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Syntheses and potential anti-prostate cancer activities of ionone-based chalcones

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ABSTRACT

We report the SAR studies of 43 ionone-based chalcones that demonstrate substantial in vitro anti-proliferative activities in LNCaP, MDA-PCa-2b, 22Rv1, C4-2B and PC-3 prostate cancer cell lines. Compound **25** with an IC_{50} value of 0.74 μM in LNCaP cells potently antagonizes DHT-induced transactivation of the wild type and the clinically relevant T877A, W741C and H874Y mutated androgen receptors, representing a novel chalcone as pan-antagonist of androgen receptor.

Androgen receptor (AR) is a critical mediator of prostate cancer.[1,2] Androgen ablation by chemical/surgical castration to suppress testicular androgen secretion and antiandrogens to antagonize the action of residual androgen causes cancer regression. However, many men eventually failed this hormonal therapy as initial response was almost always followed by a relapse to a lethal hormone-refractory state, which is also referred to as castration-resistant prostate cancer (CRPC).[3] Once prostate cancer becomes castration-resistant, further treatment is palliative and patients eventually die of the disease. It has been established that AR remains a critical target for therapeutic intervention in CRPC.[2]

The β-ionone is a phytochemical present in many fruits, vegetables and grains. It was found to exert anti-carcinogenic and antitumor activities in melanoma[4] and cancers of the colon,[5] breast[6] and prostate.[7] On the other hand, curcumin, the major pigment in the dietary spice turmeric, was found to possess anti-inflammatory, anti-oxidant, antiangiogenic and anticancer activities.[8] Numerous curcumin analogues have been synthesized and several of them possess antiandrogenic activity.[8,9] One widely used strategy for structural modification in curcumin involves truncation of the central conjugated β-diketone into a mono-carbonyl dienone, obtaining better bioavailability.[9] In our effort to develop natural product-based anticancer agents, we have synthesized 43 ionone-based chalcones by incorporating ionone and mono-carbonyl dienone into one chemical entity and evaluated them for in vitro cytotoxicity in a panel of prostate cancer cell lines, including LNCaP, MDA-PCa-2b, 22Rv1, C4-2B and PC-3, as well as a nontumorigenic prostate epithelial cell line (RWPE-1). The most potent compound has been evaluated for its anti-androgenic activity by AR-dependent reporter assays.

Three series of ionone-based chalcones (compounds **1–43)** were synthesized by condensing commercial available substituted benzaldehyde with α-ionone, β-ionone and 4,5-epoxy-β-ionone, respectively (Tables 1–3). Compounds **1, 2** and **12** which contain phenolic hydroxyl were synthesized by acid-catalyzed Adol condensation.[10] We found H_2SO_4-catalyzed reactions at room temperature gave **1**, **2** and **12** in good yields, but AcOH-catalyzed reactions gave little products. Chalcones **3**–**11** and **13**–**15** were obtained by facile Adol condensation catalyzed by sodium hydroxide in ethanol (Scheme 1).[11] Compounds **16**–**22** were obtained by Adol condensation catalyzed by sodium hydroxide in water in the presence of cetyltrimethyl ammonium bromide.[12] Compounds **23**–**43** were synthesized by Adol condensation catalyzed by sodium hydroxide pellet in methanol. The epoxide ring opening of the 4,5-epoxy-β-ionone during the reactions furnished 4-hydroxyl-α-ionone derivatives **23**–**43** (Scheme 1), which was confirmed by the formation of the 5,6-double bond as indicated by the proton NMR analyses. Purification of the crude products were achieved by silica gel CC (elutant: *n*-Hexane and EtOAc).

