Chalcones: A New Class of Antimitotic Agents

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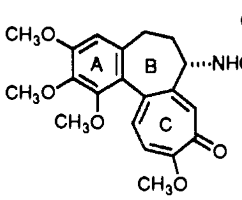
A series of chalcones was evaluated as antimitotic agents. One of these, (*E*)-1-(2,5-dimethoxyphenyl)-3-[4-(dimethylamino)phenyl]-2-methyl-2-propen-1-one) (**73**), was found to be an effective antimitotic agent at a concentration of 4 nM in an in vitro HeLa cell test system. When evaluated in experimental tumor models in vivo, this compound exhibited antitumor activity against L1210 leukemia and B_{16} melanoma.

Vincristine and vinblastine are anticancer agents which inhibit microtubule assembly by binding in an irreversible manner to tubulin.[1] With the exception of colchicine (1),



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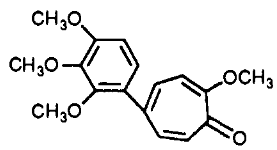

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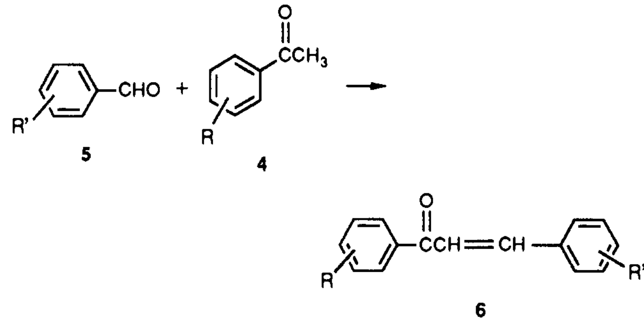
reversible inhibitors of microtubule assembly[2a-j] are generally not clinically useful. Colchicine's reversible binding site on tubulin is different from that of the vinca alkaloids.[3]

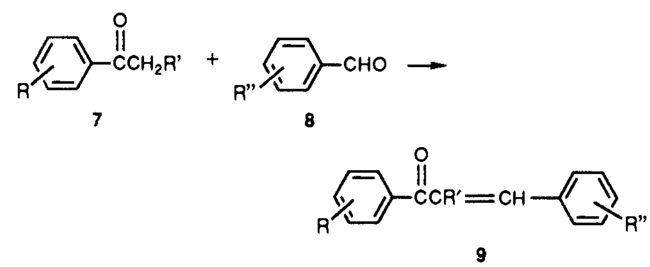
Recent reports have delineated the SAR of colchicine–tubulin binding[4a-e] and the antimitotic activity of analogues of colchicine,[5a-e] podophyllotoxin,[6a-c] and steganocin.[7a,b] These studies have shown that the important structural features of the colchicine molecule for binding to tubulin are the methoxy groups of the A ring and the carbonyl of the C ring. The combretastatins[9a] and compound **2**,[9b] with no B ring, exhibit tubulin binding of the same order as that of colchicine.

Sulfhydryl reagents also interfere with microtubule assembly[10a,b] and this effect can be inhibited by the presence of colchicine or podophyllotoxin,[11a,b] suggesting the presence of a sulfhydryl residue at the colchicine-binding site on tubulin. These results prompted us to examine com-

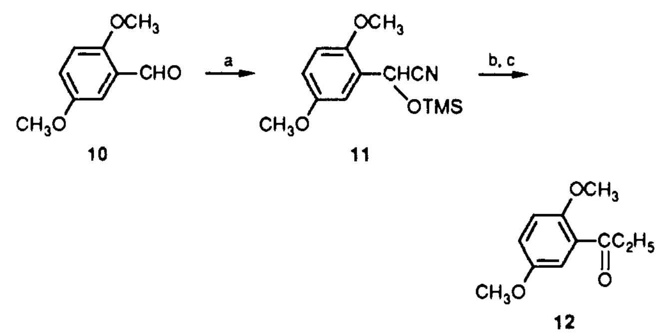
Scheme I



Scheme II



Scheme III[a]



[a] Reagents and conditions: (a) TMSCl, KCN, $ZnCl_2$; (b) LDA, C_2H_5I; (c) $N(Bu)_4F$.

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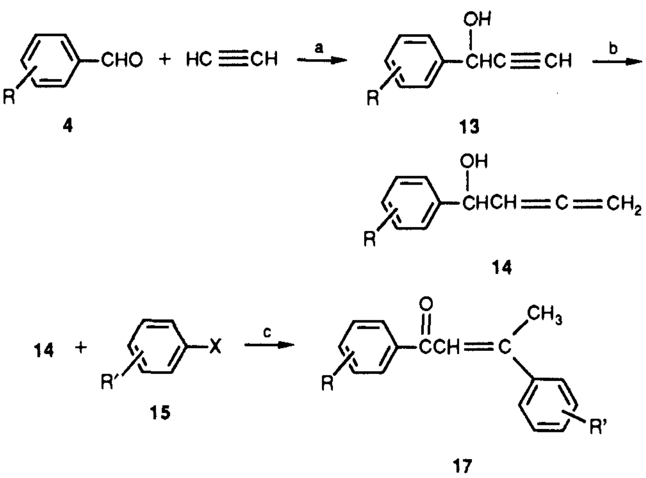
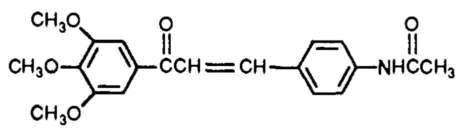
Scheme IV[a]



[a] Reagents and Conditions: (a) EtMgBr; (b) CH_2O, LDA, CuBr; (c) $Pd(OAc)_2$.

pounds incorporating in their structures both a trimethoxyphenyl group and a group capable of interaction with sulfhydryl residues as potential irreversible tubulin polymerization inhibitors. Appropriately substituted chalcones, as typified by compound **3**, were found to be potent antimitotic agents. In this paper we report the synthesis of this new class of antimitotic agents and their structure–activity relationships.



