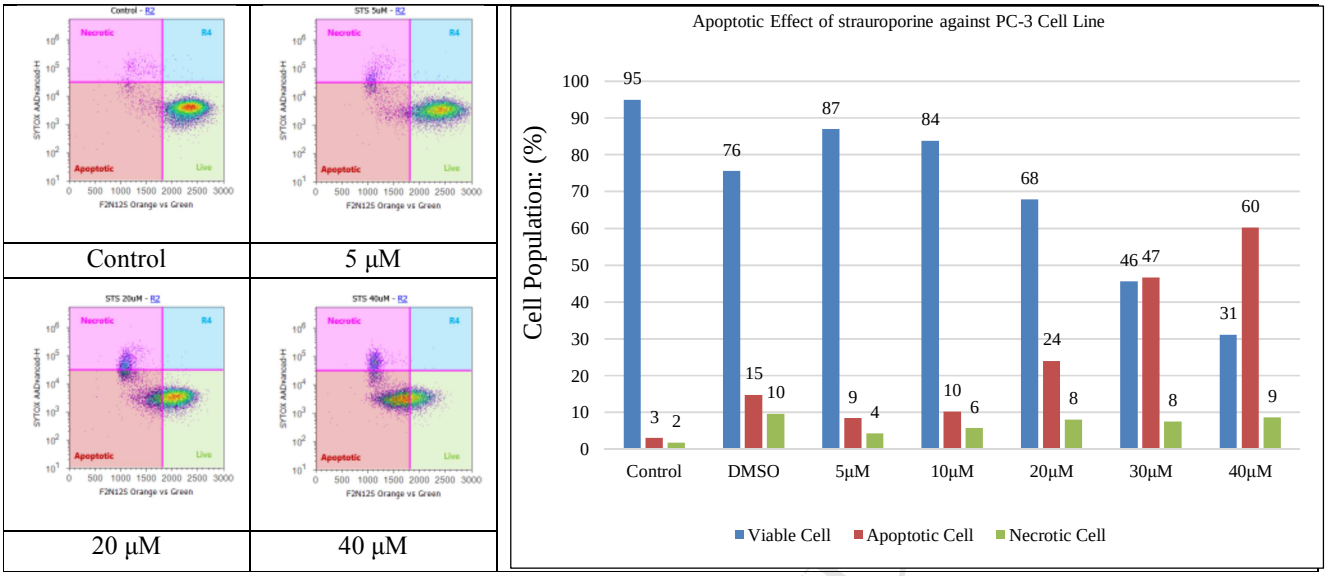


Fig. 4. Evolution of viable, apoptotic, and necrotic PC-3 cells populations in response to increasing dosages of straurosporine.

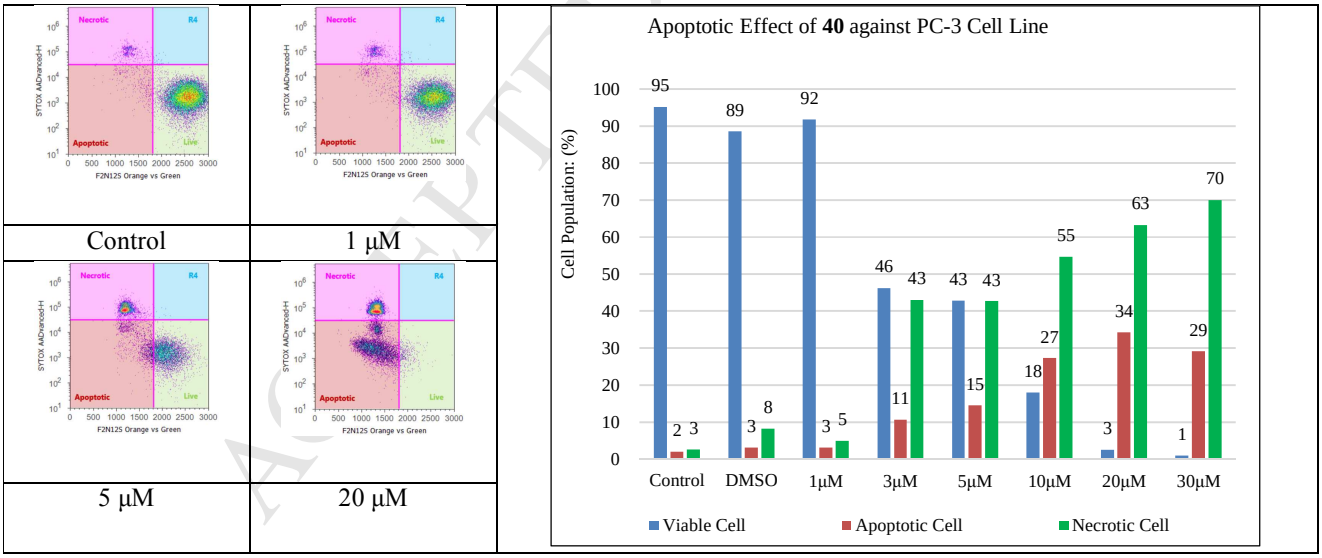


Fig. 5. Evolution of viable, apoptotic, and necrotic PC-3 cells populations in response to increasing dosages of compound **40**.

3. Conclusion

In summary, thirty five 1,7-diarylhepta-1,4,6-trien-3-ones with different terminal heteroaromatic rings have been designed and assessed for their anti-proliferative potency against three prostate cancer cell lines and one benign prostate epithelial cell line. Our findings provide further evidence that this group of curcumin-based analogs possesses promising antiproliferative activities toward both androgen-sensitive and androgen-insensitive prostate cancer cell lines. The optimal analog **40** is 82-, 67-, 39-fold more potent than curcumin against PC-3, DU-145 and LNCaP human prostate cancer cell lines. Structure-activity relationships demonstrate that the trienones with two different terminal aromatic rings possess greater potency toward three prostate cancer cell lines, but also have higher capability in suppressing the proliferation of PWR-1E benign human prostate epithelial cells, as compared to those with two identical terminal rings. The terminal aromatic rings are a determining factor of this scaffold in the ability to suppress proliferation and to induce apoptosis in prostate epithelial cells. Considering both potency towards prostate cancer cells and selectivity between prostate cancer cells and non-neoplastic cells, compounds **2-6** and **42** are identified as the optimal 1,7-diaryl-1,4,6-heptatrien-3-ones for further investigation.

4. Experimental

4.1 General Procedures. HRMS were obtained on an Orbitrap mass spectrometer with electrospray ionization (ESI). NMR spectra were obtained on a Bruker Fourier 300 spectrometer in $CDCl_3$. 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 loaded with silica gel 60 GF254 (EMD Millipore Corporation). Curcumin was synthesized by Claisen-Schmidt condensation of aromatic aldehyde with acetylacetone according to the procedure described in the literature [17]. 1,3-Bis(diethylphosphonato)acetone (**49**) was synthesized using the procedure illustrated in the literature [18]. (*E*)-3-(aryl)-acryladehydes (**48**) and (*E*)-diethyl(2-oxo-4-aryl-but-3-en-1-yl)phosphonates (**47**) were synthesized according to the procedure described in the literature [8]. To compare the anti-

proliferative activity toward PWR-1E prostate non-neoplastic epithelial cells, seven known 1,7-diheteroarylhepta-1,4,6-trien-2-ones (**2-11**) with two identical terminal aromatic rings have also been synthesized according to the procedure illustrated in the literature [8].

4.2 General procedure for the synthesis of diethyl ((3*E*,5*E*)-2-oxo-6-(aryl)hexa-3,5-dien-1-yl)phosphonates (50-53).

To the solution of tetraethyl (2-oxopropane-1,3-diyl)bis(phosphonate) (**49**) (0.5 mmol) in ethanol (0.4 mL), potassium carbonate (1 mmol) in water (1.0 mL) was added dropwise at 4 ^{o}C. After 30 min at room temperature, (*E*)-(aryl)acrylaldehyde (**48**, 0.8 mmol) in ethanol (0.4 mL) was added dropwise to the solution. The inorganic solids were removed by filtration after 2 h, and the filtrate was diluted with water (10 mL) and 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 the PTLC purification using dichloromethane/methanol (100/3, v/v) as eluent to give the respective product. The yields and NMR data for each product were listed below:

4.2.1 Diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (50): Yellow oil, 40%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 3.2 Hz, 1H), 7.41–7.31 (m, 1H), 7.35 (d, J = 3.2 Hz, 1H), 7.22–7.08 (m, 2H), 6.51 (d, J = 15.2 Hz, 1H), 4.20–4.04 (m, 4H), 3.24 (d, J = 22.7 Hz, 2H), 1.31 (q, J = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, CDCl3) δ 189.9 (d, J = 6.0 Hz), 164.1, 143.6, 141.6, 132.3, 130.6, 130.3, 119.4, 61.8 (d, J = 6.4 Hz), 40.3 (d, J_{CP} = 126.8 Hz), 15.4 (d, J = 6.2 Hz, 1H).

