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Synthesis, Antifungal Activity, and Structure Activity Relationships of Coruscanone A Analogs

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Abstract

Coruscanone A, a plant derived cyclopentenone derivative, showed potent *in vitro* antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, comparable to amphotericin B and fluconazole. A series of analogs have been synthesized by modification of the cyclopentenone ring, the enolic methoxy functionality, and the side chain styryl moiety of this natural product lead. A structurally close 1,4-benzoquinone analog was also prepared. All the compounds were examined for their *in vitro* activity against major opportunistic fungal pathogens including *C. albicans*, *C. neoformans* and *Aspergillus fumigatus*, and fluconazole-resistant *C. albicans* strains, with several analogs demonstrating potent antifungal activity. Structure activity relationship studies indicate that the 2-methoxymethylene-cyclopent-4-ene-1,3-dione structural moiety is the pharmacophore responsible for the antifungal activity of this class of compounds, while the side chain styryl-like moiety plays an important complementary role, presumably contributing to target binding.

Introduction

The limitations of current antifungal drugs, increased incidence of systemic fungal infections, and rapid development of drug resistance have highlighted the need for the discovery of new antifungal agents, preferably with novel mechanisms of action.¹ While synthetic efforts remain the mainstream in antifungal drug discovery, as evidenced by the fact that 18 out of 23 antifungal drugs approved from 1980–2002 are synthetic (83% belong to the single azole class), unique antifungal natural products have demonstrated remarkable success in this regard.² For example, two major drug classes currently in use, represented by amphotericin B, the ‘gold standard’ in the antifungal armamentarium, and the lipopeptide caspofungin, the most important antifungal drug approved in recent years, are derived from natural products.¹

In the course of our search for prototype antifungal agents from natural sources, we discovered the antifungal lead, coruscanone A (**1**), from the ethanol extract of the piperaceous plant *Piper coruscans*.³ Coruscanone A possesses a 2-methoxymethylenecyclopent-4-ene-1,3-dione skeleton with a styryl moiety (designated as side chain below) attached to the methylene at C-2. This unique structural property facilitates the coexistence of two geometrical isomers along the C-2 *exo*-double bond in solution, via photoisomerization.^{3,4} It must be pointed out that all the compounds of this class described herein have two geometrical isomers in a ratio of almost 1:1 in solution; however, for convenience sake the structure of only one isomer is

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presented. Coruscanone A showed strong antifungal activity against two major opportunistic pathogens *C. albicans* and *C. neoformans* associated with immuno-compromised patients (AIDS, cancer, or organ transplant), comparable to the positive controls amphotericin B and fluconazole. The lack of antibacterial activity of **1** against *Staphylococcus aureus* indicated a good selectivity. Coruscanone A represents the most fungicidal plant metabolite that is active against *C. albicans*.³ In addition, **1** showed acceptable in vitro cytotoxicity against mammalian Vero cells, comparable to amphotericin B, and is readily accessible by total synthesis.³ Although the cyclopent-4-ene- and cyclopentane-1,3-dione structural moieties are present in a large number of synthetic compounds with therapeutic applications or potential, the functional groups in these compounds are di- or triketonic in nature,⁵ in contrast to the unique diketone enol ether form in **1**. Also, little is known about the mechanisms of action of these compounds. Therefore, this drug-like, small molecule natural product is an ideal structural template for the synthesis of a series of analogs in order to explore their structure activity relationships (SAR), thus affording the information for further lead optimization of this class of compounds as potential new antimycotics.

Results and Discussion

Chemistry

To develop a robust pharmacophoric model and understand the basis for the antifungal activity of coruscanone A (**1**), its structure can be subdivided into three key structural moieties: cyclopentenenedione ring (A), enolic methoxy functionality (S1), and the side chain styryl moiety (S2) (Fig. 1). Each structural moiety can be modified independently in order to facilitate the systematic refinement of the search for increasingly effective coruscanone A analogs.

The general synthetic methodology for the preparation of coruscanone A analogs is a modified version of a method developed by Clemo *et al.*,⁶ and successful synthesis of coruscanone A has demonstrated its feasibility.³ As outlined in Scheme 1, the synthetic sequence starts with Wittig condensation between a maleic anhydride derivative, e.g., 2-methylmaleic anhydride (**2**), 2,3-dimethylmaleic anhydride (**3**) or 2-phenylmaleic anhydride (**4**) and a phosphorane (**16–21**, refer to Experimental section for their structures) prepared from the corresponding α -methyl ketone in a three step sequence, via brominated α -methyl ketone (**5–9**) and phosponium salt (**10–15**). The key step is the base-catalyzed rearrangement of the resulting 4-ylidenebutenolide (**22–29**), affording the corresponding cyclopent-4-ene-1,3-dione (**30–37**). Alkylation or acylation of the cyclopentenenediones yields the enolic hydroxy substituted product (**38–52**).

1. Structural Modifications of the Cyclopentenenedione Ring (A) of 1—Our first modification of the cyclopentenenedione ring of coruscanone A involved the synthesis of 2-(1-methoxy-3-phenylpropenylidene)-4,5-dimethylcyclopent-4-ene-1,3-dione (**38**), since this compound would only have one isomer in solution due to the symmetric nature of the cyclopentenenedione ring. It was thus prepared from 2,3-dimethylmaleic anhydride (**3**) and 4-phenyl-1-(triphenylphosphoranylidene)-(3*E*)-buten-2-one (**16**) that was available from styryl methyl ketone as shown in Scheme 1. The second compound, 2-[(*E*)-1-methoxy-3-phenylallylidene]-4-phenylcyclopent-4-ene-1,3-dione (**39**), represented replacement of the methyl substituent on the cyclopentenenedione ring of **1** with a phenyl group, which was prepared from 4-phenylmaleic anhydride (**4**) and phosphorane **16**. It was postulated that introduction of the bulky phenyl group which extends conjugation might have a significant impact on the antifungal activity of the compound.

It should be pointed out that Wittig condensation of a monosubstituted maleic anhydride derivative, e.g. **2** or **4**, with a phosphorane always results in a regional- and stereoselective formation of an *E*-butenolide as the predominant product, as observed in **22** and **24**. In the case

of disubstituted 2,3-dimethylmaleic anhydride (**3**), the intermediate butenolide **23** as the major product possesses Z-configuration at the C-4 double bond due to less steric hindrance between the olefinic proton and the cyclic methyl group. However, both types of butenolides can be readily converted to corresponding cyclopentenones via a non-stereoselective base-catalyzed rearrangement.^{3,6}

2. Structural Modification of the Enolic Methoxy Functionality of 1 by Alkylation and Acylation (S1)—Our previous study has indicated that *O*-methylation of the enolic hydroxy group plays a key role in the antifungal activity of coruscanone A (**1**) when compared to its demethylated precursor, coruscanone B (**30**), which shows only marginal antifungal activity.³ Thus, modification of the enolic methoxy functionality of **1** would have a significant impact on the antifungal activity of newly synthesized analogs. When **30** was alkylated with Et₂SO₄/K₂CO₃ in acetone and acetylated with acetic anhydride/pyridine, compounds **47** and **49** were obtained, respectively. Similarly, ethylation of cyclopentenone **31** with Et₂SO₄/K₂CO₃ in acetone yielded **48**. Acylation of **31** with acetic anhydride/pyridine, benzoyl chloride/THF/DMAP and cinnamoyl chloride/pyridine/DMAP afforded **50–52**, respectively.

3. Structural Modification of the Styryl Side Chain (S2) of 1—Selection of α -methyl ketones to construct the side chain of synthetic coruscanone A analogs was based on minor modification of the styryl moiety of **1**, with the aim of achieving improved antifungal potency and better selectivity, thus arriving at meaningful SAR information. With the retention of the structural moieties A and S1 of **1**, six analogs (**40–43**, **45** and **46**) were prepared according to the general synthetic approach illustrated in Scheme 1. While the preparation of **40–43** was fairly straightforward, the synthesis of **45** and **46** involved protection/deprotection of a phenolic hydroxy group at appropriate stages (see Experimental section). The side chains in these compounds vary from a phenyl group in **43**, to heteroaromatic furanyl and thiophene moieties in **41** and **42**, respectively, and additional substitution at the phenyl group of the styryl moiety in **40**, **45** and **46**.

The last, simplest synthetic entity in this series is 2-(1-hydroxyethylidene)-4-methylcyclopent-4-ene-1,3-dione (**53**) in which a methyl group replaces the styryl group in **1**. It was prepared by an alternative method via reaction of 2-methylmaleic anhydride (**2**) and α -methylvinyl acetate in AlCl₃/C₂H₄Cl₂,⁷ followed by methylation with Me₂SO₄/K₂CO₃ in acetone.

4. Synthesis of 2-Methoxy-5-methyl-3-styryl-1,4-benzoquinone (60)—It is well-known that some 1,4-benzoquinones are reported to have good antifungal activity.⁸ Since coruscanone A (**1**) shows some similarity to 1,4-benzoquinone derivatives, a structurally close analog, 2-methoxy-5-methyl-3-styryl-1,4-benzoquinone (**60**), was synthesized for SAR studies. This would also indicate whether any correlation exists between the two classes of compounds regarding their biological activity. Thus, starting from commercially available methoxyhydroquinone (**54**), its methoxymethyl protected ether **55** was treated with pyridinium hydrobromide perbromide in MeOH to give the brominated product **56**, which was subjected to bromine/lithium exchange in MeLi/THF. The lithio derivative was quenched with MeI, and the resultant C-methyl derivative **57** was converted to the lithium salt with BuLi/THF in a DoM-type reaction,⁹ and then coupled with phenylacetaldehyde to give benzyl alcohol **58**. Dehydration and deprotection of **58** using mineral acid yielded stilbene **59**, which was oxidized by DDQ to furnish **60** (Scheme 2). In view of the limited number of available methods for the synthesis of styryl benzoquinones,¹⁰ it is worthwhile mentioning that direct coupling of phenylacetaldehyde with di-*ortho*-oxygenated benzenes, followed by oxidation, is a facile method for preparation of 2-hydroxy (or methoxy)-3-styryl benzoquinone derivatives.

Biological Activity

Synthetic coruscanone A (**1**) and its analogs (**30–43**, and **45–53**) as well as the 1,4-benzoquinone **60** were evaluated for their in vitro antifungal activity against three major opportunistic fungal pathogens *C. albicans*, *C. neoformans*, and *Aspergillus fumigatus* using the positive control drugs amphotericin B and fluconazole for comparison. Compounds showing 50% growth inhibition (IC₅₀) at the concentration of less than 20 µg/ml were considered to be active and were further evaluated for their antifungal activity against clinical isolates of fluconazole-susceptible and resistant *C. albicans* (isolates #1 and #17, respectively) from AIDS patients during fluconazole therapy.¹¹ The results indicated that compounds **1**, **33**, **39**, **40–43**, **45–47** and **49** showed varying degrees of antifungal activity (Table 1), while other compounds were inactive at 20 µg/mL. The in vitro cytotoxicity of the antifungal compounds against mammalian kidney cells (Vero and LLC-PK-1) was determined and compared with the anticancer drug doxorubicin. While coruscanone A remains the most potent antifungal compound among the synthetic derivatives, several analogs (**40–42**) demonstrated potent, but slightly weaker activity than **1**. In addition, compounds that were active against *C. albicans* retained equivalent activity against fluconazole-resistant *C. albicans* isolate #17, which involves several resistance mechanisms, including overexpression of efflux pumps CDR1 (p-glycoprotein) and MDR1 (major facilitator) mRNA, as well as overexpression of fluconazole's target gene, ERG11.^{11b} Since overexpression of these genes leads to inefficacy of fluconazole via efflux pump activity and/or overproduction of ERG11's product, 14 α -lanosterol demethylase, a key enzyme in the biosynthesis of ergosterol,^{11a} coruscanone A and its analogs would target something other than ERG11, and/or are not a substrate for the CDR1 or MDR1 efflux pumps. Meaningful SAR information is summarized as follows:

1. Modifications of the cyclopentenone ring: Compound **38** with only one additional methyl group on the cyclopentenone ring when compared to **1**, and its derivatives **31**, **48**, and **50–52** surprisingly lack antifungal activity. This indicates that the antifungal mechanism of coruscanone A regarding its reactive site to the target would be on the cyclopentenone ring that may be considered as the 'warhead' of the molecule. It is thus postulated that **1** may act like maleimide derivatives as a Michael acceptor,¹² reacting at C-5 of the cyclopentenone ring with a nucleophilic site of the target (enzyme). Additional methyl substitution at C-5 such as in **38** would preclude such a reaction due to steric hindrance. When the methyl group in **1** is replaced by a large phenyl group in **39**, the activity decreases significantly, presumably due to decreased affinity to the target.

