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Synthesis and biological evaluation of retinoid-chalcones as inhibitors of colon cancer cell growth

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ABSTRACT

Based on the observed anticancer activity of chalcones and retinoids, a novel class of retinoid-chalcone hybrids was designed and synthesized. As part of our ongoing studies to discover natural product based anticancer compounds, the retinoid-chalcone hybrids were tested against the colon cancer cell line HT-29. Retinoid like moiety was introduced through Friedel–Crafts alkylation of toluene. Among the synthesized compounds, the cyano derivative (*E*)-3-(3-oxo-3-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)prop-1-enyl)benzonitrile **8** showed submicromolar inhibitory activity with an IC_{50} of 0.66 μM.

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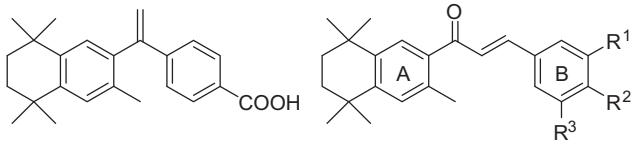
Chalcones are secondary metabolite precursors of flavonoids and isoflavonoids, which are commonly found in edible plants. The presence of flavonoids in fruits and vegetables has been related to the health benefits of this food group. A good safety profile, possibility of oral administration,[1] and easy synthesis are the major factors contributing to the increasing interest in exploring the pharmacological activities of chalcones. In addition, chalcones have been reported to exhibit several biological activities, including antitumor,[2,3] anti-inflammatory,[4,5] immunomodulatory,[6] antibacterial,[7] antimalarial,[8] antileishmanial,[9] trypanocidal,[10] and nitric oxide inhibitory activity.[11] Chalcones comprise one of the main classes of natural small molecules with very promising anticancer activity, related to their ability to inhibit tubulin polymerization.[12] Several groups have focused on the antitumor activity of this class of compounds. There are a number of reports on the activity of chalcones against several cell lines including prostate[13] and breast cancer[14] in low nanomolar concentration.

Retinoids are derivatives of vitamin A. Investigation of the therapeutic use of this class of compounds dates back to the 1970s.[15] However, toxicity has limited the clinical use of retinoids. This toxicity is related to the high hydrophobic nature of the compounds, and is usually presented as an over-expression of the physiological functions of the retinoids.[16] Retinoids are regulators of important biological events, including cell differentiation and proliferation;[12] thus, they have been developed as anticancer agents. One example of a drug belonging to this class is targretin, a synthetic retinoid available in the market for treatment of cutaneous T-cell lymphoma.[17]

In an attempt to improve anticancer activity, a retinoid moiety was introduced into chalcone template, generating a class of retinoid-chalcone hybrids. Similar work has been done by Kagechika et al. in which the authors synthesized hybrid chalcones with strong ability to induce differentiation of HL-60 cells into mature granulocytes.[18] In another study, Romagnoli et al. have reported increased antiproliferative activity of hybrid α-bromoacryloylamido chalcones compared to the corresponding amino chalcones.[2]

In this work, a series of retinoid-chalcone hybrids (Fig. 1) was synthesized. The tetramethylcyclohexane group on ring A was fixed, and diversity was created by introducing different substituents on ring B. The rationale for fixing ring A was based on the previously mentioned work of Kagechika et al.,[18] where the authors reported the synthesis of chalcones containing a tetramethylcyclohexane group in ring A with stronger activity than retinoic acid.

All molecules were tested for inhibition of growth of HT-29 cells. The HT-29 cell line was chosen as a representative human



Targretin Retinoid-chalcone hybrids

Figure 1. Chemical structure of targretin and retinoid-chalcone hybrids.

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colon cancer cell line; these colon carcinoma cells are *Apc*-null and contain wild-type β-catenin protein, and they respond well to compounds to induce E-cadherin protein levels and decrease c-Myc and cyclin D1.[19–22]

Synthesis of retinoid-chalcone hybrids is shown in Scheme 1. Toluene **1** underwent Friedel–Crafts alkylation[23] using 2,5-dichloro-2,5-dimethylhexane and aluminum chloride in dichloromethane to afford tetrahydronaphthalene **2**. Friedel–Crafts acylation[23] of **2** in the presence of acetyl chloride gave **3**. Coupling of acetophenone **3** with different aldehydes was accomplished using NaOH in ethanol and afforded the retinoid-chalcone hybrids. Reduction of the nitro derivative **20** using sodium dithionite[24] gave amino compound **15**. Prenylated chalcones **16** and **17** were synthesized from O-alkylation of chalcones-bearing hydroxyl groups using potassium carbonate according to procedures by Johnston et al.[25]

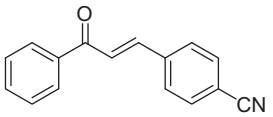
The retinoid-chalcone derivatives were tested for their growth inhibitory effect on HT-29 cell lines.[21] The cells were incubated with varying concentrations of the test compounds for 3 days at 37 °C. The results of the assay are shown in Table 1.

