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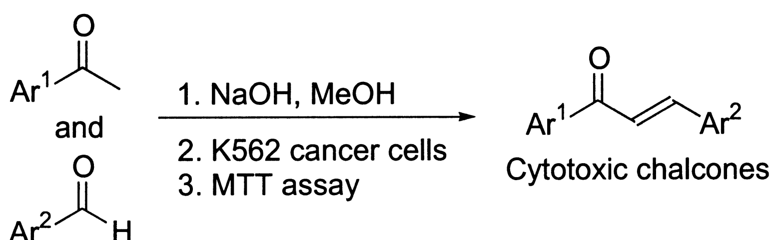

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Linked Parallel Synthesis and MTT Bioassay Screening of Substituted Chalcones

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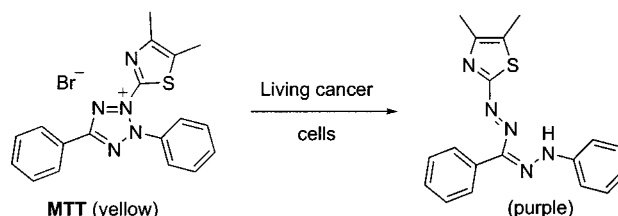
A 644-membered library of chalcones was prepared by parallel synthesis using the Claisen−Schmidt base-catalyzed aldol condensation of substituted acetophenones and benzaldehydes. The cytotoxicity of these chalcones was conveniently determined upon the crude products directly in 96-well microtiter test plates by the conventional MTT assay. This method revealed seven chalcones of IC_{50} less than 1 μM of which 4′-hydroxy-2,4,6,3′-tetramethoxychalcone (**5a**) was the most active [IC_{50} (K562), 30 nM]; it causes cell cycle arrest at the G_2/M point and binds to tubulin at the colchicine binding site.

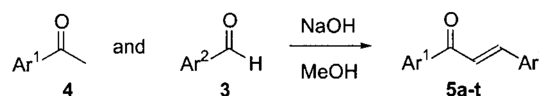
The use of combinatorial[1,2] and parallel synthesis[3] as tools for the discovery of new therapeutic agents is now widespread. Reports are now beginning to appear that detail the combinatorial approach to the development of new anticancer drugs.[4] These include peptide antagonists of TNF-α,[5] cytotoxic peptides,[6] tyrosine kinase inhibitors,[7] microtubule stabilizing agents sarcodictyins[8] and epothilones,[9] and platinum drugs.[10] Anticancer drugs are often developed using cell cytotoxicity as the primary screening method. We now show, in principle, that this approach can be used in a meaningful way to determine the in vitro anticancer activity of libraries prepared via parallel solution-phase synthesis. One of the most common ways of determining in vitro cell cytotoxicity uses the MTT assay.[11] This important assay is very widely used to determine the cytotoxicity of anticancer drugs. Cultured cancer cells are grown in the presence of the putative anticancer agent. The amount of viable cells remaining can then be determined spectrophotometrically. This is based on the reduction of the yellow MTT [3-(4′,5′-dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenases of metabolically active cancer cells to a purple-blue formazan (Scheme 1). The activity is most often reported as its IC_{50} concentration—the concentration that results in a 50% decrease in cell growth relative to an untreated control.

We recently described the use of the MTT assay in the determination of the anticancer activity of various α,β-unsaturated ketones prepared by serial synthesis.[12] The anticancer properties of chalcones, one of the classes of ketones investigated, have also been reported by Ikeda et al.[13] and Edwards et al.[14] One of the compounds we prepared, the α-methyl-substituted chalcone **1**, based on the structure of the natural product combretastatin A-4,[15] was highly cytotoxic; its IC_{50} (K562) was 0.21 nM. The related chalcone **2** lacking the α-methyl group (Figure 1) is also potent (IC_{50} 4.3 nM). Chalcone **2** was prepared in excellent yield by the Claisen−Schmidt condensation of 3,4,5-trimethoxyacetophenone with isovanillin. This method for the preparation of chalcones **5** is attractive because it specifically generates the (*E*)-isomer, normally in high yield, from substituted benzaldehydes **3** and acetophenones **4**, a large number of which are commercially available and inexpensive (Scheme 2). The ease of preparation and the biological activity of chalcones clearly showed that as a class of anticancer agent, they deserved greater attention. We therefore chose chalcones as a simple class of compounds to illustrate the potential and simplicity of the MTT assay as a partly quantitative screen for solution-phase parallel libraries of moderate size. In addition to proving the validity of this approach, we also hoped to reveal additional active chalcones.[16] We now report the results of this study.

