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Original article

Design, synthesis, and evaluation of novel heteroaromatic analogs of curcumin as anti-cancer agents

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ABSTRACT

To improve the potential of curcumin to treat advanced hormone-refractory prostate cancer, three series (A–C) of heteroaromatic analogs (thirty two compounds) with different monoketone linkers have been synthesized and evaluated for cytotoxicity against two human androgen-independent prostate cancer cell lines (PC-3 and DU-145). Among them, thirty analogs are more potent than curcumin against PC-3 cells, and twenty one analogs are more cytotoxic towards DU-145 cells relative to curcumin. The most potent compounds (**44**, **45**, **51**, and **52**) also showed impressive cytotoxicity against three other metastatic cancer cell lines (MDA-MB-231, HeLa, and A549), with IC_{50} values ranging from 50 nM to 390 nM. All four most potent analogs exhibited no apparent cytotoxicity towards the MCF-10A normal mammary epithelial cells. Taken together, selective enhancement of cell death in prostate cancer cell lines and other aggressive cancer cell lines suggests that nitrogen-containing heteroaromatic rings are promising bioisosteres of the substituted phenyl ring in curcumin.

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1. Introduction

Prostate cancer has the highest incidence and the second highest cancer mortality in American men. The American Cancer Society estimates that 238,590 new cases of prostate cancer will be diagnosed and 29,720 men will die of prostate cancer in the United States in 2013 [1]. Current therapies (radical prostatectomy, chemotherapy, local radiotherapy, or hormonotherapy) are successful in treating localized, androgen-dependent, prostate cancer. However, treatment of hormone-refractory prostate cancer remains hindered by inevitable progression of resistance to first-line treatment with docetaxel. Consequently, novel drugs are needed to treat advanced hormone-resistant prostate cancer [2,3].

Curcumin or diferuloylmethane (**1**, Table 1), a polyphenolic molecule extracted from the rhizome of the plant *Curcuma longa* (turmeric), is a yellow spice used as curry ingredient and has been used for centuries in Ayurvedic, Chinese, and Hindu medicine systems. There is a huge difference in the rate of incidence of prostate cancer between Western (120 per 100,000 in Northern America) and East Asian countries (less than 10 per 100,000 in Asia) [4]. The increased risk of prostate cancer in the first generation of Asian men emigrating to the United States suggests a chemopreventive effect of Asian traditional food. Recent preclinical and clinical studies have demonstrated that curcumin has a number of anticancer properties [5,6]. The potential of curcumin to treat both androgen-dependent and androgen-independent prostate cancer has been demonstrated by the *in vitro* and *in vivo* studies [7,8]. A new philosophy that favors multitargeted drugs has recently gained momentum [9]. Curcumin serves as a good example of a class of compounds that is able to target multiple enzymes with a “magic shotgun” [10]. The anticancer effects of curcumin are associated with its influence on numerous growth factors within the cell [11,12]. The effect of curcumin on any particular growth factor is small, but its aggregate effect is significant. This characteristic is especially valuable for diseases like cancer that are complex, inflammation associated, and often evolve mutations in multiple genes. Because of its potential ability to treat hormone-refractory prostate cancer, its low molecular weight, lack of toxicity, and its mechanism of action against multiple targets, curcumin could be an ideal candidate as an androgen-independent agent against prostate cancer. However, its clinical development has been limited by its suboptimal pharmacokinetics and poor bioavailability caused by poor solubility in water and rapid *in vivo*

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Table 1
In vitro cytotoxicity (IC_{50}, μM)[a] of the compounds against human cell lines.

Comp. No.	Series	Het	IC_{50} (μM)		IC_{50} (curcumin)/ IC_{50} (analog)	
			DU-145[b]	PC-3[c]	DU-145	PC-3
Curcumin	–	–	0.30	1.98	1	1
21	**A**	**a**	0.13	0.093	2.3	21
22	**A**	**b**	0.14	0.11	2.1	18
23	**A**	**c**	0.01	6.63	30	0.3
24	**A**	**e**	1870	76	0.0002	0.03
25	**A**	**f**	1.64	1.02	0.18	1.9
26	**A**	**g**	0.046	0.42	6.5	4.7
27	**A**	**h**	0.43	0.80	0.7	2.5
28	**A**	**i**	0.076	0.31	3.9	6.4
29	**A**	**j**	0.034	0.14	8.8	14
30	**A**	**k**	0.69	0.13	0.4	15
31	**B**	**a**	0.63	0.094	0.5	21
32	**B**	**b**	0.10	0.16	3	12
33	**B**	**c**	0.25	0.83	1.2	2
34	**B**	**d**	0.36	0.47	0.8	4.2
35	**B**	**e**	90	130	0.003	0.015
36	**B**	**f**	0.73	0.26	0.41	7.6
37	**B**	**g**	0.12	0.11	2.5	18
38	**B**	**h**	0.075	0.12	4	16.5
39	**B**	**i**	1.65	1.97	0.18	1
40	**B**	**j**	0.07	0.071	4.3	28
41	**B**	**k**	0.34	0.054	0.9	37
42	**C**	**a**	0.057	0.11	5.3	18
43	**C**	**b**	0.054	0.089	5.6	22
44	**C**	**c**	0.035	0.063	8.6	31
45	**C**	**d**	0.057	0.046	5.3	43
46	**C**	**f**	0.16	0.13	1.9	15
47	**C**	**g**	0.055	0.068	5.5	29
48	**C**	**h**	0.096	0.094	3	21
49	**C**	**i**	0.75	0.84	0.4	2.4
50	**C**	**j**	0.042	0.25	7.1	7.9
51	**C**	**k**	0.016	0.041	18.8	48
52	**C**	**l**	0.020	0.033	15	60

[a] IC_{50} is the drug concentration effective in inhibiting 50% of the cell viability measured by the trypan blue exclusion assay after 5 days exposure.
[b] Human androgen-independent prostate cancer cell line.
[c] Human androgen-independent prostate cancer cell line.

metabolism [13]. It has been found that, with oral administration at the dose of 450–3600 mg/day in a phase I trial, the blood concentration of curcumin in plasma and target tissues falls under the detection limit [14].

Curcumin has extensively been used as a lead compound to design and synthesize analogs for the potential treatment of prostate cancer [15–28]. Some analogs, such as JC-9 [22], FLLL11, and FLLL12 (Fig. 1) [19] were found to be more potent than curcumin towards PC-3 prostate cancer cell line. The reported studies focused mainly on changes in the β-diketone structure and aryl substitution pattern of curcumin. It is believed from reported studies that the β-diketone moiety in the structure of curcumin appears to be a specific substrate of a series of aldo-keto reductases and can be decomposed rapidly *in vivo* [29,30]. It has been evidenced that monoketone analogs generally have improved pharmacokinetic profiles over curcumin, and that some monoketone analogs with the acetone or cyclohexanone spacer confer increased cytotoxicity towards PC-3 cell lines [16,19].