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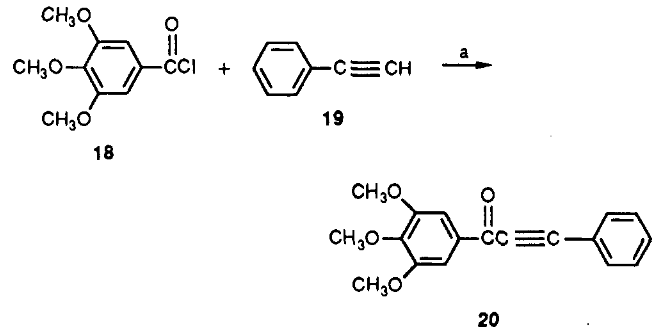
Chemistry

The majority of the title compounds were synthesized by a base-catalyzed condensation of appropriately substituted aldehyde **5** and ketone **4**[12] (Scheme I, procedure A; see compound **61** in the Experimental Section). The α-alkyl chalcones of general structure **9** were prepared in a similar manner with piperidinium acetate as catalyst and molecular sieves to remove water from the reaction solvent (Scheme II, procedure B; see compound **73** in the Experimental Section). Ketones (**7**) not commercially available were prepared as described by Hünig[13] from aldehyde **10** as exemplified by 2,5-dimethoxypropiophenone (**12**) (Scheme III).

Reaction of an α-unsubstituted chalcone with Br_2 or SO_2Cl_2 followed by base-catalyzed elimination afforded the corresponding α-halogenated derivatives[14] **65–70** (procedures C and D). Palladium-assisted reaction[15] of an allene (**14**) and a halogen-substituted benzene (**15**) produced β-alkyl chalcones **17** (Scheme IV, procedure E). Coupling of phenylacetylene (**19**) and 3,4,5-trimethoxybenzoyl chloride (**18**) produced acetylenic ketone **20**[16] (Scheme V). Selective reduction of either the carbonyl group ($NaBH_4$) or double bond (Pd/C, H_2) of **36** was readily accomplished to prepare compounds **80** and **81**, respectively. Displacement of the aromatic fluoride of **24** by morpholine or *n*-butylamine provided products **48** and **49**[17] (procedure F).

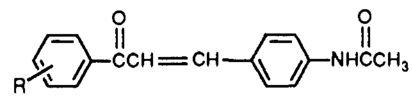
NMR analysis of the chalcones obtained by procedures A and B indicated formation of only the expected *E* isomers. In the case of compound **73**, an X-ray study[18] confirmed the assignment of *E* stereochemistry.

Scheme V[a]



[a] Reagents and conditions: (a) $PdCl_2P(Ph_3)_2$, CuI.

Table I. Physical Properties and Equivalent Dose of Selected Chalcones in the 6-h HeLa Cell Test[a]



compd[b]	R	6-h test ED, μg/mL	mp, °C
3	3,4,5-$(OCH_3)_3$	3.1	190–191
21	3-C_2H_5	>100	119–120
22	4-OCH_3	3.1	185.5–186.5
23	3-CF_3	25	179–180
24	4-*t*-Bu	0.25	183–184
25	4-CN	25	214
26	4-C(O)NH_2	3.1	298–299
27	3-Cl	>100	
28	2,5-$(OCH_3)_2$	6.25	154–155[c]
29	2,4,6-$(OCH_3)_3$	3.1	207–208
30	2,3,4-$(OCH_3)_3$	3.1	113–114
colchicine		0.05	

[a] Cells were exposed to test compound continuously for 6 h. The mitotic index no. of cells in mitosis/no. of cells counted was determined after a total of approximately 300 cells from three fields had been counted. The concentration of test compound giving the same mitotic index as colchicine (0.05 μg/mL) is given as the equivalent dose (ED). [b] All compounds were prepared by procedure A. [c] Anal. C: calcd, 70.14; found, 69.54.

Results and Discussion

To identify compounds with antimitotic activity, an in vitro assay system (HeLa cells) was used. In this asynchronous line, the cell cycle is 24 h and mitosis requires 1 h, so about 4% of the cells are in mitosis at any given time. Colchicine, a known antimitotic agent, was used as a standard. Colchicine does not prevent cells from entering mitosis but prevents completion of cell division. It arrested mitosis at a concentration of 0.05 μg/mL, i.e. it increased the number of mitotic cells in the culture ~4% every hour so that on completion of a 6-h incubation 20–24% of the cells in culture were arrested in mitosis (6 h × 4%/h). The activity of test compounds is reported as the equivalent dose (ED) which is the concentration of test compound found to be equivalent to 0.05 μg/mL colchicine. To evaluate irreversible activity, test compounds or vinblastine (0.06 μg/mL) were incubated with HeLa cells for 1 h, the cells were washed free of drug, and after 24 h the per-

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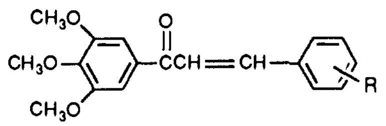
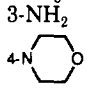
(18) X-ray determined by Dr. J. C. Huffman at Indiana University, Department of Chemistry.

