Antimitotic and Antiproliferative Activities of Chalcones: Forward Structure–Activity Relationship

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A series of 59 chalcones was prepared and evaluated for the antimitotic effect against K562 leukemia cells. The most active chalcones were evaluated for their antiproliferative activity against a panel of 11 human and murine cell cancer lines. We found that three chalcones were of great interest as potential antimitotic drugs. In vivo safety studies conducted on one of the most active chalcones revealed that the compound was safe, allowing further in vivo antitumor evaluation.

Introduction

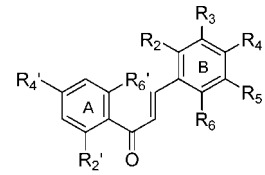
Interfering with the dynamic instability of microtubules, spindle poisons arrest dividing cells in G2/M phase of the cell cycle, causing apoptotic cell death. Many clinically successful anticancer drugs acting as antimitotics are being used worldwide. Most of these molecules are derived from naturally occurring compounds and act by stabilization or destabilization of microtubules.[1] Among the natural products affecting microtubule dynamics are colchicines, the vinca alkaloids, combrestatin A4, epothilone, and taxanes.[2] Flavonoids are naturally occurring polyphenols possessing a variety of biological activities.[3] The health benefits of fruits and vegetables is partially due to the presence of flavonoids in substantial amounts.[4] The anticancer potential of flavonoids and their biogenetic precursors have been investigated.[5] In this regard, the example of flavopiridol, a synthetic flavone acting as a cyclin-dependent kinase inhibitor with potent activity in chronic lymphocytic leukemia, is illustrative.[6] Chalcones that are flavone precursors have been investigated for their antiproliferative effect.[7] Recent and evident structure–activity relationship studies are being emerged.[8–10]

As part of a subsequent study to determine the important features of flavone precursors influencing their anticancer activity, we disclose here additional structural requirements for the antimitotic activity of chalcones. Combined with recently reported data, this study will aid in the design of more active, selective, and safe chalcones.[8–10]

The activity of chalcones was found to be dependent on the presence, the number, and the positions of hydroxy and methoxy groups in both A and B rings.[11–17] The present article is mostly focused on the synthesis of chalcones bearing hydroxy, methoxy, and halogens and effects on cell cycle and cell growth to address additional elements of structure−anticancer activity (Figure 1).

Chemistry

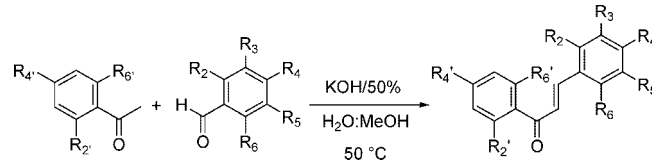
Chalcones are prepared by the Claisen−Schmidt condensation of an acetophenone derivative with substituted benzaldehydes



R_2-$R_{6'}$ = H, OH, OMe, OEt, F, Cl, NH_2

Figure 1. General structure of targeted chalcones.

Scheme 1. Synthesis of Chalcones; R2−R6 are Shown in Tables 1 and 2



in the presence of KOH (50%) (Scheme 1). The 4′-*N*-acetyl-2′,6′-dimethoxyacetophenone needed for the preparation of chalcone **56** (Table 1) was prepared according to an earlier report.[18] Chalcones bearing ethoxy groups were prepared by condensation of the appropriate ethoxyacetophenone with the required ethoxybenzaldehyde. In this case, ethoxyacetophenones and ethoxybenzaldehydes were obtained by alkylation of hydroxylated derivatives with bromoethane in the presence of NaH in DMF.