To identify new curcumin analogs with improved bioavailability and potential to treat hormone-refractory prostate cancer, we replaced the substituted phenyl rings in curcumin with two identical basic *N*-containing heteroaromatic rings. We focused on basic nitrogen heteroaromatics to take advantage of their ability to exist in both the protonated and neutral form, allowing both solubility in aqueous media and enhanced potential to cross cellular membranes. It has been reported that pyridine analogs had better potency against MDA-MB-231 cancer cells [31], head and neck squamous cell carcinoma [32], and PC-3 prostate cancer cell line [20,33]. To the best of our knowledge, there is no cytotoxic study of *N*-containing five-membered heteroaromatic analogs against prostate cancer cells.

We have synthesized twenty-nine new compounds and three known compounds (Fig. 2), which are classified as three series according to their different linkers: 1-methylpiperidone (series A), cyclohexanone (series B), and acetone (series C). Among them, thirty one are five-membered heteroaromatic analogs and only one is six-membered analog (**52**). In this paper, we describe the synthesis of these curcumin analogs and the *in vitro* evaluation of their anticancer activities.

2. Results and discussion

To engineer more effective analogs of curcumin for potential clinical use in treating hormone-refractory prostate cancer, three series of heteroaromatic curcumin analogs with three different monoketone linkers have been designed by replacing the two substituted phenyl rings in curcumin with two identical *N*-containing heteroaromatic rings. All these three series compounds are symmetrical monoketone curcumin analogs with *N*-methylpiperidone, cyclohexanone, or acetone as a linker, respectively (Fig. 2). Twenty nine of them are new, and three analogs (**21**, **31**, and **52**) are known. Analogs **21** and **31** have been synthesized by Yadav and co-workers for the evaluation of their cytotoxicity against ER-negative breast cancer cell line MDA-MB-231 [31]. The cytotoxicity of analog **52** towards colorectal carcinoma HCT 116/p53+/+ cells has been investigated [34]. However, no cytotoxicity of these three known compounds towards prostate cancer cell lines has been reported. Each of them was synthesized through a Claisen–Schmidt condensation of the corresponding aromatic aldehyde with the appropriate ketone. The structures of these analogs have been determined by interpretation of their NMR and HR-MS data. The cytotoxicity of all synthesized analogs has been evaluated against two human androgen-independent prostate cancer cell lines (PC-3 and DU-145). Most of these curcumin analogs exhibited significantly more potent cytotoxicity than curcumin towards PC-3 and DU-145 prostate cancer cell lines.

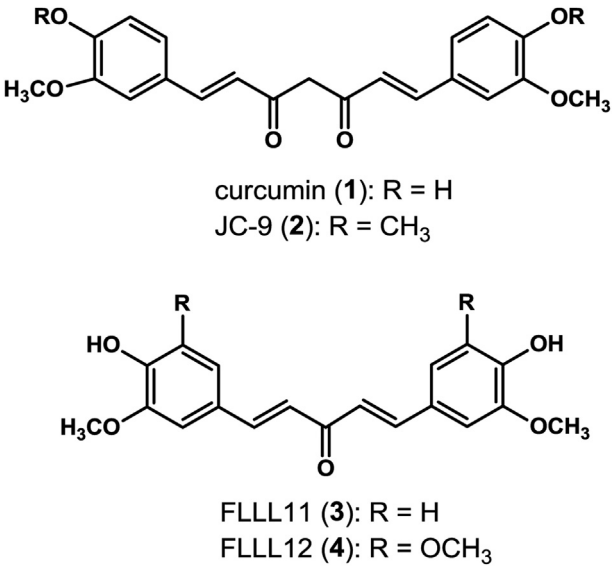


Fig. 1. Curcumin and its monoketone analogs.

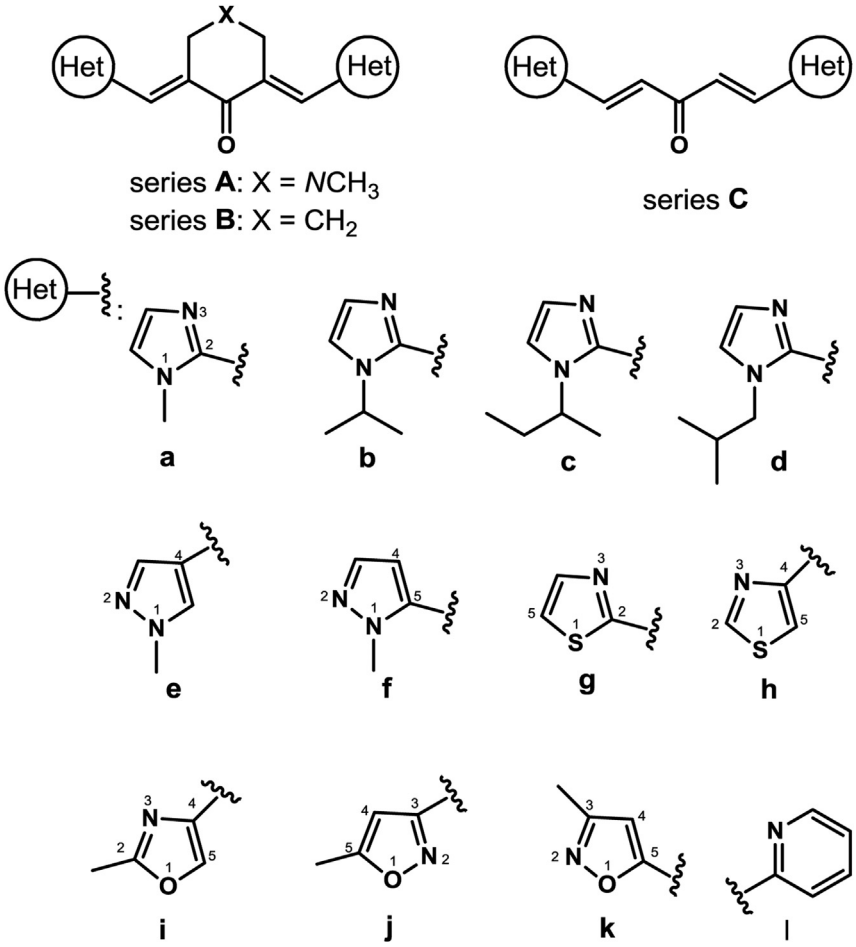


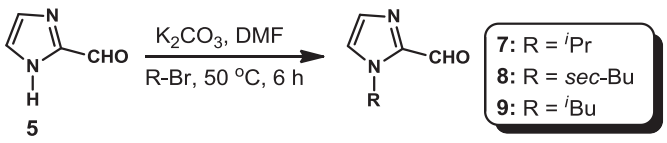
Fig. 2. Structures of synthesized monoketone curcumin analogs.

