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A new class of hybrid anticancer agents inspired by the synergistic effects of curcumin and genistein: Design, synthesis, and anti-proliferative evaluation

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ABSTRACT

Inspired by the synergistic effects of dietary natural products with different scaffolds on the inhibition of cancer cell proliferation, incorporation of central (1*E*,4*E*)-1,4-penta-dien-3-one linker (an optimal substitute for the central metabolically unstable diketone linker of curcumin), 1-alkyl-1*H*-imidazol-2-yl (a promising bioisostere of terminal aryl group in curcumin), and chromone (the common pharmacophore in genistein and quercetin) into one chemical entity resulted in ten new hybrid molecules, 3-((1*E*,4*E*)-5-(1-alkyl-1*H*-imidazol-2-yl)-3-oxopenta-1,4-dien-1-yl)-4*H*-chromen-4-ones. They were synthesized through a three-step transformation using acid-catalyzed aldol condensation as key step. The WST-1 cell proliferation assay showed that they have greater anti-proliferative potency than curcumin, quercetin, and genistein on both androgen-dependent and androgen-independent human prostate cancer cells.

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It has been demonstrated that the additive or even significant synergistic effects can be achieved by hybridizing two or more different medicinally active scaffolds into one single chemical entity.[1] Synthesis of natural product hybrids indeed represents a promising approach in the development of leads for medicinal applications.[1] This approach not only inherits the structural complexity and intriguing bioactivities from parent natural products, but also expands the number and scaffold of lead compounds. As compared with conventional combinatorial chemistry, natural product hybrids exhibit advantage in having the superior structural diversity and inherent biological activity of parent compounds.[2]

Searching for effective chemotherapeutics for prostate cancer, especially for advanced metastatic castration-resistant prostate cancer (CRPC), is still in need because (i) no significantly effective chemotherapy is currently available for advanced, metastatic CRPC patients; and (ii) approximately 30,000 American men with advanced metastatic CRPC die each year due to the inevitable progression of resistance to the US FDA-approved first-line treatment with docetaxel.[3,4]

The increased incidence of prostate cancer in first generation immigrants from East Asian countries to the Western countries suggests a preventive and/or therapeutic effect of the diet.[5] The anti-prostate cancer potential and human safety profiles of several dietary natural products with different scaffolds, exemplified by curcumin [**1**, (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione], genistein [**2**, 5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one], and quercetin [**3**, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one] (Fig. 1), have been demonstrated by in vitro cell-based assays, preclinical and clinical studies.[6–17] More interestingly, Ide and coworkers have observed the synergistic antiproliferative effects between curcumin and isoflavones (genistein, daidzein, and glycitein) in LNCaP human prostate cancer cells.[18,19] Also, Aditya et al. has concluded that effects in suppressing PC-3 human prostate cancer cell growth in vitro were much more noticeable when treatment with curcumin and genistein in combination than alone.

Driven by our current interest in developing potent dietary natural products-based anti-prostate cancer agents, this study was proposed based on the synergistic effects of curcumin and genistein on the prostate cancer cell proliferation as described by Ide et al. and Aditya et al.[18–20] Consequently, ten new 3-((1*E*,4*E*)-5-(1-alkyl-1*H*-imidazol-2-yl)-3-oxopenta-1,4-dien-1-yl)-4*H*-chromen-4-ones (**6**–**15**, Scheme 1) were designed and synthesized for the evaluation of their anti-proliferative effects toward both androgen-dependent (LNCaP) and androgen-independent (PC-3 and