Scheme 1. Basis of the MTT Assay



Scheme 2. Claisen−Schmidt Synthesis of Chalcones **5**



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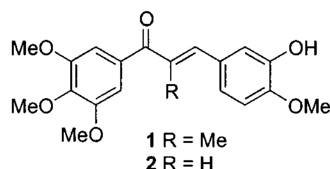


Figure 1. Structures of chalcones **1** and **2**.

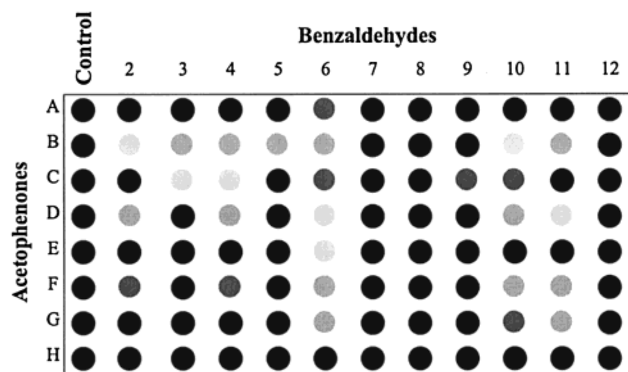


Figure 2. Typical synthesis of chalcones **5** in a 96-well microtiter plate. Wells (1A−1D) contained methanol (200 μL); wells 1E and 1F contained sodium hydroxide (10 μL of a 5 M aqueous solution) and methanol (190 μL); wells 1G and 1H contained sodium hydroxide (10 μL of a 5 M solution), hydrochloric acid (10 μL of a 5 M solution) and methanol (180 μL). Cell growth/kill is represented schematically (in comparison to cell growth of the control): (black circles) similar (i.e., negative response); (lightest-gray circles) highly active (i.e., positive response); (medium-gray and lighter-medium-gray circles) intermediate levels of activity (still considered negative).

Results and Discussion

It was clear to us that the parallel synthesis of chalcones on a small scale (<0.1 mmol) in a conventional 96-well microtiter plate should not present a problem. However, it was not apparent whether we could assay the crude reaction mixtures for their ability to inhibit or reduce cancer cell growth by the MTT assay and extract meaningful information of their IC_{50} values. Previously, the growth inhibitory activities of the chalcones were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay.[12] The IC_{50} concentration was calculated with reference to a standard curve constructed for control cells and involves incubating cells at several different concentrations (ca. 6−8) of drug, in triplicate. Only small numbers of compounds can be conveniently screened in this way. Our new approach, described below, to facilitate the assessment of larger numbers of compounds involves testing at a single concentration of drug and determines whether the IC_{50} is below or above this arbitrary concentration.

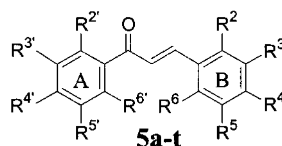
The parallel synthesis of chalcones was conducted in a conventional 96-well tissue culture test-plate. Solutions of a substituted benzaldehyde **3** (100 μL of a 0.1 M solution in methanol), substituted acetophenone **4** (100 μL of a 0.1 M solution in methanol), and sodium hydroxide (10 μL of a 5 M aqueous solution) were added to each well to give 10 μmol of substituted chalcone **5**. Eighty-eight reactions were performed on each plate by using a different ketone across each of the 8 rows (A−G), varying the aldehyde in each of the 11 columns (2−12) (Figure 2). The first column (1) was used as a control lacking both the aldehyde and ketone.[17] The test-plate was covered, the reaction mixture was left for 3 days before the lid was removed, and the solvent was left to slowly evaporate overnight in a fume hood. A reaction time of 3 days was chosen because we had found that most Claisen−Schmidt reactions are complete overnight. This cautious approach therefore makes provision for unusually slow reactions. There was no evidence of cross-well contamination in the evaporation process, and in most cases the product crystallized from solution. Addition of DMSO (200 μL) to each well gave 50 mM solutions of chalcones. Each well was diluted by a factor of 10 four times to give a final plate containing chalcones at a concentration of 5 μM. The final plate was incubated with K562 cells at 37 °C for 5 days. The number of cells in each well was then measured by the MTT assay. The wells that contained less than half the number of cells in the control wells were deemed to produce a positive bioassay response. The chalcones in these wells were therefore likely to have an IC_{50} of less than 5 μM.

In total, 23 substituted acetophenones were used in this study in combination with 28 substituted benzaldehydes (selected randomly). Sixteen wells gave a positive bioassay response; a "success" rate of 2.5%.[18] It is gratifying to note that the chalcones (**5a**−**5p**), prepared conventionally on a 10 mmol scale, corresponding to these wells were indeed active with an IC_{50} less than 5 μM (Table 1). Those close to, but greater than, the 5 μM mark that produced a negative response had an IC_{50} of >5 μM when assessed in the conventional way. The most active compounds corresponded to those wells that had the lowest optical density in the MTT assay. The reactions failed when the aldehyde possessed a hydroxyl or amine group, and this probably reflects the poorer electrophilicity under basic reaction conditions.[17] The results show very clearly that the MTT-based cytotoxicity assay is suitable for the screening of libraries of several hundred members.