Cytotoxicity of chalcones **1**–**43** in a panel of prostate cancer cell lines were evaluated by MTT assays.[13] It should be noted that com-

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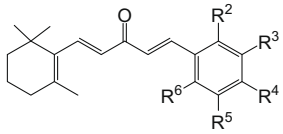
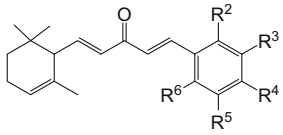
Table 1
Cytotoxicity of β-ionone-based chalcones in LNCaP and PC-3 cells



Compound	Substituent					Cytotoxicity, IC_{50}[a] (μM)	
	R^2	R^3	R^4	R^5	R^6	LNCaP	PC-3
1	H	OCH_3	OH	H	H	9.5	21.3
2	H	OH	OCH_3	H	H	9.9	37.4
3	H	OCH_3	OCH_3	H	H	9.5	42.0
4	H	OC_2H_5	OC_2H_5	H	H	8.2	>50
5	H	CF_3	H	H	H	2.8	7.7
6	H	H	CF_3	H	H	9.1	12.0
7	H	CF_3	H	CF_3	H	8.7	7.2
8	F	H	H	H	H	10.7	22.1
9	H	F	H	H	H	12.9	18.7
10	H	H	F	H	H	7.3	25.8
11	H	NO_2	H	H	H	2.7	10.0
12	H	NO_2	OH	H	H	26.8	> 50
13	H	NO_2	H	H	Cl	4.2	18.9
14	H	CH_3	H	H	H	17.8	35.0
15	H	H	Ph	H	H	14.8	47.8
β-Ionone						151.0	Inactive[b]

[a] IC_{50} is the concentration of compounds which causes a 50% inhibition as compared to the control (0.5% DMSO).
[b] Maximum tested concentration is 150 μM.

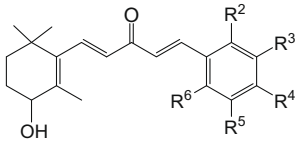
Table 2
Cytotoxicity of α-ionone-based chalcones in five prostate cancer cell lines



Compound	Substituents					Cytotoxicity, IC_{50} (μM)				
	R^2	R^3	R^4	R^5	R^6	LNCaP	PCa 2b	22Rv1	C4-2B	PC-3
16	CF_3	H	H	H	H	22.6	N.D.[a]	18.4	11.7	19.0
17	H	CF_3	H	H	H	1.0	4.4	2.9	4.4	5.2
18	H	H	CF_3	H	H	4.7	7.0	3.3	5.2	7.9
19	F	H	H	H	H	1.6	8.3	9.4	6.0	4.8
20	F	H	H	CF_3	H	1.7	3.0	3.5	4.7	3.1
21	H	NO_2	H	H	H	3.3	5.9	4.7	4.0	2.9
22	H	CH_3	H	H	H	12.2	N.D.	15.2	6.2	5.9

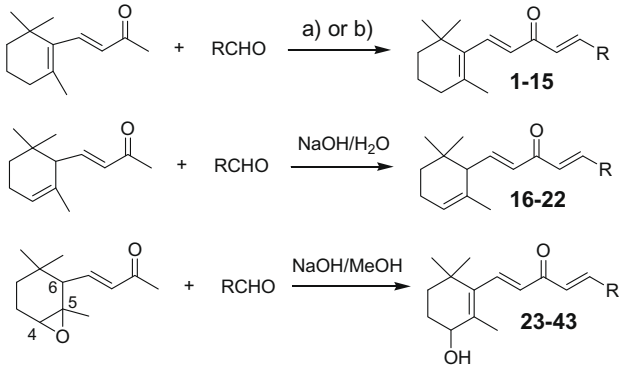
[a] N.D., not determined.

