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# Antitumor Agents. Part 214:† Synthesis and Evaluation of Curcumin Analogues as Cytotoxic Agents

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**Abstract**—Fifty-eight curcumin analogues were prepared and evaluated for in vitro cytotoxicity against a panel of human tumor cell lines. Compound **50** was the most potent analogue against several cell lines, including HOS (bone cancer) and 1A9 (breast cancer), with $ED_{50}$ values of 0.97 and $<0.63$ μg/mL, respectively.


## Introduction

The rhizome of *Curcuma longa* L. (turmeric) has been widely used as a yellow coloring agent and spice in many foods, and it has also been used in indigenous medicine for the treatment of inflammatory and other diseases.[2] Curcumin (**1**), a well-known acyclic diarylheptanoid, has been identified as the major constituent in turmeric. Recently, numerous studies have demonstrated the remarkable cancer preventive properties of curcumin (**1**).[2–13] The chemopreventive effects of curcumin have been attributed to various biological properties, including neutralization of carcinogenic free radicals[3] and anti-angiogenesis action, which limits the blood supply to rapidly growing malignant cells.[4,5] In addition, curcumin possesses other pharmacological properties, including stimulation of Phase I and Phase II detox systems, (e.g., inhibition of COX-1 and COX-2 enzymes, and stimulation of glutathione *S*-transferase).[6,7] In addition, curcumin exhibited remarkable cytotoxic effects on various cancer cells[8–10] and induced apoptotic cell death in human promyelocytic leukemia HL-60 cells and human oral squamous carcinoma HSC-4 cells.[11]

In our previous paper,[12] we reported that 13-oxomyricanol (**16**) and myricanone (**17**), two cyclic diarylheptanoids, exhibited potent antitumor promoting effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse skin carcinogenesis. Furthermore, **17** inhibited papilloma formation initiated by peroxynitrite.[13]

In a continuing search for potent and selective cytotoxic antitumor agents, we prepared 32 curcumin analogues (**1**–**32**) and evaluated their cytotoxic effects against a panel of human tumor cell lines. We also evaluated 26 β-diketone compounds (**33**–**58**) that are structurally related to curcumin (**1**). In addition, the effects of selected compounds on drug resistance were evaluated using a drug resistance reversal assay.

## Chemistry

As shown in Figure 1, compounds **1**, **2**, and **3** were obtained by column chromatography (silica gel, $CHCl_3$–MeOH) of commercially available (Aldrich) curcumin, which contained **2** and **3** as minor components. Compounds **4**–**6** were prepared by heating **1**–**3** overnight with histidine hydrazide, acetic acid, and *p*-TsOH. Acetylation of **1** gave **7**. Compound **8** was obtained by demethylation of **1** with $AlCl_3$ and pyridine in $CH_2Cl_2$. Compound **9** was obtained by treating **1** with methyl iodide. Hydrogenation of **1**, **9**, and **50** with Pd/C gave **10**–**13** and **51**. Preparation of **14**–**23** was described in a previous paper.[12] Compounds **24**–**32** were synthesized from the appropriate benzaldehyde

†For Paper 213, See ref 1.

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and 2,4-pentanedione by the method reported by Pedersen et al.[14] Compounds **33–50** and **52–58** were obtained from Aldrich Inc. (Milwaukee, WI, USA).

## Results and Discussion

### Cytotoxicity of diarylheptanoids

Thirty-two curcumin derivatives (**1–32**) were tested for cytotoxicity against a panel of human tumor cell lines. Selected results for active compounds are summarized in Table 1. Curcumin hydrazide (**4**) showed a broad cytotoxicity spectrum ($ED_{50}$ values from 1.0 to 3.9 μg/mL against KB, A549, CAKI-1, MCF-7, 1A9, HCT-8, SK-MEL-2, U-87-MG, HOS, PC-3, KB-VIN, HepG2, and LNCaP (clone FGC) cell lines. Demethylcurcumin (**8**) was selectively active against KB cells with an $ED_{50}$ value of 1.0 μg/mL, and trimethylcurcumin (**9**) was also active against a narrow spectrum of cell lines (1A9, U87-MG, and HOS). All hydrogenated (**10–13**) and cyclic (**14–23**) diarylheptanoids were inactive. Compound **26**, which is *o*-fluorinated on each benzene ring, had a broad cytotoxicity spectrum, but borderline activity, while the remaining fluorinated diarylheptanoids (**24**, **25**, and **27–32**) were inactive.