The saving in time was impressive. The library was prepared and evaluated in approximately 20 days. We estimate that it would take approximately 500 days to conventionally prepare and test the same number of chalcones. The most active compound **5a** is impressively cytotoxic (IC_{50} 30 nM) and presents a compound of great potential. The effects of **5a** upon the cell cycle were measured by flow cytometry. The results in Table 2 show that **5a** causes significant arrest of the cell cycle at the G_2/M point, relative to the untreated control, consistent with the behavior of a tubulin-binding agent.[19] Further experiments, described previously,[20] to determine the origin of its antimitotic effect reveal that it inhibits the polymerization of tubulin (IC_{50} 1.5 μM). The impressive activity is comparable with that of the tubulin-binding anticancer agent combretastatin A-4 (IC_{50} 0.5[21]−2[22] μM). The chalcone **5a** displaces tubulin-bound colchicine, indicating that it binds to or close to the colchicine binding site of tubulin. It is the ability of combretastatin A-4 to damage tumor vasculature, thereby effectively starving tumors of nutrients, which makes agents of this type such exciting molecules with considerable clinical potential.[23]

In conclusion, we have shown that the Claisen−Schmidt reaction can be used to generate chalcones that can be conveniently assessed for cytotoxicity in situ by the MTT

Table 1. Cell Growth Inhibitory Properties of Chalcones Prepared by Conventional Methods Originating from the Parallel Synthesis Study Tested against the K562 Cell Line



compound	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	$R^{6'}$	R^2	R^3	R^4	R^5	R^6	yield (%)	IC_{50} (μM)	bioassay
5a	H	OMe	OH	H	H	OMe	H	OMe	H	OMe	57	0.03	+
5b	OMe	H	H	OMe	H	OMe	H	OMe	H	OMe	81	0.04	+
5c	H	OMe	OMe	OMe	H	H	H	OMe	H	H	67	0.1	+
5d	F	H	OMe	H	H	F	H	H	Br	H	95	0.1	+
5e	H	OMe	OMe	OMe	H	H	H	NMe_2	H	H	67	0.2	+
5f	H	OMe	OMe	OMe	H	OMe	H	OMe	H	OMe	79	0.2	+
5g	H	H	Me	H	H	H	H	Cl	H	H	86	0.6	+
5h	H	H	NO_2	H	H	H	OMe	H	OMe	H	70	1.2	+
5i	H	CF_3	H	H	H	OMe	OMe	H	H	H	65	1.3	+
5j	F	H	OMe	H	H	H	OMe	H	OMe	H	87	1.4	+
5k	OMe	H	OMe	H	H	H	F	F	H	H	84	1.4	+
5l	H	H	H	H	H	H	H	Cl	H	H	83	2.1	+
5m	H	H	OMe	H	H	H	F	F	H	H	79	2.7	+
5n	Me	H	Me	H	Me	OMe	H	H	H	H	42	2.8	+
5o	H	OMe	OMe	H	H	OMe	H	OMe	H	H	63	4.7	+
5p	H	OMe	OMe	H	H	F	H	Br	H	H	51	5.0	+
5q	OMe	H	H	OMe	H	OMe	OMe	OMe	H	H	79	5.9	−
5r	H	H	Cl	H	H	OMe	H	H	OMe	H	80	6.4	−
5s	F	H	OMe	H	H	F	H	Br	H	H	61	8.9	−
5t	F	H	OMe	H	H	OMe	H	OMe	H	H	67	21.0	−

Table 2. Effects upon the Cell Cycle[a]

compound	G_0-G_1 (%)	S phase (%)	G_2/M (%)
control	25.3	47.2	27.5
5a	2.8	10.7	86.5

[a] Percentage of cells in each phase of the cell cycle as measured by flow cytometry (according to previously described methods[24]).

assay without the need for purification or workup. We hope that this general approach will find use elsewhere in the search and design of future anticancer drugs.

Experimental Section

The 200 MHz ^{1}H NMR spectra were recorded using a Bruker AC 200 NMR spectrometer, while all 300 MHz ^{1}H spectra were recorded using a Bruker AC 300 spectrometer. Spectra were recorded using $CHCl_3$ as an internal standard. Chemical ionization (CI) and electron impact (EI) mass spectra were recorded using a Kratos MS25 mass spectrometer; fast atom bombardment (FAB) mass spectra were recorded using a Kratos MS50 mass spectrometer, using a *m*-nitrobenzyl alcohol matrix. Accurate mass determinations were performed using a Kratos Concept IS mass spectrometer. Elemental analysis was performed using a Carlo-Ebra 1106 elemental analyzer. Infrared spectra were recorded using a Phillips Analytical PU9625 pulsed FT spectrometer. All melting points were determined using a Büchi 510 melting point apparatus and were not corrected. Column chromatography was conducted using silica gel 60, 230−400 mesh (Merck & Co.), and silica TLC was conducted on precoated aluminum sheets (60 F_{254}) with a 0.2 mm thickness (Aldrich Chemical Co.).