Table II. Physical Properties and Equivalent Dose of Chalcones,[a] 6-h Test



compd	R	ED, μg/mL	prep.[b]	mp, °C
3	4-$NHCOCH_3$	3.1		190–191
31	3-$NHCOCH_3$	>100	*c*	164–165
32	4-*t*-Bu	2.5		100–101
33	H	0.15		87–88
34	4-SCH_3	0.3		88–89
35	4-$SOCH_3$	>100	*d*	131–132
36	4-$N(CH_3)_2$	0.015		148–149
37	3-$N(CH_3)_2$	>100	*e*	198–200
38	4-NH_2	0.15		
39	3-NO_2	>100		147–148
40	4-F	>25	*f*	115–116
41	4-CN	3.1	*g*	198–199.5
42	4-*O*-*i*Pr	>25		112–113
43	4-Br	0.015		88–91
44	4-CF_3	6.25		136.5–138
45	4-NO_2	25		190–191
46	6-CF_3	100		237–238
47	3-NH_2	0.15	*h*	198–203
48		6.25	*i*	187–188
49	4-NHC_4H_9	6.25	*i*	125–127
50	4-OC_4H_9	>25		117–118
51	4-$NHCO_2CH_3$	3.1		178–179
52	4-OC_2H_5	0.15		90–91
53	4-$N(C_2H_5)_2$	0.0075		140–141
colchicine		0.05		
vinblastine		0.0015		

[a] See footnote *a*, Table I. [b] Prepared by procedure A, unless stated otherwise. [c] Anal. N: calcd, 3.94; found, 4.38. [d] Prepared by oxidation of **34** with *m*-chloroperbenzoic acid. HCl salt·H_2O: Anal. N: calcd, 3.58; found, 4.69. [f] Anal. C: calcd, 68.34; found, 67.69. [g] Anal. C: calcd, 70.58; found, 70.03. [h] HCl salt; Anal. C: calcd, 61.80; found, 61.05. [i] Prepared from compound **40** and the appropriate amine (procedure F).

centage of cells in mitosis was determined. Again, the data are reported as ED—that concentration of test compound equivalent to 0.06 μg/mL vinblastine.

A systematic program of structural variation of lead compound **3** was undertaken. Substitution of the trimethoxyphenyl and acetamidophenyl rings was varied and substituents were added on the double bond at positions α and β to the carbonyl. The data in Table I examine the effect of substitution on the trimethoxyphenyl ring. In general, these variations did not greatly enhance activity relative to parent compound **3**. Interestingly, meta substitution (**21**, **23**, **27**) greatly diminished activity. The three trimethoxy analogues (**3**, **29**, **30**) were essentially equipotent. Structural variations involving the acetamido-substituted ring resulted in considerable improvement in ED values when the substituent was 4-dimethylamino (**36**) or diethylamino (**53**) (Table II). Again, meta substituents dramatically diminished the activity (compare compound **3**, **31**; **36**, **37**).

The data in Table III are for analogues with methoxy positional variations and substitution (alkyl, halogen) on the double bond. Several compounds were active in the 6-h assay at the nanogram level and selected compounds were found to bind in an irreversible manner, as demonstrated by activity in the 1-h test.

Data for a group of miscellaneous chalcone analogues are presented in Table IV. From these data it can be seen that substitution of the dimethylaminobenzene ring by heterocyclic rings is detrimental to biological activity; reduction of the carbonyl group (**80**) or the double bond (**81**) diminished activity. It should be noted that β-alkyl groups (**87**, **88**, **90**) do not enhance activity. The butadiene analogue (**83**) of **3** and the analogue of **3** with the substituents on the two rings reversed (**84**) each demonstrated modest activity.

The three most potent analogues in the 1-h assay were evaluated in experimental animal tumor models and the results are found in Table V. These compounds were of comparable activity to that of vinblastine at the optimum doses tested. Higher doses of these compounds relative to vinblastine were needed to obtain this activity. Higher doses of vinblastine were toxic. In summary, we report a new class of antimitotic agents, some of which are as active in vivo in experimental tumor test systems in mice as vinblastine. In addition, data presented elsewhere[19] show one of these compounds (**73**) to be inhibitory in vitro against both a murine leukemia cell line resistant to adriamycin and vinblastine and a Chinese hamster ovary tumor resistant to vinblastine. When evaluated in a bovine brain tubulin assay, the respective IC_{50} values were colchicine, 6 μM; vinblastine, 0.6 μM; nocardazole, 3 μM; and compound **73**, 1 μM.[20a] In PtK2 cell culture, **73** was reported to show superior affinity for tubulin compared to that of the vinca alkaloids.[20b]

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analysis was performed by the Merrell Dow Research Institute Analytical Department and, unless otherwise indicated, agree with theoretical values within ±0.4%. NMR spectra were obtained on a Varian VXR-300 or a Varian EM-360L spectrometer. Chemical shifts are reported downfield from TMS in spectra obtained in $CDCl_3$. IR spectra were obtained on a Perkin-Elmer 1800 FT IR spectrometer. MS were obtained on a Finnigan MAT 4600 spectrometer. All spectra were consistent with structure. Thin-layer chromatography (TLC) was done on Merck silica gel 60 F254 analytical plates, visualized with I_2 and/or UV.

3-Phenyl-1-(3,4,5-trimethoxyphenyl)-2-propyn-1-one (20). A mixture of 3,4,5-trimethoxybenzoyl chloride (4.6 g, 0.02 mol), phenylacetylene (2.04 g, 0.02 mol), cuprous iodide (20 mg), bis(triphenylphosphine)palladium chloride (20 mg), and triethylamine (40 mL) was stirred at ambient temperature under a N_2 atmosphere for 18 h. The reaction mixture was taken up in ethyl acetate (250 mL) and the solution was extracted with 1 N HCl, water, and brine. The organic layer was separated, dried ($MgSO_4$), and evaporated. The residue was chromatographed (35% EtOAc/hexane) and the purified material was recrystallized (hexane/EtOAc) to give 2.2 g (37%) of a yellow solid: mp 94–95 °C; NMR ($CDCl_3$) δ 7.70–7.25 (m, 7 H), 3.85 (s, 9 H); IR (KBr) 2940, 2215, 1660, 1585 cm^{-1}; MS (CI/CH_4) *m/e* (M + H) 297. Anal. $C_{18}H_{16}O_4$ C, H.