The lack of antifungal activity of the benzoquinone derivative **60** further indicates that the cyclopentenone structural moiety of coruscanone A analogs is selective to fungal pathogens, although a similar Michael addition reaction mechanism may also occur on the benzoquinone skeleton of **60**.

2. Modifications to the enolic functionality: Different substitutions at the enolic hydroxy group affect the activity. Similar to coruscanone B (**30**), all the synthetic cyclopentenones with a free enolic hydroxy group (**31**, **32**, **34–36**) are inactive except for the chlorine-containing compound **33** which shows moderate activity against *C. albicans* and fluconazole-resistant *C. albicans* strains. Compound **49** with *O*-acetyl substitution is only moderately active against *C. albicans* and marginally active against *C. neoformans*, and **47** with *O*-ethyl substitution is about 10-fold (MIC value) less active against *C. albicans* than **1**. The weak activity of the two compounds, particularly **47**, can not be simply explained by the reaction mechanism of Michael addition mentioned above. The binding affinity or selectivity of the structures of the compounds to the reactive site of the target (enzyme) must play an important role. At this point, the enolic *O*-methyl group remains the optimum functional group among all the synthetic analogs.

3. Modifications to the styryl side chain: The side chain plays an important role for the activity of coruscanone A analogs. Compounds **40** and **46** that have aromatic chloro- and methoxy substituents show a slightly weaker activity than **1**. It is noteworthy that compounds **41** and **42** possessing five-membered heteroaromatic rings instead of a benzene ring, display significant activity against *C. albicans* and *C. neoformans* with enhanced activity against *A. fumigatus* compared to **1**. However, when the styryl moiety is replaced by a phenyl group as in **43** or a methyl group as in **53**, the activity is decreased drastically for **43** or completely lost for **53**, implying the double bond of the styryl moiety or the intact styryl moiety (e.g., in **1**) or styryl-like moiety (e.g., in **41** and **42**) are necessary for the activity. This strongly suggests that the side chain is associated with binding affinity for the target.
4. Separation of antifungal activity from cytotoxicity: Although the above antifungal compounds show moderate in vitro cytotoxicity to the mammalian cells Vero (IC₅₀s from 3.3 to >10 µg/mL) and LLC-PK-1 (IC₅₀s from 3.1 to >10 µg/mL), their cytotoxicity does not seem to be necessarily correlated to their antifungal activity. For example, compound **47**, which exhibits a cytotoxicity similar to **1** (IC₅₀ of 4.8 v.s. 4.9 µg/mL against Vero cells, Table 1), is only moderately active against *C. albicans* (MIC of 20 v.s. 2.08 µg/mL). Compound **49**, which is even less antifungal than **47**, also shows cytotoxicity similar to **1** (IC₅₀ of 5.5 v.s. 4.9 µg/mL against Vero cells, Table 1). In contrast, the most cytotoxic compounds, **41**, **42** and **45**, are not the most potent antifungal compounds. This indicates that these compounds may act via different mechanisms of action for their antifungal activity against *C. albicans* and cytotoxicity against mammalian cells, or that minor structural changes afford important alterations in fungal cell permeability/transport, etc. This would render an opportunity to improve the antifungal activity or therapeutic index of the analogs by further chemical modification of the lead structure without effecting cytotoxicity.

It was recently demonstrated that a synthetic cyclopentenone derivative **61** showed a potent inhibition of human chymase, a potential drug target associated with cardiovascular diseases and chronic inflammation following fibrosis.¹³ A possible mechanism for inhibition of the enzyme by **61** was proposed as shown in Scheme 3, i.e., the enzyme interacts with the inhibitor at C-6 due to its strong electrophilicity, while the phenyl group of the inhibitor is enclosed by the residue of the S1 pocket.¹³ However, the reactive site of coruscanone A and its analogs to the target enzyme is apparently different from that of **61**. Coruscanone A may act as a Michael acceptor reacting at C-5 of the cyclopentenone ring with a nucleophile of the active site residue of the target (enzyme) to form an irreversible covalent adduct,¹⁴ while its styryl and methoxy groups are enclosed by other residues of the target (Scheme 4). The irreversible covalent nature of the adduct may also explain the fungicidal activity of coruscanone A. There is no doubt that Michael acceptors are associated with certain levels of toxicity towards mammalian cells, as observed in coruscanone A analogs (Table 1). However, numerous reports/patents have described the therapeutic potential of Michael acceptors from the perspective of drug discovery. For example, it is suggested that Michael acceptors can be structurally modified, so that they can react selectively with target nucleophiles or can be converted into selective Michael acceptors during metabolism for anticancer drug discovery. Representative natural cytotoxic compounds containing Michael acceptors include menadione and shikonin analogs, while the anticancer drug mitomycin is transformed by reductive activation into a conjugated dienone Michael acceptor.¹⁵ Another anticancer drug mitoxantrone acts via oxidative activation involving a Michael acceptor intermediate.¹⁶ Recent work has demonstrated that Michael acceptors are a new class of inhibitors specific for caspases and other Clan cysteine proteases. A large number of aza-peptides composed of a 2-pyridone-containing peptidomimetic binding determinant and an α,β -unsaturated Michael acceptor "warhead" have been synthesized for the development of orally bioavailable inhibitors for the 3C protease, which were tested against rhinoviruses.¹⁷ Moreover, extensive studies

have demonstrated that Michael acceptors are an important class of compounds that can induce phase 2 detoxification enzymes [e.g., glutathione transferases, NAD(P)H:quinone reductase, glucuronosyl transferases, epoxide hydrolase] and thus possess anticarcinogenic properties.¹⁸ Moderately reactive Michael acceptors are receiving growing interest for the design of chemoprotective drugs.¹⁹ Therefore, there is great potential for the development of coruscanone A analogs carrying a Michael acceptor “warhead” as therapeutic agents.

In conclusion, this study has demonstrated that the 2-methoxymethylenecyclopent-4-ene-1,3-dione structural moiety is responsible for the antifungal activity of coruscanone A analogs, with the styryl-like moiety being an ideal supplemental group, presumably contributing to enzyme binding. The putative pharmacophore may act like maleimides as a Michael acceptor¹² reacting with the target to form an irreversible covalent adduct, while additional experimental evidence is needed to support this hypothesis. Although compounds with improved antifungal activity have not yet been found, a meaningful SAR for coruscanone A analogs has been established based on the limited number of compounds synthesized by a rationally designed classical medicinal chemistry approach. This has laid a solid foundation for further lead optimization of this class of compounds by a systematic chemical modification including the synthesis of water-soluble compounds to improve their overall pharmaceutical properties. An effort aimed at elucidating the antifungal mechanism of action of coruscanone A analogs using genomic profiling and biochemical approaches is also underway in our group.

Experimental Section

Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on Bruker DPX-300, DRX-400 or Varian Mercury-400BB instruments. Chemical shifts are expressed in ppm relative to the solvent residue signals. ESI-FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. High resolution TOFMS were measured on an Agilent series 1100 SL equipped with an ESI source. Flash column chromatography was done on silica gel (40 μ m, J. T. Baker) and reversed-phase silica gel (RP-18, 40 μ m, J. T. Baker). TLC was performed on silica gel sheets (Silica Gel 60 F₂₅₄, Merck, Germany) and reversed-phase plates (RP-18 F₂₅₄S, Merck, Germany). Analytical HPLC used to determine the purity of target compounds was conducted on an ODS (Discovery®) column (250 \times 4.6 mm, 10 μ m) using two solvents systems, (a) 70% CH₃CN in H₂O and (b) 80% MeOH in H₂O, and UV detection at 254 nm for a 20-minutes run. The results are expressed by a retention time in minutes and a relative content in percentage. All the reagents were obtained from the commercial vendors in appropriate grades and were used without further purification.

General Procedure for Preparation of Bromomethyl Ketones 5–8—To a solution of the α -methyl ketone (2 mmol) in dry THF (20 mL) at room temperature under nitrogen was slowly added a solution of the pyrrolidone hydrotribromide (2.4 mmol) in dry THF (40mL) in 1 hr. The mixture was continued to stir at room temperature for 24 hr. After completion of the reaction, Excess pyrrolidone hydrotribromide was removed by filtration. The filtrate was concentrated to dryness. The resulting residue was dissolved in Et₂O, washed with brine and dried (Na₂SO₄). Removal of the solvents afforded crude product, which was chromatographed on silica gel eluting with Et₂O-hexanes (1:6) to give the bromomethyl ketone.

Bromomethyl Styryl Ketone (5)—Yield, 77% from styryl methyl ketone, see ref. ³ for spectroscopic data.

(E)-1-Bromo-4-(4-chlorophenyl)but-3-en-2-one (6)—Yield, 70% from 4-(4-chlorophenyl)but-3-en-2-one, pale yellow oil; ¹H NMR (400 MHz, CDCl₃) δ 7.65 (1H, d, *J* =

16 Hz, β -CH=), 7.51 (2H, d, J = 8 Hz, H-3, 5), 7.38 (2H, d, J = 8 Hz, H-2, 6), 6.92 (1H, d, J = 16 Hz, α -CH=), 4.06 (2H, s, $\underline{\text{CH}_2\text{-Br}}$).

(*E*)-1-Bromo-4-(furan-2-yl)but-3-en-2-one (7)—Yield, 59% from 4-(furan-2-yl)but-3-en-2-one, colorless oil; ^1H NMR (400 MHz, CDCl_3) δ 7.55 (1H, br s, H-5), 7.48 (1H, d, J = 16 Hz, β -CH=), 6.87 (1H, d, J = 16 Hz, α -CH=), 6.75 (1H, d, J = 3.2 Hz, H-3), 6.53 (1H, m, H-4), 4.05 (2H, s, $\underline{\text{CH}_2\text{-Br}}$).

(*E*)-1-Bromo-4-(thiophen-2-yl)but-3-en-2-one (8)—Yield, 59% from 4-(thiophen-2-yl)but-3-en-2-one, colorless oil; ^1H NMR (400 MHz, CDCl_3) δ 7.78 (1H, dd, J = 16, 3.2 Hz, β -CH=), 7.43 (1H, br s, H-5), 7.32 (1H, br s, H-3), 7.06 (1H, br d, H-4), 6.71 (1H, dd, J = 16, 3.2 Hz, α -CH=), 4.02 (2H, s, $\underline{\text{CH}_2\text{-Br}}$).

(*E*)-1-Bromo-4-[4-*O*-(*t*-butyldimethylsilyl) phenyl] but-3-en-2-one (9)—Yield, 65% from TBS- protected (*E*)-4-hydroxyphenylbut-3-en-2-one that was prepared by a standard procedure,²⁰ colorless oil; ^1H NMR (400 MHz, CDCl_3): δ 7.64 (1H, d, J = 16 Hz, β -CH=), 7.47 (2H, d, J = 8 Hz, H-3, 5), 6.84 (2H, d, J = 8 Hz, H-2, 6), 6.80 (1H, d, J = 16 Hz, α -CH=), 4.05 (2H, s, $\underline{\text{CH}_2\text{Br}}$), 0.97 (9H, s, 3 \times Me), 0.21 (6H, s, 2 \times Me).

General Procedure for Preparation of Phosphonium Salts 10–15—To a refluxed solution of the triphenylphosphine (1 mmol) in dry THF (25 ml) under nitrogen was slowly added bromomethyl ketone derivative (1 mmol). The reaction mixture was refluxed for 2–4 hr. After completion, reaction was cooled to room temperature. The white precipitate was collected by the filtration and washed with Et_2O .