Parallel Synthesis of Chalcones and Cytotoxicity Assessment. Eighty-eight separate reactions were performed in each 96-well microtiter plate. A substituted ketone (100 μL of a 0.1 M solution in methanol) was added across a row comprising 11 wells (see Figure 2). This was repeated using different ketones for seven other rows. Similarly, 11 different aldehydes (100 μL of a 0.1 M solution in methanol) were added down 11 columns. Sodium hydroxide (10 μL of a 5 M aqueous solution) was added to each reaction well. The first column (1) was left free as a control [wells 1A−1D contained methanol (200 μL); wells 1E and 1F contained sodium hydroxide (10 μL of a 5 M solution) and methanol (190 μL); wells 1G and 1H contained sodium hydroxide (10 μL of a 5 M solution), hydrochloric acid (10 μL of a 6 M solution), and methanol (180 μL)]. After the samples were left standing for 48 h at room temperature, the lid of the plate was removed; if either the aldehyde or ketone possessed a phenolic hydroxyl group, hydrochloric acid (10 μL of a 6 M solution) was added to the well. The solvent was left to evaporate overnight. DMSO (200 μL) was added to each well of the plate to dissolve the crude reaction product. A 10-fold dilution was achieved by the addition of each reaction solution (20 μL) to DMSO (180 μL) to give mixtures at 5 mM concentration. This was repeated to give putative chalcones at 0.5 mM concentration. A portion (20 μL) of each well solution was added to RPMI cell culture medium (180 μL). These solutions (20 μL) were added to RPMI medium containing K562 cells (180 μL); the final concentration of the putative chalcone was 5 μM. The plates were incubated at 37 °C for 5 days in an atmosphere of 5% carbon dioxide in air.

Cytotoxicity Testing. A K562 human leukaemia cell line was cultured as described previously.[25] The cell line was mycoplasma-free, and cytotoxicity tests were carried out using the MTT assay described by Edmondson and co-

workers.[11] The bioassay was considered positive if the optical density of the well was less than half that of the wells in the control column. The IC_{50} value for chalcones **5a**–**5t** was calculated by reference to a standard curve constructed for control cells.

General Method for the Preparation of Chalcones 5 (Scheme 2). Sodium hydroxide (1 mL of a 50% w/v aqueous solution) was added to a stirred solution of substituted benzaldehyde (10.0 mmol) and substituted acetophenone (10.0 mmol) in methanol (30 mL). The mixture was stirred overnight at room temperature. When a solid formed, the chalcone was purified by filtration and recrystallization. When no solid had formed, the mixture was neutralized with hydrochloric acid (1 N) and extracted with chloroform (2 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography.

4′-Hydroxy-2,4,6,3′-tetramethoxychalcone (5a). Yellow crystals: 57% yield; mp 169–171 °C; IR (KBr disk) 1550 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.91 (s, 3H), 3.97 (s, 6H), 3.98 (s, 3H), 6.07 (s, 1H), 6.14 (s, 2H), 6.97 (d, J = 8.0 Hz, 1H), 7.61 (m, 2H), 7.89 (d, J = 15.8 Hz, 1H), 8.23 (d, J = 15.8 Hz, 1H); FABMS m/z 345 [MH^+, 100%]. Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.3; H, 5.9. Found: C, 66.4; H, 5.9. HRMS (EI) Calcd for $C_{19}H_{20}O_6$ (M^+): 344.1260; found 344.1254.

2,4,6,2′,5′-Pentamethoxychalcone (5b). Yellow crystals: 81% yield; mp 114–116 °C; IR (KBr disk) 1650 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.79 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.86 (s, 6H), 6.11 (s, 2H), 6.91 (d, J = 9.0 Hz, 1H), 6.98 (dd, J = 9.0 and 2.9 Hz, 1H), 7.14 (d, J = 2.9 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 8.07 (d, J = 16.0 Hz, 1H); FABMS m/z 359 [MH^+, 100%]. Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.0; H, 6.2. Found: C, 67.1; H, 6.1. HRMS (EI) Calcd for $C_{20}H_{22}O_6$ (M^+): 358.1416; found 358.1409.

4,3′,4′,5′-Tetramethoxychalcone (5c). Yellow solid, 67% yield; mp 98–99 °C [lit.[26] 100–101 °C]. Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.5; H, 6.1. Found: C, 69.5; H, 6.3.

