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Synthesis and selective cytotoxic activity of novel hybrid chalcones against prostate cancer cells

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ABSTRACT

A new class of hybrid chalcones (**17a–l** & **18a–l**) was synthesized by Claisen–Schmidt condensation. All compounds were characterized by ^{1}H NMR, IR and mass spectral analysis and tested for their cytotoxic activity against PC-3 (prostate cancer), HT-29 (colon cancer), B-16 (mouse macrophages) and NCI-H460 (lung cancer) cell lines. Three compounds **18i**, **18j** and **18l** (IC_{50} = 8.4, 7.9 & 5.9 μM) showed significant activity against PC-3 cell line.

1,3-diaryl-2-propen-1-ones (Chalcones **1**), which are cancer preventive components found in fruits and vegetables, constitute an important class of natural products.[1] They are defined chemically as open chain flavonoids consisting of two aromatic rings joined by a three carbon α,β-unsaturated carbonyl system. The natural and synthetic derivatives of this class of compounds display anticancer activity on various tumor cells.[1,2] They cause cell cycle arrest and apoptosis by interfering with tubulin polymerization into microtubules, which are essential in the process of cell division, trafficking of vesicles and proteins within the cell, and regulation of cell motility.[2d] Natural as well as synthetic 2,3-dihydrobenzofurans (**5–8**) have been identified to possess cytotoxic activities.[3] It is interesting to note that flavonoids and xanthenones bearing a fused dihydrofuro moiety (**8**) display a myriad of biological properties like antifungal, antitumor and aromatase inhibitory activities.[4] Pyrazoles are an important class of bio-active drug targets in the pharmaceutical industry. They are the core structure of numerous anticancer compounds.[5] In addition, pyrazole ring as B-ring of the chalcone moiety (**2**) has been reported to enhance biological activities (Fig. 1).[6]

Design and synthesis of new types of pharmacologically interesting hybrid chalcone analogs for drug discovery have gained much attention during recent years. Several structural modifications to the chalcone template, particularly replacement of either A or B-phenyl ring or both with heterocyclic groups, have been shown to enhance their biological profiles (2–4).[6,7] Even though dihydrofuro- and pyrano-chalcones are also available in nature along with their prenyl- counterparts with good biological properties, less attention was drawn towards these classes of compounds.[8] In nature, the dihydrofuro and -pyrano fused chalcones are believed to be formed by oxidative cyclization of the prenyl analogs. The nature prefers six membered pyrano fused structures to five membered furo- fused ones as exemplified by the relatively high abundance of natural pyrano- fused chalcones. The chalcone derivative (**9**) (Fig. 1), an example of less abundant furo fused chalcone, has been reported to possess antioxidant and antimicrobial properties in addition to being chemo preventive and cytotoxic.[9] Against this backdrop, we designed and synthesized a new class of chalcones with dihydrobenzofuran moiety as A-ring and either substituted phenyl or pyrazole moiety as B-ring joined together by a three carbon chain (2-propen-1-one) and evaluated their cytotoxic activity (Fig. 2).

Synthesis of the ethanones **14** and **16** was started from resacetophenone **10** which could be accessed easily from resorcinol.[10] Mono-allylation of **10** with 3-chloro-2-methylpropene in acetone in the presence of anhydrous potassium carbonate and catalytic sodium iodide gave **11**.[11] Claisen rearrangement of the allyl–aryl ether **11** at 220 °C in *N*,*N*-diethyl aniline afforded **12** in good yield.[12] Treatment of the rearranged product **12** with catalytic amount of *p*-TSA in chloroform at room temperature gave the benzofuran **13** (90%).[13] Benzylation of the phenol **13** with benzyl bromide afforded the ethanone **14** in very good yield. Compound **16**, the regioisomer of **14**, was prepared by benzylation of the

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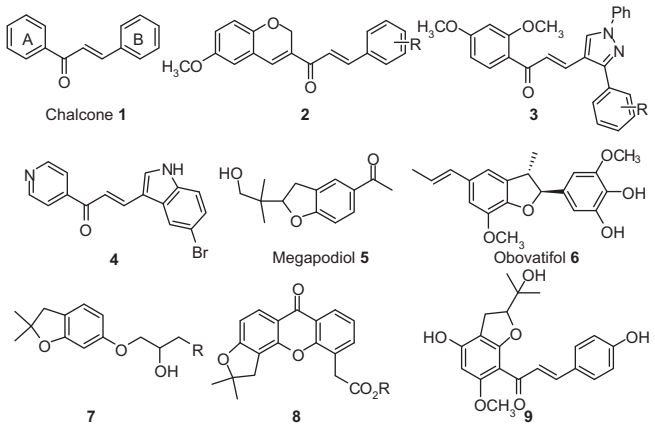


Figure 1. Chemical structures of chalcone, bio-active hybrid chalcones and dihydrobenzofuran derivatives.