4.2.2 Diethyl ((3*E*,5*E*)-6-(2-methyl-4-(trifluoromethyl)thiazol-5-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (51): Yellow oil, 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (dd, J = 15.3, 11.0 Hz, 1H), 7.22 (d, J = 15.3 Hz, 1H), 6.62 (dd, J = 15.3, 11.0 Hz, 1H), 6.45 (d, J = 15.3 Hz, 1H), 4.17–4.07 (m, 4H), 3.23 (d, J = 22.7 Hz, 2H), 2.70 (s, 3H), 1.31 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.8, 166.4, 142.5, 141.8 (q, J_{CF} = 35.1 Hz), 137.6, 132.5, 131.3, 128.2, 121.0 (q, J_{CF} = 270.3 Hz), 62.8, 41.2 (d, J_{CP} = 126.8 Hz), 19.6, 16.4.

4.2.3 Diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (52): Yellow oil, 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (d, J = 4.0 Hz, 1H), 7.68 (td, J = 7.7, 1.8 Hz, 1H), 7.50–7.31

(overlapped, 3H), 7.20 (ddd, *J* = 7.5, 4.8, 1.0 Hz, 1H), 7.02 (d, *J* = 14.0 Hz, 1H), 6.50 (d, *J* = 14.0 Hz, 1H), 4.23–4.07 (m, 4H), 3.26 (d, *J* = 22.7 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.0 (d, J_{CP} = 6.5 Hz), 153.9, 149.9, 143.6, 140.9, 136.6, 130.9, 130.1, 123.4, 123.3, 62.5 (d, *J* = 6.3 Hz), 40.8 (d, *J* = 127.0 Hz), 16.2 (d, *J* = 6.1 Hz).

4.2.4 Diethyl ((3*E*,5*E*)-6-(3-fluoropyridin-4-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (53): Light yellow oil, 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.35 (d, *J* = 4.0 Hz, 1H), 7.40–7.25 (overlapped, 2H), 7.09 (dd, *J* = 15.7, 10.2 Hz, 1H), 6.99 (d, *J* = 15.7 Hz, 1H), 6.49 (d, *J* = 15.3 Hz, 1H), 4.15–4.06 (m, 4H), 3.22 (d, *J* = 22.7 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.0 (d, *J* = 6.0 Hz), 146.0 (d, *J* = 5.1 Hz), 143.0, 139.1 (d, *J* = 24.2 Hz), 133.2, 133.1, 131.9, 131.3 (d, *J* = 2.2 Hz), 130.7 (d, *J* = 9.7 Hz), 121.1, 62.7 (d, *J* = 6.5 Hz), 40.2 (d, *J* = 126.7 Hz), 16.3 (d, *J* = 6.2 Hz).

4.3 General procedure for the synthesis of the (1*E*,4*E*,6*E*)-1,7-diarylhepta-1,4,6-trien-3-ones (12-46):

Method A: A 5 mL flask was charged with the appropriate (*E*)-diethyl (2-oxo-4-aryl-but-3-en-1-yl)phosphonate (**47**, 0.25 mmoL) and the appropriate (*E*)-3-aryl-acrylaldehyde (**48**, 0.25 mmoL). To the flask was added a solution of potassium carbonate (0.035 g, 0.25 mmoL) in water (0.2 mL) and ethanol (0.15 mL). The biphasic mixture was stirred rapidly at cold room (4 ^{o}C) overnight before being extracted with dicloromethane (10 mL × 3). The combined extracts were dried over anhydrous magnesium sulfate and concentrated. The residue was purified over repetitive PTLC eluting with dicloromethane/methanol (100:5, v/v), hexane/EtOAc (1:1, v/v), and EtOAc/methanol (100:5, v/v) to furnish the respective final product.

Method B: A 5 mL flask was charged with the appropriate (3*E*,5*E*)-2-oxo-6-arylhexa-3,5-dien-1-yl)phosphonate (**50**-**53**, 0.6 mmol) and the appropriate heteroaromatic carboxaldehyde (**54**, 0.5 mmol). To the flask was added a solution of potassium carbonate (1.5 mmol) in water (5 mL) and ethanol (5.0 mL). The subsequent biphasic mixture was stirred rapidly at cold room (4 ^{o}C) overnight. The reaction was quenched by adding water (10 mL), and the mixture was extracted with dichloromethane (10 mL × 3). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to PTLC purification eluting with dichloromethane/methanol (5% methanol in dichloromethane, v/v) to give the respective product.

4.3.1 (1*E*,4*E*,6*E*)-7-(1-Ethyl-1*H*-imidazol-2-yl)-1-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (**12**). This compound was obtained in 7% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(thiazol-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-ethyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 3.2 Hz, 1H), 7.76 (d, J = 15.7 Hz, 1H), 7.54 (dd, J = 14.7, 11.6 Hz, 1H), 7.47 (d, J = 3.2 Hz, 1H), 7.40 (dd, J = 14.7, 11.6 Hz, 1H), 7.32 (d, J = 15.7 Hz, 1H), 7.16 (d, J = 0.7 Hz, 1H), 6.99 (d, J = 1.0 Hz, 1H), 6.81 (d, J = 14.6 Hz, 1H), 6.65 (d, J = 14.8 Hz, 1H), 4.06 (q, J = 7.3 Hz, 2H), 1.45 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.2, 164.3, 145.1, 143.9, 143.5, 133.7, 130.5, 130.1, 129.8, 129.2, 125.5, 121.8, 121.1, 41.1, 16.7. IR (film) ν_{max}: 1647, 1609, 1577, 1478, 1077 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{15}H_{16}N_3OS$ $[M+H]^+$: 286.1014. Found: 286.1008.

4.3.2 (1*E*,4*E*,6*E*)-7-(1-Propyl-1*H*-imidazol-2-yl)-1-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (**13**). This compound was obtained in 15% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(thiazol-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-propyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 3.2 Hz, 1H), 7.76 (d, J = 15.7 Hz, 1H), 7.54 (dd, J = 14.9, 11.6 Hz, 1H), 7.47 (d, J = 3.2 Hz, 1H), 7.40 (dd, J = 14.6, 11.6 Hz, 1H), 7.32 (d, J = 15.7 Hz, 1H), 7.15 (s, 1H), 6.97 (d, J = 0.9 Hz, 1H), 6.80 (d, J = 14.6 Hz, 1H), 6.64 (d, J = 14.8 Hz, 1H), 3.96 (t, J = 7.2 Hz, 2H), 1.85–1.74 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 164.3, 145.1, 144.1, 143.6, 133.7, 130.4, 130.0, 129.7, 129.1, 125.6, 121.9, 121.8, 47.9, 24.8, 11.32. IR (film) ν_{max}: 1646, 1609, 1577, 1263, 1077 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{16}H_{18}N_3OS$ $[M+H]^+$: 300.1171. Found: 300.1168.

4.3.3 (1*E*,4*E*,6*E*)-7-(1-Isopropyl-1*H*-imidazol-2-yl)-1-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (**14**). This compound was obtained in 17% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(thiazol-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-isopropyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 3.2 Hz, 1H), 7.77 (d, J = 15.7 Hz, 1H), 7.56 (dd, J = 14.5, 11.6 Hz, 1H), 7.48 (d, J = 3.2 Hz, 1H), 7.44 (dd, J = 14.5, 11.6 Hz, 1H), 7.33 (d, J = 15.7 Hz, 1H), 7.19 (s, 1H), 7.07 (d, J = 0.9 Hz, 1H), 6.87 (d, J = 14.4 Hz, 1H), 6.66 (d, J = 14.7 Hz, 1H), 4.59–4.49 (m, 1H), 1.50 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.2, 164.3, 145.0, 143.6, 133.75, 130.6, 130.0, 129.8, 129.2, 125.7, 121.8, 117.6, 47.57, 23.78. IR (film) ν_{max}: 1647, 1609, 1577, 1463, 1078 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{16}H_{18}N_3OS$ $[M+H]^+$: 300.1171. Found: 300.1163.