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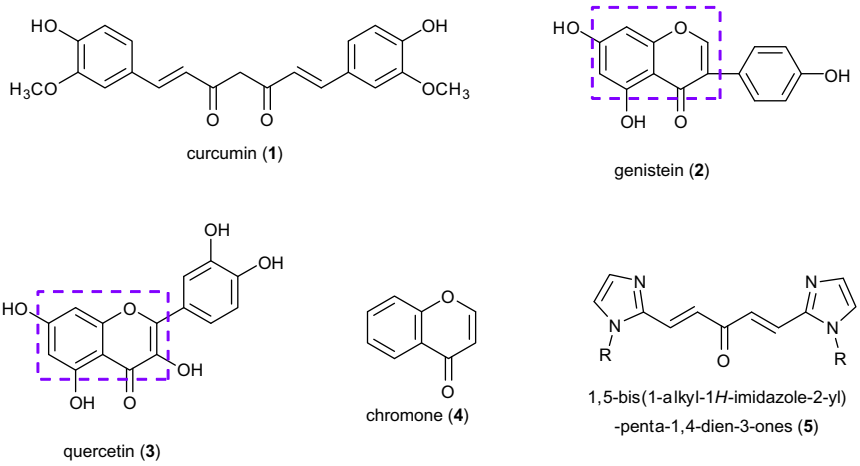
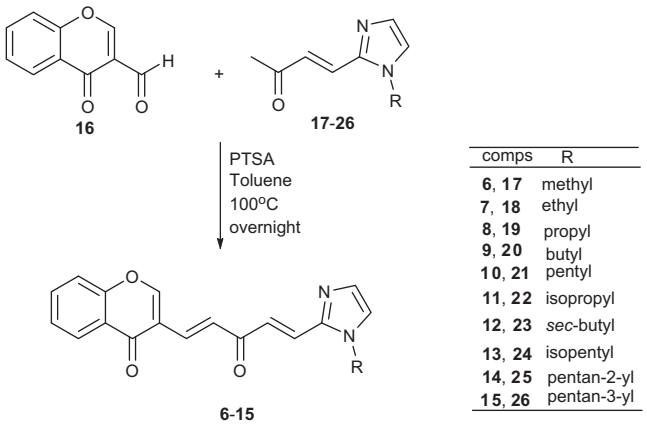


Figure 1. Structures of curcumin, genistein, quercetin, chromone, and 1,5-bis(1-alkyl-1*H*-imidazole-2-yl)-penta-1,4-dien-3-ones.



Scheme 1. Synthesis of 1,5-bis(1-alkyl-1*H*-imidazole-2-yl)-penta-1,4-dien-3-ones (**6**–**15**).

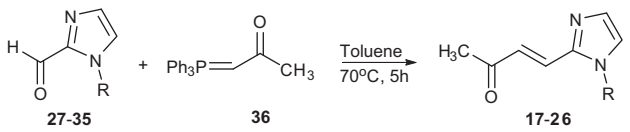
DU145) human prostate cancer cell lines. These target compounds were designed to integrate one terminal chromone moiety, one 1-alkyl-1*H*-imidazol-2-yl, and a central dienone linear linker. The central 1,4-pentadien-3-one linker was selected because it has been verified, by the structure-activity relationship data acquired in our previous research, as an optimal substitute for the central metabolically unstable diketone linker of curcumin as promising anti-prostate cancer agents.[21,22] The nitrogen-containing hetero aromatic rings, 1-alkyl-1*H*-imidazol-2-yl, have also been established by us as one of the optimal bioisosteric replacements for the phenols in curcumin.[22] Chromone was chosen due to the fact that it serves as pharmacophore for genistein and quercetin, as well as other dietary natural products and that it has been valued as a privileged heterocyclic scaffold with attractive anticancer drug-like properties.[23,24] In addition to having a nitrogen-containing heteroaromatic ring, these hybrid molecules, with molecular weight ranging from 306 to 362 and *Clog P* ranging from 1.36–3.35, may have a high probability of good oral bioavailability according to Lipinski's rule of five.[25–27]