By comparing the cytotoxicity results in Table 1, the following structure–activity relationships (SARs) were drawn. (a) Converting the keto-enol moiety (**1–3**) to the corresponding pyrazole (**4–6**) led to increased cytotoxicity against various cell lines. Compound **4** was active in all cell lines, whereas, compounds **5** and **6** exhibited selective activity against the 1A9 cell line. Thus, the ring substituents affected the activity in the pyrazole derivatives. (b) Demethylation or methylation of **1** to form the dihydroxy (**8**) and trimethyl (**9**) derivatives, respectively, significantly increased cytotoxic activity against 1A9, KB and HCT-8 cell lines. Thus, the presence of catechol or 3,4-dimethoxyphenyl constituents enhanced the cytotoxic properties. (c) Saturation of the olefinic bonds of **1** and **9** to give **10–13** abolished activity. In addition, all cyclic diarylheptanopids (**14–23**) were inactive. Therefore, the presence of the conjugated β-diketone in the acyclic carbon chain appears to play an important role for cytotoxicity in this class. (d) Fluorination at the *ortho* position of both benzene rings (**26**) increased activity. Introducing a methoxy group at the 4- (**31**) or 6- (**32**) position of both fluorinated rings decreased cytotoxicity. Additional fluorination (**30**) or fluorination solely (**24**) at the *para* position abolished activity. Thus, the position and nature of substituents on the benzene rings seem to modulate antitumor activity.

### Cytotoxicity of β-diketones (1,3-diaryl-1,3-diketo-propane)

Twenty-six 1,3-diaryl-1,3-diketo-propane and related analogues (**33–58**) were also tested for cytotoxicity against the human tumor cell line panel. The $ED_{50}$ values of the active compounds are summarized in Table 1. Compound **38** (β-diketone) displayed moderate cytotoxicity, whereas **34** (β-triketone) and **36** (β-tetraketone) were inactive. Compound **44** (4-chlorophenyl) was also more active than **35**, the corresponding triketone. Thus, it would appear that the β-diketone moiety enhances the cytotoxic properties. Compound **40** (4-*tert*-butylated phenyl) was more potent than **38** (unsubstituted) and **39** (3,5-dimethylphenyl) against 1A9 cells selectively. Compound **41** (2,4-dimethoxyphenyl) showed no activity. Thus, introducing the *tert*-butyl group (an electron-donating substituent) on the phenyl ring led to increased cytotoxicity against the 1A9 cell

**Table 1.** Selected in vitro cytotoxicity of curcumin analogues

| Compd | $ED_{50}$ (μg/mL)[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KB[b] | A549 | CAKI-1 | MCF-7 | 1A9 | HCT-8 | SK-MEL-2 | U-87-MG | HOS | PC-3 | KB-VIN | HepG2 | LNCaP clone FGC |
| **1** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | ND | ND |
| **4** | 1.0 | 1.7 | 2.4 | 3.9 | 1.8 | 1.9 | 2.4 | 2.4 | ND | 2.8 | ND | 2.5 | 2.0 |
| **5** | 9.0 | >20 | ND | 8.8 | 4.2 | 7.0 | 13.6 | 14.0 | 10.2 | 13.0 | >20 | ND | ND |
| **6** | 10.7 | 20 | ND | 13.5 | 1.2 | 10.0 | 14.1 | >20 | 10.5 | 15.0 | 9.5 | ND | ND |
| **8** | 1.0 | 8.0 | >10 | 9.5 | 3.8 | ND | >10 | ND | ND | ND | ND | ND | ND |
| **9** | 7.9 | 7.5 | ND | 6.5 | 1.1 | 2.2 | 4.6 | 3.0 | 4.0 | 5.2 | 6.7 | 5.4 | 13.0 |
| **26** | 4.3 | 6.0 | 7.6 | 4.7 | 3.9 | 6.3 | 7.4 | 6.1 | 4.9 | 5.8 | 6.1 | 6.9 | ND |
| **38** | >20 | 20 | ND | 20 | 6.0 | 7.5 | >20 | >20 | <10 | >20 | >20 | ND | ND |
| **39** | >20 | 20 | ND | 14.5 | 4.8 | 7.5 | >20 | >20 | <10 | >20 | >20 | ND | ND |
| **40** | >20 | >20 | ND | >20 | 3.9 | 10.0 | >20 | >20 | 16.0 | >20 | >20 | ND | ND |
| **42** | 16.5 | >20 | ND | 15 | 4.0 | 12.0 | 16.0 | 16.3 | 8.0 | 16.0 | 16.5 | ND | ND |
| **43** | 20 | 16.5 | ND | 13.5 | <10 | 5.8 | >20 | >20 | >10 | >20 | >20 | ND | ND |
| **44** | 19.5 | >20 | ND | >20 | 5.0 | 8.0 | >20 | >20 | 9.0 | >20 | 19.5 | ND | ND |
| **45** | 19.7 | 17.5 | ND | 17.5 | <2.5 | 9.7 | >20 | >20 | 10.0 | >20 | >20 | ND | ND |
| **46** | 15.5 | 18 | ND | 15.0 | 3.9 | 6.0 | 7.0 | 15.0 | 12.0 | 14 | 15.0 | ND | ND |
| **50** | 3.8 | 4.4 | ND | 7.0 | <0.63 | 1.8 | 2.0 | 3.8 | 0.97 | 5.0 | 4.5 | ND | ND |
| **51** | 14.4 | 18.8 | ND | 16.7 | 13.6 | 18.3 | >20 | 18.8 | 18.0 | >20 | >20 | ND | ND |
| **57** | 4.5 | 8.9 | ND | 4.0 | 2.1 | 3.0 | 6.0 | 7.7 | <2.5 | 8.0 | 7.4 | ND | ND |