[2-Oxo-4-phenyl-(3*E*)-butenyl]triphenyl Phosphonium Bromide (10)—Yield, 86% from **5**, see ref. ³ for spectroscopic data.

[2-Oxo-4-(4-chlorophenyl)-(3*E*)-butenyl]triphenyl Phosphonium Bromide (11)—Yield, 90% from **6**, white powder; ^1H NMR (400 MHz, CDCl_3) δ 8.46 (1H, d, J = 16 Hz, β -CH=), 7.90–7.64 (15H, m, Ph), 7.71 (2H, d, J = 8 Hz, H-3, 5), 7.31 (2H, d, J = 8 Hz, H-2, 6), 7.04 (1H, d, J = 16 Hz, α -CH=), 5.91 (2H, d, J = 12 Hz, $\underline{\text{CH}_2\text{-P}}$).

[2-Oxo-4-(furan-2-yl)-(3*E*)-butenyl]triphenyl Phosphonium Bromide (12)—Yield, 56% from **7**, white powder; ^1H NMR (400 MHz, CDCl_3) δ 8.31 (1H, d, J = 16 Hz, β -CH=), 7.89–7.58 (15H, m, Ph), 7.48 (1H, br s, H-5), 7.03 (1H, d, J = 3.6 Hz, H-3), 6.70 (1H, dd, J = 16, 2.4 Hz, α -CH=), 6.45 (1H, m, H-4), 5.82 (2H, d, J = 12 Hz, $\underline{\text{CH}_2\text{-P}}$).

[2-Oxo-4-(thiophen-2-yl)-(3*E*)-butenyl]triphenyl Phosphonium Bromide (13)—Yield, 56% from **8**, white powder; ^1H NMR (400 MHz, CDCl_3) δ 8.75 (1H, d, J = 16 Hz, β -CH=), 7.89–7.63 (17H, m), 7.42 (1H, br d, J = 4 Hz, H-3), 7.03 (1H, br s, H-4), 6.64 (1H, d, J = 16 Hz, α -CH=), 5.82 (2H, d, J = 12 Hz, $\underline{\text{CH}_2\text{-P}}$).

(2-Oxo-2-phenylethyl)triphenyl Phosphonium Bromide (14)—Yield, 62% from commercially available 2-bromo-1-phenylethanone, white powder; ^1H NMR (400 MHz, CDCl_3) δ 8.35 (2H, d, J = 7 Hz, H-2, 6), 7.89–7.95, 7.72, 7.56, 7.48 (18H, m), 6.34 (2H, d, J = 12 Hz, $\text{CH}_2\text{-P}$).

[2-Oxo-4-(4-*t*-butyldimethylsilyloxyphenyl)-(3*E*)-butenyl]triphenyl Phosphonium Bromide (15)—Yield, 75% from **9**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.28 (1H, d, J = 16 Hz, β -CH=), 7.90–7.63, (17H, m), 6.96 (1H, d, J = 16 Hz,

α -CH=), 6.81 (2H, d, J = 8 Hz, H-2, 6), 5.83 (2H, d, J = 12 Hz, $\underline{\text{CH}_2\text{-P}}$), 0.95 (9H, s, Me \times 3), 0.19 (6H, s, Me \times 2).

General Procedure for Preparation of Phosphoranes 16–21—To a suspension of the above phosphonium salt **6** (1 mmol) in H₂O and MeOH (1:1) was added a solution of 2N NaOH (10 mL). The mixture was stirred at room temperature overnight. After evaporation of the MeOH, the suspension was extracted with CHCl₃. The organic layer was washed with the brine, dried (Na₂SO₄), and concentrated to dryness to give corresponding phosphorane.

4-Phenyl-1-(triphenylphosphoranylidene)-(3E)-buten-2-one (16)—Yield, 91% from **10**, yellow crystal, see ref. ³ for spectroscopic data.

4-(4-Chlorophenyl)-1-(triphenylphosphoranylidene)-(3E)-buten-2-one (17)—Yield, 80% from **11**, colorless oil; ¹H NMR (400 MHz, CDCl₃) δ 7.71-7.40 (17H, m), 7.34 (1H, d, J = 16 Hz, β -CH=), 7.26 (2H, d, J = 8 Hz, H-2, 6), 6.85 (1H, d, J = 16 Hz, α -CH=), 4.06 (1H, br d, J = 23 Hz, CH=P).

4-(Furan-2-yl)-1-(triphenylphosphoranylidene)-(3E)-buten-2-one (18)—Yield, 93% from **12**, pale yellow powder; ¹H NMR (400 MHz, CDCl₃): δ 7.70-7.31 (17H, m), 7.18 (1H, d, J = 16 Hz, β -CH=), 6.81 (1H, d, J = 16 Hz, α -CH=), 6.36 (1H, m, H-4), 3.98 (1H, br d, J = 24 Hz, CH=P).

4-(Thiophen-2-yl)-1-(triphenylphosphoranylidene)-(3E)-buten-2-one (19)—Yield, 93% from **13**, pale yellow powder; ¹H NMR (400 MHz, CDCl₃) δ 7.71-7.47 (16H, m), 7.16 (1H, br d, H-5), 7.06 (1H, br d, H-3), 6.95 (1H, m, H-4), 6.71 (1H, d, J = 16 Hz, α -CH=), 3.97 (1H, br d, J = 24 Hz, CH=P).

2-Phenyl-(triphenylphosphoranylidene)ethan-2-one (20)—Yield, 90% from **14**, pale yellow powder; ¹H NMR (400 MHz, CDCl₃) δ 7.97 (2H, br d, H-2, 6), 7.75-7.35 (18H, m, Ph, H-3, 4, 5), 4.43 (1H, d, J = 24 Hz, CH=P).

4-(4-*t*-Butyldimethylsilyloxyphenyl)-1-(triphenylphosphoranylidene)-(3E)-buten-2-one (21)—Yield, 80% from **15**, yellow oil; ¹H NMR (400 MHz, CDCl₃): δ 7.68-7.37 (19H, m, Ph, H-3, 5, α -CH=, β -CH=), 6.77 (2H, br d, H-2, 6), 3.97 (1H, br d, CH=P), 0.97 (9H, s, Me \times 3), 0.18 (6H, s, Me \times 2).

General Procedure for Preparation of Butenolides 22–29—A solution of maleic anhydride derivative (1.2 mmol) in benzene (10 mL) was added to a refluxed solution of a phosphorane (1 mmol) in benzene (50 mL) under nitrogen. After completion of the reaction (monitored by TLC), the reaction mixture was evaporated to dryness and the residue was chromatographed on silica gel eluting with hexane-acetone (4:1) to yield the desired product.

(5E)-3-Methyl-5-[(E)-2-oxo-4-phenylbut-3-enylidene]furan-2(5H)-one (22)—Yield, 73% from 2-methylmaleic anhydride (**2**) and **16**, see ref. ³ for spectroscopic data.

(5Z)-3,4-Dimethyl-5-[(E)-2-oxo-4-phenylbut-3-enylidene]furan-2(5H)-one (23)—Yield, 96% from 2,3-dimethylmaleic anhydride (**3**) and **16**, yellow powder; ¹H NMR (400 MHz, CDCl₃) δ 7.72 (1H, d, J = 16 Hz, H-4'), 7.65 (2H, m, H-6', 10'), 7.56 (1H, d, J = 16 Hz, H-3'), 7.42 (3H, m, H-7', 8', 9'), 5.78 (1H, s, H-1'), 2.13 (3H, s, Me), 2.03 (3H, s, Me); ¹³C NMR (100 MHz, CDCl₃) δ 187.5, 168.9, 155.8, 148.4, 144.0, 134.7, 130.7, 129.0 (2C), 128.4, 128.7 (2C), 125.2, 106.2.

(5E)-5-[(E)-2-Oxo-4-phenylbut-3-enylidene]-3-phenylfuran-2(5H)-one (24)—

Yield, 56% from 2-phenylmaleic anhydride (**4**) and **16**, yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.57 (1H, s, H-3), 8.01 (2H, br d), 7.68 (1H, d, $J = 16$ Hz, H-4'), 7.61 (2H), 7.47-7.43 (6H), 6.92 (1H, d, $J = 16$ Hz, H-3'), 6.56 (1H, s, H-1').

(5E)-5-[(E)-4-(4-Chlorophenyl)-2-oxobut-3-enylidene]-3-methylfuran-2(5H)-one (25)—

Yield, 56% from **2** and **17**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.09 (1H, s, H-3), 7.58 (1H, d, $J = 16$ Hz, H-4'), 7.51 (2H, d, $J = 8$ Hz, H-7',9'), 7.38 (2H, d, $J = 8$ Hz, H-6',10'), 6.83 (1H, d, $J = 16$ Hz, H-3'), 6.43 (1H, s, H-1'), 2.10 (3H, s, Me).

(5E)-5-[(E)-4-(Furan-2-yl)-2-oxobut-3-enylidene]-3-methylfuran-2(5H)-one (26)—

Yield, 50% from **2** and **18**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (1H, s, H-3), 7.53 (1H, br d, H-8'), 7.41 (1H, d, $J = 16$ Hz, H-4'), 6.76 (1H, d, $J = 16$ Hz, H-3'), 6.74 (1H, br d, H-7'), 6.51 (1H, br d, H-6'), 6.37 (1H, s, H-1'), 2.09 (3H, s, Me); HRESIMS m/z $[\text{M} + \text{H}]^+$ 231.0652 (calcd for $\text{C}_{13}\text{H}_{11}\text{O}_4$, 231.0652).

(5E)-3-Methyl-5-[(E)-2-oxo-4-(thiophen-2-yl)but-3-enylidene]furan-2(5H)-one (27)—

Yield, 50% from **2** and **19**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (1H, s, H-3), 7.76 (1H, d, $J = 15.6$ Hz, H-4'), 7.44 (1H, br d, H-8'), 7.35 (1H, br d, H-7'), 7.08 (1H, brd, H-6'), 6.67 (1H, d, $J = 15.6$ Hz, H-3'), 6.39 (1H, s, H-1'), 2.10 (3H, s, Me); HRESIMS m/z $[\text{M} + \text{H}]^+$ 247.0424 (calcd for $\text{C}_{13}\text{H}_{11}\text{O}_3\text{S}$, 247.0423).

(5E)-3-Methyl-5-(2-oxo-2-phenylethylidene)furan-2(5H)-one (28)—

Yield, 56% from **2** and **20**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (1H, s, H-3), 7.95 (2H, d, $J = 7$ Hz), 7.59 (1H, m), 7.51 (2H, d, $J = 7$ Hz), 6.90 (1H, s, H-1'), 2.11 (3H, s, Me); HRESIMS m/z $[\text{M} + \text{H}]^+$ 215.0703 (calcd for $\text{C}_{13}\text{H}_{11}\text{O}_3$, 215.0703).

(5E)-5-[(E)-4-(*t*-Butyldimethylsilyloxyphenyl)-2-oxobut-3-enylidene]-3-methylfuran-2(5H)-one (29)—

Yield, 40% from **2** and **21**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (1H, s, H-3), 7.59 (1H, d, $J = 15$ Hz, H-4'), 7.48 (2H, br d), 6.86 (2H, d, $J = 8$ Hz), 6.75 (1H, d, $J = 15$ Hz, H-3'), 6.45 (1H, s, H-1'), 2.10 (3H, s, Me), 0.98 (9H, s, Me \times 3), 0.22 (6H, s, Me \times 2).

General Procedure for Preparation of Cyclopentenones 30–37—A solution of the butenolide (1 mmol) in dry MeOH (20 mL) was added to a solution of NaOMe (10 mmol) in MeOH (5 mL) and the resulting orange solution was stirred at room temperature for 3–6 hr and then poured into ice water (100 mL) and acidified to pH 1.0 with 2 M HCl. The methanol was removed by evaporation, and the suspension was extracted with ether. Evaporation of the dried extracts gave the crude products, which was generally crystallized from MeOH to afford the product.