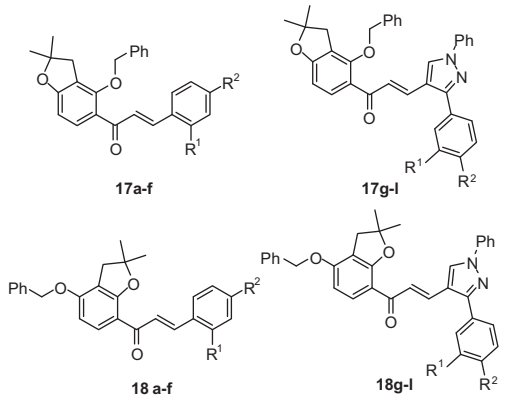
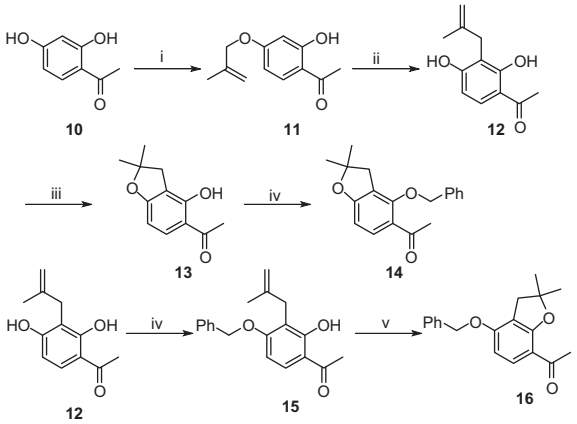


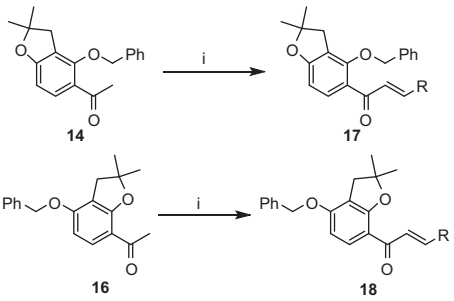
Figure 2. Chemical structures of new hybrid chalcones **17a–i**, and **18a–i**.

4-hydroxy group of **12** followed by cyclization with catalytic amount of *p*-TSA in refluxing toluene scheme 1.

Finally the target 1,3-disubstituted-2-propen-1-ones (**17a–l** and **18a–l**) were prepared by the Claisen–Schmidt condensation[14] of ethanone **14** or **16** with various benzaldehydes and pyrazolaldehydes[15] in ethanolic NaOH (Scheme 2). Our selection of diaryl pyrazoles was based on the excellent biological profiles reported for



Scheme 1. Reaction conditions: (i) 3-Chloro-2-methylprop-1-ene, K_2CO_3,NaI, acetone, reflux, 6 h, 65%; (ii) *N*,*N*-diethylaniline, 220 °C, 4 h, 85%; (iii) *p*-TSA, $CHCl_3$, rt, 6 h, 90%; (iv) $C_6H_5CH_2Br$, K_2CO_3, acetone, reflux, 6 h, (**14**–94%, **15**–90%); (v) *p*-TSA, toluene, reflux, 3 h, 96%.



Scheme 2. Reaction conditions: (i) RCHO, 20%NaOH, EtOH, rt, then 1 M HCl.

Table 1
New hybrid chalcones (Fig. 2)

Entry no.	Compound no.	R^1	R^2	Yield[a] (%)	mp[b] (°C)
1	**17a**	H	H	80	114–116
2	**17b**	H	F	82	138–140
3	**17c**	H	Cl	80	152–154
4	**17d**	H	Br	76	150–152
5	**17e**	H	OCH_3	76	124–126
6	**17f**	Cl	Cl	81	102–104
7	**17g**	H	H	87	120–122
8	**17h**	H	CH_3	79	124
9	**17i**	H	Cl	88	134–136
10	**17j**	H	OCH_3	81	128–130
11	**17k**	H	NO_2	85	152–154
12	**17l**	NO_2	H	87	140
13	**18a**	H	H	75	72–76
14	**18b**	H	F	79	94–96
15	**18c**	H	Cl	89	98–100
16	**18d**	H	Br	79	114–116
17	**18e**	H	OCH_3	83	86–88
18	**18f**	Cl	Cl	86	92–94
19	**18g**	H	H	89	110–112
20	**18h**	H	CH_3	78	164
21	**18i**	H	Cl	87	154
22	**18j**	H	OCH_3	82	134–136
23	**18k**	H	NO_2	91	168
24	**18l**	NO_2	H	86	178–180