As shown in Scheme 1, the ten hybrid molecules (**6**–**15**) have been synthesized through a three-step transformation using acid-catalyzed aldol reaction as a critical step. Specifically, 1,3-((1*E*,4*E*)-5-(1-alkyl-1*H*-imidazol-2-yl)-3-oxopenta-1,4-dien-1-yl)-4*H*-chromen-4-ones (**6**–**15**) were successfully achieved by aldol condensation of 3-formylchromone (**16**) with the appropriate (*E*)-4-(1-alkyl-1*H*-imidazol-2-yl)but-3-en-2-one (**17**–**26**) catalyzed by *p*-toluenesulfonic acid (PTSA) in toluene at 100 °C for 48 h.[28] The PTSA instead of base was selected to catalyze the aldol condensation is because the acid conditions were more amenable to chromone even with relatively high temperatures (100–110 °C) and the basic conditions may promote the ring C opening.[29] We initiated our synthesis with the same procedure described in the literature[28] by treating 1 equiv of ketone with 1 equiv of aldehyde in the present of 0.5 equiv of PTSA for 3 h at 100 °C. The TLC plate showed a newly formed product but with very low conversion rate. In order to optimize the yields, various experiments were carried out with varying amount of 3-formylchromone (**16**) and different reaction time. It was established that 3 equiv of 3-formylchromone (**16**) and 48 h reaction time are the optimal reaction conditions. The excess of 3-formylchromone (**16**) can be easily recycled.

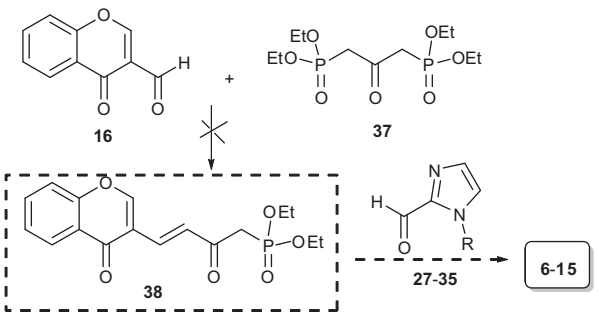
The (*E*)-4-(1-alkyl-1*H*-imidazol-2-yl)but-3-en-2-ones (**17**–**26**) were readily synthesized by the Wittig reaction of 1-alkyl-1*H*-imidazole-2-carbaldehydes (**27**–**36**) with 1-(triphenyl-phosphanylidene)propan-2-one (**36**) (Scheme 2) in toluene at 75 °C for 12 h. The aldehydes (**27**–**36**) were synthesized according to the procedure described in the literature.[21,22,30]

Considering the generally low yields for the hybrid molecules **6**–**15** through the aldol condensation reaction, we have attempted to synthesize them by two sequential Horner–Wadsworth–Emmons (HWE) reactions (Scheme 3). However, this synthetic method was not successful because treatment of 1 equiv of 3-formylchromone (**16**) with same equivalent of tetraethyl (2-oxopropane-1,3-diyl)bis(phosphonate) (**37**) using NaH or K_2CO_3 as base failed to provide the desired intermediate **38**.

The in vitro antiproliferative activity of 1,3-((1*E*,4*E*)-5-(1-alkyl-1*H*-imidazol-2-yl)-3-oxopenta-1,4-dien-1-yl)-4*H*-chromen-4-ones (**6**–**15**) was evaluated using WST-1 cell proliferation assay against both androgen-independent (PC-3 and DU145) and androgen-dependent (LNCaP) human prostate cancer cell lines. These three cell lines represent the most common cell-based models for in vitro assessment of potency and efficacy of antiprostate cancer agents. Curcumin, genistein, and quercetin were used for comparison in the parallel experiments, and DMSO was used as negative control.



Scheme 2. Synthesis of (*E*)-4-(1-alkyl-1*H*-imidazol-2-yl)but-3-en-2-ones (**17**–**26**).



Scheme 3. Attempt to synthesize compounds **6**–**15** by HWE reactions.