Results

Overall, 59 chalcones were obtained and tested in vitro for the antimitotic activity on human leukemic K562 cell line. Their structures and the corresponding cell cycle arrest are presented in Tables 1 and 2. Cells were exposed to test compounds at a concentration of 10 μM for 24 h and stained with propidium iodide and analyzed by flow cytometry to determine the distribution of the total population in the different phases (G0/G1, S, and G2/M). Compounds inducing G2/M arrest equal to or higher than our reference compound, vincristine (VCR) were evaluated for the antiproliferative effect against a panel of cell lines representing different types of cancer. Chalcones **3**, **6**, **13**, and **39** induce G2/M arrest

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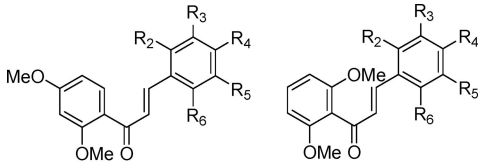
higher than vincristine, whereas chalcones **52** and **56** are equally potent than the reference compound. Based on the results obtained from cell cycle arrest, chalcones **6**, **13**, and **39** were selected and screened for their ability to inhibit cell growth using the MTT assay on a series of 11 human and murine cell lines representative of various solid tumors and hematological malignancies (Table 3). The IC_{50} concentration was calculated as the drug concentration resulting in 50% loss of cell viability with reference to untreated cells after 24 h incubation.

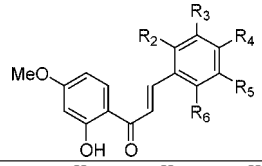
Chalcone **13** was selected for in vivo studies to check its toxicity on healthy animals before proceeding to efficacy testing. The compound was formulated in a H_2O−PEG system to reach a solubility of 0.35 $g \cdot L^{-1}$. Toxicity studies were performed on groups of five animals treated at different dose levels. Intraperitoneal injections were administered three times a week up to a total of 8 injections. This molecule was well tolerated up to the maximum dose level tested, 1 mg/kg. Tolerance was considered satisfactory because none of the animals died and animal weight did not vary by more than 10%.

Table 1. Cell Cycle Arrest Induced by Chalcones **1–49** at 10 μM against K562 Cell Line[a]

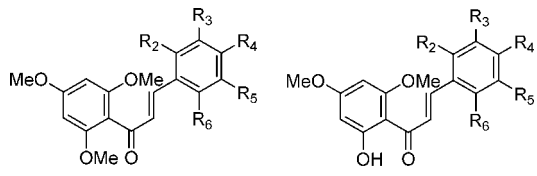


1-4 **5-22**

cmpd	R_2	R_3	R_4	R_5	R_6	G2/M	ClogP
1	H	F	H	H	H	32	4.01
2	Cl	H	H	H	H	42	4.58
3	OMe	H	OMe	H	OMe	**86**	3.87
4	OMe	H	OMe	H	H	25	3.88
5	H	H	H	H	H	45	3.87
6	OMe	H	H	H	OMe	**78**	3.88
7	F	H	H	H	H	38	4.01
8	Cl	H	H	H	H	23	4.58
9	H	OMe	H	H	H	29	3.79
10	H	F	H	H	H	27	4.01
11	H	Cl	H	H	H	19	4.58
12	OMe	H	H	H	H	18	3.79
13	OMe	H	OMe	H	OMe	**84**	3.87
14	Cl	H	H	H	Cl	39	5.3
15	H	H	CF_3	H	H	23	4.75
16	OEt	H	OEt	H	OEt	12	5.46
17	OMe	OMe	OMe	H	H	19	3.17
18	OMe	H	OMe	OMe	H	49	3.52
19	H	OMe	OMe	OMe	H	25	3.17
20	OMe	F	OMe	F	OMe	46	3.61
21	Me	H	Me	H	Me	23	5.37
22	OMe	H	OMe	H	H	22	3.88