4.3.4 (1*E*,4*E*,6*E*)-7-(1-(sec-Butyl)-1*H*-imidazol-2-yl)-1-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (**15**). This compound was synthesized in 19% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(thiazol-2-

yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-(*sec*-butyl)-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 3.2 Hz, 1H), 7.76 (d, J = 15.7 Hz, 1H), 7.56 (dd, J = 14.5, 11.6 Hz, 1H), 7.46 (d, J = 14.5 Hz, 1H), 7.44 (dd, J = 14.5, 11.6 Hz, 1H), 7.33 (d, J = 15.7 Hz, 1H), 7.20 (s, 1H), 7.03 (d, J = 1.0 Hz, 1H), 6.86 (d, J = 14.5 Hz, 1H), 6.65 (d, J = 14.5 Hz, 1H), 4.33–4.18 (m, 1H), 1.85–1.72 (m, 2H), 1.48 (d, J = 6.7 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.2, 164.3, 145.0, 144.1, 143.7, 133.6, 130.8, 129.9, 129.8, 129.2, 125.8, 121.8, 117.8, 53.5, 31.0, 21.8, 10.8. IR (film) ν_{max}: 1647, 1610, 1577, 1459, 1264, 1078, 904 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{17}H_{20}N_3OS$ $[M+H]^+$: 314.1327. Found: 314.1325.

4.3.5 (1*E*,4*E*,6*E*)-7-(2-Methyl-4-trifluoromethyl-thiazol-5-yl)-1-thiazol-2-yl-hepta-1,4,6-trien-3-one (16). This compound was synthesized in 39% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(thiazol-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(2-methyl-4-(trifluoromethyl)thiazol-5-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 3.1 Hz, 1H), 7.76 (d, J = 15.7 Hz, 1H), 7.49 (d, J = 3.5 Hz, 1H), 7.45 (dd, J = 15.3, 11.0 Hz, 1H), 7.35–7.24 (overlapped, 2H), 6.69 (dd, J = 15.2, 11.0 Hz, 1H), 6.61 (d, J = 15.3 Hz, 1H), 2.72 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.0, 166.4, 164.0, 145.1, 142.3, 140.9 (q, J = 35.1 Hz), 137.7, 134.1, 132.9, 131.2, 128.4, 128.0, 122.1, 120.0 (q, J_{CF} = 270.1 Hz). IR (film) ν_{max}: 1650, 1612, 1481, 1363, 1125, 1001 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{15}H_{12}F_3N_2OS_2$ $[M+H]^+$: 357.0343. Found: 357.0333.

4.3.6 (1*E*,4*E*,6*E*)-1-(1-Ethyl-1*H*-imidazol-2-yl)-7-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (17): This compound was synthesized in 40% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-ethyl-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J = 3.2 Hz, 1H), 7.55–7.53 (overlapped, 2H), 7.51 (dd, J = 15.3, 10.2 Hz, 1H), 7.36 (d, J = 3.2 Hz, 1H), 7.28–7.18 (overlapped, 2H), 7.14 (d, J = 15.4 Hz, 1H), 7.06 (d, J = 1.0 Hz, 1H), 6.60 (d, J = 15.3 Hz, 1H), 4.13 (q, J = 7.3 Hz, 2H), 1.46 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 165.4, 144.5, 143.0, 141.8, 133.3, 132.6, 132.1, 130.9, 126.7, 125.8, 122.2, 120.2, 41.2, 16.9. IR (film) ν_{max}: 1646, 1613, 1579, 1244, 1082 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{15}H_{16}N_3OS$ $[M+H]^+$: 286.1014. Found: 286.1009.

4.3.7 (1*E*,4*E*,6*E*)-1-(1-Propyl-1*H*-imidazol-2-yl)-7-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (**18**). This compound was synthesized in 71% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-propyl-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 3.2 Hz, 1H), 7.60–7.43 (overlapped, 3H), 7.34 (d, J = 3.2

Hz, 1H), 7.26–7.16 (overlapped, 2H), 7.12 (d, *J* = 15.3 Hz, 1H), 7.03 (d, *J* = 1.0 Hz, 1H), 6.57 (d, *J* = 15.3 Hz, 1H), 4.01 (t, *J* = 7.2 Hz, 2H), 1.86–1.72 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 165.4, 144.5, 143.2, 141.8, 133.3, 132.5, 132.0, 130.6, 126.6, 125.8, 123.0, 120.2, 48.0, 24.9, 11.2. IR (film) ν_{max}: 1644, 1611, 1578, 1442, 1277, 1077, 993 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{16}H_{18}N_3OS$ $[M+H]^+$: 300.1171. Found: 300.1166.

4.3.8 (1*E*,4*E*,6*E*)-1-(1-Propyl-1*H*-imidazol-2-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (**19**). This compound was synthesized in 63% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 1-propyl-1*H*-imidazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (d, *J* = 4.7 Hz, 1H), 7.68 (td, *J* = 7.7, 1.8 Hz, 1H), 7.63–7.41 (overlapped, 4H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 5.8 Hz, 1H), 7.19 (d, *J* = 6.5 Hz, 1H), 7.01 (d, *J* = 14.5 Hz, 2H), 6.59 (d, *J* = 14.9 Hz, 1H), 4.03 (t, *J* = 7.2 Hz, 2H), 1.87–1.74 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 154.4, 150.1, 143.3, 142.9, 140.3, 136.8, 132.8, 131.0, 130.5, 126.3, 126.0, 123.6, 123.4, 122.9, 48.0, 24.9, 11.2. IR (film) ν_{max}: 1646, 1605, 1467, 1080 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{20}N_3O$ $[M+H]^+$: 294.1606. Found: 294.1602.

4.3.9 (1*E*,4*E*,6*E*)-1-(1-Isopropyl-1*H*-imidazol-2-yl)-7-(thiazol-2-yl)-hepta-1,4,6-trien-3-one (**20**). This compound was synthesized in 35% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-isopropyl-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, *J* = 3.2 Hz, 1H), 7.74 (d, *J* = 15.0 Hz, 1H), 7.59 (dd, *J* = 15.1, 10.8 Hz, 1H), 7.57 (d, *J* = 14.6 Hz, 1H), 7.37 (d, *J* = 3.2 Hz, 1H), 7.28–7.15 (overlapped, 4H), 6.59 (d, *J* = 15.4 Hz, 1H), 4.74–4.64 (m, 1H), 1.50 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 165.4, 144.5, 142.7, 141.7, 133.3, 132.5, 132.1, 131.0, 126.8, 125.89, 120.2, 118.7, 47.8, 24.0. IR (film) ν_{max}: 1646, 1612, 1461, 1265, 1081, 905, 725 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{16}H_{18}N_3OS$ $[M+H]^+$: 300.1171. Found: 300.1170.

4.3.10 (1*E*,4*E*,6*E*)-1-(1-Isopropyl-1*H*-imidazol-2-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (**21**). This compound was synthesized in 64% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 1-isopropyl-1*H*-imidazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (d, *J* = 4.2 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 1H), 7.62–7.58 (overlapped, 2H), 7.56 (d, *J* = 15.8 Hz, 1H), 7.44 (dd, *J* = 14.8, 11.4 Hz, 1H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.24–7.14 (overlapped, 2H), 7.11 (s, 1H), 7.00 (d, *J* = 14.9 Hz, 1H), 6.58 (d, *J* = 15.1 Hz, 1H), 4.70–4.61 (m, 1H), 1.46 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 154.3, 150.1, 142.9, 142.7, 140.2, 136.8,

132.7, 131.0, 130.7, 126.3, 126.0, 123.6, 123.4, 118.6, 47.7, 23.9. IR (film) ν_{max}: 1646, 1605, 1462, 1269, 1081 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{20}N_3O$ $[M+H]^+$: 294.1606. Found: 294.1605.

4.3.11 (1*E*,4*E*,6*E*)-1-(1-Isopropyl-1*H*-imidazol-2-yl)-7-(2-methyl-4-(trifluoromethyl)thiazol-5-yl)hepta-1,4,6-trien-3-one (**22**). This compound was synthesized in 45% yield as a yellow oil from diethyl ((3*E*,5*E*)-6-(2-methyl-4-(trifluoromethyl)thiazol-5-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (**51**) and 1-isopropyl-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, J = 15.0 Hz, 1H), 7.58 (s, 1H), 7.49 (dd, J = 15.0, 10.6 Hz, 1H), 7.28–7.14 (overlapped, 3H), 6.70 (dd, J = 15.2, 11.0 Hz, 1H), 6.52 (d, J = 15.5 Hz, 1H), 4.73–4.64 (m, 1H), 2.73 (s, 3H), 1.50 (d, J = 6.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 166.3, 142.7, 141.8, 140.8 (q, J_{CF} = 35.1 Hz), 137.8, 133.1, 132.9, 131.1, 127.6, 126.9, 125.5, 121.1 (q, J_{CF} = 270.4 Hz), 118.8, 47.8, 23.9, 19.7. IR (film) ν_{max}: 1647, 1613, 1489, 1364, 1126, 1083 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{19}F_3N_3OS$ $[M+H]^+$: 382.1201. Found: 382.1200.