ND, not determined.

[a]Cytotoxicity as $ED_{50}$ for each cell line, the concentration of compound that causes 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay.

[b]The human cell line panel consisted of epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A549), renal cancer (CAKI-1), breast cancer (MCF-7), ovarian cancer (1A9), ileocecal carcinoma (HCT-8), melanoma (SK-MEL-2), glioblastoma (U-87-MG), bone cancer (HOS), liver cancer (HepG2), and prostate cancer (PC-3, LNCaP clone FGC). The human KB drug-resistant sub-line panel included KB-VIN (expressing P-glycoprotein).

line. Replacing the hydrogen atom with fluorine (an electron-withdrawing substituent) at the *para* position on the benzene rings (**45**) increased activity against 1A9 cells compared with unsubstituted **38** and 4-chlorophenyl compounds (**43** and **44**). β-Bromination between the keto groups (**42**, **46**, and **50**) led to enhanced activity compared with the unsubstituted compounds (**39**, **38**, and **49**) against 1A9 cells. However, nitroso (**47**), benzoyl methyl (**53**), and furan (**56**) substitution at this position abolished activity.

Compound **50**, which has an α-bromo substituent and 4-nitro and 4-methoxy groups on separate benzene rings, demonstrated the strongest cytotoxic effects against HOS and 1A9 cells with $ED_{50}$ values of 0.97 and $<0.63$ μg/mL, respectively. However, compound **51**, which has a 4-amino rather than the 4-nitro group, showed decreased activity, and compound **48**, which has 4-nitro groups on both benzene rings, was inactive. Thus, asymmetrical substitution led to enhanced activity and different electronegative aryl substituents (4-nitro and 4-methoxy) led to increased activity against 1A9 and HOS cells selectively. Compound **50** is highly electrophilic and would be expected to be a DNA-interactive agent.

In addition, replacing the phenyl groups in **46** with thiophenyl groups (**57**) increased cytotoxicity against HOS and 1A9 cell lines ($ED_{50}$ values of $<2.5$ and 2.1 μg/mL, respectively).

### Inhibitory activity of curcumin analogues on P-glycoprotein mediated drug resistance

Six compounds (**1**, **2**, **3**, **4**, **17**, and **21**) were also assayed for vincristine toxicity potentiation in tumor cells (KB-VIN) possessing P-glycoprotein mediated drug resistance. Interestingly, the cyclic ketal **21** inhibited P-glycoprotein in a dose-dependent manner in the presence of vincristine; the corresponding ketone (**17**) was less active. Although **21** was less active than verapamil as judged by potentiation of vincristine toxicity (Table 2), it was also less toxic. Additional studies are planned with this initial novel lead for potential modulation of multidrug resistance.

**Table 2.** Effects of co-treatment with verapamil or **21** on vincristine toxicity

| Treatment | $ED_{50}$ (μM)[a] | Potentiation index[b] |
|---|---|---|
| Verapamil | 30.5 | NA[c] |
| **21** | >40.0 | NA |
| Verapamil + 0.125 μM Vincristine[d] | 2.2 | 13.9 |
| **21** + 0.125 μM Vincristine | 20 | >2 |

[a]Concentration for 50% inhibition of KB-VIN cell growth in presence or absence of a non-growth inhibitory concentration of vincristine.
[b]The ratio of $ED_{50}$ without vincristine treatment to the $ED_{50}$ with vincristine.
[c]Not applicable. The isolation and characterization of KB-VIN (expressing P-glycoprotein) are described in ref 16.
[d]0.125 μM Vincristine is not toxic to this cell line.

## Conclusions

In summary, modification of curcumin produced compounds with potential for further development as anticancer agents. Based on these preliminary screening results, compounds **45**, **50**, and **57** showed significant activity in certain cancer cell lines and have been targeted for further studies. Additional research, including mode of action studies, is planned to accurately establish relative activity for SAR and rational design.