2.1. Chemistry

The starting aldehydes, **6**, and **10**–**16**, are commercially available. 1-Alkyl-1*H*-imidazole-2-carbaldehydes (**7**–**9**) were prepared from 1*H*-imidazole-2-carbaldehydes (**5**) using potassium carbonate as base (Scheme 1) according to the procedure described in the literature [35].

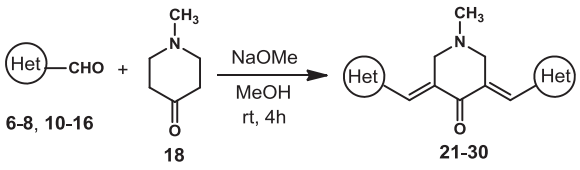
The general synthetic scheme for series A with *N*-methylpiperidone as a linker is shown in Scheme 2. The synthesis of this scaffold with various basic (*N*-containing) heteroaromatic rings was carried out via double aldol condensation of two equivalents of the appropriate aldehyde (**6**–**8** and **10**–**16**) with *N*-methylpiperidone using sodium methoxide as base following the procedure reported in the literature [31].

The synthetic strategy for series B with cyclohexanone as linker is shown in Scheme 3. The synthesis of **31**–**41** was carried out via double aldol condensation of two equivalents of the appropriate aldehyde (**6**–**16**) with cyclohexanone using sodium methoxide as base according to the procedure described in the literature [31].



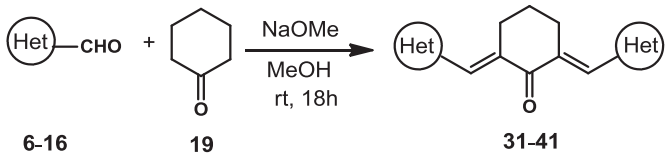
Scheme 1. Synthesis of 1-alkyl-1*H*-imidazole-2-carbaldehyde (**7**–**9**).

As shown in Scheme 4, eleven curcumin analogs with acetone linker were prepared in two different methods. The imidazole analogs **42** and **43** can be synthesized from the respective aldehyde **6** and **7** using the same procedure as described in the literature [31]. However, the aldol condensation of acetone with aldehydes **8**–**17** using sodium methoxide as base only gave messy products. The mechanistic reason for the formation of **52** was postulated to be that water elimination is hindered by the neighboring nitrogen atom through an inductive effect which results in electron withdrawal from the carbon atom bearing the hydroxyl group [36]. Consequently, the analogs **44**–**52** were prepared from the corresponding aldehyde and acetone at 70 °C, using potassium carbonate as base and toluene–ethanol–water (4:4:2) as solvent, following the procedure reported by Long and



Comp.	6	7	8	9	10	11	12	13	14	15	16	17
Het	a	b	c	d	e	f	g	h	i	j	k	l

Scheme 2. Synthesis of curcumin analogs with *N*-methylpiperidone as a linker (**21**–**30**).



Scheme 3. Synthesis of curcumin analogs with cyclohexanone as a linker (**31**–**41**).

co-workers [34]. We observed that not all compounds of this scaffold can be synthesized via this method. An improved method is currently explored in our laboratory and will be reported in due course.

2.2. Cytotoxicity towards human androgen-independent prostate cancer cell lines

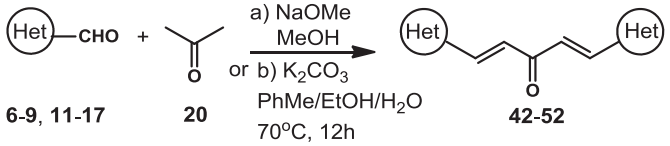
To determine the *in vitro* cytotoxicities of the synthesized curcumin analogs, we performed prostate cancer cell viability assays in which the ability of the curcumin analogs to inhibit growth of PC-3 and DU-145 cell lines was measured. Both PC-3 and DU-145 cell lines are androgen-independent human prostate cancer cells. Curcumin and DMSO were used as positive and negative control, respectively.

Among thirty two heteroaromatic analogs of curcumin that have been prepared and evaluated, thirty analogs are more potent than curcumin against PC-3 cells, and twenty one analogs are more cytotoxic towards DU-145 cells than curcumin. As shown in Table 1, the IC_{50} values of these twenty one analogs against PC-3 cells and DU-145 cells are significantly lower than those of curcumin.

The analogs of series C in particular are more potent than parent curcumin in their cytotoxicity against PC-3 and DU-145 androgen-independent prostate cancer cell lines. Among the three scaffolds of analogs that have been prepared and evaluated, all compounds that contain scaffold C with acetone linker (only exception is **49**) showed excellent cytotoxicity against both PC-3 and DU-145 prostate cancer cell lines with optimum IC_{50} value as 16 nM against DU-145 cells and 33 nM against PC-3 cells. They are 19 times and 60 times, respectively, more potent than curcumin.

2.3. Cytotoxicity towards aggressive human cancer cell lines

To further evaluate the effects of the analogs on other types of aggressive cancers, the four most promising curcumin analogs (**44**, **45**, **51**, and **52**) were selected for further evaluation of their cytotoxicities towards a metastatic breast cancer cell line (MDA-MB-231), an aggressive cervical cell line (HeLa), and a metastatic non-small cell lung cancer cell line (A549). As shown in Table 2, these four curcumin analogs are 7–9 times more potent than curcumin against MDA-MB-231 breast cancer cell line, 32–203 times better than curcumin against HeLa cervical cell line, and 94–294 folds more potent than curcumin against A549 non-small cell lung cancer line.



Scheme 4. Synthesis of curcumin analogs with acetone as a linker (**42**–**52**).

Table 2
In vitro cytotoxicity (IC_{50}, μM)[a] of selective curcumin analogs against other three aggressive cancer cell lines.

Comp. No.	Series	Het	IC_{50} (μM)				
			DU-145[b]	PC-3[c]	MDA-MB-231[d]	HeLa[e]	A549[f]
Curcumin	–	–	0.30	1.98	0.88	12.6	15.0
44	**C**	**c**	0.035	0.063	0.13	0.062	0.10
45	**C**	**d**	0.057	0.046	0.15	0.39	0.11
51	**C**	**k**	0.016	0.041	0.156	0.19	0.16
52	**C**	**l**	0.020	0.033	0.097	0.12	0.051

[a] IC_{50} is the drug concentration effective in inhibiting 50% of the cell viability measured by the trypan blue exclusion assay after 5 days exposure.
[b] Human androgen-independent prostate cancer cell line.
[c] Human androgen-independent prostate cancer cell line.
[d] Metastatic breast cancer cell line.
[e] Aggrasive cervical cancer cell line.
[f] Metastatic non small cell lung cancer cell line.