4.3.12 (1*E*,4*E*,6*E*)-1-(1-(sec-Butyl)-1*H*-imidazol-2-yl)-7-(thiazol-2-yl)hepta-1,4,6-trien-3-one (**23**). This compound was synthesized in 35% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-(*sec*-butyl)-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J = 3.2 Hz, 1H), 7.64–7.56 (overlapped, 2H), 7.52 (dd, J = 15.4, 10.1 Hz, 1H), 7.36 (d, J = 3.2 Hz, 1H), 7.24 (d, J = 0.8 Hz, 1H), 7.23 (dd, J = 15.4, 10.2 Hz, 1H), 7.14 (d, J = 15.4 Hz, 1H), 7.09 (d, J = 1.1 Hz, 1H), 6.59 (d, J = 15.3 Hz, 1H), 4.45–4.34 (m, 1H), 1.83–1.76 (m, 2H), 1.47 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 165.5, 144.5, 143.3, 141.7, 133.4, 132.5, 132.1, 131.2, 126.9, 125.8, 120.2, 119.0, 53.6, 31.0, 22.0, 10.7. IR (film) ν_{max}: 1646, 1610, 1459, 1245, 1085, 967 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{17}H_{20}N_3OS$ $[M+H]^+$: 314.1327. Found: 314.1320.

4.3.13 (1*E*,4*E*,6*E*)-1-(1-(*sec*-Butyl)-1*H*-imidazol-2-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (**24**). This compound was synthesized in 62% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 1-(*sec*-butyl)-1*H*-imidazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (d, J = 4.4 Hz, 1H), 7.70–7.51 (overlapped, 4H), 7.44 (dd, J = 14.7, 11.3 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.23–7.14 (m, 2H), 7.07 (s, 1H), 7.00 (d, J = 14.9 Hz, 1H), 6.58 (d, J = 14.9 Hz, 1H), 4.44–4.29 (m, 1H), 1.86–1.69 (m, 2H), 1.44 (d, J = 6.7 Hz, 3H), 0.82 (t, J =

7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 154.3, 150.1, 143.2, 142.9, 140.2, 136.8, 132.8, 131.0, 130.9, 126.5, 126.0, 123.6, 123.4, 118.8, 53.5, 31.0, 22.0, 10.7. IR (film) ν_{max}: 1646, 1605, 1459, 1263, 1081, 906 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{19}H_{22}N_3O$ $[M+H]^+$: 308.1763. Found: 308.1758.

4.3.14 (1*E*,4*E*,6*E*)-1-(1-(Pentan-2-yl)-1*H*-imidazol-2-yl)-7-(thiazol-2-yl)hepta-1,4,6-trien-3-one (25). This compound was synthesized in 75% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-(pentan-2-yl)-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J* = 3.2 Hz, 1H), 7.57 (s, 2H), 7.51 (dd, *J* = 15.4, 10.1 Hz, 1H), 7.34 (d, *J* = 3.2 Hz, 1H), 7.24–7.03 (overlapped, 4H), 6.58 (d, *J* = 15.3 Hz, 1H), 4.53–4.38 (m, 1H), 1.72 (q, *J* = 7.7 Hz, 2H), 1.44 (d, *J* = 6.7 Hz, 3H), 1.28–1.07 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 165.4, 144.5, 143.1, 141.8, 133.3, 132.5, 132.0, 131.0, 126.7, 125.9, 120.2, 118.9, 52.0, 40.0, 22.4, 19.4, 13.8. IR (film) ν_{max}: 1645, 1610, 1456, 1264, 1082, 996 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{22}N_3OS$ $[M+H]^+$: 328.1483. Found: 328.1480.

4.3.15 (1*E*,4*E*,6*E*)-1-(1-(Pentan-2-yl)-1*H*-imidazol-2-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (26). This compound was synthesized in 52% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 1-(pentan-2-yl)-1*H*-imidazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (d, *J* = 4.7 Hz, 1H), 7.66 (td, *J* = 7.7, 1.7 Hz, 1H), 7.62–7.49 (m, 3H), 7.44 (dd, *J* = 14.7, 11.3 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.22–7.12 (m, 2H), 7.07 (d, *J* = 0.9 Hz, 1H), 6.99 (d, *J* = 14.9 Hz, 1H), 6.58 (d, *J* = 14.8 Hz, 1H), 4.53–4.37 (m, 1H), 1.76–1.68 (m, 2H), 1.43 (d, *J* = 6.7 Hz, 3H), 1.30–1.06 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 154.3, 150.1, 143.2, 142.8, 140.2, 136.8, 132.8, 131.1, 131.0, 126.5, 125.9, 123.5, 123.3, 118.8, 51.9, 40.0, 22.4, 19.4, 13.8. IR (film) ν_{max}: 1646, 1604, 1458, 1258, 1080 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{20}H_{24}N_3O$ $[M+H]^+$: 322.1919. Found: 322.1912.

4.3.16 (1*E*,4*E*,6*E*)-1-(1-Isopentyl-1*H*-imidazol-2-yl)-7-(thiazol-2-yl)hepta-1,4,6-trien-3-one (27). This compound was synthesized in 60% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-(isopentyl)-1*H*-imidazole-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, *J* = 3.2 Hz, 1H), 7.56–7.53 (overlapped, 2H), 7.52 (dd, *J* = 15.4, 10.1 Hz, 1H), 7.36 (d, *J* = 3.2 Hz, 1H), 7.28–7.19 (overlapped, 2H), 7.15 (d, *J* = 15.3 Hz, 1H), 7.04 (d, *J* = 1.0 Hz, 1H), 6.60 (d, *J* = 15.3 Hz, 1H), 4.07 (t, *J* = 7.2 Hz, 2H), 1.70–1.58 (m, 3H), 0.96 (d,

J = 6.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 165.5, 144.5, 143.2, 141.8, 133.3, 132.6, 132.1, 130.7, 126.6, 125.7, 122.8, 120.3, 44.8, 40.4, 25.8, 22.4. IR (film) ν_{max}: 1649, 1614, 1264, 730 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{22}N_3OS$ $[M+H]^+$: 328.1484. Found: 328.1475.

4.3.17 (1*E*,4*E*,6*E*)-1-(1-isopentyl-1*H*-imidazol-2-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (28). This compound was synthesized in 57% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 1-isopentyl-1*H*-imidazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (dd, *J* = 4.8, 0.9 Hz, 1H), 7.68 (td, *J* = 7.7, 1.8 Hz, 1H), 7.58 (d, *J* = 15.0 Hz, 1H), 7.58 (dd, *J* = 15.0, 11.3 Hz, 1H), 7.51 (d, *J* = 14.9 Hz, 1H), 7.45 (dd, *J* = 14.8, 11.3 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.23–7.16 (m, 2H), 7.03 (s, 1H), 7.01 (d, *J* = 14.9 Hz, 1H), 6.59 (d, *J* = 14.8 Hz, 1H), 4.07 (t, *J* = 7.2 Hz, 2H), 1.71–1.55 (m, 3H), 0.96 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 154.4, 150.1, 143.2, 142.9, 140.3, 136.8, 132.7, 131.0, 130.6, 126.3, 126.0, 123.6, 123.4, 122.7, 44.8, 40.4, 25.7, 22.4. IR (film) ν_{max}: 1674, 1605, 1468, 1274, 1081 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{20}H_{23}N_3O$ $[M+H]^+$: 322.1919. Found: 322.1918.