Recently, the cancer chemopreventive effects of curcumin have been intensively investigated. Curcumin exhibited pronounced antitumor activity by triggering apoptosis in human tumor cells.[13] Studies are underway to investigate the apoptosis-inducing activity of compounds found to be cytotoxic in this study.

## Experimental

### General

Melting points were determined on a Fisher–Johns melting apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Varian Gemini-300 spectrometer. The chemical shifts are presented in terms of ppm with TMS as the internal reference. MS spectra were recorded on an HP5989A instrument. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, USA

**Curcumin (1).** Yellow needles, mp 181–183 °C (EtOH) (lit.[15] 184–185 °C).

**Demethoxycurcumin (2).** Orange crystalline powder, mp 173–175 °C ($CHCl_3$) (lit.[16] 175–177 °C).

**Bisdemethoxycurcumin (3).** Orange solid, mp 216–218 °C (lit.[17] 209–211 °C). The structures of **1**–**3** were confirmed by comparing their physical and spectral data with those reported in the literature.[15–17]

**General procedure for the preparation of pyrazole derivatives (4–6).** To a solution of starting material in butanol and EtOH were added histidine hydrazide, HOAc, and TsOH. The solution was refluxed for 24 h, then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography and plc.

**3,5-Bis[β-(4-hydroxy-3-methoxyphenyl)-ethynyl]pyrazole (4).** Yellow amorphous solid (yield 22%), mp 215 °C (lit.[18] 211–214 °C). The structure of **4** was confirmed by comparing experimental and literature physical and spectral data.[18]

**Compound 5.** Yellow amorphous solid (yield 10%), mp 184–185 °C; $^1$H NMR (500 MHz, acetone-$d_6$) δ 3.78 (3H, s, $OCH_3$), 6.53 (1H, s, pyrazole 4-H), 6.70 (1H, d, $J=8.1$ Hz, aryl-6′), 6.72 (2H, d, $J=8.6$ Hz, aryl-3′,5′), 6.83 (1H, d, $J=16.4$ Hz, *CH*=CH–aryl), 6.86 (1H, d, $J=16.4$ Hz, *CH*=CH–aryl), 6.86 (1H, bd, aryl 5′), 6.99 (1H, d, $J=16.4$ Hz, CH=*CH*–aryl), 7.00 (1H, d,