Table 3
Cytotoxicity of 4-hydroxy-β-ionone-based chalcones in five prostate cancer cell lines and a normal prostate epithelial cell line (RWPE-1)



Compound	Substituents					Cytotoxicity, IC_{50} (μM)					
	R^2	R^3	R^4	R^5	R^6	LNCaP	PCa-2b	22Rv1	C4-2B	PC-3	RWPE-1
23	H	H	H	H	H	5.8	12.6	8.0	1.8	15.5	N.D.
24	CF_3	H	H	H	H	2.9	2.9	2.3	N.D.[a]	3.7	N.D.
25	H	CF_3	H	H	H	0.74	2.6	1.7	1.4	3.5	0.73
26	H	H	CF_3	H	H	3.0	9.3	3.2	N.D.	10.2	N.D.
27	CF_3	H	H	CF_3	H	3.2	4.0	N.D.	N.D.	1.9	N.D.
28	H	CF_3	H	CF_3	H	2.1	3.5	2.2	N.D.	7.0	N.D.
29	F	H	H	H	H	2.9	N.D.	3.3	N.D.	10.4	N.D.
30	H	F	H	H	H	4.4	N.D.	5.6	N.D.	16.0	N.D.
31	H	H	F	H	H	3.9	N.D.	7.5	4.0	6.2	N.D.
32	H	CF_3	F	H	H	2.2	10.0	3.2	7.3	7.3	N.D.
33	H	CF_3	H	F	H	1.1	N.D.	2.4	N.D.	3.0	N.D.
34	F	CF_3	H	H	H	1.1	N.D.	N.D.	N.D.	2.1	N.D.
35	F	H	CF_3	H	H	2.6	N.D.	3.8	N.D.	4.6	N.D.
36	F	H	H	CF_3	H	1.7	N.D.	2.0	N.D.	3.6	N.D.
37	F	H	H	H	CF_3	4.9	N.D.	8.9	N.D.	8.8	N.D.
38	H	CH_3	H	H	H	4.1	19.0	4.4	N.D.	6.2	N.D.
39	H	CN	H	H	H	1.8	N.D.	2.2	N.D.	7.2	1.9
40	H	NO_2	H	H	H	2.0	6.0	1.9	N.D.	5.8	1.9
41	H	H	NO_2	H	H	4.2	N.D.	4.1	N.D.	15.0	N.D.
42	H	OCH_3	H	H	H	4.9	N.D.	N.D.	N.D.	6.5	N.D.
43	H	$CH(OEt)_2$	H	H	H	1.8	N.D.	N.D.	N.D.	4.7	N.D.

[a] N.D., not determined.



Scheme 1. Preparation of ionone-based chalcones **1**–**43**, where RCHO is a substituted benzaldehyde. Reagents and conditions: (a) H_2SO_4, MeOH, room temperature (for compounds **1**–**2** and **12**); and (b) NaOH, EtOH, room temperature (for compounds **3**–**11** and **13**–**15**).

pounds **3**, **10** and **21** are known compounds, but their cytotoxicity in prostate cancer cell lines have not been evaluated.[12,14] The IC_{50} values were determined from cell survival curves and reported in Tables 1–3.

The β-ionone-based chalcones **1**–**15** show considerable cytotoxicity in LNCaP cell line and modest activity in PC-3 cells (Table 1). Compound **5** and **11**, both of which have electron-withdrawing group at the *meta* position, are the two most potent β-ionone-based chalcones. Moving trifluomethyl group (–CF_3) from the *meta* position to the *para* position or substituting –CF_3 with –CH_3 group have substantially weakened the cytotoxicity in prostate cancer cells (compare **5**, **6** and **14**). Substitution of *meta*-CF_3 with *meta* fluoro (-F) decreases the cytotoxicity in LNCaP cells (compare **5** and **9**). This indicates the electron-withdrawing –CF_3 or –NO_2 at the *meta* position is critical for the cytotoxicity of β-ionone-based chalcones in LNCaP cell line. However, we found some β-ionone-based chalcones are not stable enough. We have therefore focused the studies on the other two series (Tables 2 and 3). Among the α-ionone-based chalcones **16**–**22** (Table 2), compound **17** with a *meta*-CF_3 is the most potent compound in the series and replacement of the *meta*-CF_3 group with a *meta*-CH_3 has severely reduced its activity. Chalcones **19** and **20,** which contain an *ortho*-F substitution and the *ortho*-F and *meta*-CF_3 double substitutions, respectively, demonstrated dose-dependent cytotoxicity in LNCaP cells with an IC_{50} value similar to that of compound **17**. The importance of an electron-withdrawing group at the *meta* position has also been observed among the 4-hydroxy-β-ionone-based chalcones **23**–**43** (Table 3). In particular, compounds **25**, **33**, **34**, **36**, **39** and **40**, which contain an electron-withdrawing group at the *meta* position, such as *meta*-CF_3, –CN or –NO_2, are much more potent than compounds **23**, **38** and **42**, which contain –H, –CH_3 and –OCH_3 at the *meta* position, respectively. Interestingly, compound **43** with