2-[(E)-1-Hydroxy-3-phenylallylidene]-4-methylcyclopent-4-ene-1,3-dione (30)—

Yield, 62% from **22**, see ref. ³ for physical and spectroscopic data including NMR signal assignments.

2-[(E)-1-Hydroxy-3-phenylallylidene]-4,5-dimethylcyclopent-4-ene-1,3-dione (31)—

Yield, 82% from **23**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 12.05 (1H, br s, OH), 7.73 (2H, br s), 7.58 (2H, br d), 7.37 (3H, br s), 1.96 (6H, br s, 2 \times Me); ^{13}C NMR (100 MHz, CDCl_3) δ 200.9, 192.1, 166.2, 151.4, 147.4, 142.3, 134.9, 130.5, 128.9 (2C), 128.5 (2C), 117.7 and 102.8 [for C-1, 3, 6, 4, 5, 8, 9, 12, 11 (13), 10 (14), 7, 2, respectively], 9.0 (Me), 8.4 (Me); HRESIMS m/z $[\text{M} + \text{H}]^+$ 255.1014 (calcd for $\text{C}_{16}\text{H}_{15}\text{O}_3$, 255.1016).

2-[(E)-1-Hydroxy-3-phenylallylidene]-4-phenylcyclopent-4-ene-1,3-dione (32)—Yield, 47% from **24**, yellow powder; ^1H NMR (400 MHz, benzene- d_6 , *Z/E*-isomers) δ 12.65 (br s), 8.13/8.06 (1H each, d, J = 16 Hz, H-7), 7.82 (1H \times 2, d, J = 16 Hz, H-8), 7.76 (2H \times 2, m, H-10, 14), 7.34 (2H \times 2, m), 7.07 (3H \times 2, m), 6.98 (3H \times 2, m), 6.54 (1H \times 2, s, H-5); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 200.6, 199.8, 191.2, 191.1, 169.0, 168.9, 153.7, 150.8, 143.5, 143.4, 137.7, 134.9, 134.8, 134.5, 131.1 (2C), 131.0 (2C), 130.4, 130.0 (2C), 129.4, 129.2 (2C), 129.0 (2C), 128.9 (2C), 128.8 (4C), 128.7 (4C), 117.7, 117.5, 104.4, 104.2; HRESIMS m/z $[\text{M}+\text{H}]^+$ 303.1001 (calcd for $\text{C}_{20}\text{H}_{15}\text{O}_3$, 303.1016).

2-[(E)-3-(4-Chlorophenyl)-1-hydroxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (33)—Yield, 55% from **25**, yellow powder; ^1H NMR (400 MHz, benzene- d_6 , *Z/E*-isomers) δ 12.70/12.40 (1H each, br s, OH), 7.91/7.84 (1H each, d, J = 16 Hz, H-7), 7.58 (1H \times 2, d, J = 16 Hz, H-8), 6.94 (2H \times 2, br d), 6.90 (2H \times 2, br d), δ 5.98 (1H \times 2, s, H-5), 1.53 and 1.47 (3H each, s, Me-4); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 201.1, 200.5, 192.1, 191.6, 167.4, 167.1, 158.2, 154.3, 141.5, 141.4 (2C), 140.9, 137.1 (2C), 136.6, 133.3, 129.7 (4C), 129.2 (4C), 118.1, 118.0, 103.5, 103.3, 10.5, 10.3; HRESIMS m/z $[\text{M}+\text{H}]^+$ 275.0470 (calcd for $\text{C}_{15}\text{H}_{12}\text{O}_3\text{Cl}$, 275.0470).

2-[(E)-3-(Furan-2-yl)-1-hydroxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (34)—Yield, 50% from **26**, yellow powder; ^1H NMR (400 MHz, benzene- d_6 , *Z/E*-isomers) δ 12.64/12.35 (1H each, br s, OH), d_6 , *Z/E*-isomers) δ 8.03/7.98 (1H each, d, J = 16 Hz, H-7), 7.48 (1H \times 2, d, J = 16 Hz, H-8), 6.82 (1H \times 2, br d, H-12), 6.05 (1H \times 2, br d, H-10), 5.95 (1H \times 2, br d, H-11), 5.85 (1H \times 2, s, H-5), 1.52 and 1.45 (3H each, s, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 201.1, 200.6, 192.0, 191.5, 167.5, 167.3, 157.9, 153.9, 151.7 (2C), 145.7, 145.6, 140.6, 136.7, 129.0, 128.9, 116.03, 115.99, 115.4, 115.3, 112.8 (2C), 103.2, 103.0, 11.3, 10.5; HRESIMS m/z $[\text{M}+\text{H}]^+$ 231.0641 (calcd for $\text{C}_{13}\text{H}_{11}\text{O}_4$, 231.0652).

2-[(E)-1-Hydroxy-3-(thiophen-2-yl)allylidene]-4-methylcyclopent-4-ene-1,3-dione (35)—Yield, 50% from **27**, yellow powder; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 12.08/11.98 (1H each, br s, OH), 7.90 (1H \times 2, d, J = 16 Hz, H-7), 7.51/7.49 (1H each, brd, J = 16 Hz, H-8), 7.50 (1H \times 2, br s, H-12), 7.37 (1H \times 2, br d, H-10), 7.11 (1H \times 2, m, H-11), 6.68/6.60 (1H each, s, H-5), 2.11/2.12 (3H each, s, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 201.1, 200.5, 192.1, 191.6, 167.5, 167.3, 157.9, 154.0, 140.6 (2C), 136.8 (2C), 135.6, 135.5, 131.6, 131.5, 130.0, 129.9, 128.4 (2C), 116.4, 116.3, 103.0, 102.8, 11.3, 10.5; HRESIMS m/z $[\text{M}+\text{H}]^+$ 247.0411 (calcd for $\text{C}_{13}\text{H}_{11}\text{O}_3\text{S}$, 247.0423).

2-[Hydroxy(phenyl)methylene]-4-methylcyclopent-4-ene-1,3-dione (36)—Yield, 52% from **28**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 8.06 (2H \times 2, d, J = 8 Hz, H-8, 12), 7.59 (1H \times 2, t, J = 8 Hz, H-10), 7.50 (2H \times 2, t, J = 8 Hz, H-9, 11), 6.74/6.59 (1H each, q, J = 1.2 Hz, H-5), 2.12/2.09 (3H each, s, J = 1.2 Hz, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 202.7, 202.1, 190.3, 189.8, 173.8, 173.5, 157.5, 153.0, 140.4, 136.2, 133.0, 131.2, 129.9 (2C), 129.7 (4C), 128.0 (4C), 103.2, 102.9, 11.4, 10.4; HRESIMS m/z $[\text{M}+\text{H}]^+$ 215.0681 (calcd for $\text{C}_{13}\text{H}_{11}\text{O}_3$, 215.0703).

2-[(E)-3-[4-O-(*t*-Butyldimethylsilyl)]phenyl-1-methoxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (37)—Yield, 50% from **29**, pale yellow oil; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 12.1/12.0 (1H each, br s, OH), 7.76 (1H \times 2, d, J = 16 Hz, H-7), 7.61/7.58 (1H each, d, J = 16 Hz, H-8), 7.56 (2H \times 2, dd, J = 8, 2 Hz, H-10, 14), 6.89 (2H \times 2, d, J = 8 Hz, H-11, 13), 6.67/6.68 (1H each, q, J = 1.6 Hz, H-5), 2.12/2.11 (3H each, d, J = 1.6 Hz, Me), 1.02 (6H \times 2, s, Me), 0.25 (6H \times 2, s, 2 \times Me).

General Procedure for Alkylation of Cyclopentenediones 30–37—To a solution of the cyclopentenedione (1 mmol) in dry acetone (20 mL) under argon was added K_2CO_3 (1.5 mmol). After refluxing for 5 minutes, Me_2SO_4 or Et_2SO_4 (1.2 mmol) was added to the deep yellow-colored solution, and reflux was continued for 1–3 hr. After completion of the reaction, K_2CO_3 was removed by filtration. The filtrate was concentrated to dryness and the residue was subjected to reversed phase RP-18 column chromatography using aqueous MeOH (60–80%) to give the desired product.

2-[(E)-1-Methoxy-3-phenylallylidene]-4-methylcyclopent-4-ene-1,3-dione (1, Coruscanone A)—Yield, 93% from methylation of **30**, see ref. ³ for physical and spectroscopic data including NMR signal assignments. Anal. HPLC [t_R (min) (content)]: 5.65 (97.7%) from solvent a, or 5.72 (99.0%) from solvent b.

2-[(E)-1-Methoxy-3-phenylallylidene]-4,5-dimethylcyclopent-4-ene-1,3-dione (38)—Yield, 60% from methylation of **31**, yellow powder; 1H NMR (400 MHz, $CDCl_3$) δ 8.04 (1H, d, $J = 16$ Hz, H-7), 7.61 (2H, br d), 7.55 (1H), 7.33 (3H, m), 4.18 (3H, s, OMe), 1.99 (6H, br s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.6, 192.2, 167.3, 150.8, 150.3, 141.9, 135.6, 130.1, 128.9 (2C), 128.4 (2C), 121.3, 108.6, 64.5, 9.02 (2C); HRESIMS m/z [$M+H$]⁺ 269.1171 (calcd for $C_{17}H_{17}O_3$, 269.1172); Anal. HPLC [t_R (min) (content)]: 6.56 (99.5%) from solvent a, or 5.72 (99.6%) from solvent b.

2-[(E)-1-Methoxy-3-phenylallylidene]-4-phenylcyclopent-4-ene-1,3-dione (39)—Yield, 92% from methylation of **32**, yellow powder; 1H NMR (400 MHz, benzene- d_6 , Z/E -isomers) δ 8.48/8.35 (1H each, d, $J = 16$ Hz, H-7), 7.84 (2H \times 2, m, H-2', 6'), 7.62 (1H \times 2, d, $J = 16$ Hz, H-8), 7.45 (3H \times 2, m), 7.11 (3H \times 2, m), 7.02 (3H \times 2, m), 6.78/6.76 (1H each, s, H-5), 4.0/3.92 (3H each, s, OMe); ^{13}C NMR (100 MHz, $CDCl_3$, Z/E -isomers) δ 194.0, 193.4, 191.3, 191.1, 170.0, 169.9, 153.0, 152.8, 143.2, 143.0, 138.4, 138.1, 135.5 (2C), 130.8, 130.7, 130.4 (2C), 130.0, 129.1 (4C), 129.0 (3C), 128.9 (4C), 128.8 (2C), 128.6 (4C), 121.4, 121.2, 110.0, 109.8, 64.8, 64.7; HRESIMS m/z [$M+H$]⁺ 317.1160 (calcd for $C_{21}H_{17}O_3$, 317.1172); Anal. HPLC [t_R (min) (content)]: 9.56 (99.5%) from solvent a, or 11.70 (99.7%) from solvent b.

2-[(E)-3-(4-Chlorophenyl)-1-methoxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (40)—Yield, 95% from methylation of **33**, yellow powder; 1H NMR (400 MHz, benzene- d_6 , Z/E -isomers) δ 8.30/8.23 (1H each, d, $J = 16$ Hz, H-7), 7.39 (1H \times 2, d, $J = 16$ Hz, H-8), 7.08 (2H \times 2, d, $J = 7.6$ Hz, H-11, 13), 6.94 (2H \times 2, d, $J = 7.2$ Hz, H-10, 14), 6.22/6.19 (1H each, s, H-5), 3.98/3.93 (3H each, s, OMe), 1.60 (6H, br s, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, Z/E -isomers) δ 195.2, 194.0, 192.4, 191.6, 168.4, 168.2, 157.0, 156.5, 141.0 (2C), 140.7, 140.4, 136.1 (2C), 134.0 (2C), 129.6 (2C), 129.5 (2C), 129.2 (4C), 121.9, 121.7, 108.9, 108.8, 64.8, 64.7, 11.2, 11.1; HRESIMS m/z [$M+H$]⁺ 289.0611 (calcd for $C_{16}H_{14}O_3Cl$, 289.0626); Anal. HPLC [t_R (min) (content)]: 7.68 (96.7%) from solvent a, or 7.46 (99.4%) from solvent b.