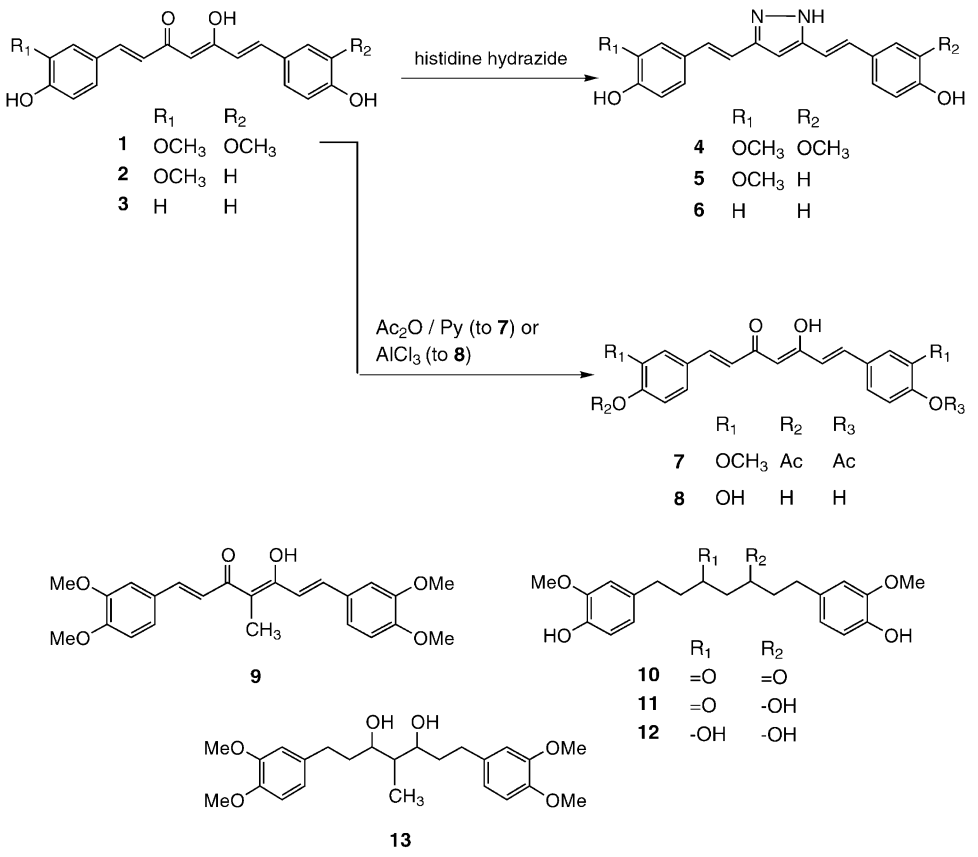



**Figure 1.**

$J = 16.4$ Hz, CH = *CH*–aryl), 7.07 (1H, d, $J = 1.9$ Hz, aryl 2′), 6.99 (2H, d, $J = 8.8$ Hz, aryl-2′,6′); EI–MS *m*/*z*: 334 ($M^+$), HR FABMS *m*/*z* 334.1316 ($M^+$) (calcd for $C_{20}H_{18}N_2O_3$: 334.1317).

**Compound 6.** White amorphous solid (yield 7%), mp 272–273 °C; $^1$H NMR (500 MHz, acetone-$d_6$) δ 6.53 (1H, s, 1-H), 6.72 (4H, d, $J = 8.5$ Hz, aryl-3bm5,5′), 6.83 (2H, d, $J = 16.5$ Hz, *CH* = CH–aryl), 6.99 (2H, d, $J = 16.5$ Hz, CH = *CH*–aryl), 7.28 (4H, d, $J = 8.5$ Hz, aryl 2′,6′); EI–MS *m*/*z*: 304 ($M^+$); HR FABMS *m*/*z* 304.1203 ($M^+$) (calcd for $C_{19}H_{16}N_2O_2$: 304.1212).

**Diacetylcurcumin (7).** A solution of curcumin in anhydrous pyridine was treated with anhydrous $Ac_2O$ and stirred overnight. After a general workup procedure, a yellow amorphous solid was obtained. Compound **7** was identified on the basis of $^1$H NMR data comparison with literature values.[15]

**1,7-Bis(3,4-dihydroxyphenyl)-11,6-heptadiene-3,5-dione (8).** Yellow powder, compound **8** was prepared by heating **1** with $AlCl_3$, pyridine in $CHCl_2$ by the method in ref. 19. Compound **8** was identified on the basis of $^1$H NMR data comparison with literature values.[19]

**Trimethylcurcumin (9).** Curcumin in dry acetone with methyl iodide was refluxed over anhydrous $K_2CO_3$ for 48 h with stirring. After filtration, the solvent was evaporated and the residue purified by silica gel column chromatography to yield 9 as a yellow amorphous solid (55%). Mp 142–145 °C. The structure of **9** was confirmed by comparing experimental and literature physical and spectral data.[15]

**Hydrogenation of curcumin (1).** A solution of 1 (55.3 mg) in MeOH (2 mL) was hydrogenated for 4 h at room temperature and 1 atm over 10% Pd/C catalyst. The filtered solution was concentrated and separated by silica gel column chromatography [benzene/ AcOEt (8:1)→(1:1)] and plc [$CHCl_3$/MeOH (6:1)] to yield 10, 11, and 12. The structures of **10**–**12** were confirmed by comparing their physical and spectral data with those reported in the literature.[15]

**Tetrahydrocurcumin (10).** White amorphous solid (yield 14%), mp 92–93 °C (lit.[21] 95–96 °C).

**Hexahydrocurcumin (11).** White amorphous solid (yield 9%), mp 87–88 °C (lit.[21] 78–80 °C).

**Octahydrocurcumin (12).** Colorless oil (yield 5%).

**Compound 13.** A solution of **9** (546 mg) in EtOAc (100 mL) was hydrogenated overnight at room temperature and 45 psi over 10% Pd/C catalyst. The filtered solution was concentrated and separated by silica gel column chromatography [benzene/ EtOAc (10:1)] and plc [$CHCl_3$/ MeOH (6:1)] to yield **13**. Colorless oil (yield 7%); $^1$H NMR (300 MHz, $CDCl_3$) δ 0.95 (3H, d, CH*CH*$_3$), 1.52 (1H, m, *CH*CH$_3$), 1.84 (2H, m, *CHa*Hb-CHOH), 2.67 (6H, m, CHa*HbCH*$_2$), 3.83 (14H, bs,