a *meta*-$CH(OEt)_2$ group, which has hydrogen bond acceptors and possesses weak electron-withdrawing capability, shows potent activity in LNCaP cells. This underscores the important role of the hydrogen bond acceptors in the *meta*-CF_3, –CN and $–NO_2$ groups. In addition, we have tested compounds **25**, **39** and **40** in a nontumorigenic androgen-dependent prostate epithelial cell line (RWPE-1) (Table 3). Compounds **25**, **39** and **40** showed similar cytotoxicity in both RWPE-1 and LNCaP cell lines.

Among the 43 chalcones, compound **25** demonstrated submicromolar or low micromolar IC_{50} values in LNCaP, MDA-PCa-2b, 22Rv1, C4-2B and PC-3 cell lines (Table 3). Both LNCaP and MDA-PCa-2b cell lines are androgen-dependent and express mutated ARs, with the T877A mutated AR in LNCaP and the T877A and L701H double mutated AR in MDA-PCa-2b cells. The 22Rv1 cells express the H874Y mutated AR, and the growth of this cell line is weakly stimulated by dihydrotestosterone (DHT). The C4-2B cells are androgen-independent, and express the T877A mutated AR. PC-3 cells, which lack endogenous AR, are androgen-independent. Therefore, compound **25** has demonstrated potent cytotoxicity in both androgen-dependent and androgen-independent prostate cancer cells (Table 3).

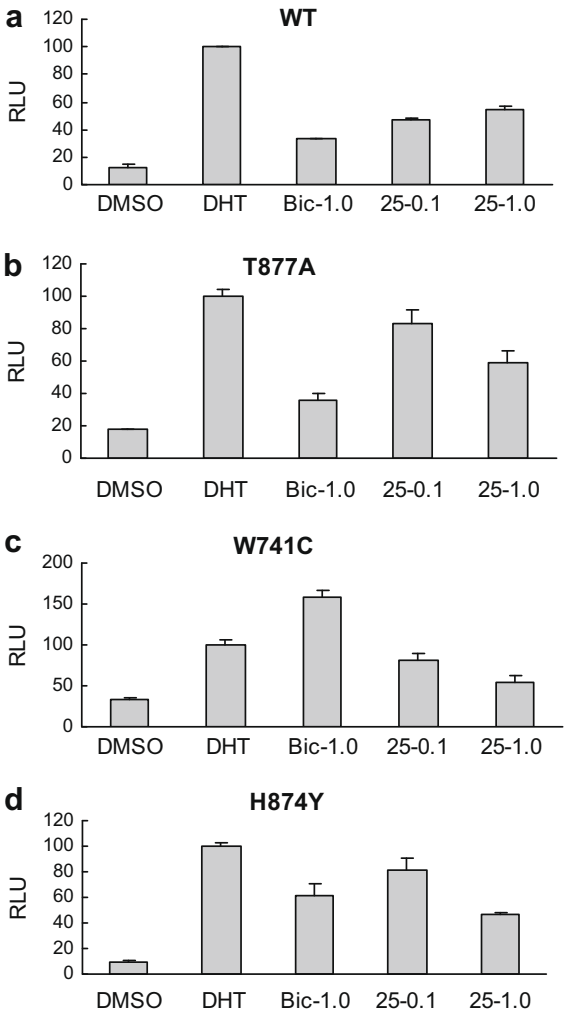


Figure 1. Effect of bicalutamide (1.0 μM) and compound **25** (0.1 and 1.0 μM) on DHT-induced transactivation of the wild type and the mutated ARs (a–d). PC-3 cells were transiently transfected with pCMV-MMTV-Luc, Renilla null luciferase and AR expressing plasmids. The cells were treated with 0.1 nM DHT with and without test compound for 24 h. Relative luciferase activity was determined by dual luciferase assay kit (Promega), standardized to Renilla luciferase control and normalized to 0.1 nM DHT without test compound (100%). Bic, bicalutamide; RLU, relative luciferase unit.