2-[(E)-3-(Furan-2-yl)-1-methoxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (41)—Yield, 90% from methylation of **34**, yellow powder; 1H NMR (400 MHz, benzene- d_6 , Z/E -isomers) δ 8.42/8.35 (1H each, d, $J = 16$ Hz, H-7), 7.33 (1H \times 2, d, $J = 16$ Hz, H-8), 6.89 (1H \times 2, brd, H-12), 6.20 (1H \times 2, br d), 6.15 (1H \times 2, br d), 5.91 (1H \times 2, br s, H-5), 3.94/3.89 (3H each, s, OMe), 1.59/1.58 (3H each, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$, Z/E -isomers) δ 195.0, 193.9, 192.5, 191.7, 168.6, 168.4, 156.6, 156.4, 152.2 (2C), 145.3 (2C), 140.4, 140.2, 128.8 (2C), 119.2, 119.0, 115.2 (2C), 112.7 (2C), 108.7, 108.6, 64.6, 64.5, 11.1, 11.0; HRESIMS m/z [$M+H$]⁺ 245.0793 (calcd for $C_{14}H_{13}O_4$, 245.0809); Anal. HPLC [t_R (min) (content)]: 4.22 (98.7%) from solvent a, or 3.88 (98.0%) from solvent b.

2-[(E)-1-Methoxy-3-(thiophen-2-yl)allylidene]-4-methylcyclopent-4-ene-1,3-dione (42)—Yield, 90% from methylation of **35**, yellow powder; ^1H NMR (400 MHz, benzene- d_6 , *Z/E*-isomers) δ 8.29/8.23 (1H each, d, J = 16 Hz, H-7), 7.66 (1H, d, J = 16 Hz, H-8), 6.85 (1H \times 2, br d), 6.71 (1H \times 2, br d), 6.54 (1H \times 2, br d), 6.19/6.16 (1H each, s, H-5), 3.94/3.89 (3H each, s, OMe), 1.60/1.59 (3H each, s, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 195.2, 194.0, 192.5, 191.7, 168.6, 168.4, 156.7, 156.3, 141.3 (2C), 140.5, 140.2, 135.3, 135.2, 130.9, 130.8, 129.3 (2C), 128.3 (2C), 120.4, 120.2, 108.4 (2C), 64.7, 64.6, 11.15, 11.1; HRESIMS m/z $[\text{M}+\text{H}]^+$ 231.0564 (calcd for $\text{C}_{14}\text{H}_{13}\text{O}_3\text{S}$, 261.0580); Anal. HPLC [t_R (min) (content)]: 4.77 (95.8%) from solvent a, or 4.64 (96.0%) from solvent b.

2-[Methoxy(phenyl)methylene]-4-methylcyclopent-4-ene-1, 3-dione (43)—Yield, 88% from methylation of **36**, pale yellow powder; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 7.50 (3H \times 2, m), 7.29 (2H \times 2, m), 6.70/6.57 (1H each \times 2, s, H-5), 3.77/3.76 (3H each \times 2, s, OMe), 2.06/1.95 (3H each \times 2, s, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 193.7, 193.5, 193.0, 192.3, 171.7, 171.5, 156.6, 156.4, 140.4, 140.3, 131.1, 131.0, 130.8, 130.7, 128.9 (4C), 128.7 (4C), 107.8 (2C), 59.4, 59.2, 11.22, 11.17; HRESIMS m/z $[\text{M}+\text{H}]^+$ 229.0839 (calcd for $\text{C}_{14}\text{H}_{13}\text{O}_3$, 229.0859); Anal. HPLC [t_R (min) (content)]: 2.98 (97.7%) from solvent a, or 2.95 (98.1%) from solvent b.

2-[(E)-3-[4-O-(*t*-Butyldimethylsilyl)phenyl]-1-methoxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (44)—Yield, 85% from methylation of **37**, yellow oil; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 7.83/7.81 (1H each, d, J = 16 Hz, H-7), 7.58 (1H \times 2, d, J = 16 Hz, H-8), 7.52 (2H \times 2, d, J = 8 Hz, H-11, 13), 6.84 (2H \times 2, d, J = 8 Hz, H-10, 14), 6.68/6.66 (1H each, s, H-5), 4.18 (3H \times 2, s, OMe), 2.08 (3H \times 2, s, Me), 0.98 (9H \times 2, s, 3 \times Me), 0.22 (6H \times 2, s, 2 \times Me).

2-[(E)-3-(4-Hydroxyphenyl)-1-methoxyallylidene]-4-methylcyclopent-4-ene-1,3-dione (45)—To a solution of **44** (20 mg) in dry THF (10 mL) tetra-*t*-butyl ammonium fluoride (TBAF) was added under N_2 . After stirring for 3 hr at room temperature, the reaction mixture was extracted with Et_2O . The organic layers were separated, dried (Na_2SO_4) and evaporated to give the crude product, which was purified by column chromatography on silica gel using 30% EtOAc in hexanes as pale orange powder (11.2 mg in a yield of 80%); ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 7.84/7.83 (1H each, d, J = 16 Hz, H-7), 7.61 (1H \times 2, d, J = 16 Hz, H-8), 7.56 (2H \times 2, d, J = 8 Hz, H-10, 14), 6.96 (2H \times 2, m, H-11, 13), 6.70/6.68 (1H each, s, H-5), 6.63/6.47 (1H each, br s, OH), 4.21 (3H \times 2, s, OMe), 2.10/2.09 (3H each, s, Me); HRESIMS m/z $[\text{M}+\text{H}]^+$ 271.0935 (calcd for $\text{C}_{16}\text{H}_{15}\text{O}_4$, 271.0965); Anal. HPLC [t_R (min) (content)]: 3.21 (96.4%) from solvent a, or 3.84 (98.8%) from solvent b.

2-[(E)-1-Methoxy-3-(4-methoxyphenyl)allylidene]-4-methylcyclopent-4-ene-1,3-dione (46)—To a solution of **45** (1 mmol) in dry acetone (20 mL) under argon was added K_2CO_3 (1.5 mmol). After refluxing for 5 min, MeI (1.2 mmol) was added to the deep yellow-colored solution, and reflux continued for 3 hr. After filtration, the filtrate was concentrated to dryness to give a residue, which was purified by flash chromatography using 30% EtOAc in hexanes to give **46** as yellow powder in a yield of 90%; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 7.87/7.85 (1H each, d, J = 16 Hz, H-7), 7.62 (1H \times 2, d, J = 16 Hz, H-8), 7.59 (2H \times 2, d, J = 8 Hz, H-10, 14), 6.93 (2H \times 2, d, J = 8 Hz, H-11, 13), 6.70/6.68 (1H each, q, J = 1.6 Hz, H-5), 4.21 (3H \times 2, s, OMe), 3.86 (3H \times 2, s, Ar-OMe), 2.10/2.09 (3H each, s, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 195.4, 194.3, 192.6, 191.9, 169.6, 169.4, 161.6 (2C), 156.5, 156.1, 142.78, 142.76, 140.2, 140.0, 130.3 (4C), 128.3 (2C), 118.9, 118.8, 114.4 (4C), 108.2, 108.1, 64.6, 64.5, 55.4 (2C), 11.1, 11.0; HRESIMS m/z $[\text{M}+\text{H}]^+$ 285.1088 (calcd for $\text{C}_{17}\text{H}_{17}\text{O}_4$, 285.1121); Anal. HPLC [t_R (min) (content)]: 5.31 (97.7%) from solvent a, or 5.67 (99.3%) from solvent b.

2-[(E)-1-Ethoxy-3-phenylallylidene]-4-methylcyclopent-4-ene-1,3-dione (47)—

Yield, 92% from ethylation of **30**, yellow needles (MeOH), mp 75–76 °C; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 8.02/8.00 (1H each, d, J = 16 Hz, H-7), 7.61 (3H \times 2, m, H-8, 10, 14), 7.36 (3H \times 2, m, H-11, 12, 13), 6.67/6.66 (1H each, s, H-5), 4.45 (2H \times 2, m, OCH_2CH_3), 2.06 (3H \times 2, s, Me), 1.45 (3H \times 2, m, OCH_2CH_3); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 195.7, 194.5, 192.9, 192.1, 168.1, 167.9, 156.9, 156.5, 143.0 (2C), 140.7, 140.4, 135.7 (2C), 130.5 (2C), 129.1 (4C), 128.7 (4C), 122.0, 121.9, 109.4, 109.3, 74.0, 73.9, 16.0 (2C), 11.41, 11.36; HRESIMS m/z $[\text{M}+\text{H}]^+$ 269.1155 (calcd for $\text{C}_{17}\text{H}_{17}\text{O}_3$, 269.1172); Anal. HPLC [t_R (min) (content)]: 5.82 (97.4%) from solvent b.

2-[(E)-1-Ethoxy-3-phenylallylidene]-4, 5-dimethylcyclopent-4-ene-1,3-dione (48)

—Yield, 83% from ethylation of **31**, yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.08 (1H, d, J = 16 Hz, H-7), 7.68 (1H, d, J = 16 Hz, H-8), 7.58 (2H, m, H-10, 14), 7.36 (3H, m, H-11, 12, 13), 4.42 (2H, m, OCH_2CH_3), 1.99 (3H, s, Me), 1.45 (3H, t, OCH_2CH_3); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 194.7, 192.3, 166.2, 150.6, 150.2, 141.9, 135.7, 130.0, 128.8 (2C), 128.4 (2C), 121.9, 109.2, 73.4, 15.7, 8.9; Anal. HPLC [t_R (min) (content)]: 9.11 (96.7%) from solvent a, or 9.50 (99.0%) from solvent b.

General Procedure for Acylation of Cyclopentenediones **30 and **31****—A solution of the cyclopentenedione (50 mg) in pyridine (2 mL) and Ac_2O (2 mL) was kept at room temperature for 24 hr. In the case of using the acylating reagents benzoyl chloride and cinnamoyl chloride, the solution of cyclopentenedione and a catalytic amount of DMAP in pyridine was first cooled to 0°C. After addition of the acylating reagent, the reaction mixture was brought to room temperature and kept for 24 hr. The reaction mixture was poured into ice water and extracted with Et_2O . The Et_2O layer was washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel eluting with hexane-acetone (6:1) to afford the product.

2-[(E)-1-Acetoxy-3-phenylallylidene]-4-methylcyclopent-4-ene-1, 3-dione (49)—

Yield, 76% from **30**, yellow powder; ^1H NMR (400 MHz, CDCl_3 , *Z/E*-isomers) δ 8.14/8.13 (1H each, d, J = 16 Hz, H-7), 7.61 (2H \times 2, m, H-10, 14), 7.45–7.39 (4H \times 2, m, H-11, 12, 13, H-8), 6.88/6.82 (1H each, q, J = 1.2 Hz, H-5), 2.49/2.48 (3H each, s, OAc), 2.12/2.08 (3H each, d, J = 1.2 Hz, Me); ^{13}C NMR (100 MHz, CDCl_3 , *Z/E*-isomers) δ 193.3, 192.0 (2C), 190.6, 167.52, 167.47, 159.2, 158.9, 155.7, 155.5, 143.2, 143.1, 142.7, 142.3, 134.9 (2C), 130.8 (2C), 129.0 (4C), 128.72 (2C), 128.68 (2C), 119.4, 119.3, 113.4 (2C), 20.91, 20.88, 11.4, 11.3; HRESIMS m/z $[\text{M}+\text{H}]^+$ 283.0964 (calcd for $\text{C}_{17}\text{H}_{15}\text{O}_4$, 283.0965); Anal. HPLC [t_R (min) (content)]: 5.06 (97.8%) from solvent a, or 4.67 (100%) from solvent b.