3-[4-(4-Morpholinyl)phenyl]-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one (48) (Procedure F). A mixture of compound **40** (3.16 g, 10 mmol), morpholine (0.85 g, 11 mmol), and potassium carbonate (1.52 g, 11 mmol) in DMF (12 mL) was heated at reflux for 18 h. The mixture was cooled and diluted with EtOAc (400 mL), and the solution was extracted with aqueous $NaHCO_3$ and brine. The organic layer was separated, dried, and evaporated. Chromatography (50% EtOAc/hexane) followed by recrystallization (EtOAc/hexane) gave 0.7 g (17%) of a yellow solid: mp 187–188 °C; NMR ($CDCl_3$) δ 7.75 (d, *J* = 11 Hz, 1 H), 7.60 (d, *J* = 7 Hz, 2 H), 7.35 (d, *J* = 11 Hz, 1 H), 7.30 (s, 2 H), 6.90 (d, *J* = 7 Hz, 2 H), 3.95 (s, 9 H), 3.85 (m, 4 H), 3.25 (m, 4

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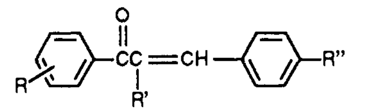
Table III. Physical Properties and Equivalent Dose of Chalcones



compd	R	R'	R''	ED, μg/mL 6 h[a]	1 h[b]	prep.	mp, °C
36	3,4,5-$(OCH_3)_3$	H	$N(CH_3)_2$	0.015	12.5	A	148–149
54	2-OCH_3	H	$N(CH_3)_2$	0.15	6.25	A	165–169
55	2,4,6-$(OCH_3)_3$	H	$N(CH_3)_2$	12.5		A	149–150
56	2,5-$(OCH_3)_2$	H	$N(CH_3)_2$	0.0075	1.5	A	108–109
57	3,4-$(OCH_3)_2$	H	$N(CH_3)_2$	3.1		A	83–84
58	2,4-$(OCH_3)_2$	H	$N(CH_3)_2$	6.25		A	124–125
59	2,3,4-$(OCH_3)_3$	H	$N(CH_3)_2$	0.015		A	86–88
60	3,5-$(OCH_3)_2$, 4-OH	H	$N(CH_3)_2$	3.1		A	202–203
61	2,3,4-$(OCH_3)_3$	H	$N(C_2H_5)_2$	0.15		A	95–96
62	2,3,4-$(OCH_3)_3$	CH_3	$N(C_2H_5)_2$	25		B	78–79
63	3,4,5-$(OCH_3)_3$	CH_3	$N(C_2H_5)_2$	0.0075		B	220–225
64	2,5-$(OCH_3)_2$	CH_3	$N(C_2H_5)_2$	0.15		B	60–61
65	2,5-$(OCH_3)_2$	Br	$N(CH_3)_2$	0.0075		C[c]	74–75
66	1,3,4-$(OCH_3)_3$	Br	$N(CH_3)_2$	0.15	6.25	C	139–140
67	3,4,5-$(OCH_3)_3$	Br	$N(CH_3)_2$	0.0038	1.5	C	111–113
68	2,5-$(OCH_3)_2$	Cl	$N(CH_3)_2$	0.015		D[d]	205–210
69	2,3,4-$(OCH_3)_3$	Cl	$N(CH_3)_2$	0.015		D	125–126
70	3,4,5-$(OCH_3)_3$	Cl	$N(CH_3)_2$	3.1		D	86–87
71	2,3,4-$(OCH_3)_3$	H	2-F			A	71–73
72	2,3,4-$(OCH_3)_3$	C_2H_5	$N(CH_3)_2$	0.15		B	260
73	2,5-$(OCH_3)_2$	CH_3	$N(CH_3)_2$	0.0038	0.06	B	85–86
74	2,3,4-$(OCH_3)_3$	CH_3	H		6.25	B	oil
75	2,3,4-$(OCH_3)_3$	CH_3	$N(CH_3)_2$	1.5		B	97–98
76	3,4,5-$(OCH_3)_3$	CH_3	$N(CH_3)_2$	0.019	0.3	B	113–114
colchicine				0.05	inactive		
vinblastine				0.0015	0.06		

[a] Six-hour test—see footnote *a*, Table I. [b] HeLa cells were exposed to test compound for 1 h. The cells were washed in fresh medium and incubated in compound-free medium for 18 h. The mitotic index was determined as previously described. That concentration of test compound which gave the same mitotic index as vinblastine (0.06 μg/mL) is listed as the equivalent dose (ED). [c] Prepared by addition of Br_2 to unsubstituted precursor (procedure C). [d] Prepared by reaction of unsubstituted precursor with sulfuryl chloride (procedure D).

H); IR (KBr) 3440, 1650, 1570 cm^{-1}; MS (EI) *m/e* (M^+) 383. Anal. $C_{22}H_{25}NO_5$ C, H, N.

(*E*)-3-[4-(Diethylamino)phenyl]-1-(2,3,4-trimethoxyphenyl)-2-propen-1-one (61) (Procedure A). A mixture of ethanol (50 mL), 2,3,4-trimethoxyacetophenone (3.1 g, 0.015 mol), 4-(diethylamino)benzaldehyde (2.5 g, 0.015 mol), and 50% aqueous NaOH (1 mL) was stirred 18 h at ambient temperature. The mixture was evaporated and the residue was chromatographed on a flash silica gel column (toluene/ethyl acetate, 10/1). The purified material from the column was recrystallized from ethanol to give the product (1.7 g, 31%) as bright yellow needles: mp 95–96 °C; NMR ($CDCl_3$) δ 1.2 (t, *J* = 9 Hz, 6 H), 3.4 (q, *J* = 9 Hz, 4 H), 3.8 (s, 9 H), 6.5–6.75 (m, 3 H), 7.25–7.5 (m, 5 H); IR (KBr) 1650, 1595, 1575, 1550, 1520, 1460, 1410, 1360, 1290, 1250, 1185, 1160, 1100, 1075, 1020, 810 cm^{-1}; MS (EI) *m/e* (M^+) 369. Anal. $C_{25}H_{27}NO_4$ C, H, N.