4.3.18 (1*E*,4*E*,6*E*)-1-(2-Methyl-4-trifluoromethyl-thiazol-5-yl)-7-thiazol-2-yl-hepta-1,4,6-trien-3-one (29). This compound was synthesized in 84% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 2-methyl-4-(trifluoromethyl)-thiazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 16.2 Hz, 1H), 7.88 (d, *J* = 6.3 Hz, 1H), 7.47 (dd, *J* = 15.0, 9.6 Hz, 1H), 7.38 (d, *J* = 3.2 Hz, 1H), 7.25 (dd, *J* = 15.0, 9.6 Hz, 1H), 7.18 (d, *J* = 15.1 Hz, 1H), 6.73 (d, *J* = 16.2 Hz, 1H), 6.67 (d, *J* = 15.5 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.2, 167.7, 165.2, 144.6, 143.4 (q, J_{CF} = 35.6 Hz), 142.5, 136.3, 133.2, 131.5, 130.9, 130.5, 129.6, 120.8 (q, J_{CF} = 270.8 Hz), 120.5, 19.8. IR (film) ν_{max}: 1664, 1608, 1476, 1324, 1076, 1001 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{15}H_{12}F_3N_2OS_2$ $[M+H]^+$: 357.0343. Found: 357.0341.

4.3.19 (1*E*,4*E*,6*E*)-1-(2-Methyl-4-trifluoromethyl-thiazol-5-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (30). This compound was synthesized in 86% yield as a yellow solid from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 2-methyl-4-trifluoromethyl-thiazol-5-carbaldehyde using Method B. Yellow solid, 86% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (d, *J* = 4.7 Hz, 1H), 7.87 (dd, *J* = 15.6, 0.9 Hz, 1H), 7.67 (td, *J* = 7.7, 1.8 Hz, 1H), 7.57–7.38 (m, 2H), 7.34 (d, *J* =

7.8 Hz, 1H), 7.23–7.16 (m, 1H), 7.03 (d, *J* = 14.4 Hz, 1H), 6.72 (d, *J* = 15.6 Hz, 1H), 6.63 (d, *J* = 14.3 Hz, 1H), 2.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.5, 167.6, 154.2, 150.2, 143.5, 143.3 (q, J_{CF} = 35.2 Hz), 141.0, 136.8, 136.4, 131.0, 130.5, 130.0, 129.3, 126.2, 123.5, 120.8 (q, J_{CF} = 270.7 Hz), 19.7. IR (film) ν_{max}: 1650, 1604, 1585, 1345, 1165 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{17}H_{14}F_3N_2OS$ $[M+H]^+$: 351.0779. Found: 351.0777.

4.3.20 (1*E*,4*E*,6*E*)-7-(3-Fluoro-pyridin-4-yl)-1-(2-methyl-4-trifluoromethyl-thiazol-5-yl)-hepta-1,4,6-trien-3-one (**31**). This compound was synthesized in 76% yield as a yellow solid from diethyl ((3*E*,5*E*)-6-(3-fluoropyridin-4-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (**53**) and 2-methyl-4-trifluoromethyl-thiazol-5-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (d, *J* = 2.1 Hz, 1H), 8.41 (d, *J* = 5.0 Hz, 1H), 7.89 (d, *J* = 15.6 Hz, 1H), 7.47 (dd, *J* = 15.1, 10.6 Hz, 1H), 7.39 (t, *J* = 5.7 Hz, 1H), 7.20 (dd, *J* = 15.7, 10.7 Hz, 1H), 7.06 (d, *J* = 15.7 Hz, 1H), 6.72 (d, *J* = 15.9 Hz, 1H), 6.67 (d, *J* = 15.9 Hz, 1H), 2.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.3, 167.8, 156.9 (d, J_{CF} = 259.6 Hz), 146.1 (d, J_{CF} = 5.2 Hz), 143.5 (q, J_{CF} = 35.3 Hz), 142.9, 139.4, 139.1, 136.3, 133.4 (d, J_{CF} = 6.0 Hz), 131.5 (d, J_{CF} = 2.1 Hz), 130.9, 130.8, 129.7, 121.2, 120.7 (q, J_{CF} = 270.7 Hz), 19.8. IR (film) ν_{max}: 1650, 1607, 1583, 1489, 1417, 1122, 1055 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{17}H_{13}F_4N_2OS$ $[M+H]^+$: 369.0684. Found: 369.0675.

4.3.21 (1*E*,4*E*,6*E*)-7-(1-Propyl-1*H*-imidazol-2-yl)-1-pyridin-2-yl-hepta-1,4,6-trien-3-one (**32**). This compound was synthesized in 33% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(pyridin-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-propyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 4.7 Hz, 1H), 7.72 (td, *J* = 7.7, 1.8 Hz, 1H), 7.65 (d, *J* = 15.6 Hz, 1H), 7.55 (d, *J* = 15.5 Hz, 1H), 7.54 (dd, *J* = 14.9, 11.5 Hz, 1H), 7.46 (d, *J* = 5.6 Hz, 1H), 7.40 (dd, *J* = 14.7, 11.5 Hz, 1H), 7.28 (dd, *J* = 4.8, 1.1 Hz, 1H), 7.15 (s, 1H), 6.95 (d, *J* = 1.0 Hz, 1H), 6.78 (d, *J* = 14.6 Hz, 1H), 6.67 (d, *J* = 14.8 Hz, 1H), 3.95 (t, *J* = 7.2 Hz, 2H), 1.85–1.73 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.2, 153.4, 150.3, 144.2, 143.1, 141.4, 137.0, 130.8, 130.1, 130.1, 128.5, 125.3, 125.1, 124.5, 121.8, 47.9, 24.8, 11.3. IR (film) ν_{max}: 1649, 1617, 1578, 1430, 1080, 995 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{20}N_3O$ $[M+H]^+$: 294.1606. Found: 294.1601.

4.3.21 (1*E*,4*E*,6*E*)-7-(1-Isopropyl-1*H*-imidazol-2-yl)-1-pyridin-2-yl-hepta-1,4,6-trien-3-one (**33**). This compound was synthesized in 29% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(pyridin-2-

yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-isopropyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (dd, *J* = 4.7, 0.9 Hz, 1H), 7.72 (td, *J* = 7.7, 1.8 Hz, 1H), 7.65 (d, *J* = 15.6 Hz, 1H), 7.55 (dd, *J* = 15.1, 11.5 Hz, 1H), 7.54 (d, *J* = 15.5 Hz, 1H), 7.48–7.37 (overlapped, 2H), 7.32–7.24 (m, 1H), 7.16 (s, 1H), 7.04 (d, *J* = 1.1 Hz, 1H), 6.84 (d, *J* = 14.6 Hz, 1H), 6.67 (d, *J* = 14.6 Hz, 1H), 4.56–4.47 (m, 1H), 1.47 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.2, 153.4, 150.3, 143.7, 143.2, 141.4, 137.0, 130.7, 130.5, 130.0, 128.6, 125.3, 124.5, 117.4, 47.5, 23.8. IR (film) ν_{max}: 1649, 1617, 1578, 1264, 1080, 906 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{18}H_{20}N_3O$ $[M+H]^+$: 294.1606. Found: 294.1600.

4.3.23 (1*E*,4*E*,6*E*)-7-(1-(sec-Butyl)-1*H*-imidazol-2-yl)-1-pyridin-2-yl-hepta-1,4,6-trien-3-one (34). This compound was synthesized in 36% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(pyridin-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-(*sec*-butyl)-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, *J* = 4.6 Hz, 1H), 7.71 (td, *J* = 7.7, 1.8 Hz, 1H), 7.64 (d, *J* = 15.5 Hz, 1H), 7.54 (dd, *J* = 14.9, 11.5 Hz, 1H), 7.54 (d, *J* = 15.5 Hz, 1H), 7.47–7.36 (m, 2H), 7.30–7.23 (m, 1H), 7.17 (s, 1H), 7.00 (d, *J* = 1.1 Hz, 1H), 6.82 (d, *J* = 14.5 Hz, 1H), 6.65 (d, *J* = 14.6 Hz, 1H), 4.29–4.16 (m, 1H), 1.83–1.70 (m, 2H), 1.45 (d, *J* = 6.7 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.2, 153.4, 150.3, 144.2, 143.2, 141.3, 137.0, 130.64, 130.62, 130.0, 128.5, 125.4, 125.3, 124.4, 117.7, 53.4, 30.9, 21.8, 10.8. IR (film) ν_{max}: 1651, 1617, 1578, 1460, 1080 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{19}H_{22}N_3O$ $[M+H]^+$: 308.1763. Found: 308.1761.