2-[(E)-1-Acetoxy-3-phenylallylidene]-4, 5-dimethylcyclopent-4-ene-1, 3-dione (50)—

Yield, 90% from **31**, yellow powder, mp 214–216 °C; ^1H NMR (400 MHz, CDCl_3) δ 8.18 (1H, d, J = 16 Hz, H-7), 7.62 (2H, m, H-10, 14), 7.42–7.39 (4H, m, H-8, 11, 12, 13), 2.50 (3H, s, OAc), 2.06/2.02 (3H each, s, Me); ^{13}C NMR (100 MHz, CDCl_3): δ 192.7, 191.5, 167.7, 154.3, 153.4, 153.0, 142.3, 135.1, 130.6, 129.0 (2C), 128.6 (2C), 119.5, 113.0, 20.9, 9.24, 9.15. HRESIMS m/z $[\text{M}+\text{H}]^+$ 297.1125 (calcd for $\text{C}_{18}\text{H}_{17}\text{O}_4$, 297.1121); Anal. HPLC [t_R (min) (content)]: 5.94 (99.6%) from solvent a, or 5.72 (99.6%) from solvent b.

2-[(E)-1-Benzoyloxy-3-phenylallylidene]-4, 5-dimethylcyclopent-4-ene-1, 3-dione (51)—

Yield, 77% from **31**, yellow powder; ^1H NMR (400 MHz, CDCl_3) δ 8.30 (1H, m, d, J = 16 Hz, H-7), 8.27 (2H, d, J = 7.2 Hz, H-2', 6'), 7.71 (1H, t, J = 7.2 Hz), 7.63–7.56 (4H, m), 7.45 (1H, d, J = 16 Hz, H-8), 7.39 (4H, m), 2.09/2.00 (3H each, s, Me); ^{13}C NMR (100 MHz, CDCl_3) δ 192.7, 191.2, 163.5, 154.6, 153.5, 153.2, 142.4, 135.1, 133.9, 130.6, 130.5 (2C), 129.0 (2C), 128.8 (3C), 128.6 (2C), 119.6, 113.1, 9.3, 9.2; HRESIMS m/z $[\text{M}+\text{H}]^+$ 359.1263

(calcd for $C_{23}H_{19}O_4$, 359.1278); Anal. HPLC [t_R (min) (content)]: 10.11 (99.5%) from solvent a, or 10.79 (99.5%) from solvent b.

2-[(E)-1-Cinnamoyloxy-3-phenylallylidene]-4, 5-dimethylcyclopent-4-ene-1, 3-dione (52)—Yield, 78% from **31**, yellow powder; 1H NMR (400 MHz, $CDCl_3$) δ 8.23 (1H, d, J = 16 Hz, H-7), 7.94 (1H, d, J = 16 Hz, β -H of cinnamoyl), 7.63 (3H, m, H-10, 14), 7.44-7.38 (8H, m), 6.74 (1H, d, J = 16 Hz, α -H of cinnamoyl), 2.07/2.00 (3H each, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 191.5, 163.7, 154.5, 153.5, 153.3, 148.0, 142.5, 135.4, 134.2, 131.1, 130.8, 129.2 (4C), 128.8 (4C), 119.8, 116.5, 113.4, 9.5, 9.4; Anal. HPLC [t_R (min) (content)]: 12.33 (97.6%) from solvent a, or 10.49 (97.9%) from solvent b.

2-(1-Methoxyethylidene)-4-methyl-cyclopent-4-ene-1,3-dione (53)—To a solution of 2-methylmaleic anhydride (**2**) (8.0 g, 71.4 mmol) in dry 1,2-dichloroethane (200 mL) at room temperature under N_2 is added anhydrous $AlCl_3$ (32.3 g, 133.4 mmol). The suspension was cooled to 0 °C and α -methylvinyl acetate (10.7 g, 107.1 mmol) was added dropwise over 15 minutes. The mixture was subsequently refluxed for 1.5 hr. After cooling, the mixture was poured to ice water containing 10% HCl (300 mL). The aqueous layer was extracted with $CHCl_3$ (150 mL \times 3). The combined organic layers were washed with brine (150 mL) and H_2O (200 mL \times 4) to neutral, dried (Na_2SO_4), and concentrated to dryness to yield a residue (8.5 g), which was chromatographed on silica gel eluting with hexane-EtOAc (1:2) to afford 2-(1-hydroxyethylidene)-4-methylcyclopent-4-ene-1,3-dione (5.97 g, 55%) as pale yellow powder, 1H NMR (400 MHz, $CDCl_3$, *E/Z*-isomers) δ 11.8 (br s, OH), 6.50/6.42 (br s, H-5), 2.22 (s, Me), 1.91 (s, Me-4); ^{13}C NMR (100 MHz, $CDCl_3$, *E/Z*-isomers) δ 200.4/199.6/192.1/191.7 (CO), 176.9/176.7 (C-6), 157.8/153.4 (C-4), 140.5/136.2 (C-5), 104.2 (2C, C-2), 17.9 (vinylic Me), 11.2/10.2 (Me-4); HRTOFMS m/z [$M + H$] $^+$ 153.0585 (calcd for $C_8H_9O_3$, 153.0546). Following the general procedure for methylation of cyclopentenenediones, the title compound was obtained from the above compound in a yield of 46% as yellow powder; 1H NMR (400 MHz, $CDCl_3$, *E/Z*-isomers) δ 6.61/6.59 (br s, H-5), 4.01 (s, OMe), 2.62 (br s, vinylic Me), 2.03/2.05 (s, Me-4); ^{13}C NMR (100 MHz, $CDCl_3$, *E/Z*-isomers) δ 195.4/194.5/193.0/192.5 (C=O), 173.8/173.5 (C-6), 155.8/155.2 (C-4), 139.7/139.1 (C-5), 107.2 (2C, C-2), 56.0/55.9 (OMe), 14.05/13.99 (vinylic Me), 11.0/10.8 (Me-4); HRTOFMS m/z [$M + H$] $^+$ 167.0682 (calcd for $C_9H_{11}O_3$, 167.0703). Anal. HPLC [t_R (min) (content)]: 2.51 (97.6%) from solvent a, or 2.66 (98.5%) from solvent b.

2-Methoxy-1,4-bis-methoxymethoxybenzene (55)—A solution of methoxyhydroquinone (**54**) (1 g, 7.14 mmol) in dry THF (30 mL) was added to an ice-cooled, stirred suspension of NaH (0.68 g of 60% dispersion in mineral oil, 17.00 mmol) in dry THF (100 mL). After stirring for 10 min at 0 °C, chloromethoxymethyl ether (2.29 g, 28.44 mmol) was added and stirring continued for 24 hr. Crushed ice and water (200 mL) were added to the reaction mixture. The aqueous phase was extracted with Et_2O (200 mL \times 3). The organic layers were combined, washed with H_2O (150 mL), dried (Na_2SO_4), and concentrated to dryness. The residue was chromatographed on silica gel using $CHCl_3$ as solvent to give **55** (1.23 g, yield, 75%) as colorless oil, 1H NMR ($CDCl_3$, 400 MHz) δ 7.03 (1H, d, J = 8.7 Hz), 6.63 (1H, d, J = 2.6 Hz), 6.56 (1H, dd, J = 8.7, 2.6 Hz), 5.13 (2H, s), 5.11 (2H, s), 3.84 (3H, s), 3.50 (3H, s), 3.47 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.5 (s), 151.2 (s), 141.8 (s), 118.2 (d), 107.5 (d), 102.4 (d), 96.6 (t), 95.5 (t), 56.5 (q), 56.3 (q), 56.2 (q).

1-Bromo-4-methoxy-2,5-bis-methoxymethoxybenzene (56)—To a stirred solution of **55** (1 g, 4.386 mmol) and $NaHCO_3$ (368 mg, 4.386 mmol) in MeOH (100 mL) at room temperature was added slowly a solution of pyridium hydrobromide perbromide ($C_5H_5N + HBr_3^-$) (1.47 g, 4.596 mmol) in MeOH (50 mL) in 25 min. The reaction was complete immediately after the addition. The reaction mixture was diluted with ice water (150 mL) and

extracted with Et₂O (125 mL × 4). The combined organic layers were washed with brine and H₂O, dried (MgSO₄), and concentrated to dryness to give **56** (1.2 g, yield, 89%) as a purple powder, ¹H NMR (CDCl₃, 400 MHz) δ 7.30 (1H, s), 6.78 (1H, s), 5.16 (2H, s), 5.12 (2H, s), 3.84 (3H, s), 3.52 (3H, s), 3.49 (3H, s); ¹³C NMR (CDCl₃, 100 MHz) δ 150.2 (s), 149.6 (s), 142.2 (s), 121.5 (d), 102.7 (d), 102.5 (s), 96.4 (t), 96.3 (t), 56.6 (q), 56.4 (q), 56.3 (q).

1-Methoxy-2,5-bis-methoxymethoxy-4-methylbenzene (57)—To a solution of **56** (0.88 g, 2.857 mmol) in dry THF (30 mL) at –78 °C was added MeLi (2.45 mL of 1.4 M in Et₂O, 3.43 mmol). After stirring at –78 °C for 1 hr, MeI (2.03 g, 14.296 mmol) was added to the resultant white cloudy suspension. The solution was stirred at –78 °C for 1 hr, and then warmed to room temperature. The reaction mixture was diluted with saturated aqueous NH₄Cl (20 mL) and water (20 mL). The organic layer was separated and the aqueous layer was extracted with Et₂O (50 mL × 3). The combined organic layers were washed with brine and H₂O, dried (MgSO₄), and concentrated to dryness to yield **57** (0.62 g, yield, 90%) as a colorless oil, ¹H NMR (CDCl₃, 300 MHz) δ 6.88 (1H, s), 6.68 (1H, s), 5.08 (4H, s), 3.78 (3H, s), 3.46 (3H, s), 3.44 (3H, s), 2.12 (3H, s); ¹³C NMR (CDCl₃, 75 MHz) δ 151.1 (s), 148.9 (s), 141.3 (s), 120.3 (d), 119.5 (s), 101.5 (s), 96.6 (t), 95.8 (t), 56.5 (q), 56.4 (q), 56.2 (q), 15.8 (q).

1-(2-Methoxy-3,6-bis-methoxymethoxy-5-methylphenyl)-2-phenylethanol (58)—To a solution of **57** (0.59 g, 2.438 mmol) in dry THF (5 mL) at –78 °C was added BuLi (1.46 mL of 2.5 M in hexane, 3.657 mmol). After stirring at –78 °C for 1 hr, the solution was warmed to 0 °C and stirring continued at 0 °C for 5 hr. The solution was again warmed to 25 °C and stirred for 3 hr. The resultant white cloudy suspension was cooled to 0 °C. Phenylacetaldehyde (0.37 g, 3.657 mmol) was added slowly over 10 min and stirring continued for 30 min. The reaction was quenched with saturated aqueous NH₄Cl (20 mL). The organic layer was separated and the aqueous layer was extracted with Et₂O (30 mL × 3). The combined organic layers were washed with brine and H₂O, dried (MgSO₄), and concentrated to dryness. The residue was chromatographed on silica gel using CH₂Cl₂ and then CH₂Cl₂–MeOH (50:1) to yield **58** (0.55 g, yield, 66%) as an off-white oil, ¹H NMR (CDCl₃, 300 MHz) δ 7.25 (5H, m, H-2''-6''), 6.92 (1H, s, H-4'), 5.28 (1H, dd, *J* = 8.4, 5.5 Hz, H-1), 5.16 (2H, s, MeOCH₂O-3'), 4.96/4.89 (1H each, ABq, *J* = 5.6 Hz, MeOCH₂O-6'), 3.90 (3H, s, MeO-2'), 3.60 (3H, s, MeOCH₂O-6'), 3.52 (3H, s, MeOCH₂O-4'), 3.29 (1H, dd, *J* = 13.6, 8.4 Hz, H-2a), 3.18 (1H, dd, *J* = 13.7, 5.5 Hz, H-2b); ¹³C NMR (CDCl₃, 75 MHz) δ 149.2 (s, C-6'), 147.1 (s, C-2'), 146.9 (s, C-3'), 139.8 (s, C-1''), 130.8 (s, C-1'), 129.9 (2C, d, C-3'',5''), 128.6 (2C, d, C-2'',6''), 127.0 (s, C-5'), 126.6 (s, C-4''), 118.6 (d, C-4'), 100.4 (d, MeOCH₂O-6'), 95.9 (d, MeOCH₂O-3'), 70.3 (d, C-1), 61.6 (q, MeO-2'), 57.9 (q, MeOCH₂O-6'), 56.7 (q, MeOCH₂O-3'), 44.6 (t, C-2), 17.4 (q, Me-5'); the above NMR signal assignments were facilitated by a 2D HMBC experiment.