(*Z*)-2-Bromo-3-[4-(dimethylamino)phenyl]-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one (67) (Procedure C). A solution of compound **36** (20.3 g, 0.054 mol) in CCl_4 (650 mL) was stirred at ambient temperature while a solution of bromine (8.6 g, 0.054 mol) in CCl_4 (100 mL) was added dropwise over 45 min. The solvent was removed at reduced pressure and the residue was chromatographed (35% EtOAc/hexane). The purified material was recrystallized from ethyl acetate/hexane to give 4.2 g (23%) of yellow powder: mp 111–113 °C; NMR ($CDCl_3$) δ 7.85 (d, *J* = 9 Hz, 2 H), 7.20 (s, 1 H), 6.95 (s, 2 H), 7.70 (d, *J* = 9 Hz, 2 H), 3.90 (s, 3 H), 3.80 (s, 6 H), 3.05 (s, 6 H); IR (KBr) 3440, 1700, 1630, 1570 cm^{-1}. Anal. $C_{20}H_{22}BrNO_4$ C, H, N, Br.

(*Z*)-2-Chloro-3-[4-(dimethylamino)phenyl]-1-(2,3,4-trimethoxyphenyl)-2-propen-1-one (69) (Procedure D). A solution of compound **59** (1.4 g, 7.2 mmol) in CCl_4 (20 mL)/CH_2Cl_2 (10 mL) was chilled to 0 °C. A solution of sulfuryl chloride (1.06 g, 7.8 mmol) in CH_2Cl_2 (4 mL) was added dropwise. The mixture was stirred for 1 h at 0 °C and poured into saturated $NaHCO_3$, and the organic layer was separated, dried ($MgSO_4$), and evaporated. Chromatography (25% EtOAc/hexane) followed by two recrystallizations (Et_2O/hexane) gave 0.630 g (23%) of yellow solid: mp 125–126 °C; NMR ($CDCl_3$) δ 7.80 (d, *J* = 9 Hz, 2 H), 7.35 (s, 1 H), 7.05 (d, *J* = 8 Hz, 1 H), 7.20 (d, *J* = 8 Hz, 1 H), 7.15 (d, *J* = 9 Hz, 2 H), 3.85 (s, 9 H), 3.00 (s, 6 H); IR (KBr) 3440, 3089, 1641, 1616, 1594, 1301 cm^{-1}; MS (CI/CH_4) *m/e* (M^+H) 376. Anal. $C_{20}H_{22}ClNO_4$ C, H, N, Cl.

(*E*)-1-(2,5-Dimethoxyphenyl)-3-[4-(dimethylamino)phenyl]-2-methyl-2-propen-1-one (73) (Procedure B). A solution of 2,5-dimethoxypropiophenone (14.8 g, 0.076 mol), 4-(dimethylamino)benzaldehyde (11.6 g, 0.078 mol), piperidine (15 mL), and acetic acid (7.5 mL) in ethanol (80 mL) was heated at reflux. The ethanol was dried by passing the distillate through 3-Å sieves by use of a Soxhlet apparatus. After 18 h the solvent was removed and the residue was chromatographed on a flash silica gel column using 25% EtOAc in hexane as eluant. The product was recrystallized from ether to give 17.2 g (70%) of a bright yellow solid: mp 85–86 °C; NMR ($CDCl_3$) δ 7.30 (d, *J* = 9 Hz, 2 H), 7.10 (br s, 1 H), 6.85–6.50 (m, 5 H), 3.75 (s, 3 H), 3.70 (s, 3 H), 2.95 (s, 6 H), 2.25 (br s, 3 H); IR (KBr) 3420, 2900, 1630, 1590 cm^{-1}; MS (CI/CH_4) *m/e* (M^+H) 326. Anal. $C_{20}H_{23}NO_3$ C, H, N.

(*E*)-[2-[4-(Dimethylamino)phenyl]ethenyl](3,4,5-trimethoxyphenyl)methanol (80). Sodium borohydride (0.5 g, 13 mmol) was added to a solution of compound **36** (2 g, 5.2 mmol) in ethanol (100 mL) and the mixture was stirred at ambient temperature for 18 h. The solvent was removed and the residue was taken up in EtOAc (200 mL). The solution was extracted with aqueous Na_2CO_3; the organic layer was separated, dried, and evaporated. The residue was chromatographed (50% EtOAc/hexane) and the purified material was recrystallized from ether to yield 0.07 g (3.5%) of **80**: mp 69–70 °C; NMR ($CDCl_3$) δ 7.30–7.0 (m, 3 H), 6.75–6.40 (m, 5 H), 3.75 (s, 9 H), 3.50 (m, 1 H), 2.85 (s, 6 H); IR (KBr) 3440, 2940, 1610, 1590, 1175 cm^{-1}. Anal. $C_{20}H_{25}NO_4$ H, N; C: calcd, 69.95; found, 69.51.

3-[4-(Dimethylamino)phenyl]-1-(3,4,5-trimethoxyphenyl)-1-propanone (81). A solution of compound **36** (2.4 g, 7 mmol) in acetic acid (600 mL) was hydrogenated at atmospheric pressure in the presence of 10% Pd/C (0.6 g) until the theoretical

Table IV. Physical Properties and Equivalent Dose (ED) of Analogues with HeLa Cells, 6-h Test