2.4. Cytotoxicity towards MCF-10A normal mammary epithelial cells

The four most promising curcumin analogs (**44**, **45**, **51**, and **52**) were also selected for further evaluation of their toxicity towards normal cells. As shown in Fig. 3, all these four analogs demonstrate no apparent cytotoxicity towards MCF-10A normal mammary epithelial cells up to 1 μM.

3. Conclusion

In summary, we have prepared a panel of curcumin analogs in which both the central and terminal sectors of the molecule were modified. The central diketone moiety was replaced with three different monoketone linkers. The terminal oxygenated aromatic rings in curcumin were substituted with two identical basic heteroaromatic rings; most of them are five-membered heteroaromatic rings (only one exception). Three scaffolds, comprising twenty nine new compounds, of basic curcumin analogs have been evaluated for cytotoxic potency against two androgen-independent human prostate cancer cell lines (DU-145 and PC-3) by the trypan blue dye exclusion method. A number of important findings resulted from this study are: i) thirty analogs are more potent than curcumin against PC-3 cells, and twenty one analogs are more cytotoxic towards DU-145 cells relative to curcumin; ii) the scaffold containing two identical basic heteroaromatic rings with a dienone linker showed excellent cytotoxicity against both PC-3 and DU-145 prostate cancer cell lines; iii) the four most promising curcumin analogs are more potent than curcumin against three other metastatic human cancer cell lines: MDA-MB-231, HeLa, and A549; and

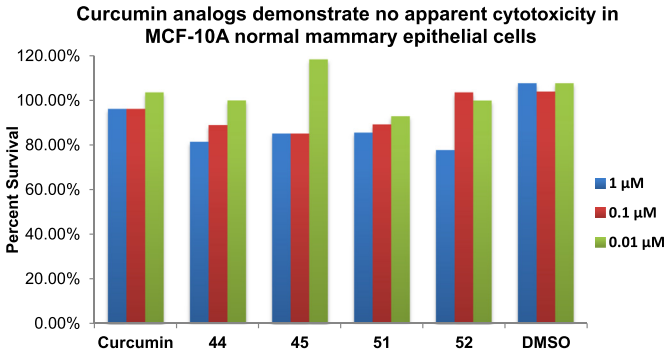


Fig. 3. Cytotoxicity of curcumin analogs towards MCF-10A normal mammary epithelial cells.

iv) these four most potent analogs demonstrate no apparent cytotoxicity towards MCF-10A normal mammary epithelial cells. The structure-activity data acquired indicate the combination of two identical basic heteroaromatic rings with a dienone linker constitutes a promising scaffold to design novel curcumin analogs with promising cytotoxicity against aggressive prostate cancer cells but with no apparent toxicity towards normal mammary cells. Synthesis of more analogs for better understanding their structure–activity relationships is undergoing in our laboratory. Additional research is needed on mechanism study and the *in vivo* activity of potential compounds on tumor growth in appropriate animal models.

4. Experimental

4.1. General synthetic procedures

NMR spectra were obtained on a Bruker Fourier 300 spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6. The chemical shifts are given in δ (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 μ). Preparative thin-layer chromatography (PTLC) separations were carried out on 1000 μ AnalTech thin layer chromatography plates (Lot No.13401). Curcumin was synthesized by Claisen–Schmidt condensation of aromatic aldehyde with acetylacetone according to the procedure described in the literature [37].

4.2. General procedure for the synthesis of 1-alkyl-1H-imidazole-2-carbaldehyde [35]

To a solution of 1*H*-imidazole-2-carbldehyde (13 mmol) and potassium carbonate (16 mmol) in DMF (13 mL) was added alkyl bromide (16 mmol), and the reaction mixture was stirred at 50 °C for 6 h. The inorganic solids were removed by filtration, and the filtrate was diluted with water and extracted with diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulfate, and the volatile components were evaporated under vacuum to give the respective product.

4.2.1. 1-Isopropyl-1H-imidazole-2-carbaldehyde (**7**)

Yellow oil, 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (d, J = 7.0 Hz, 6H), 5.41–5.55 (m, 1H), 7.31–7.33 (overlapped, 2H), 9.83 (s, 1H).

4.2.2. 1-sec-Butyl-1H-imidazole-2-carbaldehyde (**8**)

Yellow oil, 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.84 (t, J = 7.4 Hz, 3H), 1.46 (d, J = 7.0 Hz, 3H), 1.79 (quin, J = 7.0 Hz, 2H), 5.35 (sex, J = 7.0 Hz, 1H), 7.20 (s, 1H), 7.22 (s, 1H), 9.86 (s, 1H).

4.2.3. 1-Isobutyl-1H-imidazole-2-carbaldehyde (**9**)

Yellow oil, 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.927 (d, J = 3.0 Hz, 6H), 2.08 (s, 1H), 4.22 (d, J = 4.4 Hz, 2H), 7.14 (s, 1H), 7.30 (s, 1H), 9.84 (s, 1H).

4.3. General procedure for the synthesis of mono-ketone curcumin analogs

Method A [31]: To a solution of the starting aldehyde (1.5 mmol) and ketone (0.75 mmol) in methanol (10 mL) was added the solution of sodium methoxide in methanol (5.4 M, 0.14 mL, 0.75 mmol), and the mixture was stirred for 4–18 h and monitored with TLC. When the reaction was completed, the following two work-up procedures were applied. Procedure 1: if precipitate was observed, the precipitate was filtered and rinsed with cold methanol. Procedure 2: if no precipitate was observed, then saturated solution of ammonium chloride was added, and the subsequent mixture was extracted with dichloromethane. The organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated under vacuum to give a crude product, which was purified by preparative TLC (3–5% methanol in dichloromethane) or column chromatography (2% methanol in dichloromethane).

Method B [34]: The reaction mixture of aldehyde (4 mmol), acetone (116 mg, 2 mmol) and K_2CO_3 (1.1 g, 4 mmol) in the mixed solvent of toluene–ethanol–water (10 mL + 4.0 mL + 2.0 mL) was stirred at 70 °C for 12 h. After cooling down to room temperature, the solvent was evaporated *in vacuo*. The resulting residue was partitioned between dichloromethane and water. The aqueous phase was further extracted with dichloromethane twice. The combined organic extracts were rinsed with brine and dried over anhydrous magnesium sulfate. The organic solvent was removed under vacuum to give a residue, which was purified by preparative TLC (5% methanol in dichloromethane) or column chromatography (2% methanol in dichloromethane).