5-Bromo-2,2′-difluoro-4′-methoxychalcone (5d). White crystals: 95% yield; mp 118–124 °C; IR (KBr disk) 1660 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.68 (dd, J = 13.1 and 2.4 Hz, 1H), 6.82 (dd, J = 8.7 and 2.4 Hz, 1H), 7.04 (dd, J = 9.9 and 8.8 Hz, 1H), 7.45–7.50 (m, 1H), 7.55 (dd, J = 15.8 and 2.9 Hz, 1H), 7.77 (dd, J = 6.5 and 2.4 Hz 1H), 7.83 (dd, J = 15.8 and 2.0 Hz, 1H), 7.95 (t, J = 8.7 Hz, 1H); FABMS m/z 353 [MH^+, 100%]. Anal. Calcd for $C_{16}H_{11}O_2F_2Br$: C, 54.4; H, 3.1; F, 10.8. Found: C, 54.2; H, 3.0; F, 10.5. HRMS (EI) Calcd for $C_{16}H_{11}O_2F_2{}^{79}Br$ (M^+): 351.9910; found 351.9908.

4-(*N,N*-Dimethylamino)-3′,4′,5′-trimethoxychalcone (5e). Yellow crystals: 67% yield; mp 146–147 °C [lit.[15] 148–149 °C]. Anal. Calcd for $C_{20}H_{23}O_4N$: C, 70.4; H, 6.8; N, 4.1. Found: C, 70.5; H, 6.9; N, 4.1.

2,4,6,3′,4′,5′-Hexamethoxychalcone (5f). Yellow crystals: 79% yield; mp 136–140 °C [lit.[27] 144 °C]. Anal. Calcd for $C_{21}H_{24}O_7$: C, 64.9; H, 6.2. Found: C, 65.1; H, 6.3.

4-Chloro-4′-methylchalcone (5g). Yellow crystals: 86% yield; mp 66–68 °C; IR (KBr disk) 1655 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.60 (s, 3H), 7.45 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 9.4 Hz, 2H), 7.60 (d, J = 15.8 Hz, 1H), 7.72 (d, J = 9.4 Hz, 2H), 7.91 (d, J = 15.8 Hz, 1H), 8.08 (d, J = 8.5 Hz, 2H); FABMS m/z 257 [MH^+, 100%]. Anal. Calcd for $C_{16}H_{13}OCl$: C, 74.9; H, 5.1; Cl, 13.8. Found: C, 74.6; H, 5.0; Cl, 13.9.

3,5-Dimethoxy-4′-nitrochalcone (5h). Yellow crystals: 70% yield; mp 166–168 °C; IR (KBr disk) 1670 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 6H), 6.57 (s, 1H), 6.78 (s, 2H), 7.42 (d, J = 15.8 Hz, 1H), 7.75 (d, J = 15.8 Hz, 1H), 8.13 (d, J = 8.5 Hz, 2H), 8.35 (d, J = 8.5 Hz, 2H); FABMS m/z 314 [MH^+, 75%]. Anal. Calcd for $C_{17}H_{15}O_5N$: C, 65.2; H, 4.8; N, 4.5. Found: C, 65.2; H, 4.7; N, 4.4.

2,3-Dimethoxy-3′-(trifluoromethyl)chalcone (5i). Pale-yellow crystals: 65% yield; mp 78–82 °C; IR (KBr disk) 1670 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.89 (s, 3H), 3.90 (s, 3H), 6.99 (dd, J = 8.1 and 1.4 Hz, 1H), 7.11 (t, J = 8.1 Hz, 1H), 7.29 (dd, J = 8.1 and 1.4 Hz, 1H), 7.56 (d, J = 15.8 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 15.8 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.26 (s, 1H); FABMS m/z 337 [MH^+, 100%]. Anal. Calcd for $C_{18}H_{15}O_3F_3$: C, 64.3; H, 4.5; F, 16.9. Found: C, 64.2; H, 4.6; F, 17.0. HRMS (EI) Calcd for $C_{18}H_{15}O_3F_3$ (M^+): 336.0973; found 336.0972.

2′-Fluoro-3,5,4′-trimethoxychalcone (5j). Pale-yellow crystals: 87% yield; mp 87–89 °C; IR (KBr disk) 1650 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.83 (s, 6H), 3.87 (s, 3H), 6.51 (t, J = 2.2 Hz, 1H), 6.65 (dd, J = 12.9 and 2.2 Hz, 1H), 6.71 (d, J = 2.2 Hz, 2H), 6.75 (dd, J = 8.5 and 2.3 Hz, 1H), 7.42 (dd, J = 15.3 and 2.8 Hz, 1H), 7.71 (dd, J = 15.3 and 2.0 Hz, 1H), 7.90 (t, J = 8.5 Hz, 1H); FABMS m/z 317 [MH^+, 100%]. Anal. Calcd for $C_{18}H_{17}O_4F$: C, 68.3; H, 5.4; F, 6.0. Found: C, 68.4; H, 5.5; F, 6.3. HRMS (EI) Calcd for $C_{18}H_{17}O_4F$ (M^+): 316.1111; found 316.1109.