23	Cl	H	H	H	H	67	4.74
24	H	H	Cl	H	H	10	4.74
25	Cl	H	H	H	OMe	45	4.8
26	Cl	H	H	H	F	19	4.89
27	H	F	H	H	H	40	4.17
28	H	OMe	H	H	H	26	3.95
29	OMe	OMe	H	H	H	22	3.69
30	H	OMe	OMe	H	H	18	3.69
31	OMe	H	OMe	H	H	18	4.04
32	OMe	H	H	OMe	H	32	4.04
33	OMe	H	H	H	OMe	18	4.04
34	H	OMe	H	OMe	H	40	4.04
35	OMe	OMe	OMe	H	H	20	3.33
36	OMe	H	OMe	OMe	H	15	3.68
37	OMe	H	OMe	H	OMe	19	4.03
38	H	OMe	OMe	OMe	H	20	3.33

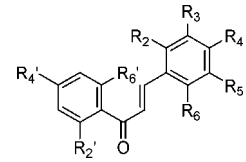


39-41 **42-49**

39	OMe	H	OMe	H	OMe	**86**	3.83
40	OMe	H	OMe	H	H	15	3.83
41	OMe	H	H	H	OMe	64	3.83
42	OMe	H	H	H	OMe	12	4.01
43	Cl	H	H	H	H	43	4.72
44	H	F	H	H	H	48	4.15
45	H	OMe	H	OMe	H	11	4.01
46	H	H	H	H	H	28	4
47	Cl	H	H	H	Cl	26	5.43
48	OMe	H	H	H	H	41	3.92
49	H	OMe	H	H	H	52	3.92

[a] Compounds that induced equal or higher G2/M arrest than vincristine are in bold.

Table 2. Cell Cycle Arrest Induced by Chalcones **50–59** at 10 μM against K562 Cell Line



cmpd	$R_{2'}$	$R_{4'}$	$R_{6'}$	R_2	R_3	R_4	R_5	R_6	G2/M	ClogP
50	H	H	H	OMe	H	H	H	OMe	34	3.63
51	3′-Cl	Cl	H	OMe	H	OMe	H	OMe	30	5.03
52	3′-OMe	OMe	H	OMe	H	OMe	H	OMe	**72**	3.52
53	OEt	H	OEt	OMe	H	OMe	H	OMe	10	4.93
54	OEt	H	OEt	OMe	H	H	H	OMe	14	4.94
55	OEt	H	OEt	OEt	H	OEt	H	H	11	6
56	OMe	NH_2	OMe	OMe	H	OMe	H	OMe	**74**	2.95
57	OEt	OEt	OEt	OEt	H	OEt	H	OEt	18	7
58	H	H	H	Cl	H	H	H	Cl	28	5.05
59	OH	OMe	OMe	3,4-methylendioxy					13	3.97
VCR									**72**	4.01

Discussion

The present article is focused on the synthesis of specific chalcones, notably methoxylated and to less extent hydroxylated derivatives. This choice was motivated by a recent report disclosing a natural chalcone, 2′-hydroxy-2,3,4′,6′-tetramethoxychalcone as an agent against the cancer cell.[19] On the other hand, a polymethoxyphenyl moiety is frequently met in the number of naturally occurring anticancer agents such as colchicine and combretastatin A. In an effort to contribute to robust SAR studies and develop potential anticancer drugs, we targeted chalcones possessing hydroxy and methoxy groups at the 2′,4′,6′-positions of the A-ring and methoxy groups at different positions of the B-ring. The results shown in Tables 1 and 2 indicate that the most potent chalcones belong to the series with an optimum of lipophilicity (CLOGP around 4), suggesting that higher lipophilicity lowered the cell permeation and thus reduced dramatically the cell arrest activity of the resulting derivatives. This phenomenon is particularly evident for the replacement of the methoxy groups by ethoxyl or methyl groups (**13** vs **16** and **21** or **6** vs **54**). We also observed a clear correlation between the cell cycle arrest and the methoxylation pattern. The methoxylation did not affect greatly the lipophilicity of examined chalcones but reflected modifications in their pharmacodynamic interactions with biological targets. Indeed, it appeared that dimethoxylation and trimethoxylation at 2′,4′,6′-carbons are highly beneficial (compounds **3**, **6**, **13**, **39**, **41**, **52**). The