4.3.24 (1*E*,4*E*,6*E*)-7-(1-(Pentan-2-yl)-1*H*-imidazol-2-yl)-1-pyridin-2-yl-hepta-1,4,6-trien-3-one (35). This compound was synthesized in 36% yield as a yellow oil from (*E*)-diethyl (2-oxo-4-(pyridin-2-yl)but-3-en-1-yl)phosphonate and (*E*)-3-(1-(pentan-2-yl)-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (dd, *J* = 4.7, 0.9 Hz, 1H), 7.72 (td, *J* = 7.7, 1.8 Hz, 1H), 7.65 (d, *J* = 15.5 Hz, 1H), 7.55 (dd, *J* = 15.1, 11.8 Hz, 1H), 7.54 (d, *J* = 15.5 Hz, 1H), 7.46 (d, *J* = 7.4 Hz, 1H), 7.55 (dd, *J* = 14.6, 11.6 Hz, 1H), 7.30–7.25 (m, 1H), 7.17 (d, *J* = 0.9 Hz, 1H), 7.01 (d, *J* = 1.1 Hz, 1H), 6.83 (d, *J* = 14.5 Hz, 1H), 6.67 (d, *J* = 14.6 Hz, 1H), 4.40–4.24 (m, 1H), 1.77–1.69 (m, 2H), 1.45 (d, *J* = 6.7 Hz, 3H), 1.28–1.16 (m, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.2, 153.4, 150.3, 144.1, 143.2, 141.3, 137.0, 130.7, 130.0, 128.6, 125.3, 125.3, 124.5, 117.7, 51.7, 40.0, 22.2, 19.5, 13.8. IR (film) ν_{max}: 1651, 1617, 1579, 1461, 1085, 907 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{20}H_{23}N_3O$ $[M+H]^+$: 322.1919. Found: 322.1914.

4.3.25 (1*E*,4*E*,6*E*)-7-(2-Methyl-4-trifluoromethyl-thiazol-5-yl)-1-pyridin-2-yl-hepta-1,4,6-trien-3-one (**36**). This compound was synthesized in 55% yield as a yellow oil from diethyl ((3*E*,5*E*)-6-(2-methyl-4-(trifluoromethyl)thiazol-5-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (**51**) and pyridin-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 4.2 Hz, 1H), 7.77–7.70 (m, 1H), 7.67 (d, J = 15.5 Hz, 1H), 7.55 (d, J = 15.5 Hz, 1H), 7.52–7.41 (overlapped, 2H), 7.31–7.23 (m, 2H), 6.70 (dd, J = 15.2, 11.0 Hz, 1H), 6.62 (d, J = 15.7 Hz, 1H), 2.72 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.0, 166.3, 153.2, 150.4, 142.0, 141.9, 141.0 (q, J_{CF} = 34.9 Hz), 137.8, 137.1, 133.1, 131.9, 127.9, 127.6, 125.4, 124.6, 121.1 (q, J_{CF} = 270.2 Hz), 19.7. IR (film) ν_{max}: 1652, 1620, 1584, 1363, 1164 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{17}H_{14}F_3N_2OS$ $[M+H]^+$: 351.0779. Found: 351.0773.

4.3.26 (1*E*,4*E*,6*E*)-1-(4-Methyl-2-pyridin-4-yl-thiazol-5-yl)-7-thiazol-2-yl-hepta-1,4,6-trien-3-one (**37**). This compound was synthesized in 73% yield as a yellow oil from (diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 4-methyl-2-pyridin-4-yl-thiazol-5-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 (d, J = 2.9 Hz, 2H), 7.87 (d, J = 2.6 Hz, 1H), 7.84 (d, J = 15.0 Hz, 1H), 7.79 (d, J = 5.5 Hz, 2H), 7.48 (dd, J = 15.1, 9.8 Hz, 1H), 7.37 (d, J = 3.1 Hz, 1H), 7.25 (dd, J = 15.9, 9.2 Hz, 1H), 7.17 (d, J = 15.2 Hz, 1H), 6.75 (d, J = 15.3 Hz, 1H), 6.64 (d, J = 15.1 Hz, 1H), 2.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.4, 165.3, 164.5, 158.5, 150.8, 144.6, 141.8, 139.9, 132.8, 132.3, 131.8, 131.7, 131.3, 127.0, 120.5, 120.4, 16.1. IR (film) ν_{max}: 1644, 1595, 1573, 1408, 1325, 1084, 904 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{19}H_{16}N_3OS_2$ $[M+H]^+$: 366.0735. Found: 366.0730.

4.3.27 (1*E*,4*E*,6*E*)-1-(4-Methyl-2-pyridin-4-yl-thiazol-5-yl)-7-pyridin-2-yl-hepta-1,4,6-trien-3-one (**38**). This compound was synthesized in 58% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(pyridin-2-yl)hexa-3,5-dien-1-yl)phosphonate (**52**) and 4-methyl-2-pyridin-4-yl-thiazol-5-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (d, J = 4.5 Hz, 1H), 8.72 (d, J = 4.5 Hz, 1H), 8.63 (dd, J = 4.7, 0.8 Hz, 1H), 7.84 (d, J = 15.3 Hz, 1H), 7.80 (d, J = 4.5 Hz, 1H), 7.79 (d, J = 4.5 Hz, 1H), 7.69 (td, J = 7.7, 1.8 Hz, 1H), 7.58–7.43 (m, 2H), 7.36 (d, J = 7.8 Hz, 1H), 7.24–7.19 (m, 1H), 7.05 (d, J = 14.4 Hz, 1H), 6.77 (d, J = 15.3 Hz, 1H), 6.65 (d, J = 14.4 Hz, 1H), 2.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.8, 164.4, 158.3, 154.3, 150.9, 150.1, 142.9, 140.5, 139.9, 136.9, 132.0, 131.3, 131.2, 130.8, 127.2, 123.6, 123.5, 120.5, 16.0. IR (film) ν_{max}: 1645, 1595, 1468, 1274, 1000 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{21}H_{18}N_3OS$ $[M+H]^+$: 360.1170. Found: 360.1165.

4.3.28 (1*E*,4*E*,6*E*)-7-(3-Fluoro-pyridin-4-yl)-1-(4-methyl-2-pyridin-4-yl-thiazol-5-yl)-hepta-1,4,6-trien-3-one (**39**). This compound was synthesized in 79% yield as a yellow solid from diethyl ((3*E*,5*E*)-6-(3-fluoropyridin-4-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (**53**) and 4-methyl-2-pyridin-4-yl-thiazol-5-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (dd, J = 4.5, 1.6 Hz, 2H), 8.48 (d, J = 2.3 Hz, 1H), 8.40 (d, J = 5.1 Hz, 1H), 7.85 (d, J = 15.3 Hz, 1H), 7.78 (dd, J = 4.5, 1.6 Hz, 2H), 7.48 (dd, J = 15.2, 10.7 Hz, 1H), 7.42– 7.36 (m, 1H), 7.19 (dd, J = 15.7, 10.6 Hz, 1H), 7.05 (d, J = 15.7 Hz, 1H), 6.75 (d, J = 15.3 Hz, 1H), 6.65 (d, J = 15.2 Hz, 1H), 2.62 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.5, 164.6, 158.6, 156.9 (d, J = 259.5 Hz), 150.9, 146.1 (d, J = 5.3 Hz), 142.2, 139.7, 139.2 (d, J = 24.8 Hz), 133.6 (d, J = 6.0 Hz), 132.2 (d, J = 27.0 Hz), 131.2, 131.1, 130.9 (d, J = 9.8 Hz), 126.8, 121.2, 120.4, 16.1. IR (film) ν_{max}: 1645, 1602, 1415, 1085 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{21}H_{17}FN_3OS$ $[M+H]^+$: 378.1076. Found: 378.1070.

4.3.29 (1*E*,4*E*,6*E*)-1-(1-Isopropyl-1*H*-benzo[d]imidazol-2-yl)-7-(2-methyl-4-(trifluoromethyl) thiazol-5-yl)hepta-1,4,6-trien-3-one (**40**). This compound was synthesized in 55% yield as a yellow oil from diethyl ((3*E*,5*E*)-6-(2-methyl-4-(trifluoromethyl)thiazol-5-yl)-2-oxohexa-3,5-dien-1-yl)phosphonate (**51**) and 1-isopropyl-1*H*-benzo[d]imidazol-2-carbaldehyde using Method B. Yellow oil, 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91–7.76 (m, 3H), 7.63–7.48 (m, 2H), 7.35–7.23 (m, 3H), 6.70 (dd, J = 15.2, 10.9 Hz, 1H), 6.54 (d, J = 15.5 Hz, 1H), 4.98 (hept, J = 7.0 Hz, 1H), 2.72 (s, 3H), 1.70 (d, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.0, 166.5, 147.7, 143.9, 142.6, 141.0 (q, J_{CF} = 35.0 Hz), 137.7, 134.5, 133.0, 132.6, 130.1, 128.1, 127.8, 123.8, 123.3, 121.0 (q, J_{CF} = 270.6 Hz), 120.7, 112.2, 48.2, 22.1, 19.7. IR (film) ν_{max}: 1651, 1615, 1586, 1384, 1158 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{22}H_{21}F_3N_3OS$ $[M+H]^+$: 432.1357. Found: 432.1350.