Table 1
Antiproliferative activities (IC_{50}[a]) of the hybrid molecules (**6**–**15**) against prostate cancer cell lines

Compd	PC-3[b]	DU-145[b]	LNCaP[c]
Curcumin	25.4 ± 2.2	26.2 ± 0.7	12.1 ± 0.7
Genistein	68.6 ± 3.2	>100	37.4 ± 2.6
Quercetin	>100	>100	45.5 ± 1.3
6	2.8 ± 0.3	4.5 ± 0.2	2.3 ± 0.2
7	1.8 ± 0.3	3.0 ± 0.5	1.0 ± 0.2
8	2.4 ± 0.3	4.6 ± 0.2	2.1 ± 0.9
9	2.0 ± 0.1	4.3 ± 0.1	2.0 ± 0.3
10	2.1 ± 0.3	2.7 ± 0.4	1.8 ± 0.9
11	2.3 ± 1.2	3.3 ± 0.6	1.2 ± 0.6
12	1.8 ± 0.4	3.3 ± 0.4	1.3 ± 0.2
13	3.6 ± 0.1	5.8 ± 0.1	4.0 ± 1.0
14	3.2 ± 0.1	1.4 ± 0.3	3.9 ± 0.5
15	2.9 ± 0.5	5.2 ± 0.5	2.8 ± 0.7

See Supplementary data for experimental details associated with this assessment.
[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 error of the mean.
[b] Human androgen-independent prostate cancer cell line.
[c] Human androgen-dependent prostate cancer cell line.

Curcumin was prepared by Claisen–Schmidt condensation of aromatic aldehyde with acetylacetone according to the procedure described in the literature.[31] The procedure for the WST-1 cell proliferation assay was described in Supplementary data and the IC_{50} value for each compound was determined. The results were summarized in Table 1, which shown that all ten 1,3-((1*E*,4*E*)-5-(1-alkyl-1*H*-imidazol-2-yl)-3-oxopenta-1,4-dien-1-yl)-4*H*-chromen-4-ones (**6**–**15**) were appraised as promising anti-prostate cancer agents by comparing their IC_{50} values with those of curcumin, genistein, and quercetin (Table 1). They are 3–19 times, 9–74 times, 11–74 times, respectively, more potent than curcumin, genistein, and quercetin in three human prostate cancer cell lines. These data indicate that the scaffold with a chromone and a 1-alkyl-1*H*-imidazo-2-yl as terminal aromatic rings and with linear dienone as central linker is a potent template worthy of further development for prostate cancer chemotherapy.

Theses ten hybrid molecules share same scaffold with the only structural difference in having distinct alkyl groups on the nitrogen of imidazole moiety. They show similar IC_{50} values ranging from 1.0 to 5.8 μM for cell proliferation inhibition toward the three prostate cancer cell lines. Elongation of the hybrid alkyl chain did not significantly alter anti-proliferative effect by comparing the IC_{50} values of compound **6** to those of **7**, **8**, **9**, and **10**. Linear alkyl hybrids (**8**, **9**, and **10**) exhibited similar IC_{50} values to their branched versions (**11**, **12**, **13**, **14**, and **15**). All these data suggested that the length and steric hindrance of the alkyl groups on the nitrogen of terminal imidazole cannot contribute considerable effects to the proliferation suppression of prostate cancer cells.

In summary, ten asymmetrical hybrid molecules inspired by the synergistic effects of dietary curcumin and genistein were designed and synthesized through a three-step transformation using PTSA-catalyzed aldol condensation as key step. Their structures are characteristic of a chromone and an imidazole as two different terminal aromatic rings and of a linear five-carbon dienone as the central linker. These hybrid molecules are significantly more potent than curcumin and genistein on inhibiting cell proliferation toward both androgen-dependent and androgen-independent human prostate cancer cells. These findings warrant further investigations to explore the therapeutic potential of this scaffold for the treatment of prostate cancer.

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Supplementary data

Supplementary data (synthetic procedures and structural characterization) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.08.064.

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