Table 3. Cytotoxicity of Chalcones **6**, **13**, and **39** against Different Cell Lines

	chalcone (IC_{50}, μM)[a]		
cell line	**6**	**13**	**39**
MCF7	75	60	52
N2A	55	2.2	4
NIH3T3	60	30	30
SW48	10	0.25	0.8
HNO150	62	1.3	10
HCT116	9	0.45	1
Messa	10	1	2.2
CEM	6	0.65	1.9
K562	80	50	50
RL	40	0.8	0.9
L1210	34	7	8.5

[a] IC_{50} was determined with reference to a standard curve constructed for control cells and represents the concentration that results in a 50% decrease in cell growth after 24 h incubation.

importance of methoxylation on the A-ring was previousley reported and discussed by Ducki and co-workers.[10,14] Indeed, a number of potent anticancer chalcones having three methoxy groups at 3′,4′,5′-positions were reported (IC_{50} within the nM range). The presence of a hydrogen-bond donor such as an NH_2 at 4′-position (compound **56**) did not affect the cell cycle arrest showing that this position may tolerate a variety of substituents. The beneficial effect of a NH_2 group at 4′-position has been recently pointed out by Robinson and co-workers.[11] The enhanced activity of 2′,6′-dimethoxy derivatives indicates that the conformation of the acyl substituent is an important parameter for the binding to biological targets because di-*ortho*-substitution drives by electrostatic repulsion the dihedral angle between the A-ring and the carbonyl group to 90°. The issue related to the conformation of chaclcones versus anticancer activity has been adressed by Ducki et al.[14,20]

The hydroxylation at 2′ is generally undesirable (e.g., **41** vs **42** and **3** vs **37**). It is conceivable that the negative effect of a hydroxy group at 2′-position is due to a flat geometry induced by the intramolecular hydrogen bond between the hydroxyl and carbonyl groups. This observation is in contrast with the results reported by Rao and co-workers,[13] showing high cytotoxic 2′-hydroxylated chalcones against Jurkat and U937 cancer cells. In the case of dimethoxylated chalcones on the A-ring, it is shown that the most suitable positions for methoxylation are 2′,4′ or 2′,6′ (derivatives **3** and **13** vs **52**).

At the B-ring, it is clear that 2-, 4-, and 6-positions are the most suitable positions for substitution (compounds **3**, **6**, **13**, **39**, **41**, **52**, **56**). The substitution pattern can include two or three methoxy groups at the above positions. For dimethoxylated derivatives on the B-ring, the methoxy groups should preferably be linked to carbons 2 and 6 (**6** vs **22**). However, the topological requirements for methoxy substitution on the B-ring are less demanding than those of the A-ring, suggesting that this phenyl ring is not a strong pharmacophoric element for the pharmacodynamic behavior of these compounds. Several attempts to drive 2D or 3D quantitative relationships were performed using a wide panel of recently developed in silico methods. Unfortunately, no predictive model was obtained with regard to the low internal predictive power. We believe that, to interfere with the different phases of the cell cycle, the compounds have to permeate the cell and the nucleus membranes. Variations of molecular structure of examined chalcones influence differently pharmacodynamics and permeation mechanisms, and these complex mechanisms, quantified by cell growth inhibition results, are presumably responsible for the lack of global QSAR models.

Chalcone **6**, one of the most active chalcones reported by Bowen and co-workers,[12] was prepared and its growth inhibition against 11 cancer cell lines was compared to chalcones **13** and **39**. As shown in Table 3, it is clear that higher antimitotic activity is correlated to strong antiproliferative effect. The IC_{50} values observed, in the micromolar range, are in keeping with those observed in human serum with a number of commercially active anticancer agents, such as cytarabine, methotrexate, or cyclophosphamide.

Although additional data, such as clearance studies, are required, the preliminary toxicity data obtained on chalcone **13** suggest that it is a good candidate for further in vivo development.