The physical and spectroscopic data of mono-ketone curcumin analogs were listed below:

4.3.1. (3E,5E)-1-Methyl-3,5-bis((1-methyl-1H-imidazol-2-yl) methylene)piperidin-4-one (**21**)

This compound was prepared by method A in 94% yield as a yellow solid: mp. 163–164 °C. IR (neat) ν_{max}: 3096, 2941, 1614, 1578, 1475, 1262 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3H), 3.80 (s, 6H), 4.12 (s, 4H), 7.22 (s, 2H), 7.41 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.3, 45.8, 57.2, 118.3, 125.0, 130.8, 133.6, 143.3, 186.2.

4.3.2. (3E,5E)-1-Methyl-3,5-bis((1-isopropyl-1H-imidazole-2-yl) methylene)piperidin-4-one (**22**)

This compound was prepared by method A as a yellow-orange solid in 95% yield; mp. 133–137 °C. IR (neat) ν_{max}: 3104, 2977, 1655, 1610, 1577, 1461, 1255, 1156 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.52 (d, J = 6.6 Hz, 12 H), 2.56 (s, 3H), 4.15 (s, 4H), 4.80 (sep, J = 6.6 Hz, 2H), 7.28 (s, 2H), 7.47 (s, 2H), 7.69 (s, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ: 22.3, 46.8, 56.7, 118.8, 119.0, 130.4, 134.0, 141.8, 186.3. HR-MS (ESI) m/z: calcd for $C_{20}H_{28}N_5O$ [M + H]: 354.2294; found 354.2288.

4.3.3. (3E,5E)-3,5-Bis((1-(sec-butyl)-1H-imidazol-2-yl)methylene)-1-methylpiperidin-4-one (**23**)

This compound was prepared by method A in 90% yield as a yellow oil. IR (neat) ν_{max}: 2969, 2934, 1652, 1551, 1459, 1259 cm^{-1}. ^{1}H NMR (300 MHz, MeOD) δ: 0.82 (t, J = 7.3 Hz, 6H), 1.51 (d, J = 6.6 Hz, 6H), 1.79–1.93 (m, 4H), 2.56 (s, 3H), 4.16 (s, 4H), 4.55 (sex, J = 6.7 Hz, 2H), 7.31 (s, 2H), 7.44 (s, 2H), 7.69 (s, 2H). ^{13}C NMR (75 MHz, MeOD) δ: 9.4, 20.6, 30.3, 44.4, 53.9, 56.6, 119.1, 119.2, 130.5, 133.9, 142.5, 186.3. HR-MS (ESI) m/z: calcd for $C_{22}H_{32}N_5O$ [M + H]: 382.2607; found 382.2599.

4.3.4. (3E,5E)-1-Methyl-3,5-bis((1-methyl-1H-pyrazol-4-yl) methylene)piperidin-4-one (**24**)

This compound was prepared by method A in 43% yield as a yellow solid: mp. 179–180 °C. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ: 2.20 (s, 3H), 3.32 (s, 4H), 3.54 (s, 6H), 7.21 (s, 2H), 7.26 (s, 2H), 7.41 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD) δ: 37.8, 44.7, 56.0, 117.1, 126.8, 128.7, 132.3, 140.5, 185.4. HR-MS (ESI) m/z: calcd for $C_{16}H_{20}N_5O$ [M + H]: 298.1668; found 298.1674.

4.3.5. (3E,5E)-1-Methyl-3,5-bis((1-methyl-1H-pyrazol-5-yl) methylene)piperidin-4-one (**25**)

This compound was prepared by method A in 92% yield as a yellow crystal: mp. 155–157 °C. IR (neat) ν_{max}: 2944, 1672, 1612, 1583, 1453, 1267, 1187, 925, 608 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ: 2.56 (s, 3H), 3.77 (br.s, 4H), 4.00 (s, 6H), 6.56 (d, $J = 2.1$ Hz, 2H), 7.57 (d, $J = 2.1$ Hz, 2H), 7.69 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 35.7, 44.5, 56.0, 108.6, 120.7, 133.3, 136.8, 138.3, 185.3. HR-MS (ESI) m/z: calcd for $C_{16}H_{20}N_5O$ [M + H]: 298.1668; found 298.1662.

4.3.6. (3E,5E)-1-Methyl-3,5-bis(thiazole-2-yl methylene)piperidin-4-one (**26**)

This compound was prepared by method A in 71% yield as a yellow solid: mp. 110–111 °C. IR (neat) ν_{max}: 3078, 2939, 1670, 1608, 1582, 1481, 1271, 1180 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.61 (s, 3H), 4.16 (s, 4H), 7.54 (d, $J = 3.0$ Hz, 2H), 7.76 (s, 2H), 8.03 (d, $J = 3.0$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 45.9, 57.3, 122.4, 124.9, 135.7, 145.3, 163.1, 186.7. HR-MS (ESI) m/z: calcd for $C_{14}H_{14}N_3OS_2$ [M + H]: 304.0578; found 304.0566.

4.3.7. (3E,5E)-1-Methyl-3,5-bis(thiazol-4-ylmethylene)piperidin-4-one (**27**)

This compound was prepared by method A in 65% yield as a yellow crystal: mp. 131–132 °C. IR (neat) ν_{max}: 3101, 2940, 1671, 1616, 1581, 1471, 1263, 1180 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.43 (s, 3H), 4.01 (s, 4H), 7.58 (s, 2H), 8.24 (s, 2H), 9.27 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 46.1, 57.4, 126.1, 126.4, 133.7, 152.6, 155.6, 187.8. HR-MS (ESI) m/z: calcd for $C_{14}H_{14}N_3OS_2$ [M + H]: 304.0578; found 304.0577.

4.3.8. (3E,5E)-1-Methyl-3,5-bis((2-methyloxazol-4-yl)methylene) piperidin-4-one (**28**)

This compound was prepared by method A in 6% yield as a yellow crystal: mp. 138–139.5 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.51 (s, 6H), 2.63 (s, 3H), 4.14 (s, 4H), 7.51 (s, 2H), 7.77 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 44.9, 56.2, 124.3, 131.6, 137.6, 141.4, 162.1, 185.8. HR-MS (ESI, M + H) m/z: calcd for $C_{16}H_{18}N_3O_3$ [M + H]: 300.1348; found 300.1351.