[a] Isolated yields after purification.
[b] Uncorrected.

such structures in the literature.[6] Additionally, these compounds could be prepared easily from readily available diaryl pyrazolaldehydes, making the synthesis very facile. In compounds **17a–l**, the A-ring is 2,2-dimethyl-2,3-dihydrobenzofuran with the hydrophobic benzyloxy group *ortho*- to the propenone moiety, while in derivatives **18a–l** A-ring is 2,2-dimethyl-2,3-dihydrobenzofuran with the benzyloxy group *para*- to the propenone moiety. B-ring in both the cases is, either substituted phenyl group (**17a–f** & **18a–f**) or 1-phenyl-3-(substituted phenyl)-pyrazolyl group (**17g–l** & **18g–l**) (Table 1).

The cytotoxic potential of all newly synthesized hybrid chalcones was evaluated in vitro against a panel of four tumor cell lines - prostate cancer (PC-3), colon cancer (HT-29), lung cancer (NCI-H460) and mouse macrophages (B-16)—using MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product.[16] Doxorubicin was used as the reference drug. The results are summarized in Table 2.

The cytotoxicities of these compounds were found to be dependent on the nature as well as substitution patterns of both the rings as shown in Table 2. The new class of compounds showed moderate to significant cytotoxic activity on PC-3 cell lines (IC_{50} = 5.9–50 μM). Compound **17b** with fluorine substituent on the B-ring exhibited very good activity on HT-29 cell lines with IC_{50} value 23.0 μM. In fact, this is the only compound which

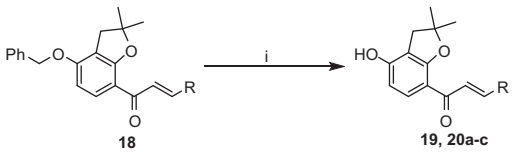
Table 2
Cytotoxic activities (IC_{50}, μM[a]) of hybrid chalcones in vitro[b]

Entry no.	Compound no.	PC-3	HT-29	B-16	NCI-H460
1	**17a**	>100[c]	>100	>100	>100
2	**17b**	44.2	23.0	>100	75.8
3	**17c**	>100	>100	na[d]	>100
4	**17d**	>50	na	na	>100
5	**17e**	48.9	na	>100	>100
6	**17f**	n.a	>100	>100	>100
7	**17g**	>100	>100	>100	na
8	**17h**	24.2	>100	na	>100
9	**17i**	26.0	>100	na	>100
10	**17j**	18.4	>100	na	>100
11	**17k**	62.3	na	na	na
12	**17l**	>100	>100	>50	>100
13	**18a**	na	na	30.3	>100
14	**18b**	na	na	>100	22.3
15	**18c**	>100	na	>100	na
16	**18d**	>100	>100	na	>100
17	**18e**	13.5	na	na	>100
18	**18f**	>100	na	na	na
19	**18g**	n.a	na	>100	na
20	**18h**	>100	na	>100	na
21	**18i**	8.4	na	na	na
22	**18j**	7.9	>100	na	na
23	**18k**	>100	>100	>100	>100
24	**18l**	5.9	na	na	>100
25	Doxorubicin	5.9	19.3	0.03	5.6

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.
[c] IC_{50} > 100 μM.
[d] Not active (<10% inhibition at 100 μM).

showed notable activity on this cell line. Only compounds **17l** and **18a** displayed moderate activity on B-16 cell lines and all other derivatives showed very low or no activity. The isomeric structures, with fluorine substituent on B-ring, **17b** and **18b** only showed cytotoxicity on NCI-H460 cell lines, the latter one with IC_{50} value 22.3 μM.