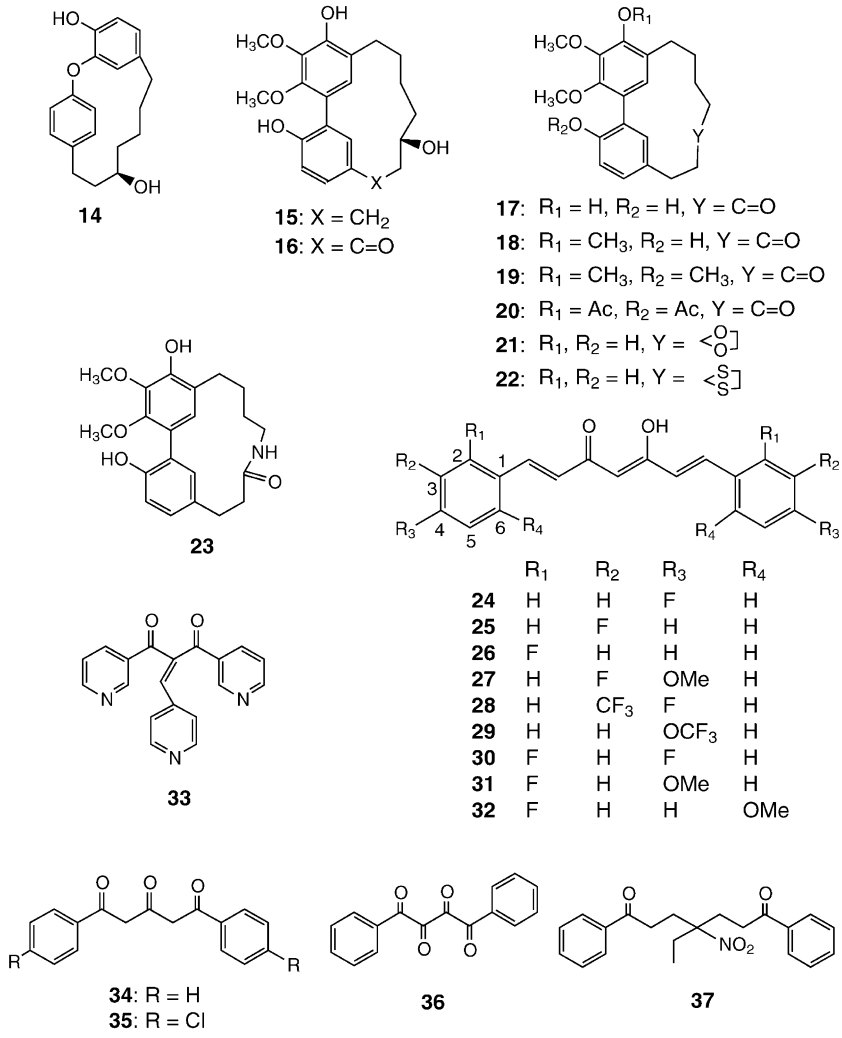



**Figure 2.**

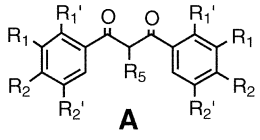
$OCH_3$, *CH*OH), 6.78 (6H, m, aryl H). EI–MS *m*/*z*: 418 ($M^+$); HR FABMS *m*/*z*: 418.236618 ($M^+$) (calcd for $C_{24}H_{34}O_6$: 418.2355392).

**Compound 51.** A solution of **50** (70 mg) in EtOAc (50 mL) was hydrogenated overnight at room temperature and 45 psi over 10% Pd/C catalyst. The filtered solution was concentrated and separated by silica gel column chromatography [benzene/EtOAc (100:1)→(10:1)] and plc [benzene/EtOAc (10:1)] to yield **51**. White powder (yield 31%); $^1$H NMR (300 MHz, $CDCl_3$) δ 4.00 (1H, s, CHBr), 4.02 (3H, s, $OCH_3$), 6.83 (2H, d, $J$ = 9 Hz, aryl 3′,5′), 7.11 (2H, d, $J$ = 9 Hz, aryl 3′,5′), 7.98 (2H, d, $J$ = 9 Hz, aryl 2′,6′), 8.09 (2H, d, $J$ = 9 Hz, aryl 2′,6′); EI–MS *m*/*z*: 348 ($M^+$), HR FABMS *m*/*z*: 348.0299 (calcd for $C_{16}H_{15}O_3NBr$: 348.2035).

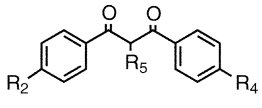
**Preparation of fluorinated diarylheptanoids (24–32).** The procedure reported by Pedersen et al.[14] was applied with compounds **24**–**32**. In general, 2,4-pentanedione (0.5 equiv) and boric anhydride (0.35 equiv), dissolved in 10 mL of EtOAc, were stirred for 30 min at 40 °C. Benzaldehyde (1 equiv) and tributylborate (1 equiv) were added, and the mixture was stirred for 30 min. Butylamine (0.75 equiv), dissolved in 10 mL of EtOAc, was added dropwise over 15 min. Stirring was continued for 18 h at 40 °C. The mixture was hydrolyzed by adding 10 mL of 1 N HCl and heating at 60 °C for 1 h. Two layers were separated, and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed until neutral, dried ($Na_2SO_4$), and the solvent was removed in vacuo. The crude products were purified by silica gel column chromatography using an *n*-hexane–EtOAc solvent system.