4.3.9. (2E,6E)-2,6-Bis((5-methylisoxazol-3-yl)methylene) cyclohexanone (**29**)

This compound was prepared by method A in 49% yield as a yellow crystal: mp. 155–156 °C. IR (neat) ν_{max}: 3129, 2943, 1685, 1636, 1598, 1426, 1267, 1181, 910, 783 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.47 (s, 6H), 2.52 (s, 3H), 3.90 (s, 4H), 6.11 (s, 2H), 7.42 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.2, 45.7, 57.5, 103.6, 121.9, 138.1, 158.8, 169.9, 186.5. HR-MS (ESI) m/z: calcd for $C_{16}H_{18}N_3O_3$ [M + H]: 300.1348; found 300.1345.

4.3.10. (3E,5E)-1-Methyl-3,5-bis((3-methylisoxazol-5-yl) methylene)piperidin-4-one (**30**)

This compound was prepared by method A in 36% yield as a yellow crystal: mp. 162–164 °C. IR (neat) ν_{max}: 3135, 2935, 1679, 1631, 1605, 1600, 1446, 1412, 1273, 1185 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.37 (s, 6H), 3.02 (s, 3H), 4.07 (s, 4H), 6.36 (s, 2H), 7.48 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.3, 45.6, 56.6, 109.5, 118.6, 136.0, 160.2, 166.0, 185.9. HR-MS (ESI) m/z: calcd for $C_{16}H_{18}N_3O_3$ [M + H]: 300.1348; found 300.1347.

4.3.11. (2E,6E)-2,6-Bis((1-methyl-1H-imidazol-2-yl)methylene) cyclohexanone (**31**)

This compound was prepared by method A in 72.5% yield as a yellow solid: mp. 190–192 °C. IR (neat) ν_{max}: 3129, 3105, 3042, 2942, 1663, 1605, 1567, 1505, 1477, 1266, 1176, 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.89 (quin, $J = 6.3$ Hz, 2H), 3.37 (t, $J = 5.4$ Hz, 4H), 3.82 (s, 6H), 7.02 (s, 2H), 7.32 (s, 2H), 7.55 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.5, 28.5, 33.3, 118.6, 123.0, 130.3, 138.7, 144.3, 190.1.

4.3.12. (2E,6E)-2,6-Bis((1-isopropyl-1H-imidazole-2-yl)methylene) cyclohexanone (**32**)

This compound was prepared by method A in 74% yield as a yellow–orange solid: mp. 158.5–160 °C. IR (neat) ν_{max}: 3104, 2977, 2933, 1660, 1608, 1567, 1463, 1248, 1157 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.52 (d, $J = 6.6$ Hz, 6H), 1.78 (quin, $J = 6.0$ Hz, 2H), 3.28 (t, $J = 5.5$ Hz, 4H), 4.62 (sep, $J = 6.6$ Hz, 2H), 7.03 (s, 2H), 7.20 (s, 2H), 7.54 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.7, 23.7, 28.6, 47.5, 117.3, 119.3, 130.6, 138.2, 143.0, 190.2. HR-MS (ESI) m/z: calcd for $C_{20}H_{27}N_4O$ [M + H]: 339.2185; found 339.2193.

4.3.13. (2E,6E)-2,6-Bis((1-(sec-butyl)-1H-imidazol-2-yl)methylene) cyclohexanone (**33**)

This compound was prepared by method A in 72% yield as a yellow-orange solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.84 (t, $J = 7.3$ Hz, 6H), 1.45 (d, $J = 6.6$ Hz, 6H), 1.74–1.88 (m, 6H), 3.35 (t, $J = 5.1$ Hz, 4H), 4.43 (sex, $J = 6.8$ Hz, 2H), 7.06 (s, 2H), 7.31 (s, 2H), 7.61 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 10.6, 21.8, 28.7, 30.9, 53.4, 117.6, 119.5, 130.7, 138.3, 143.6, 190.3. HR-MS (ESI) m/z: calcd for $C_{22}H_{31}N_4O$ [M + H]: 367.2498; found 367.2494.

4.3.14. (2E,6E)-2,6-Bis((1-isobutyl-1H-imidazol-2-yl)methylene) cyclohexanone (**34**)

This compound was prepared by method A in 72% yield as a yellow-orange solid. 75% yield. IR (neat) ν_{max}: 3103, 2960, 1663, 1605, 1570, 1281, 1168, 1131 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (d, $J = 6.6$ Hz, 12H), 1.85 (quin, $J = 6.3$ Hz, 2H), 2.06 (m, $J = 6.7$ Hz, 2H), 3.35 (t, $J = 5$ Hz, 4H), 3.87 (d, $J = 7.4$ Hz, 4H), 6.97 (s, 2H), 7.26 (s, 2H), 7.54 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.9, 21.6, 28.5, 30.4, 53.8, 119.4, 122.3, 130.1, 138.1, 143.9, 190.1. HR-MS (ESI) m/z: calcd for $C_{22}H_{31}N_4O$ [M + H]: 367.2498; found 367.2488.

4.3.15. (2E,6E)-2,6-Bis((1-methyl-1H-pyrazol-4-yl)methylene) cyclohexanone (**35**)

This compound was prepared by method A in 83% as a yellow solid: mp. 188.5–190 °C. IR (neat) ν_{max}: 2940, 1662, 1609, 1566, 1544, 1155 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.93 (quin, $J = 6.0$ Hz, 2H), 2.98 (t, $J = 6.0$ Hz, 4H), 3.96 (s, 6H), 7.59 (s, 2H), 7.65 (s, 2H), 7.72 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.9, 28.4, 39.2, 118.8, 127.2, 131.7, 132.7, 140.9, 189.0. HR-MS (ESI) m/z: calcd for $C_{16}H_{19}N_4O$ [M + H]: 283.1559; found 283.1567.

4.3.16. (2E,6E)-2,6-Bis((1-methyl-1H-pyrazol-5-yl)methylene) cyclohexanone (**36**)

This compound was prepared by method A in 97% yield as a yellow solid: mp. 153–154 °C. IR (neat) ν_{max}: 3097, 2943, 1664, 1607, 1450, 1265, 1172, 923 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.87 (quin, $J = 6.3$ Hz, 2H), 2.84 (t, $J = 6.3$ Hz, 4H), 3.97 (s, 6H), 6.47 (s, 2H), 7.51 (s, 2H), 7.66 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.6, 28.1, 37.1, 108.3, 122.1, 136.1, 137.5, 138.5, 188.5. HR-MS (ESI) m/z: calcd for $C_{16}H_{19}N_4O$ [M + H]: 283.1559; found 283.1553.