Structure activity relationship studies were performed on the B-ring of the retinoid-chalcone hybrids. Groups such as methoxy, halogens, hydroxyl, amino and tetrazole were introduced on the 3′,4′, and 5′ positions to generate diversity. Results from the biological assay showed that, in general, electron-withdrawing groups at the *meta* position of the benzene ring are better for activity. Thus, the best compound was the cyano derivative **8** with an IC_{50} = 0.66 μM. Changes in the substitution patterns have different effects on the inhibitory activity depending on the functional group on B-ring. Shifting the cyano group from the *meta* (**8**) to the *para* (**6**) position decreased activity almost three fold, while the same change with the nitro containing hybrids (**20** and **12**) did not affect activity.

Electron donating groups at the *meta* position were not as effective as the electron-withdrawing groups. Compound **14** with a hydroxyl group was four fold-less active than cyano derivative **8**. Bulky groups at the *para* position are less favored than at the *meta* position. This is supported by comparing the activity of *meta*-substituted compound **17** (IC_{50} = 3.73 μM) and *para*-substituted compound **16** (IC_{50} = 13.33 μM). Furthermore, compound **21** with a tetrazole group was the least active. With the exception of compound **12**, the *para*-substituted compounds showed the lowest inhibitory activity, indicating that substitution at that position is not well tolerated.

When the activity of compounds **5** and **6** was compared, it was noticeable that, for this particular pair of compounds, the introduction of the retinoid moiety improved the activity. However, having the retinoid group alone does not guarantee activity; the activity varied depending upon the substituents in the benzene ring B, as evident from the IC_{50} values.

Table 1
Anticancer activity of retinoid-chalcone derivatives against HT-29 cells



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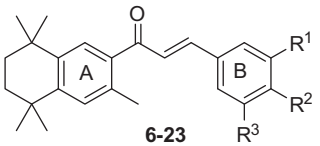


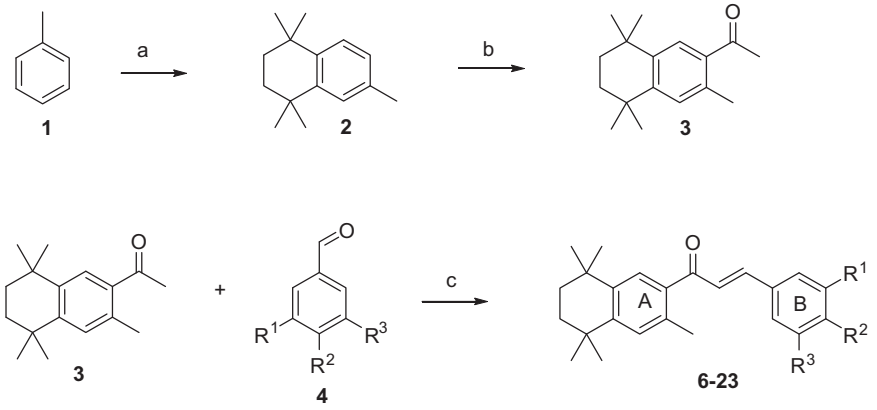
6-23

Compound	R^1	R^2	R^3	IC_{50} ± SD[a] (μM)
5				3.70 ± 0.21
6	H	CN	H	1.94 ± 0.57
7	H	H	H	2.86 ± 1.09
8	CN	H	H	0.66 ± 0.25
9	H	OCH_3	H	4.13 ± 1.06
10	H	CF_3	H	2.83 ± 0.35
11	H	Br	H	5.02 ± 0.17
12	H	NO_2	H	1.46 ± 0.22
13	H	$N(CH_3)_2$	H	6.59 ± 0.33
14	OH	H	H	2.66 ± 0.43
15	NH_2	H	H	6.99 ± 1.22
16	H	$OCH_2CHC(CH_3)_2$	H	13.33 ± 1.40
17	$OCH_2CHC(CH_3)_2$	H	H	3.73 ± 0.07
18	OCH_3	H	OCH_3	1.54 ± 0.16
19	OCH_3	OCH_3	H	8.32 ± 1.59
20	NO_2	H	H	1.45 ± 0.04
21	H	5-1H tetrazole	H	23.52 ± 1.00
22	Cl	Cl	H	8.12 ± 0.27
23	OH	OCH_3	H	1.59 ± 0.14

[a] The experiments were performed twice and the average values were obtained from two independent experiments.

For disubstituted compounds, a 3,5-substitution appeared to be better than a 3,4-substitution. Thus, 3,5-dimethoxy-substituted **18** showed better activity than 3,4-dimethoxy derivative **19** and 3,4-dichloro derivative **22**. Interestingly, however, 3,4-substituted compound **23** had better activity than either of the *meta*-monosubstituted compounds **14** and **9.** It appeared that, when together as substituents in the same molecule, the hydroxy and methoxy groups improve activity. Unsubstituted compound **7** showed moderate inhibitory activity, which was better than most of the *para*-substituted compounds.

In this work we described the synthesis and biological evaluation of a group of retinoid-chalcone hybrids. The compounds were tested against colon cancer cell lines HT-29 and the most inhibitory compound **8** showed activity in the low micromolar range. In gen-



Scheme 1. Synthesis of retinoid-chalcone hybrids. Reagents and conditions: (a) 2,5-dichloro-2,5-dimethylhexane, $AlCl_3$, DCM, 2 h, rt; (b) acetyl chloride, $AlCl_3$, DCM, 2 h, reflux; (c) NaOH, EtOH, rt, 15 h.

eral, from SAR point of view, the *meta*-substituted compounds showed better activity than the *para*-substituted compounds.

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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.2010.10.038.

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