As shown in Table 2, it is evident that the new hybrid chalcones exhibit moderate to excellent selectivity towards PC-3 cell lines. Among the two regioisomeric core structures **14** and **16**, the derivatives of **16** showed more significant cytotoxic activity. It is also worthy to note that, the derivatives with pyrazole moiety as B-ring exhibited better activity over the phenyl counterparts. **18i**, **18j** and **18l** exhibited significantly higher cytotoxicity with IC_{50} values 8.4, 7.9 and 5.9 μM respectively and most notably showed very low or no activity on the other three cell lines. Compounds **17j** and **18e** also displayed good cytotoxic activity with IC_{50} values 18.4 and 13.5 μM respectively. Derivatives with methoxy substituent **17e**, **17j**, **18e** and **18j** showed selectively against the PC-3 cell lines. To better understand the results, we compared the present results of hybrid structures with non hybrid ones available in the literature. Most of the chalcone derivatives, without the fused dihydrofuro ring, have been reported to exhibit anti-inflammatory, antimicrobial and antioxidant properties.[6a,c] In a recent report, pyrazolyl chalcone derivatives, without the fused dihydrofuro ring



Scheme 3. Reaction conditions: (i) $TiCl_4$,CH_2Cl_2, 0 ° rt, 3 h.

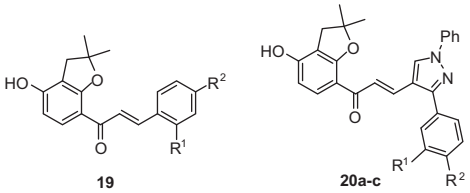


Figure 3. Chemical structures of debenzylated compounds **19**, and **20a–c**.

Table 3
Cytotoxic activity (IC_{50}, μM[a]) of debenzylated compounds in vitro[b]

Entry no.	Compound no.	R^1	R^2	Yield[c] (%)	mp[d] (°C)	PC-3
1	**19**	H	OCH_3	90	108–110	48.4
2	**20a**	H	Cl	92	158–160	18.0
3	**20b**	H	OCH_3	93	146–148	20.8
4	**20c**	NO_2	H	95	168	25.6

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.
[c] Isolated yields after purification.
[d] Uncorrected.

have been screened against 60 different human tumor cell lines and found to be active against leukemia (K-562 and SR), renal cancer (UO-31) and non-small cell lung cancer (HOP-92) cell lines.[6b] It is also interesting to note that pyrazolyl chalcones without the dihydrofuro fusion are effective inhibitors of COX-2[6c] and show antitubercular properties.[6d] Preliminary biological data of the compounds reported herein suggests that the difference in biological profiles is mainly due to the hybridization of the heterocycles in the chalcone core structure.

In order to evaluate the effect of phenolic benzyl moiety, which initially was introduced as a protecting group to enable the synthesis, we prepared the *O*-debenzyl analogs of a few selected compounds with better activity. Treatment of **18e**, **18i**, **18j** and **18l** with $TiCl_4$ in dry CH_2Cl_2 gave the free phenolic products **19** and **20a–c** respectively in very good yields (Scheme 3, Fig. 3 & Table 3). These four compounds were tested for their cytotoxic activity against PC-3 cell lines. They showed less activity compared to the corresponding benzylated counterparts. The IC_{50} values of **18e**, **18i**, **18j** and **18l** (13.5, 8.4, 7.9 and 5.9 μM) are higher than the corresponding debenzylated derivatives **19** and **20a–c** (38.4, 18.0, 20.8 and 25.6 μM) against PC-3 cell lines (Table 3). This could plausibly due to the lower hydrophobicity of the debenzylated compounds, compared to their benzylated counterparts.

In the present work, we have focused our attention on the synthesis and preliminary cytotoxic activity of a new class of hybrid-chalcones against four cell lines (PC-3, HT-29, B-16 and NCI-H460). Most of the synthesized compounds are found to be selective against PC-3 cell line. A comparison with the literature available data indicates that fusing dihydrofuro moiety indeed imparts difference in activity. As the selectivity of the drug is an important parameter in cancer chemotherapy, the newly synthesized compounds could be potential anticancer drug candidates after further structure optimization. Compounds **18i**, **18j** and **18l** (IC_{50} = 8.4, 7.9 & 5.9 μM) in particular showed significant activity. Further studies involving the structural modifications and their cytotoxic activity studies on PC-3 cell lines are currently in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.016.

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13. This reaction when carried out with *p*-TSA in refluxing toluene gave a mixture of regioisomers **13** and debenzyl derivative of **16** in 85:15 ration. Both the isomers were separated by column chromatography and characterized their structures (**13** & **13a**). (See Supplementary data).
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