2-Methoxy-5-methyl-3-styrylbenzene-1,4-diol (59)—A solution of **58** (100 mg, 0.291 mmol) and 3N HCl (3 mL) in dioxane (10 mL) was stirred under argon at 50 °C for 8 hr. The reaction mixture was diluted with H₂O (20 mL) and then extracted with Et₂O (30 mL × 3). The combined organic layers were washed with brine and H₂O, dried (MgSO₄), and concentrated to dryness to give **59** (71 mg, yield, 95%) as an off-white powder, ¹H NMR (CDCl₃, 300 MHz) δ 7.55 (2H, br d, *J* = 7.2 Hz, H-2'',6''), 7.39 (2H, br t, *J* = 7.1 Hz, H-3'',5''), 7.32 (1H, d, *J* = 7.5 Hz, H-4''), 7.29 (1H, d, *J* = 16.3 Hz, H-1'), 7.20 (1H, d, *J* = 16.9 Hz, H-1'), 6.71 (1H, s, H-5), 3.76 (3H, s, MeO-2), 2.22 (3H, s, Me-5).

2-Methoxy-5-methyl-3-styryl-1,4-benzoquinone (60)—A solution of **59** (70 mg, 0.273 mmol) and DDQ (160 mg, 0.705 mmol) in CH₂Cl₂ (15 mL) and H₂O (2 mL) was stirred under argon at room temperature for 12 hr. The reaction mixture was diluted with H₂O (20 mL) and then extracted with CH₂Cl₂ (30 mL × 3). The combined organic layers were washed with brine and H₂O, dried (MgSO₄), and concentrated to dryness. The residue was chromatographed on

silica gel using CH_2Cl_2 as solvent to yield **60** (45 mg, yield, 65%) as red powder; UV (CH_3CN), λ_{max} (log ϵ) 278 (4.35), 292 (4.35) nm; IR (KBr) ν_{max} , 1652, 1611, 1559, 1448, 1336, 1305, 1230, 1135, 965, 885, 750, 692 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 7.87 (1H, d, J = 16.6 Hz, H-8), 7.60 (2H, d, J = 7.2 Hz, H-11,15), 7.43 (2H, t, J = 7.2 Hz, H-12,14), 7.35 (1H, t, J = 7.2 Hz, H-14), 7.25 (1H, d, J = 16.6 Hz, H-9), 6.54 (1H, q, J = 1.5 Hz, H-6), 4.19 (3H, s, OMe), 2.13 (3H, d, J = 1.4 Hz, H-7); ^{13}C NMR (CDCl_3 , 75 MHz) δ 188.0 (s, C-1), 183.6 (s, C-4), 154.3 (s, C-2), 146.0 (s, C-5), 138.9 (d, C-9), 138.0 (s, C-10), 132.1 (s, C-6), 128.9 (d, C-12,14), 128.8 (d, C-13), 127.3 (d, C-11,15), 126.2 (C-4), 117.6 (C-8), 61.3 (OMe), 16.2 (C-7); ESIMS m/z $[\text{M} (\text{C}_{15}\text{H}_{11}\text{O}_3) + \text{H}]^+$ 255.

In Vitro Antifungal Susceptibility Testing—A modified version of the CLSI (formerly NCCLS) methods was used for susceptibility testing.²¹ Organisms were obtained from either the American Type Culture Collection (Manassas, VA) [*Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906] or provided by Spencer Redding/Ted White – *C. albicans* isolates #1 (fluconazole-susceptible) and #17 (fluconazole-resistant).¹¹ Samples were serially-diluted in 20% DMSO/saline and transferred in duplicate to 96 well flat bottom microplates. Inocula were prepared by correcting the OD₆₃₀ of microbe suspensions in incubation broth [RPMI 1640/2% dextrose/0.03% glutamine/MOPS at pH 6.0 (Cellgro) for *C. albicans*, Sabouraud Dextrose for *C. neoformans*, and 5% Alamar Blue™/RPMI 1640 broth (2% dextrose, 0.03% glutamine, buffered with 0.165M MOPS at pH 7.0) for *A. fumigatus*] to afford final target inocula of: *C. albicans*: 1.0×10^4 , *C. neoformans*: 1.0×10^5 , and *A. fumigatus*: 3.0×10^4 CFU/ml. Drug controls [amphotericin B (ICN Biomedicals, Ohio) and fluconazole (Sequoia Research Products Ltd., UK) were included in each assay. All organisms were read at 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont) prior to and after incubation (*C. albicans* at 37 °C for 18–24 h; *C. neoformans* and *A. fumigatus* at 30 °C for 72 h) except *A. fumigatus*, which was read at 544ex/590em using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany). Percent growth was calculated and plotted versus test concentration to afford the IC₅₀ (sample concentration that affords 50% growth of the organism). The minimum inhibitory concentration (MIC) is defined as the lowest test concentration that allows no detectable growth. Minimum fungicidal concentrations (MFCs) were determined by removing 5 μl from each clear (or blue) well, transferring to agar and incubating as previously mentioned. The MFC was defined as the lowest test concentration that allows no growth of the organism on agar.

In Vitro Cytotoxicity Assay—The mammalian cells Vero (African green monkey kidney fibroblast) and LLC-PK-1 (Pig kidney epithelial) used in this study were obtained from ATCC (Manassas, VA). Cytotoxicity was determined by Neutral Red method²² up to a highest concentration of 10 $\mu\text{g/mL}$. The detailed procedure was described in a previous paper.²³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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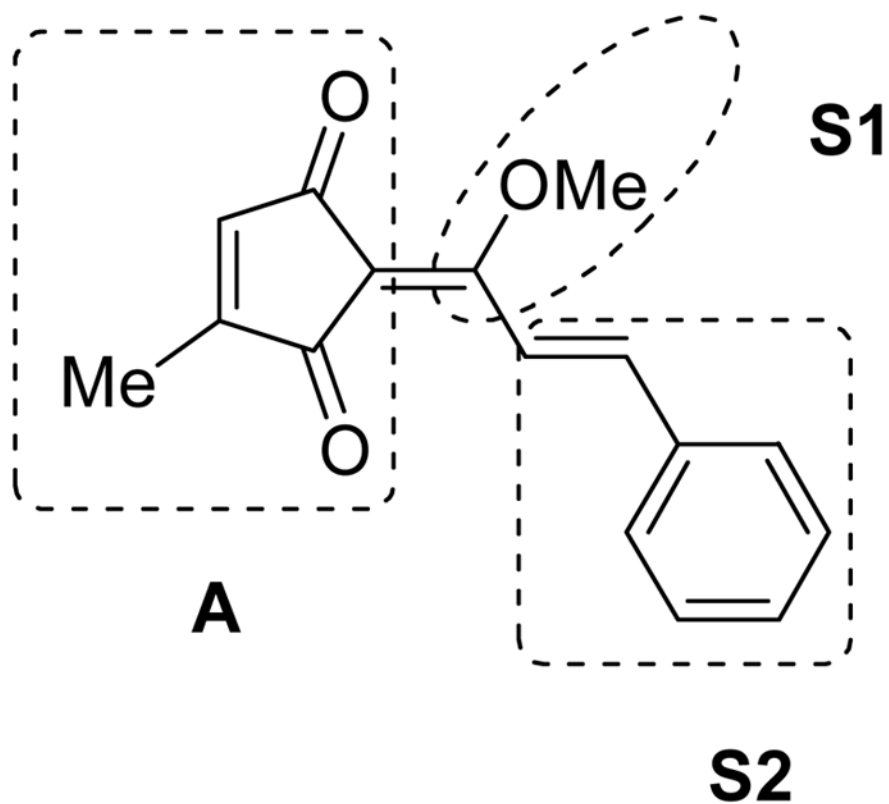
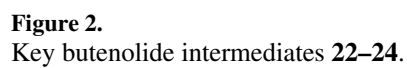


Figure 1.
Key structural moieties of coruscanone A (**1**).