To characterize antiandrogenic activity of chalcone **25**, we have investigated effect of **25** on the DHT-stimulated transactivation of the wild type and the T877A, W741C and H874Y mutated AR in transient transfection experiments, using PC-3 cells (Fig. 1).[15,16] We have included bicalutamide, an antiandrogen used in the clinics, as a control. In consistent with previous studies,[17] our reporter assays revealed that W741C mutation confers resistance to bicalutamide (Fig. 1c). As shown in Figure 1, compound **25** shows dose-dependent activity in suppressing 0.1 nM DHT-induced transactivation of the T877A, W741C and H874Y mutated ARs as well as modest antiandrogenic activity against wild type AR. This indicates that chalcone **25** is a novel pan-antiandrogen effective against the wild type and multiple mutated ARs. Importantly, the AR W741C, T877A and H874Y mutations have been characterized from patients with advanced prostate cancer.[18,19] The W741C and T877A mutations actually result in paradoxical activation by bicalutamide[17] and hydroxyflutamide,[18] respectively. Bicalutamide has been found to promote tumor growth in a novel androgen-dependent prostate xenograft model derived from a bicalutamide-treated patient.[19] The T877A mutant AR promotes prostate cancer cell growth and cell survival.[20] Consequently, compound **25** represents a novel antiandrogen that is simultaneously effective against multiple AR mutants that confer resistance to antiandrogens currently used in the clinics.

In summary, by condensing dietary ionones and substituted benzaldhydes, 43 ionone-based chalcones have been synthesized and evaluated in a panel of prostate cancer cell lines, including LNCaP, MDA-PCa-2b, 22Rv1, C4-2B and PC-3. Among the compounds synthesized, chalcone **25** shows the most potent in vitro cytotoxic activities in prostate cancer cells. However, compound **25** also shows potent cytotoxicity in an androgen-dependent normal prostate cell line (RWPE-1). The AR-dependent reporter assays revealed compound **25** is a novel antiandrogen for the wild type as well as the clinically relevant T877A, W741C and H874Y AR mutants. In addition to its antiandrogenic activity, the substantial cytotoxicity of **25** in AR-negative PC-3 cells indicates this lead compound is a multi-targeting agent. The work to identify possible additional targets for **25** is in progress in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.089.

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13. *Cell growth inhibition assay:* LNCaP, 22Rv1, C4-2B and PC-3 cells were seeded at a density of 6000–7000 cells per well in 96-well microtiter plates in RPMI 1640 supplemented with 10% FBS, 100 U/mL penicillin and 10 mg/mL streptomycin. MDA-PCa-2b cells were seeded at the same density in medium BRFF-HPC1 (Athena Enzyme Systems, Baltimore, MD, USA) supplemented with 20% FBS. RWPE-1 cells were seeded at the same density in keratinocyte serum-free medium supplemented with 0.05 mg/mL bovine pituitary extract and 5 ng/mL EGF (Cedarlane Laboratories, Burlington, ON, Canada). After overnight incubation, the cells were treated with DMSO vehicle or test compounds at various concentrations diluted from DMSO stock solution. The final DMSO concentration is 0.5%. After 72 h of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution were added to each well and incubated for another 2 h. The MTT formazan formed by viable cells was dissolved in isopropanol. The obsorbance was measured at 595 nm by a plate reader (FLUOstar OPTIMA, BMG LabTech). All experiments were performed in triplicate.
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