4.3.17. (2E,6E)-2,6-Bis(thiazole-2-yl methylene)cyclohexanone (**37**)

This compound was prepared by method A in 84% yield as a yellow solid: mp. 158.5–159 °C. IR (neat) ν_{max}: 3073, 2929, 1654, 1594, 1562, 1463, 1259, 1178, 737 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.97 (quin, $J = 6.3$ Hz, 2H), 3.22 (t, $J = 5.4$ Hz, 4H), 7.53 (s, 2H), 7.87 (s, 2H), 8.02 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.2, 28.7, 122.0, 127.4, 138.6, 144.7, 163.5, 189.2. HR-MS (ESI) m/z: calcd for $C_{14}H_{13}N_2OS_2$ [M + H]: 289.0469; found 289.0457.

*4.3.18. (2E,6E)-2,6-Bis(thiazol-4-ylmethylene)cyclohexanone (**38**)*

This compound was prepared by method A in 21% yield as a yellow solid; mp. 184.5–185 °C. IR (neat) ν_{max}: 3091, 3073, 2933, 1661, 1610, 1557, 1265, 1143, 826 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.79 (quin, J = 6.3 Hz, 2H), 3.19 (t, J = 6.3 Hz, 4H), 8.17 (d, J = 1.8 Hz, 2H), 9.24 (d, J = 1.8 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.0, 28.4, 125.3, 127.9, 136.6, 153.0, 155.1, 189.8. HR-MS (ESI) m/z: calcd for $C_{14}H_{13}N_2OS_2$ [M + H]: 289.0469; found 289.0463.

*4.3.19. (2E,6E)-2,6-Bis((2-methyloxazol-4-yl)methylene) cyclohexanone (**39**)*

This compound was prepared by method A in 13% yield as a yellow crystal: mp. 154–155 °C. IR (neat) ν_{max}: 3134, 2946, 1667, 1623, 1564, 1436, 1308, 1110 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.90 (quin, J = 6.3 Hz, 2H), 2.52 (s, 6H), 3.05 (t, J = 6.3 Hz, 4H), 7.52 (s, 2H), 7.74 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 21.7, 28.4, 124.8, 136.4, 138.0, 140.2, 161.7, 189.1. HR-MS (ESI) m/z: calcd for $C_{16}H_{17}N_2O_3$ [M + H]: 285.1239; found 285.1246.

*4.3.20. (2E,6E)-2,6-Bis((5-methylisoxazol-3-yl)methylene) cyclohexanone (**40**)*

This compound was prepared by method A in 41.5% yield as a yellow solid: mp. 188–188.5 °C. IR (neat) ν_{max}: 3112, 2960, 1681, 1590, 1451, 1428, 1309, 1258, 1168, 1138 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.88 (quin, J = 6.3 Hz, 2H), 2.47 (s, 6H), 3.07 (t, J = 6.3 Hz, 4H), 6.15 (s, 2H), 7.49 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.2, 21.4, 28.9, 103.4, 123.5, 140.9, 159.4, 169.6, 189.2. HR-MS (ESI) m/z: calcd for $C_{16}H_{17}N_2O_3$ [M + H]: 285.1239; found 285.1229.

*4.3.21. (2E,6E)-2,6-Bis((3-methylisothiazol-5-yl)methylene) cyclohexanone (**41**)*

This compound was prepared by method A in 90% yield as a yellow solid: mp. 192.5–193 °C. IR (neat) ν_{max}: 2928, 1624, 1577, 1557, 1415, 1274, 1179, 1143 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.91 (quin, J = 6.0 Hz, 2H), 2.34 (s, 6H), 3.07 (t, J = 6.0 Hz, 4H), 6.13 (s, 2H), 7.48 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.3, 21.0, 28.2, 108.7, 120.3, 139.1, 160.0, 166.7, 188.5. HR-MS (ESI) m/z: calcd for $C_{16}H_{17}N_2O_3$ [M + H]: 285.1239; found 285.1234.

*4.3.22. (1E,4E)-1,5-Bis(1-methyl-1H-imidazol-2-yl)penta-1,4-dien-3-one (**42**)*

This compound was prepared by method A in 39% yield as a yellow solid: mp. 177–178 °C. IR (neat)ν_{max} 2920, 1650, 1621, 1590, 1554, 1479, 1414, 1278 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.82 (s, 6H), 7.03 (s, 2H), 7.22 (s, 2H), 7.62 (d, J = 15.3 Hz, 2H), 7.49 (d, J = 15.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.3, 124.1, 126.6, 127.5, 130.4, 143.5, 188.1. HR-MS (ESI) m/z: calcd for $C_{13}H_{15}N_4O$ [M + H]: 243.1246; found 243.1247.

*4.3.23. (1E,3E)-1,3-Bis((1-isopropyl-1H-imidazole-2-yl)methylene) acetone (**43**)*

This compound was prepared by method A in 73% yield as a yellow–brown semi-solid. IR (neat) ν_{max}: 3106, 2979, 2932, 1648, 1616, 1462, 1269 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.47 (d, J = 6.6 Hz, 12H), 4.69 (sep, J = 6.6 Hz, 2H), 7.13 (s, 2H), 7.21 (s, 2H), 7.49 (d, J = 15.0 Hz, 2H), 7.63 (d, J = 15.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.8, 47.6, 118.6, 126.8, 127.4, 130.9, 142.4, 188.3. HR-MS (ESI) m/z: calcd for $C_{17}H_{23}N_4O$ [M + H]: 299.1872; found 299.1865.

*4.3.24. (1E,4E)-1,5-Bis(1-(sec-butyl)-1H-imidazol-2-yl)penta-1,4-dien-3-one (**44**)*

This compound was prepared by method B in 40% yield as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.84 (t, J = 7.3 Hz, 6H), 1.47 (d, J = 6.6 Hz, 6H), 1.75–1.86 (m, 4H), 4.46 (sex, J = 6.6 Hz, 2H), 7.10 (s, 2H), 7.25 (s, 2H), 7.58 (d, J = 15.0 Hz, 2H), 7.66 (d, J = 15.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 10.5, 21.9, 30.8, 53.5, 118.8, 126.6, 127.9, 130.6, 142.9, 188.3. HR-MS (ESI) m/z: calcd for $C_{19}H_{27}N_4O$ [M + H]: 327.2185; found 327.2180.

*4.3.25. (1E,4E)-1,5-Bis(1-isobutyl-1H-imidazol-2-yl)penta-1,4-dien-3-one (**45**)*

This compound was prepared by method B in 51% yield as a yellow solid. IR (neat) ν_{max}: 3106, 2962, 1648, 1618, 1594, 1474, 1445, 1301 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.93 (d, J = 6.6 Hz, 12H), 2.05 (m, J = 6.8 Hz, 2H), 3.87 (d, J = 7.4 Hz, 4H), 7.02 (s, 2H), 7.19 (s, 2H), 7.47 (d, J = 15.0 Hz, 2H), 7.57 (d, J = 15.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.9, 30.6, 53.6, 123.4, 126.9, 127.4, 130.4, 143.3, 188.1. HR-MS (ESI) m/z: calcd for $C_{19}H_{27}N_4O$ [M + H]: 327.2185; found 327.2188.