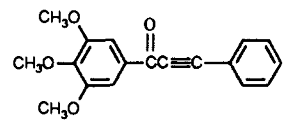
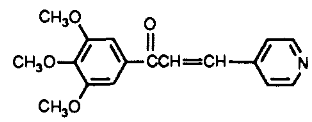
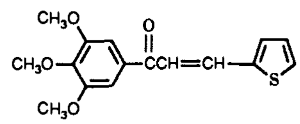
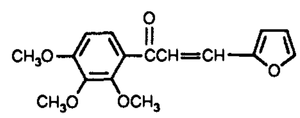
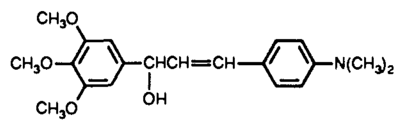
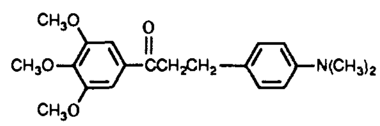
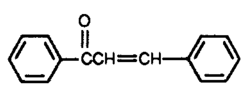
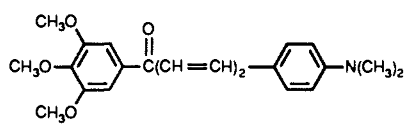
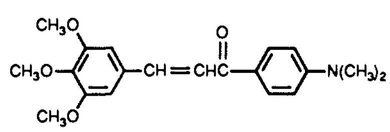
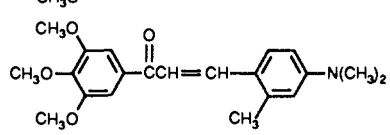
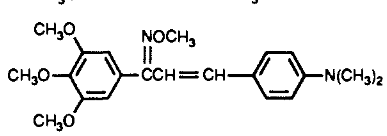
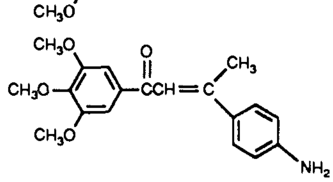
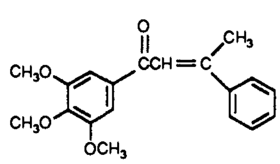
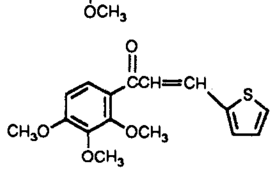
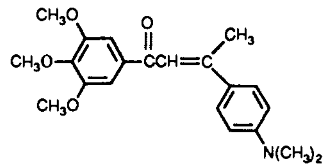
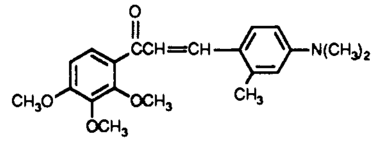
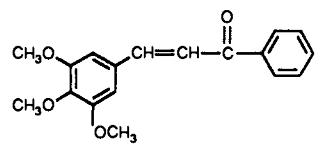
compd	structure	ED,[a] μg/mL	prep.	mp, °C
20		>6.25	E[b]	94–95
77		25	B	128–129
78		3.1	A	69–71
79		5	A	72–73
80		3.1	E[b]	69–70[c]
81		3.1	E[b]	98.5–100
82		3.1	E[b]	56–57[d]
83		3.1	A	141–142
84		0.15	A	159–160
85		0.0075	A	163–164
86		0.3	E[b]	113–114
87		0.3	F[e]	117–118
88		3.1	F[e]	oil
89		3.1	A	69–71

Table IV (Continued)

compd	structure	ED,[a] μg/mL	prep.	mp, °C
90		>100	E[b], F[e]	104–105
91		3.1	A	76–77
92		>25	A	133–134
colchicine		0.05		

[a] See footnote *a*, Table I. [b] Letter E denotes that preparation is described in the Experimental Section. [c] Anal. C: calcd, 69.96; found, 69.51. [d] Purchased from Aldrich Chemical Co. [e] Letter F denotes preparation by procedure E as represented by Scheme IV.

Table V. In Vivo Evaluation of Selected Chalcones[a]

	L1210 leukemia		B_{16} melanoma	
compd	dose, mg/kg	% T/C	dose, mg/kg	% T/C
66	25	145	100	192
	50	140		
73	6.25	126	12.5	146
	12.5	137	25	183
	25	146		
	50	154		
75	25	121	25	190
	50	138		
vinblastine	0.2	140	0.2	180

[a] Test animals were inoculated with either L1210 cells or B_{16} melanoma (1×10^5) ip on day 0. The compounds were suspended (**66**, **73**, **75**) or dissolved (vinblastine) in 5% PVP and administered ip once daily on days 1–9. The survival time was noted and an average was determined. The ratio of survival time for treated divided by survival time of control × 100 is reported as % T/C.

uptake of H_2 had occurred. The mixture was filtered and the filtrate was evaporated; the residue was taken up in ethyl acetate, and the solution was extracted with aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated. The residue was chromatographed (50% EtOAc/hexane) and the purified product was recrystallized (Et_2O) to give **81** (0.9 g, 37%): mp 98.5–100 °C; NMR ($CDCl_3$) δ 7.15 (s, 2 H), 7.10 (d, *J* = 9 Hz, 2 H), 6.70 (d, *J* = 9 Hz, 2 H), 3.80 (s, 9 H), 3.25–2.80 (m, 4 H), 2.80 (s, 6 H); IR (KBr) 3440, 2940, 1740, 1580 cm^{-1}. Anal. $C_{20}H_{25}NO_4$ C, H, N.

(*E*)-3-[4-(Dimethylamino)phenyl]-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one *O*-Methyloxime (86). A mixture of **36** (1.7 g, 5 mmol), methoxyamine hydrochloride (0.46 g, 5.5 mmol), and pyridine (1.18 g, 1.5 mmol) in ethanol (50 mL) was stirred at reflux for 18 h. The solvent was evaporated, the residue was dissolved in EtOAc (300 mL), and the solution was extracted with water, dried, and evaporated. The residue was recrystallized from EtOAc/hexane then from EtOH to give 0.38 g (21%) of product: mp 113–114 °C; NMR ($CDCl_3$) δ 7.4–7.3 (m, 3 H), 7.8–7.6 (m, 3 H), 7.75 (s, 2 H), 4.1 (s, 3 H), 3.85 (s, 9 H), 3.0 (s, 6 H); IR (KBr) 3420, 1590, 1580, 1350 cm^{-1}; MS (CI/isobutane) *m/e* (M^+H) 371. Anal. $C_{21}H_{26}N_2O_4$ C, H, N.