3,4-Difluoro-2′,4′-dimethoxychalcone (5k). White crystals: 84% yield; mp 126–128 °C; IR (KBr disk) 1670 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 3.92 (s, 3H), 6.50 (d, J = 2.5 Hz, 1H), 6.57 (dd, J = 8.7 and 2.5 Hz, 1H), 7.18 (td, J = 10.0 and 8.3 Hz, 1H), 7.31 (m, 1H), 7.41 (ddd, J = 11.0, 8.3, and 2.1 Hz, 1H), 7.45 (d, J = 15.8 Hz, 1H), 7.57 (d, J = 15.8 Hz, 1H), 7.78 (d, J = 8.7 Hz, 1H); FABMS m/z 305 [MH^+, 100%]. Anal. Calcd for $C_{17}H_{14}O_3F_2$: C, 67.1; H, 4.6; F, 12.5. Found: C, 67.2; H, 4.5; F, 12.2.

4-Chlorochalcone (5l). Cream needles: 83% yield; mp 108–110 °C [lit.[28] 112–113 °C]. Anal. Calcd for $C_{15}H_{11}$-OCl requires C, 74.2; H, 4.6; Cl, 14.7. Found: C, 74.0; H, 4.3; Cl, 15.0.

3,4-Difluoro-4′-methoxychalcone (5m). White crystals: 79% yield; mp 131–133 °C [lit.[29] 132–33 °C]. Anal. Calcd for $C_{16}H_{12}O_2F_2$: C, 70.1; H, 4.4; F, 13.9. Found: C, 70.0; H, 4.2; F, 13.9.

2-Methoxy-2′,4′,6′-trimethylchalcone (5n). Light-brown solid: 42% yield; mp 66–68 °C [lit.[30] 95 °C]. Anal. Calcd for $C_{19}H_{20}O_2$: C, 81.4; H, 7.2. Found: C, 81.5; H, 7.4.

2,4,3′,4′-Tetramethoxychalcone (5o). Pale-yellow crystals: 63% yield; mp 104–106 °C [lit.[31] 115.5 °C]; IR (KBr disk) 1650 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.85 (s, 3H), 3.90 (s, 3H), 3.96 (s, 3H), 3.95 (s, 3H), 6.48 (d, J = 2.2 Hz, 1H), 6.53 (dd, J = 8.4 and 2.2 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 15.7

Hz, 1H), 7.62 (d, $J = 1.9$ Hz, 1H), 7.67 (dd, $J = 8.2$ and 1.9 Hz, 1H), 8.04 (d, $J = 15.7$ Hz, 1H); FABMS *m/z* 329 [MH^+, 95%]. Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.4; H, 6.1. Found: C, 69.1; H, 5.9. HRMS (EI) Calcd for $C_{19}H_{20}O_5$ (M^+): 328.1311; found 328.1320.

4-Bromo-2-fluoro-3′,4′-dimethoxychalcone (5p). Pale-yellow crystals: 51% yield; mp 142−146 °C; IR (KBr disk) 1660 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.97 (s, 6H), 6.93 (d, $J = 8.3$ Hz, 1H), 7.32−7.38 (m, 2H), 7.52 (t, $J = 8.4$ Hz, 1H), 7.66 (d, $J = 15.7$ Hz, 1H), 7.61 (d, $J = 2.2$ Hz, 1H), 7.67 (dd, $J = 8.3$ and 2.2 Hz, 1H), 7.81 (d, $J = 15.7$ Hz, 1H); FABMS *m/z* 365 [MH^+, 70%]. HRMS (EI) Calcd for $C_{17}H_{14}O_3F^{79}Br$ (M^+): 364.0110; found, 364.0115.

2,3,4,2′,5′-Pentamethoxychalcone (5q). Yellow solid: 79% yield; mp 84−86 °C; IR (KBr disk) 1650 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.71 (d, $J = 8.8$ Hz, 1H), 6.93 (d, $J = 8.6$ Hz, 1H), 7.01 (dd, $J = 8.6$ and 2.9 Hz, 1H), 7.17 (d, $J = 2.9$ Hz, 1H), 7.35 (d, $J = 8.8$ Hz, 1H), 7.38 (d, $J = 16.0$ Hz, 1H), 7.84 (d, $J = 16.0$ Hz, 1H); FABMS *m/z* 359 [MH^+, 100%]. Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.0; H, 6.2. Found: C, 67.0; H, 6.2.