**Compound 24.** Yield 5%, yellow needles, mp 167–169 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) δ 5.81 (1H, enol CH), 6.54 (2H, d, $J$ = 16.0 Hz, *CH*=CH–aryl), 7.09 (4H, m, aryl 3′,5′), 7.54 (4H, m, aryl 2′,6′), 7.63 (2H, d, $J$ = 16.0 Hz, CH=*CH*–aryl). EI–MS *m*/*z* 312 ($M^+$). Anal. calcd for $C_{19}H_{14}F_2O_2$: C 73.07, H 4.52, found: C 72.81, H 4.52.

**Compound 25.** Yield 6%, mp 110–111 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) 5.84 (1H, s, enol CH), 6.60 (2H, d, $J$ = 15.0 Hz, *CH*=CH–aryl), 7.06 (2H, m, aryl 2′), 7.22–7.39 (6H, m, aryl 4′,5′,6′), 7.60 (2H, d, $J$ = 15.0 Hz, CH=*CH*–aryl). FABMS *m*/*z* 313 ($MH^+$). Anal. calcd for $C_{19}H_{14}F_2O_2$: C 73.07, H 4.52, found: C 72.87, H 4.50.




A

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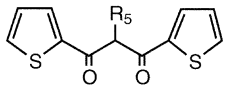



C

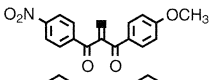
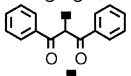
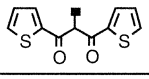
| Compound | Skeleton | $R_{1'}$ | $R_1$ | $R_2$ | $R_{2'}$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|---|
| **38** | **A** | H | H | H | H | | $H_2$ |
| **39** | **A** | H | $CH_3$ | H | $CH_3$ | | $H_2$ |
| **40** | **A** | H | H | $C(CH_3)_3$ | H | | $H_2$ |
| **41** | **A** | $OCH_3$ | H | $OCH_3$ | H | | $H_2$ |
| **42** | **A** | H | $CH_3$ | H | $CH_3$ | | Br |
| **43** | **B** | | | H | | Cl | $H_2$ |
| **44** | **B** | | | Cl | | Cl | $H_2$ |
| **45** | **B** | | | F | | F | $H_2$ |
| **46** | **B** | | | H | | H | Br |
| **47** | **B** | | | H | | H | NO |
| **48** | **B** | | | $NO_2$ | | $NO_2$ | Br |
| **49** | **B** | | | $NO_2$ | | $OCH_3$ | $H_2$ |
| **50** | **B** | | | $NO_2$ | | $OCH_3$ | Br |
| **51** | **B** | | | $NH_2$ | | $OCH_3$ | Br |
| **52** | **B** | | | $NO_2$ | | $NO_2$ | $H_2$ |
| **53** | **B** | | | H | | H | $CH_2COC_6H_5$ |
| **54** | **B** | | | $OCH_3$ | | $NO_2$ |  |
| **55** | **B** | | | H | | H |  |
| **56** | **B** | | | H | | H |  |
| **57** | **C** | | | | | | Br |
| **58** | **C** | | | | | |  |

**Figure 3.**

**Compound 26.** Yield 5%, mp 110–113 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) δ 5.89 (1H, s, enol CH), 6.75 (2H, d, $J$ = 16.0 Hz, *CH*=CH–aryl), 7.08–7.21 (4H, m, aryl CH), 7.35 (2H, m, aryl CH), 7.57 (2H, m, aryl CH), 7.78 (2H, d, $J$ = 16.0 Hz, CH=*CH*–aryl). FABMS $m/z$ 313 ($MH^+$). Anal. calcd for $C_{19}H_{14}F_2O_2$: C 73.07, H 4.52, found: C 72.89, H 4.45.

**Compound 27.** Yield 31%, mp 174–175 °C (*n*-hexane–EtOAc); $^1$H NMR (acetone-$d_6$) δ 3.92 (6H, s, $OCH_3$), 6.02 (1H, s, enol CH), 6.76 (2H, d, $J$ = 16.0 Hz, *CH*=CH-aryl), 7.18 (2H, t, $J$ = 8.4 Hz, aryl 5′), 7.43–7.55 (4H, m, aryl 2′,6′), 7.58 (2H, d, $J$ = 16.0 Hz, CH=*CH*–aryl). FABMS $m/z$ 373 ($MH^+$). Anal. calcd for $C_{21}H_{18}F_2O_4$: C 67.74, H 4.87, found: C 67.76, H 4.87.