3-[4-(Dimethylamino)phenyl]-1-(3,4,5-trimethoxyphenyl)-2-buten-1-one (90) (Procedure E). A mixture of dimethyl sulfoxide (DMSO) (30 mL), triethylamine (10 mL), 3,4,5-trimethoxy-α-1,2-propadienylbenzenemethanol[21] (2.6 g, 11.0 mmol), 4-bromo-*N*,*N*-dimethylaniline (2.0 g, 10 mmol), palladium acetate (0.11 g, 0.05 mmol), and tritolylphosphine (0.61 g, 2.0 mmol) was heated at 110 °C (bath temperature) for 20 min. The mixture was cooled and poured into EtOAc (200 mL), and the solution was extracted with water. The organic layer was dried and evaporated. The residue was chromatographed (25% EtOAc/hexane) and crystallized from Et_2O/hexane to give a yellow solid (0.4 g, 11%): mp 104–105 °C; NMR ($CDCl_3$) δ 7.50 (d, *J* = 8 Hz, 2 H), 7.20 (s, 2 H), 7.10 (br s, 1 H), 6.40 (d, *J* = 8 Hz, 2 H), 3.85 (s, 9 H), 3.00 (s, 6 H), 2.65 (br s, 3 H); IR (KBr) 3442, 1637, 1611, 1563 cm^{-1}; MS (CI/CH_4) *m/e* (M^+H) 350. Anal. $C_{21}H_{25}NO_4$ C, H, N.

Registry No. **3**, 127033-84-5; **20**, 127033-85-6; **21**, 127033-86-7; **22**, 127033-87-8; **23**, 127033-88-9; **24**, 127033-89-0; **25**, 127033-90-3; **26**, 127033-91-4; **27**, 127033-92-5; **28**, 127033-93-6; **29**, 127033-94-7; **30**, 127033-95-8; **31**, 127033-96-9; **32**, 127033-97-0; **33**, 127033-98-1; **34**, 127033-99-2; **35**, 127034-00-8; **35**·HCl, 127034-01-9; **36**, 127034-02-0; **37**, 127034-03-1; **38**, 127034-04-2; **39**, 127034-05-3; **40**, 127034-06-4; **41**, 127034-07-5; **42**, 127034-08-6; **43**, 127034-09-7; **44**, 127034-10-0; **45**, 127034-11-1; **46**, 127034-12-2; **47**, 127034-13-3; **48**, 127034-14-4; **49**, 127034-15-5; **50**, 127034-16-6; **51**, 127034-17-7; **52**, 127034-18-8; **53**, 127034-19-9; **54**, 127034-20-2; **55**, 127034-21-3; **56**, 127034-22-4; **57**, 127034-23-5; **58**, 127034-24-6; **59**, 127034-25-7; **60**, 127034-26-8; **61**, 127034-27-9; **62**, 127034-28-0; **63**, 127034-29-1; **64**, 127034-30-4; **65**, 127034-31-5; **66**, 127034-32-6; **67**, 127034-33-7; **68**, 127034-34-8; **69**, 127034-35-9; **70**, 127034-36-0; **71**, 127034-37-1; **72**, 127034-38-2; **73**, 124711-23-5; **74**, 127034-39-3; **75**, 127034-40-6; **76**, 127034-41-7; **77**, 127034-42-8; **78**, 127034-43-9; **79**, 127034-44-0; **80**, 127034-45-1; **81**, 127034-46-2; **82**, 614-47-1; **83**, 127034-47-3; **84**, 127034-48-4; **85**, 127034-49-5; **86**, 127034-50-8; **87**, 127034-51-9; **88**, 127063-58-5; **89**, 127034-52-0; **90**, 127034-53-1; **91**, 127034-54-2; **92**, 127034-55-3; *m*-EtC_6H_4Ac, 22699-70-3; *p*-$MeOC_6H_4Ac$, 100-06-1; *m*-$F_3CC_6H_4Ac$, 349-76-8; *p*-*t*-BuC_6H_4Ac, 943-27-1; *p*-NCC_6H_4Ac, 1443-80-7; *p*-$H_2NCOC_6H_4Ac$, 67014-02-2; *m*-ClC_6H_4Ac, 99-02-5; *m*-$AcNHC_6H_4CHO$, 59755-25-8; *p*-*t*-BuC_6H_4CHO, 939-97-9; PhCHO, 100-52-7; *p*-$MeSC_6H_4CHO$, 3446-89-7; *m*-$Me_2NC_6H_4CHO$, 619-22-7; *p*-$H_2NC_6H_4CHO$, 556-18-3; *m*-$O_2NC_6H_4CHO$, 99-61-6; *p*-FC_6H_4CHO, 459-57-4; *p*-NCC_6H_4CHO, 105-07-7; *p*-*i*-$PrOC_6H_4CHO$, 18962-05-5; *p*-BrC_6H_4CHO, 1122-91-4; *p*-$F_3CC_6H_4CHO$, 455-19-6; *p*-$O_2NC_6H_4CHO$, 555-16-8; *m*-$F_3CC_6H_4CHO$, 454-89-7; *m*-$H_iNC_6H_4CHO$, 1709-44-0; *p*-$BuNHC_6H_4CHO$, 89074-18-0; *p*-$BuOC_6H_4CHO$, 5736-88-9; *p*-$MeOCONHC_6H_4CHO$, 20131-81-1; *p*-$EtOC_6H_4CHO$, 10031-82-0; *o*-FC_6H_4CHO, 446-52-6; 3,4,5-$MeOC_6H_2COCH{=}CHCH_3$, 67382-46-1; *p*-$Me_2NC_6H_4COCH_3$, 2124-31-4; *p*-$BrC_6H_4NH_2$, 106-40-1; PhBr, 108-86-1; 3,4,5-trimethoxybenzoyl chloride, 4521-61-3; phenylacetylene, 536-74-3; 2,3,4-trimethoxyacetophenone, 13909-73-4; 4-(diethylamino)benzaldehyde, 120-21-8; 2,5-dimethoxypropiophenone, 5803-30-5; 4-(dimethylamino)benz-