*4.3.26. (1E,4E)-1,5-Bis(1-methyl-1H-pyrazol-5-yl)penta-1,4-dien-3-one (**46**)*

This compound was prepared by method B in 61% yield as a yellow solid: mp. 111–112 °C. IR (neat) ν_{max}: 2945, 1652, 1618, 1591, 1281 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.99 (s, 6H), 6.66 (d, J = 2.1 Hz, 2H), 6.89 (d, J = 15.3 Hz, 2H), 7.49 (d, J = 2.1 Hz, 2H), 7.64 (d, J = 15.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 37.1, 105.9, 126.8, 128.4, 138.4, 138.9, 187.3. HR-MS (ESI) m/z: calcd for $C_{13}H_{15}N_4O$ [M + H]: 243.1246; found 243.1239.

*4.3.27. (1E,4E)-1,5-Di(thiazol-2-yl)penta-1,4-dien-3-one (**47**)*

This compound was prepared by method B in 55% yield as a yellow solid: mp. 124–126 °C. IR (neat) ν_{max}: 3112, 2923, 1652, 1615, 1594, 1477, 1330, 1311, 1094 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ: 7.33 (d, J = 15.9 Hz, 2H), 7.48 (d, J = 3.0 Hz, 2H), 7.82 (d, J = 15.9 Hz, 2H), 7.95 (d, J = 3.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 122.1, 128.6, 134.7, 145.1, 163.7, 187.6. HR-MS (ESI) m/z: calcd for $C_{11}H_9N_2OS_2$ [M + H]: 249.0156; found 249.0154.

*4.3.28. (1E,4E)-1,5-Di(thiazol-4-yl)penta-1,4-dien-3-one (**48**)*

This compound was prepared by method B in 50% yield as a yellow solid: mp. 139–140 °C. IR (neat) ν_{max}: 3085, 1649, 1620, 1593, 1482, 1273, 1176, 1085 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.37 (d, J = 15.6 Hz, 2H), 7.80 (d, J = 15.6 Hz, 2H), 8.23 (d, J = 1.8 Hz, 2H), 9.23 (d, J = 1.8 Hz, 2H). IR (neat) ν_{max}: 3104, 2977, 1645, 1611, 1578, 1478, 1427, 1285, 1077 cm^{-1}. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 122.1, 127.8, 134.5, 152.9, 153.8, 189.5. HR-MS (ESI) m/z: calcd for $C_{11}H_9N_2OS_2$ [M + H]: 249.0156; found 249.0166.

*4.3.29. (1E,4E)-1,5-Bis(2-methyloxazol-4-yl)penta-1,4-dien-3-one (**49**)*

This compound was prepared by method B in 5% yield as a yellow crystal: mp. 140–141 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.52 (s, 6H), 7.20 (d, J = 15.6 Hz, 2H), 7.53 (d, J = 15.6 Hz, 2H), 7.75 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 126.6, 130.7, 137.6, 139.9, 162.7, 188.6. HR-MS (ESI) m/z: calcd for $C_{13}H_{13}N_2O_3$ [M + H]: 245.0926; found 245.0936.

*4.3.30. (1E,4E)-1,5-Bis(5-methylisoxazol-3-yl)penta-1,4-dien-3-one (**50**)*

This compound was prepared by method B in 13% yield as a yellow crystal: mp. 153–154 °C. IR (neat) ν_{max}: 3134, 1680, 1642, 1599, 1450, 974, 804 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.50 (s, 6H), 6.26 (s, 2H), 7.06 (d, J = 16.2 Hz, 2H), 7.65 (d, J = 16.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.3, 99.5, 130.5, 131.3, 160.0, 170.6, 188.0. HR-MS (ESI) m/z: calcd for $C_{13}H_{13}N_2O_3$ [M + H]: 245.0926; found 245.0919.

*4.3.31. (1E,4E)-1,5-Bis(3-methylisoxazol-5-yl)penta-1,4-dien-3-one (**51**)*

This compound was prepared by method B in 26% yield as a yellow crystal: mp. 167–169 °C. IR (neat) ν_{max}: 3112, 2925, 1677, 1642, 1609, 1573, 1414, 1091, 994 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.37 (s, 6H), 6.41 (s, 2H), 7.18 (d, $J = 15.9$ Hz, 2H), 7.50 (d, $J = 15.9$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.4, 108.1, 126.5, 128.8, 160.6, 165.4, 187.2. HR-MS (ESI) m/z: calcd for $C_{13}H_{13}N_2O_3$ [M + H]: 245.0926; found 245.0921.

*4.3.32. (1E,4E)-1,5-Di(pyridin-2-yl)penta-1,4-dien-3-one (**52**)*

This compound was prepared by method B in 89% yield as a yellow solid: mp. 79–80 °C. IR (neat) ν_{max}: 3050, 3004, 2926, 1655, 1628, 1600, 1581, 1466, 1430, 1330, 1195, 981, 785 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ: 7.44 (dd, $J = 7.0$, 3.9 Hz, 2H), 7.61 (d, $J = 15.9$ Hz, 2H), 7.77 (d, $J = 7.0$ Hz, 2H), 7.79 (d, $J = 15.9$ Hz, 2H), 7.91 (dt, $J = 7.0$, 1.2 Hz, 2H), 8.65 (d, $J = 3.9$ Hz, 2H).

4.4. Bioassay [38]

4.4.1. Cell culture

The PC-3 prostate cancer cell line was routinely cultured in RPIM-1640 medium supplemented with 10% FBS, 4 mM glutamine, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 ug/mL streptomycin and 0.25 ug/mL amphotericin. Cultures were maintained in 5% carbon dioxide at a temperature of 37 °C. The DU-145 prostate cancer cells were routinely cultured in phenol red-free DMEM supplemented with 10% FBS, 4 mM glutamine, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 ug/mL streptomycin and 0.25 ug/mL amphotericin.

4.4.2. Trypan blue dye exclusion assay

PC-3 or DU-145 cells were plated in 24-well plates at a density of 20,000 each well in 10% FBS DMED medium. The cells were then treated with curcumin, or synthesized curcumin analogs separately at 6 different doses ranging from 0.01 μM to 10 μM for 5 days, while equal treatment volumes of DMSO were used as vehicle control. Cell numbers were counted with a cell viability analyzer (Beckman-Coulter). The ratio of drug treated viable cell numbers to vehicle treated viable cell numbers was defined as percentage viability. IC_{50} values were obtained from dose–response curves for each curcumin analog.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.041.

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