4.3.30 (1*E*,4*E*,6*E*)-1-(1-Isopropyl-1*H*-benzo[d]imidazol-2-yl)-7-(thiazol-2-yl)hepta-1,4,6-trien-3-one (**41**). This compound was synthesized in 62% yield as a yellow oil from diethyl ((3*E*,5*E*)-2-oxo-6-(thiazol-2-yl)hexa-3,5-dien-1-yl)phosphonate (**50**) and 1-isopropyl-1*H*-benzo[d]imidazol-2-carbaldehyde using Method B. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74–7.56 (m, 4H), 7.45–7.35 (m, 2H), 7.17–6.96 (m, 5H), 6.43 (d, J = 15.4 Hz, 1H), 4.79 (hept, J = 6.9 Hz, 1H), 1.50 (d, J = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.0, 165.3, 147.7, 144.6, 143.5, 142.7, 134.4, 133.0, 132.9, 131.8, 130.6, 127.4, 123.9, 123.4, 120.6, 120.5, 112.2, 48.3, 22.0. IR (film) ν_{max}: 1648, 1615, 1405, 1091 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_{20}H_{20}N_3OS$ $[M+H]^+$: 350.1327. Found: 350.1326.

4.3.31 (1*E*,4*E*,6*E*)-1-(1-Isopropyl-1*H*-benzo[d]imidazole-2-yl)-7-(1-isopropyl-1*H*-imidazole-2-yl)hepta-1,4,6-trien-3-one (42): This compound was synthesized in 18% yield as a yellow oil from (*E*)-diethyl (4-(1-isopropyl-1*H*-benzo[d]imidazole-2-yl)-2-oxobut-3-en-1-yl)phosphonate and (*E*)-3-(1-isopropyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 15.0 Hz, 1H), 7.83–7.75 (m, 2H), 7.63 (dd, *J* = 15.0, 11.3 Hz, 1H), 7.58–7.54 (m, 1H), 7.44 (dd, *J* = 14.6, 11.6 Hz, 1H), 7.31–7.27 (m, 2H), 7.18 (d, *J* = 1.0 Hz, 1H), 7.06 (d, *J* = 1.1 Hz, 1H), 6.88 (d, *J* = 14.7 Hz, 1H), 6.61 (d, *J* = 15.2 Hz, 1H), 5.04–4.95 (m, 1H), 4.58–4.49 (m, 1H), 1.70 (d, *J* = 7.0 Hz, 6H), 1.50 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 148.0, 143.9, 143.6, 134.5, 131.6, 130.7, 130.6, 130.0, 127.2, 125.6, 123.7, 123.2, 120.6, 117.6, 112.1, 48.2, 47.6, 23.8, 22.0. IR (film) ν_{max}: 3051, 2977, 2933, 1648, 1608, 1575, 1461, 1383, 1271, 1183, 1074, 998, 966, 740 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{23}H_{27}N_4O$ $[M+H]^+$: 375.2185. Found: 375.2178.

4.3.32 (1*E*,4*E*,6*E*)-1-(1-(*sec*-Butyl)-1*H*-benzo[d]imidazole-2-yl)-7-(1-(*sec*-butyl)-1*H*-imidazole-2-yl)hepta-1,4,6-trien-3-one (43): This compound was synthesized in 13% yield as a yellow oil from (*E*)-diethyl (4-(1-(*sec*-butyl)-1*H*-benzo[d]imidazole-2-yl)-2-oxobut-3-en-1-yl)phosphonate and (*E*)-3-(1-(*sec*-butyl)-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, *J* = 15.0 Hz, 1H), 7.84–7.81 (m, 1H), 7.76 (d, *J* = 15.0 Hz, 1H), 7.64 (dd, *J* = 14.9, 11.5 Hz, 1H), 7.57–7.45 (m, 2H), 7.33–7.27 (m, 2H), 7.21 (s, 1H), 7.03 (d, *J* = 0.8 Hz, 1H), 6.87 (d, *J* = 14.5 Hz, 1H), 6.62 (d, *J* = 14.8 Hz, 1H), 4.74–4.58 (m, 1H), 4.30–4.20 (m, 1H), 2.29–2.09 (m, 1H), 2.07–1.92 (m, 1H), 1.85–1.74 (m, 2H), 1.68 (d, *J* = 7.0 Hz, 3H), 1.48 (d, *J* = 6.7 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 148.7, 144.1, 143.94, 143.86, 134.6, 131.6, 130.8, 130.7, 129.9, 127.3, 125.7, 123.6, 123.2, 120.6, 117.8, 112.1, 54.3, 53.5, 31.0, 28.8, 21.8, 20.4, 11.3, 10.8. IR (film) ν_{max}: 2971, 2934, 2876, 1648, 1610, 1458, 1384, 1275, 1261, 1079, 998, 968, 764, 749 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{25}H_{31}N_4O$ $[M+H]^+$: 403.2498. Found: 403.2490.

4.3.33 (1*E*,4*E*,6*E*)-1-(1-(Pentan-2-yl)-1*H*-benzo[d]imidazole-2-yl)-7-(1-(pentan-2-yl)-1*H*-imidazole-2-yl)hepta-1,4,6-trien-3-one (44): This compound was synthesized in 19% yield as a yellow oil from (*E*)-diethyl (4-(1-(pentan-2-yl)-1*H*-benzo[d]imidazole-2-yl)-2-oxobut-3-en-1-yl)phosphonate and (*E*)-3-(1-(pentan-2-yl)-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 15.0 Hz, 1H), 7.83–7.79 (m, 1H), 7.76 (d, *J* = 15.0 Hz, 1H), 7.62 (dd, *J* = 15.1, 11.5 Hz, 1H), 7.54–7.51 (m, 1H), 7.46 (dd, *J* = 14.6, 11.5 Hz, 1H), 7.32–7.24 (m, 2H), 7.19 (s, 1H), 7.02 (s, 1H), 6.86 (d, *J* = 14.6 Hz, 1H), 6.60 (d, *J* = 15.1 Hz, 1H), 4.83–4.66 (m, 1H), 4.41–4.25 (m, 1H), 2.23–2.08 (m, 1H), 1.98–1.83 (m, 1H), 1.74 (q, *J* = 7.6 Hz, 2H), 1.67 (d, *J* = 7.0 Hz, 3H),

1.47 (d, *J* = 6.7 Hz, 3H), 1.31–1.16 (m, 3H), 1.12–0.98 (m, 1H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.85 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.2, 148.5, 143.93, 143.89, 143.8, 134.6, 131.7, 130.7, 130.6, 130.2, 127.2, 125.5, 123.7, 123.2, 120.6, 117.8, 112.1, 52.7, 51.8, 40.0, 37.8, 22.2, 20.7, 20.1, 19.5, 13.8. IR (film) ν_{max}: 2959, 2932, 2873, 1648, 1611, 1578, 1458, 1384, 1275, 1080, 999, 746 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{27}H_{35}N_4O$ $[M+H]^+$: 431.2811. Found: 431.2807.

4.3.34 (1*E*,4*E*,6*E*)-1-(1-(Pentan-3-yl)-1*H*-benzo[d]imidazole-2-yl)-7-(1-(pentan-3-yl)-1*H*-imidazole-2-yl)hepta-1,4,6-trien-3-one (45): This compound was synthesized in 17% yield as a yellow oil from (*E*)-diethyl (4-(1-(pentan-3-yl)-1*H*-benzo[d]imidazole-2-yl)-2-oxobut-3-en-1-yl)phosphonate and (*E*)-3-(1-(pentan-3-yl)-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 15.0 Hz, 1H), 7.82 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.75 (d, *J* = 15.0 Hz, 1H), 7.62 (dd, *J* = 15.2, 11.5 Hz, 1H), 7.54–7.40 (m, 2H), 7.34–7.24 (m, 2H), 7.21 (s, 1H), 6.97 (d, *J* = 1.1 Hz, 1H), 6.86 (d, *J* = 14.7 Hz, 1H), 6.59 (d, *J* = 15.1 Hz, 1H), 4.45–4.29 (m, 1H), 4.01–3.92 (m, 1H), 2.26–2.09 (m, 2H), 2.07–1.96 (m, 2H), 1.93–1.81 (m, 2H), 1.79–1.67 (m, 2H), 0.80 (t, *J* = 7.5 Hz, 6H), 0.76 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.2, 145.0, 144.0, 143.9, 131.6, 131.0, 130.8, 130.0, 125.8, 125.0, 123.6, 123.2, 120.6, 117.8, 60.9, 60.0, 29.3, 27.2, 11.2, 10.8. IR (film) ν_{max}: 2967, 2934, 2876, 1648, 1610, 1578, 1457, 1385, 1265, 1176, 1079, 908, 730 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{27}H_{35}N_4O$ $[M+H]^+$: 431.2811. Found: 431.2804.