4′-Chloro-2,5-Dimethoxychalcone (5r). Yellow needles: 80% yield; mp 84−88 °C [lit.[32] 71.5 °C]. Anal. Calcd for $C_{17}H_{15}O_3Cl$: C, 67.4; H, 5.0; Cl, 11.7. Found: C, 67.5; H, 4.5; Cl, 11.7.

4-Bromo-2,2′-difluoro-4′-methoxychalcone (5s). Pale-yellow crystals: 61% yield; mp 98−102 °C; IR (KBr disk) 1660 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.86 (s, 3H), 6.65 (dd, $J = 13.2$ and 2.2 Hz, 1H), 6.79 (dd, $J = 8.8$ and 2.2 Hz, 1H), 7.27−7.35 (m, 2H), 7.49−7.58 (m, 2H) 7.82 (d, $J = 15.7$ Hz, 1H), 7.91 (t, $J = 9.1$ Hz, 1H); FABMS *m/z* 353 [MH^+, 100%]. Anal. Calcd for $C_{16}H_{11}O_2F_2Br$: C, 54.4; H, 3.1; F, 10.8; Br, 22.6. Found: C, 54.6; H, 3.0; F, 10.6; Br, 22.4. HRMS (EI) Calcd for $C_{16}H_{11}O_2F_2Br$ (M^+): 351.9910; found 351.9911.

2′-Fluoro-2,4,4′-trimethoxychalcone (5t). Yellow crystals: 67% yield; mp 99−101 °C; IR (KBr disk) 1650 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.84 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 6.45 (d, $J = 2.2$ Hz, 1H), 6.52 (dd, $J = 8.6$ and 2.2 Hz, 1H), 6.63 (dd, $J = 13.0$ and 2.3 Hz, 1H), 6.76 (dd, $J = 8.6$ and 2.3 Hz, 1H), 7.45 (dd, $J = 15.7$ and 2.8 Hz, 1H), 7.56 (d, $J = 8.6$ Hz, 1H), 7.85 (t, $J = 8.6$ Hz, 1H), 8.04 (dd, $J = 15.7$ and 2.2 Hz, 1H); FABMS *m/z* 317 [MH^+, 100%]. Anal. Calcd for $C_{18}H_{17}O_4F$: C, 68.4; H, 5.4; F, 6.0. Found: C, 68.3; H, 5.4; F, 6.4. HRMS (EI) Calcd for $C_{18}H_{17}O_4F$ (M^+): 316.1111, found 316.1107.