**Compound 28.** Yield 6%, mp 172–174 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) δ 5.86 (1H, s, enol CH), 6.60 (2H, d, $J$ = 16.0 Hz, *CH*=CH–aryl), 7.24 (2H, t, $J$ = 9.0 Hz, aryl 5′), 7.63 (2H, d, $J$ = 16.0 Hz, CH=*CH*–aryl), 7.72 (2H, m, aryl 6′), 7.79 (2H, d, $J$ = 6.6 Hz, aryl 2′). FABMS $m/z$ 449 ($MH^+$). Anal. calcd for $C_{21}H_{12}F_8O_2$: C 56.26, H 2.70, found: C 56.06, H 2.72.

**Compound 29.** Yield 10%, mp 124–126 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) δ 5.58 (1H, s, enol CH), 6.60 (2H, d, $J$ = 16.0 Hz, *CH*=CH–aryl), 7.24 (4H, m, aryl 2′6′), 7.58 (4H, m, aryl 3′,5′), 7.65 (2H, d, $J$ = 16.0 Hz, CH=*CH*–aryl). FABMS $m/z$ 445 ($MH^+$). Anal. calcd for $C_{21}H_{14}F_6O_4$: C 56.77, H 3.18, found: C 56.87, H 3.14.

**Compound 30.** Yield 3%, mp 155–157 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) δ 5.86 (1H, s, enol CH), 6.68 (2H, d, $J$ = 16.0 Hz, *CH*=CH-aryl), 6.84–6.96 (4H, m, aryl 5′,6′), 7.56 (2H, m, aryl 3′), 7.71 (2H, d, $J$ = 16.0 Hz, CH=*CH*–aryl). FABMS $m/z$ 349 ($MH^+$). Anal. calcd for $C_{19}H_{12}F_4O_2$: C 65.52, H 3.47, found: C 65.61, H 3.5.

**Compound 31.** Yield, 10%, mp 162–164 °C (*n*-hexane–EtOAc); $^1$H NMR ($CDCl_3$) δ 3.84 (6H, s, $OCH_3$), 5.82

(1H, s, enol CH), 6.62 (2H, d, $J = 16.0$ Hz, *CH* = CH–aryl), 6.62–6.75 (4H, m, aryl 5′,6′), 7.48 (2H, t, $J = 8.4$ Hz, aryl 3′), 7.71 (2H, d, $J = 16.0$ Hz, CH = *CH*–aryl). FABMS *m*/*z* 373 ($MH^+$). Anal. calcd for $C_{21}H_{18}F_2O_4$: C 67.74, H 4.87, found: C 67.58, H 4.92.

**Compound 32.** Yield 9%, mp 144–146 °C (*n*-hexane–EtOAc); $^1H$ NMR ($CDCl_3$) δ 3.82 (6H, s, $OCH_3$), 5.90 (1H, s, enol CH), 6.72 (2H, d, $J = 16.0$ Hz, *CH* = CH–aryl), 6.88 (2H, m, aryl 4′), 7.00–7.06 (4H, m, aryl 3′,5′), 7.74 (2H, d, $J = 16.0$ Hz, CH = *CH*–aryl). FABMS *m*/*z* 373 ($MH^+$). Anal. calcd for $C_{21}H_{18}F_2O_4$: C 67.74, H 4.87, found: C 67.60, H 4.96.

### Cytotoxicity and drug-resistance reversal assays

In vitro cytotoxicity assay was carried out according to the procedures described in Rubinstein et al.[20] Drug stock solutions were prepared in DMSO, and the final solvent concentration was not greater than 2% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel constituted of epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), renal cancer (CAKI-1), breast cancer (MCF-7), ovarian cancer (1A9), ileocecal carcinoma (HCT-8), melanoma cancer (SKMEL-2) and glioblastoma (U-87-MG), bone cancer (HOS), liver cancer (HepG2), and prostate cancer (PC-3 and LNCaP clone FGC). The human KB drug-resistant sub-line panel included KB-VIN (expressing P-glycoprotein).

For drug-resistance reversal, KB-VIN cells cultured in the presence or absence of 125 nM vincristine (a concentration without effect on cell growth) were used. Cells were exposed to doses of verapamil or compound **21** (2.5–40 μM) for 2 days. The $ED_{50}$ values were determined from dose–response graphs and a potentiation index (Table 2) was calculated.

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