4.3.35 (1*E*,4*E*,6*E*)-1-(1-Isobutyl-1*H*-benzo[d]imidazole-2-yl)-7-(1-isobutyl-1*H*-imidazole-2-yl)hepta-1,4,6-trien-3-one (46): This compound was synthesized in 9% yield as a yellow oil from (*E*)-diethyl (4-(1-isobutyl-1*H*-benzo[d]imidazole-2-yl)-2-oxobut-3-en-1-yl)phosphonate and (*E*)-3-1-isobutyl-1*H*-imidazol-2-yl)acrylaldehyde using Method A. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, *J* = 15.1 Hz, 1H), 7.83–7.77 (m, 1H), 7.67 (d, *J* = 15.1 Hz, 1H), 7.66 –7.57 (overlapped, 1H), 7.46–7.35 (m, 2H), 7.34–7.29 (m, 2H), 7.16 (s, 1H), 6.94 (d, *J* = 1.0 Hz, 1H), 6.81 (d, *J* = 14.8 Hz, 1H), 6.58 (d, *J* = 15.2 Hz, 1H), 4.10 (d, *J* = 7.6 Hz, 2H), 3.80 (d, *J* = 7.4 Hz, 2H), 2.27–2.15 (m, 1H), 2.11–1.96 (m, 1H), 0.96 (d, *J* = 6.7 Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.2, 148.8, 144.3, 143.9, 143.3, 136.3, 131.7, 130.2, 130.1, 129.8, 126.7, 125.6, 124.0, 123.5, 122.5, 120.3, 110.5, 53.7, 51.1, 30.7, 30.1, 20.3, 20.1. IR (film) ν_{max}: 3051, 2959, 2872, 1649, 1610, 1578, 1467, 1406, 1330, 1278, 1180, 998, 966, 734, 701 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{25}H_{31}N_4O$ $[M+H]^+$: 403.2498. Found: 403.2497.

4.4 Cell culture.

All cell lines were initially purchased from American Type Culture Collection (ATCC). 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^{o}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. The PWR-1E non-neoplastic prostate epithelial cells were routinely cultured in Keratinocyte serum free medium (K-SFM) supplemented with bovine pituitary extract and human recombinant epidermal growth factor.

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 analogues 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 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 **40** at 5 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 F2N12S 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 the test compound at different concentrations and cultured in CO_2 incubator at 37 ^{o}C for 16 h, while equal treatment volumes of DMSO were used as vehicle control. 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.

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Appendix A. Supplementary data.

Supplementary data related to this article can be found at http://.

REFERENCES

[1] R. Kuttan, P. Bhanumathy, K. Nirmala, M.C. George, Potential anticancer activity of turmeric (*Curcuma longa*), Cancer Lett. (Shannon, Ireland) 29 (1985) 197-202.

[2] T. Dorai, N. Gehani, A. Katz, Therapeutic potential of curcumin in human prostate cancer. I Curcumin induces apoptosis in both androgen-dependent and androgen-independent prostate cancer cells, Prostate Cancer Prostatic Dis. 3 (2000) 84-93.

[3] M.H. Teiten, F. Gaascht, S. Eifes, M. Dicato, M. Diederich, Chemopreventive potential of curcumin in prostate cancer. Genes Nutr. 5 (2010) 61-74.

[4] B.B. Aggarwal, A. Kumar, A.C. Bharti, Anticancer potential of curcumin: preclinical and clinical studies, Anticancer Res. 23 (2003) 363-398.

[5] Q.-H. Chen, Curcumin-based anti-prostate cancer agents, Anticancer Agents Med. Chem. 15 (2015) 138-156.

[6] N. Samaan, Q. Zhong, J. Fernandez, G. Chen, A.M. Hussain, S. Zheng, G. Wang, Q.-H. Chen, Design, synthesis, and evaluation of novel heteroaromatic analogues of curcumin as anti-cancer agents, Eur. J. Med. Chem. 75 (2014) 123-131.

[7] R. Wang, C. Chen, X. Zhang, C. Zhang, Q. Zhong, G. Chen, Q. Zhang, S. Zheng, G. Wang, Q.-H. Chen, Structure-activity relationship and pharmacokinetic studies of 1,5-diheteroarylpenta-1,4-dien-3-ones: a class of promising curcumin-based anticancer agents, J. Med. Chem. 58 (2015) 4713-4726.

[8] R. Wang, X. Zhang, C. Chen, G. Chen, Q. Zhong, Q. Zhang, S. Zheng, G. Wang, Q.-H. Chen, Synthesis and evaluation of 1,7-diheteroarylhepta-1,4,6-trien-3-ones as curcumin-based anticancer agents. Eur. J. Med. Chem. 110 (2016) 164-180.

[9] X. Zhang, R. Wang, G.R. Perez, G. Chen, Q. Zhang, S. Zheng, G. Wang, Q.-H. Chen, 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, Bioorg. Med. Chem. 24 (2016) 4692-4700.

[10] M.M. Webber, D. Bello, H.K. Kleinman, D.D. Wartinger, D.E. Williams, J.S. Rhim, Prostate specific antigen and androgen receptor induction and characterization of an immortalized adult human prostatic epithelial cell line. Carcinogenesis 17 (1996) 1641-1646.

[11] S.C. Gupta, S. Patchva, W. Koh, B.B. Aggarwal, Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clin. Exp. Pharmacol. Physiol. 39 (2012) 283-299.

[12] T. Shankar, N. Shantha, H. Ramesh, I. Murthy, V. Murthy, Toxicity studies on turmeric (Curcuma longa): acute toxicity studies in rats, guinepigs & monkeys. Indian J. Exp. Biol. 18 (1980) 73-75.

[13] S.R. Denmeade, X.S. Lin, J.T. Isaacs, Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. Prostate (N. Y.) 28 (1996) 251-265.

[14] K.H. Tsui, T.H. Feng, C.M. Lin, P.L. Chang, H.H. Juang, Curcumin blocks the activation of androgen and interlukin-6 on prostate-specific antigen in human prostatic carcinoma cells. J. Androl. 29 (2008) 661-668.

[15] R.K. Srivastava, Q. Chen, I. Siddiqui, K. Sarva, S. Shankar, Linkage of curcumin-induced cell cycle arrest and apoptosis by cyclin-dependent kinase inhibitor p21/WAF1/CIP1, Cell Cycle 6 (2007) 2953-2961.

[16] A.L. Hilchie, S.J. Furlong, K. Sutton, A. Richardson, M.R.J. Robichaud, C.A. Giacomantonio, N.D. Ridgway, D.W. Hoskin, Curcumin-induced apoptosis in PC3 prostate carcinoma cells is caspase-independent and involves cellular ceramide accumulation and damage to mitochondria, Nutr. Cancer 62 (2010) 379-389.

[17] W. Wichitnithad, U. Nimmannit, S. Wacharasindhu, P. Rojsitthisak, Synthesis, characterization and biological evaluation of succinate prodrugs of curcuminoids for colon cancer treatment. Molecules 16 (2011) 1888–1900.

[18] B. Corbel, L. Medinger, J.P. Haelters, G. Sturtz, An efficient synthesis of dialkyl 2-oxoalkanephosphonates and diphenyl-2-oxoalkylphosphine oxides from 1-chloralkyl ketones. Synthesis (1985) 1048–1051.

Highlights:

- 35 new trienones with two different heteroaromatic rings were prepared.
- Analogues showed superior potency in inhibiting prostate cancer cell proliferation.
- The optimal trienone is 39- to 82-fold more potent than curcumin.
- The utility of the trienones was further demonstrated as a potential scaffold.
- The trienones can be optimized by manipulating the terminal rings.