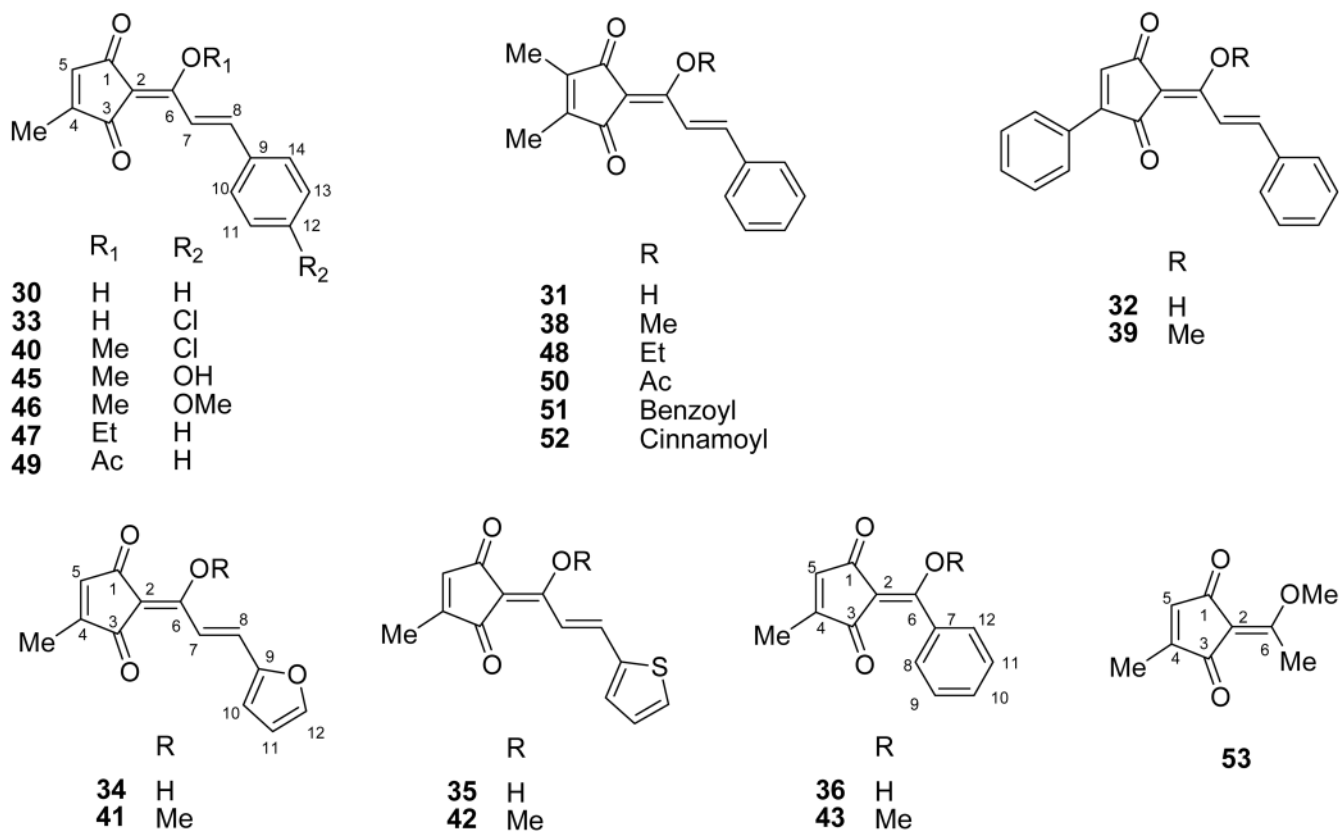
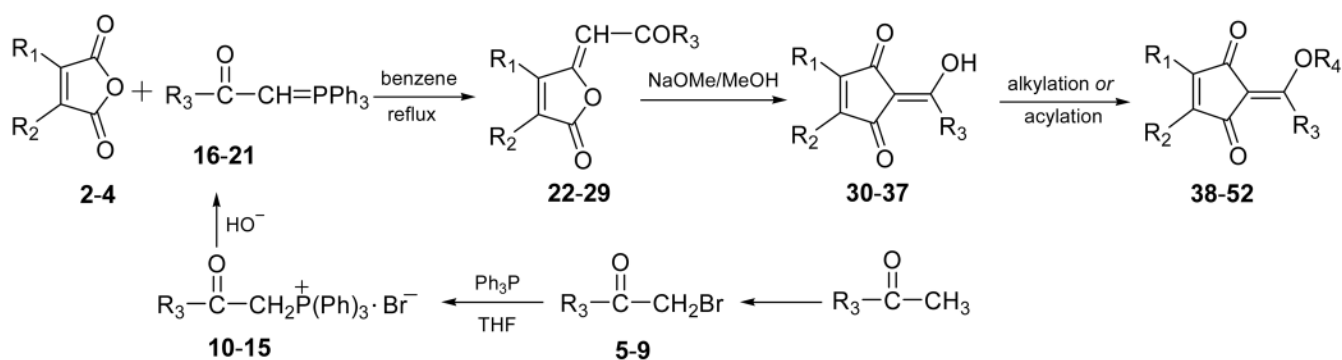
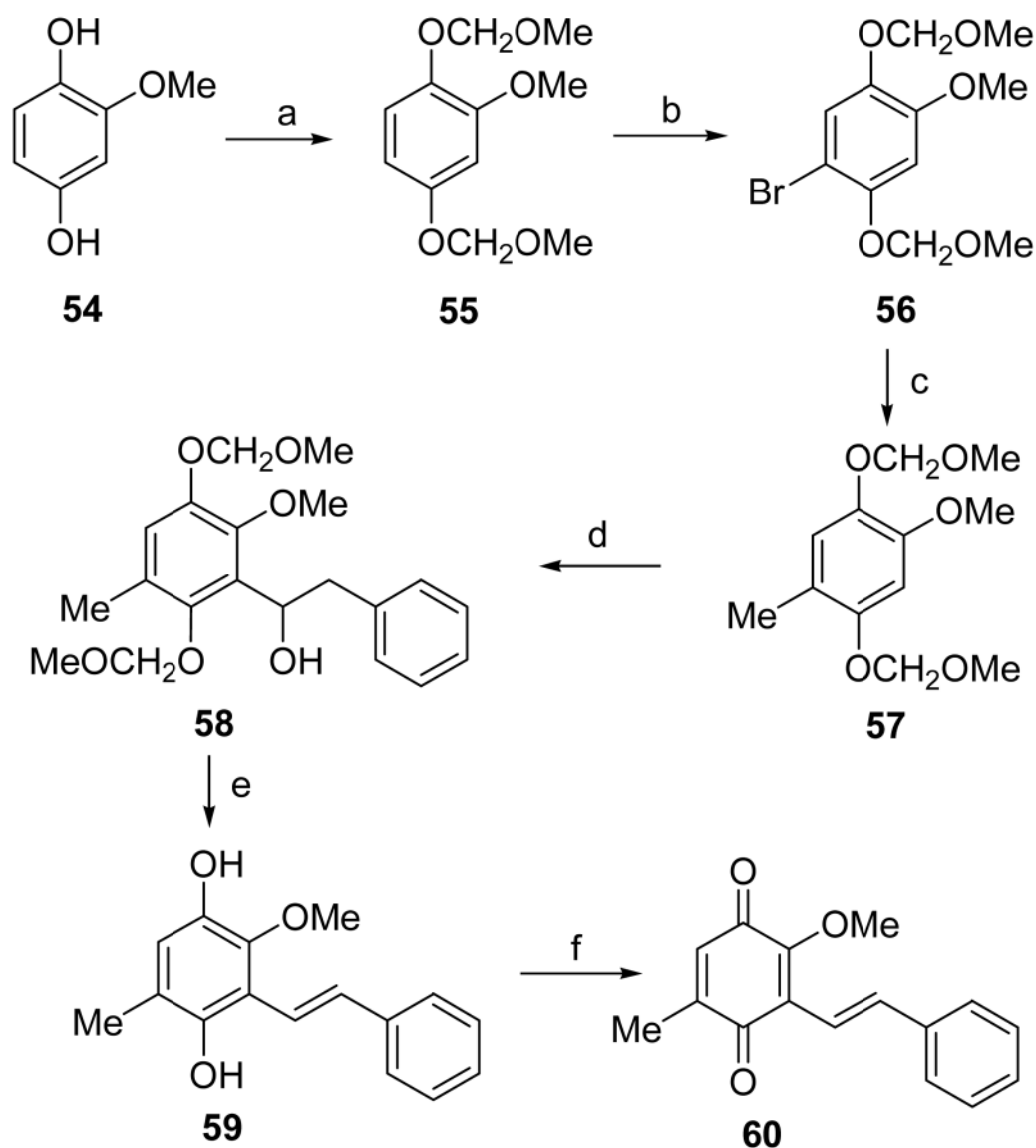


Figure 3.
 Synthetic coruscanone A analogs.

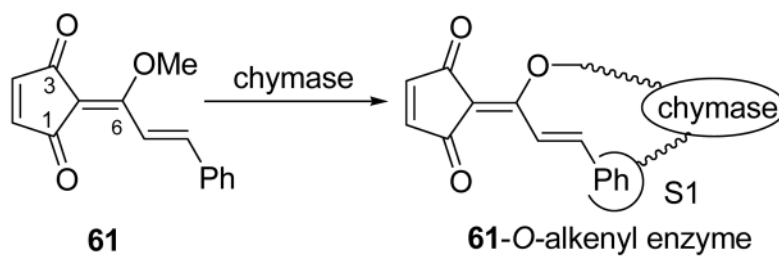
**Scheme 1.**

General Reaction Scheme for Preparation of Coruscanone A (1) Analogs

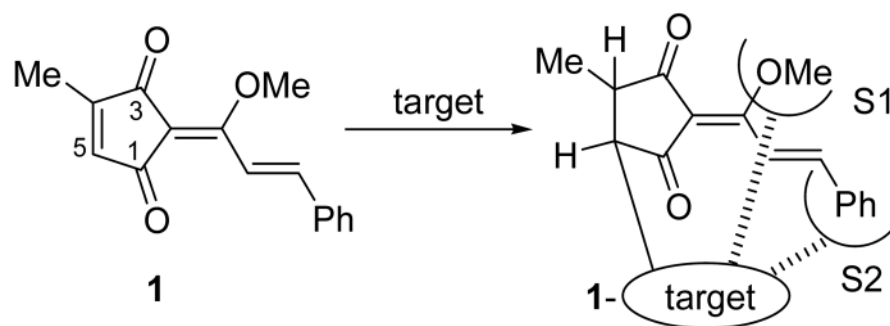
(R₁, R₂: H, CH₃, alkyl, or phenyl; R₃: aromatic, styryl, heteroaromatic, etc.; R₄: CH₃, alkyl, acyl, etc)

**Scheme 2.**Synthesis of Benzoquinone **60**^a

^a Reagents and conditions: (a) $\text{MeOCH}_2\text{Cl}/\text{NaH}$, THF, 0°C , 24 hr, 75%. (b) $\text{C}_5\text{H}_5\text{NHBr}_3/\text{MeOH}/\text{NaHCO}_3$, r.t., 25 min, 89%. (c) (i) MeLi/THF , -78°C , 1 hr; (ii) MeI , -78°C , 1 hr, 90%. (d) (i) BuLi/THF , -78°C , 1 hr, then warm to 0°C , 5 hr, r.t., 3 hr; (ii) PhCH_2CHO , 0°C , 0.5 hr; 66%. (e) $3\text{N HCl}/\text{dioxane}$, 50°C , 3 hr, 95%. (f) $\text{DDQ}/\text{CH}_2\text{Cl}_2\text{-H}_2\text{O}$, r.t., 12 hr, 65%.



Scheme 3.
Proposed Mechanism for Inhibition of Human Chymase by **61**



Scheme 4.
Possible Interaction Mechanism of **1** With the Target

Table 1
In vitro Antifungal Activity and Cytotoxicity of Coruscanone A and Its Analogs^a

| | <i>C. albicans</i> ATCC 90028 | Antifungal Activity (IC ₅₀ ^b /MIC/MFC ^d , µg/mL) | | | <i>A. fumigatus</i> ATCC 90906 | Cytotoxicity (IC ₅₀ ^b , µg/mL) | |
|------------------|-------------------------------|---|-------------------------------------|---------------------------------|--------------------------------|--|-----------|
| | | <i>C. albicans</i> #1 ^e | <i>C. albicans</i> #17 ^e | <i>C. neoformans</i> ATCC 90113 | | Vero ^f | LLC-PK-18 |
| 1 | 0.80/2.08/2.08 | 0.47/1.25/1.25 | 0.68/2.08/2.08 | 2.04/8.33/8.33 | 19/>20/>20 | 4.9 | 3.4 |
| 33 | 1.3/>20/>20 | 10/>20/>20 | 13/>20/>20 | >20/>20/>20 | >20/>20/>20 | >10 | 6.0 |
| 39 | >20/>20/>20 | 20/>20/>20 | 18/>20/>20 | >20/>20/>20 | >20/>20/>20 | >10 | 5.1 |
| 40 | 1.39/4.17/4.17 | 0.64/2.08/2.92 | 0.86/2.92/2.92 | 2.23/12/12 | 20/>20/>20 | 5.5 | 3.1 |
| 41 | 1.40/4.17/4.17 | 0.93/2.50/2.50 | 2.40/4.17/6.67 | 3.40/10/13 | 8.98/20/20 | 3.3 | 3.3 |
| 42 | 1.21/3.33/3.33 | 0.74/2.50/2.50 | 1.39/4.17/4.17 | 2.49/12/12 | 9.81/20/>20 | 3.0 | 2.8 |
| 43 | 10.6/>20/>20 | 7.88/>20/>20 | 6.04/>20/>20 | >20/>20/>20 | >20/>20/>20 | >10 | >10 |
| 45 | 3.30/10/10 | 1.86/5.00/13 | 5.35/20/20 | 6.95/>20/>20 | >20/>20/>20 | 3.7 | 3.4 |
| 46 | 3.03/10/10 | 1.56/5.00/6.67 | 4.29/10/13 | 3.41/15/17 | >20/>20/>20 | 5.0 | 3.9 |
| 47 | 5.02/20/20 | 2.49/12/17 | 4.51/13/20 | 5.02/20/20 | >20/>20/>20 | 4.8 | 4.0 |
| 49 | 8.56/>20/>20 | 3.79/>20/>20 | 5.84/>20/>20 | 20/>20/>20 | >20/>20/>20 | 5.5 | 4.9 |
| AMB ^h | 0.15/0.63/1.04 | 0.12/0.52/1.25 | 0.17/0.63/0.83 | 0.41/1.25/1.67 | 0.73/1.25/2.5 | 7.0 | 2.4 |
| FLU ^j | 0.22/0.45/>200 | 0.24/0.45/>200 | 118/>200/>200 | 5.65/37/>200 | >200/>200/>200 | >10 | >10 |
| DOX ^k | nt ⁱ | nt ⁱ | nt ⁱ | nt ⁱ | nt ⁱ | 7.5 | 0.65 |

^aMean values based on three independent experiments.

^b50% growth inhibitory concentration.

^cMinimum Inhibitory Concentration.

^dMinimum Fungicidal Concentration.

^ePatient isolates during fluconazole therapy: 1¹ #1, first isolate, azole-susceptible; #17, last isolate, azole-resistant.

^fAfrican green monkey kidney fibroblast.

^gPig kidney epithelial cells.

^hAmphotericin B.

ⁱNot tested.

^jFluconazole.

^kDoxorubicin.