(21) Synthesized by procedure of Crabbee, P.; Fillion, D. A.; Lucke, J. *J. Chem. Soc., Chem. Commun.* **1979**, 859.

aldehyde, 100-10-7; 3,4,5-trimethoxy-α-1,2-propadienylbenzenemethanol, 127034-56-4; 4-bromo-*N,N*-dimethylaniline, 586-77-6; 3,4,5-trimethoxyacetophenone, 1136-86-3; 2,5-dimethoxyacetophenone, 1201-38-3; 2,4,6-trimethoxyacetophenone, 832-58-6; 4-acetamidobenzadlehyde, 122-85-0; 2-methoxyacetophenone, 579-74-8; 3,4-dimethoxyacetophenone, 1131-62-0; 2,4-dimethoxyacetophenone, 829-20-9; 4-hydroxy-3,5-dimethoxyacetophenone, 2478-38-8; 2,3,4-trimethoxypropiophenone, 18060-58-7; 3,4,5-trimethoxypropiophenone, 5658-50-4; 2,5-dimethoxypropiophenone, 5803-30-5; 1-(2,3,4-trimethoxyphenyl)-1-butanone, 108401-78-1; 4-pyridinecarboxaldehyde, 872-85-5; 2-thiophenecarboxaldehyde, 98-03-3; trimethoxybenzaldehyde, 86-81-7; 4-(dimethylamino)-2-methylbenzaldehyde, 1199-59-3; 2-furancarboxaldehyde, 98-01-1; benzophenone, 119-61-9.

Analogues of Growth Hormone-Releasing Factor (1–29) Amide Containing the Reduced Peptide Bond Isostere in the N-Terminal Region[1]

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Previous peptide structure–activity investigations employing the $\psi[CH_2NH]$ peptide bond isostere have produced antagonists when inserted into various sequences. These include bombesin, in which the incorporation of Leu[13]$\psi[CH_2NH]$Leu[14] produced a potent antagonist, and tetragastrin, with which Boc-Trp-Leu$\psi[CH_2NH]$Asp-Phe-NH_2 is an antagonist. In this study, we chose to investigate the effect of this isostere on growth hormone-releasing factor (1–29) amide. Analogues were prepared by solid-phase synthesis and the isosteres incorporated by racemization-free reductive alkylation with a preformed protected amino acid aldehyde in the presence of $NaBH_3CN$. The aldehydes were prepared by the reduction of the protected *N,O*-dimethyl hydroxamates with $LiAlH_4$ at 0 °C. The purified analogues were assayed in a 4-day primary culture of male rat anterior pituitary cells for growth hormone (GH) release. Potential antagonists were retested in the presence of GRF(1–29)NH_2. The following results were obtained: At position 5–6, a very weak agonist was produced with $\ll$0.01% activity. Incorporation of the isostere in positions 1–2, 2–3, and 6–7 gave weak agonists with $\sim$0.1% activity. Agonists with 0.39% and 1.6% activity were produced by incorporation at 10–11 and 3–4, respectively. The analogue [Ser[9]$\psi[CH_2NH]$Tyr[10]]GRF(1–29)NH_2 was found to be an antagonist in the 10 μM range vs 1 nM GRF and had no agonist activity at doses as high as 0.1 mM.

Growth hormone-releasing factor (GRF), a 44-residue peptide, was isolated from a pancreatic tumor occurring in an acromegalic patient.[2,3] The structural characterization of GRF has resulted in numerous basic and clinical studies into this peptide's role in the control of GH secretion and its ultimate effects on growth itself. Structure-activity studies have shown that the full sequence is not required for activity and that the shortened sequence GRF(1–29)NH_2 is fully potent,[3] greatly simplifying the synthesis of analogues for further structure–activity studies. The data accumulated thus far indicate that GRF may be of value in certain clinical disorders, including childhood GH deficiency, as well as in agricultural applications pertaining to milk and meat production. In common with a number of other peptide hormones, the plasma half-life of GRF is of the order of minutes.[4] This presents a major problem in the development of potent long-acting therapeutic or agricultural agents. The most common approach to increasing the potency and duration of action of analogues has been the incorporation of unusual or D-amino acid residues in various regions of the hormone.[5-9] A less common strategy in the elaboration of structure–activity relationships is the modification of the peptide backbone by the incorporation of various peptide bond isosteres including $\psi[CH_2NH]$, $\psi[CH_2S]$, and $\psi[CH_2CH_2]$.[10] Previously, the incorporation of these isosteres required the custom synthesis of modified dipeptide units with the concomitant risk of racemization of the non-urethane-protected carboxyl terminus during activation. We have developed a racemization-free, solid-phase method for the generation of the $\psi[CH_2NH]$ isostere in situ,[11,12] which greatly facilitates the investigation of the role of the backbone in peptide activity. The method involves the reductive alkylation of the deprotected, resin-bound peptide with a protected amino acid aldehyde in the presence of $NaBH_3CN$. Structure–activity investigations with the $\psi[CH_2NH]$ peptide bond isostere have produced antagonists when inserted into various peptides. These include bombesin, in which the incorporation of Leu[13]$\psi[CH_2NH]$Leu[14] produced an antagonist,[13] and tetragastrin, in which Boc-Trp-Leu$\psi[CH_2NH]$Asp-

(1) Abbreviations used in this paper for amino acids, protecting groups, and peptides follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature and Symbols as described in *Eur. J. Biochem.* **1972**, *27*, 201 and *J. Biol. Chem.* **1975**, *250*, 3215. $\psi[CH_2NH]$, peptide bond replaced by a CH_2NH bond.

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