The present investigation allowed the identification of active chalcones with anticancer activities and brings new structural elements that will aid in the design of more active chalcones. One of the most active chalcones (chalcone **13**) was formulated and evaluated in vivo for toxicity in healthy animals. The lack of toxicity makes this compound and probably its analogs (chalcones **3** and **39**) good candidates for in vivo evaluation for antitumor chemotherapy.

Experimental Section

Chemistry. Melting points were measured on a Fisher micromelting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance 400. Mass spectra were obtained on a JEOL HX-110 spectrometer. Elemental analysis were performed by the Analytical Department of CNRS, Vernaison, France. Chemicals and reagents were obtained either from Aldrich or Acros companies. PEG 300 (Lutrol E300) was kindly donated by BASF (Germany).

Synthesis of Chalcones: Typical Procedure. To a stirred solution of acetophenone (1 mmol) and a benzladehyde derivative (1 mmol) in MeOH (10 mL) was added KOH (50% aqueous solution, 1 mL). The solution was heated at 70 °C for 3−5 h, MeOH was evaporated, and the residue was dissolved in CH_2Cl_2/H_2O (50 mL, 4:1). The organic layer was washed with brine and evaporated. After this, column chromatography was carried out over silica gel (AcOEt/hexane 1:2). In the case of fluorinated chalcones, KOH (25%) was used.

Formulation of 13 for Animal Experiments. Chalcone **13** was dissolved in a mixture of 50/50 PEG 300/injectable water (% v/v). The solution was magnetically stirred at ambient temperature in a sealed container for 4 h. A volume of 25 mL of the formulated solution was filtered using sterile 0.22 μm PVDF filtration devices (Roth Sochiel, Germany) and introduced into 50 mL glass vials, previously sterilized by autoclaving. The amount of dissolved **13** was assayed spectrophotometrically at 347 nm in absolute ethanol. Solutions without **13** were similarly prepared as references.

Flow Cytometry Analysis of Cell Cycle. Cells were treated with the test compound at 10 μM for 24 h. After drug exposure, 10^6 cells/mL were resuspended in 2 mL of propidium iodide solution (50 μL/mL), incubated at 4 °C overnight, and then analyzed by flow cytometry. The G2/M fraction of cells exposed to different compounds was performed on a FACScalibur (Becton Dickinson, San Jose, U.S.A.). Cell cycle distribution was calculated after exclusion of cell doublets and aggregates on a FL2-area/FL2-width dot plot using Modfit LT 2.0 software (Verity Software Inc., Topsham, U.S.A.).

MTT Cytotoxicity Assays. Cell viability was determined on exponentially growing K562 cells using the MTT assay, as previously described.[21] This assay is based on the conversion by metabolically active cells of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into formazan crystals, which are then dissolved in an isopropanol solution. Optical density is measured by spectrometry. Briefly, asynchronously growing cells were transferred into 96-well cultures plates (Costar, Corning Inc., NY) in 100 μL of medium, with a final cell concentration of 3×10^3 cells/well and incubated in media for 24 h. Corresponding drug concentrations were then added to each plate. After 72 h of drug exposure (10 μM), 20 μL of MTT reagent (5 mg/ml) were added

to each well. Cell viability was expressed as the percent of absorbance of treated wells relative to the untreated control wells. Assays were performed in triplicate in at least three separate experiments. Cell lines used for cytotoxicity assays included MCF7, a human breast cancer line; N2A, a murine neuroblastoma line; NIH3T3, a murine fibroblastic line; HNO150, a human ENT line; HCT 116 and SW48, human colorectal cancer lines; Messa, a human sarcoma line; K562 and CEM, human leukemic lines; RL, a human lymphoma line; and L1210, a murine leukemic line.

Supporting Information Available: Physical (melting points and elemental analysis) and spectral data (^{1}H NMR). This material is available free of charge via the Internet at http://pubs.acs.org.

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