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References and Notes

(1) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135−8173. Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Chem.*−*Eur. J.* **1997**, *3*, 1917−1920.
(2) Maehr, H. *Bioorg. Med. Chem.* **1997**, *5*, 473−491.
(3) Selway, C. N.; Terrett, N. K. *Bioorg. Med. Chem.* **1996**, *4*, 645−654.
(4) Lam, K. S. *Anti-Cancer Drug Des.* **1997**, *12*, 145−167.
(5) Partidos, C. D.; Chirinorojas, C. L.; Steward, M. W. *Immunol. Lett.* **1997**, *57*, 113−116.
(6) Salmon, S. E.; Liu-Stevens, R. H.; Zhao, Y.; Lebl, M.; Krchnak, V.; Wertman, K.; Sepetov, N.; Lam, K. S. *Mol. Diversity* **1996**, *2*, 57−63.
(7) Stratowa, C.; Baum, A.; Castanon, M. J.; Dahmann, G.; Himmelsbach, F.; Himmler, A.; Loeber, G.; Metz, T.; Schnitzer, R.; Solca, F.; Spevak, W.; Tontsch, U.; von Ruden, T. *Anti-Cancer Drug Des.* **1999**, *14*, 393−402.
(8) Nicolaou, K. C.; Pfefferkorn, J.; Xu, J.-Y.; Winssinger, N.; Ohshima, T.; Kim, S. H.; Hosokawa, S.; Vouruloumis, D.; van Delft, F.; Li, T. *Chem. Pharm. Bull.* **1999**, *47*, 1199−1213. Nicolaou, K. C.; Winssinger, N.; Vourloumis, D.; Ohshima, T.; Kim, S.; Pfefferkorn, J.; Xu, J.-Y.; Li, T. *J. Am. Chem. Soc.* **1998**, *120*, 10814−10826.
(9) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature (London)* **1997**, *387*, 268−272.
(10) Salmon, S. E.; Lam, K. S.; Felder, S.; Yeoman, H.; Schlessinger, J.; Ullrich, A.; Krchnak, V.; Lebl, M. *Int. J. Pharmacogn.* **1995**, *33*, 67−74. Sandman, K. E.; Fuhrmann, P.; Lippard, S. J. *J. Biol. Inorg. Chem.* **1998**, *3*, 74−80.
(11) Edmondson, J. M.; Armstrong, L. S.; Martinez, A. O. *J. Tissue Cult. Methods* **1988**, *11*, 15−17.
(12) Ducki, S.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; Liu, C.-Y.; McGown, A. T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3091−3094. Ducki, S.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T.; Rennison, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051−1056.
(13) Ikeda, S.; Kimura, U.; Ashizawa, T.; Gomi, K.; Saito, H. *Jpn. Kokai Tokkyo Koho* **1996**, JP 08,188,546; *Chem. Abstr.* **1996**, *125*, 221359a.
(14) Edwards, M. L.; Stemerick, D. M.; Sunkara, P. S. *J. Med. Chem.* **1990**, *33*, 1948−1954.
(15) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209−211.
(16) For recent reports on the solution- and solid-phase synthesis of chalcone libraries for other purposes see the following. Powers, D. G.; Casebier, D. S.; Fokas, D.; Ryan, W. J.; Troth, J. R.; Coffen, D. L. *Tetrahedron* **1998**, *54*, 4085−4096. Marzinzik, A. L.; Felder, E. R. *J. Org. Chem.* **1998**, *63*, 723−727. Katritzky, A. R.; Serdyuk, L.; Chassaing, C.; Toader, D.; Wang, X.; Forood, B.; Flatt, B.; Sun, C.; Vo, K. *J. Comb. Chem.* **2000**, *2*, 182−185.
(17) Controls for the aldehydes and ketones were not performed because we had previously found that those used were not cytotoxic. In principle, these could be performed by assessing the cytotoxicity at the same single value (5 μM) used for the library.
(18) Random sampling of 32 wells revealed that 60% were essentially composed of only the expected chalcone; of the other 40% most were mixtures of the benzaldehyde, acetophenone, and chalcone.
(19) Lawrence, N. J.; McGown, A. T.; Ducki, S.; Hadfield, J. A. *Anti-Cancer Drug Des.* **2000**, *15*, 135−141.
(20) Woods, J. A.; Hadfield, J. A.; Pettit, G. R.; Fox, B. W.; McGown, A. T. *Br. J. Cancer* **1995**, *71*, 705−711.
(21) Li, L.; Wang, H.-K.; Kuo, S.-C.; Wu, T.-S.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 3400−3407.

(22) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579–2588.
(23) Grosios, K.; Holwell, S. E.; McGown, A. T.; Pettit, G. R.; Bibby, M. C. *Br. J. Cancer* **1999**, *81*, 1318–1327. Tozer, G. M.; Prise, V. E.; Wilson, J.; Locke, R. J.; Vojnovic, B.; Stratford, M. R. L.; Dennis, M. F.; Chaplin, D. J. *Cancer Res.* **1999**, *59*, 1626–1634. Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829–1834. Landuyt, W.; Verdoes, O.; Darius, D. O.; Drijkoningen, M.; Nuyts, S.; Theys, J.; Stockx, L.; Wynendaele, W.; Fowler, J. F.; Maleux, G.; Van den Bogaert, W.; Anné, J.; van Oosterom, A.; Lambin, P. *Eur. J. Cancer* **2000**, *36*, 1833–1843.
(24) McGown, A. T.; Poppitt, D. G.; Swindell, R.; Fox, B. W. *Cancer Chemother. Pharmacol.* **1984**, *13*, 47–53.
(25) McGown, A. T.; Jayson, G.; Pettit, G. R.; Haran, M. S.; Ward, T. H.; Crowther, D. *Br. J. Cancer* **1998**, *77*, 216–220.
(26) Gutsche, C. D.; Jason, E. F.; Coffey, R. S.; Johnson, H. E. *J. Am. Chem. Soc.* **1958**, *80*, 5756–5767.
(27) Mayer, W.; Bauni, G.; Stolp, F. *Justus Liebigs Ann. Chem.* **1960**, *630*, 19–25.
(28) Silver, N. L.; Boykin, D. W., Jr. *J. Org. Chem.* **1970**, *35*, 759–764.
(29) Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O.; Rosenthal, P.; McKerrow, J. H. *J. Med. Chem.* **1995**, *38*, 5031–5037.
(30) Barnes, R. P.; Lucas, W. M. *J. Am. Chem. Soc*. **1942**, *64*, 2260–2261.
(31) Kauffmann, H.; Kieser, F. *Chem. Ber.* **1913**, *46*, 3788–3801.
(32) Belyaev, V. F. *J. Gen. Chem. USSR (Engl. Transl.)* **1964**, *34*, 856–858.
(33) Fletcher, D. A.; McMeeking, R. F.; Parkin, D. J. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 746–749.

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