Synthesis and Structure−Activity Relationship of Griseofulvin Analogues as Inhibitors of Centrosomal Clustering in Cancer Cells

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Griseofulvin was identified as an inhibitor of centrosomal clustering in a recently developed assay. Centrosomal clustering is an important cellular event that enables bipolar mitosis for cancer cell lines harboring supernumerary centrosomes. We report herein the synthesis and SAR of 34 griseofulvin analogues as inhibitors of centrosomal clustering. The variations in the griseofulvin structure cover five positions, namely the 4, 5, 2′, 3′, and 4′ positions. Modification of the 4 and 5 positions affords inactive molecules. The enol ether must be at the 2′ position, and the 4′ position needs to be sp^2 hybridized. The most active analogues were the 2′-benzyloxy and 2′-(4-methylbenzyloxy) analogues as well as the oxime of the former with a 25-fold increase of activity compared to griseofulvin. Comparison of the results obtained in this work with prior reported growth inhibition data for dermatophytic fungi showed both similarities and differences.

Introduction

Griseofulvin (**1**, Figure 1) is a classic antifungal agent[1,2] used clinically for the treatment of dermatomycoses. Since the isolation of griseofulvin in 1939,[3] it has attracted a lot of attention and more than a hundred papers describing analogue synthesis as well as structure−activity relationship (SAR[a]) studies relating to antifungal activity have been published. Since 1950, more than 400 analogues have been disclosed covering most positions and many have displayed significantly increased activity in published antifungal assays. More recently, griseofulvin has been the object of increased interest due to its activity toward a range of mammalian cancer cell lines.[4] Griseofulvin has been shown to be cytotoxic by itself but also to potentiate the activity of another anticancer drug, nocodazole.[5,6] The cytotoxicity of three 2′ analogues in Chinese hamster V79 cells has previously been reported by Oda.[7]

We have recently developed an assay[8] to identify small molecules that inhibit centrosomal clustering, a mechanism necessary for successful bipolar mitoses in human cancer cell lines with supernumerary centrosomes.[9,10] Healthy mammalian cells harbor two centrosomes during mitosis that function as mitotic spindle poles to ensure accurate chromosome segregation, whereas the majority of human malignancies contain multiple centrosomes. Supernumerary centrosomes can form multipolar spindles leading to aberrant mitoses with consecutive chromosome missegregation, eventually resulting in apoptosis. In most human cancer cell lines, this spindle multipolarity is overcome through centrosomal clustering, giving rise to two functional spindle poles and thereby allowing for successful mitosis.[11] As this phenotype is specific to cancer cells, there is a possibility that the compounds identified in our assay will target cancer cells selectively without interfering with healthy cells.

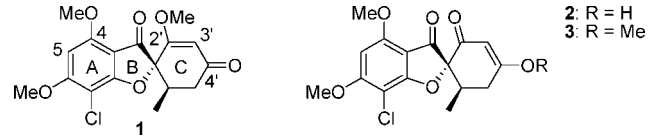

Figure 1. Structures of griseofulvin **1**, griseofulvic acid **2**, and isogriseofulvin **3**.

Microtubules are vital components of centrosomes, and compounds interacting with the dynamics of tubulin polymerization and depolymerization are a major category of anticancer agents including vinca alkaloids and taxanes.[12] Griseofulvin is known to interact with tubulin,[13] but the mode of action for this inhibition is not fully understood although a few theories have been proposed.[4,5,14]

We have tested 34 griseofulvin analogues in our assay, of which nine are novel, affording the first SAR study of this compound class with regard to anticancer activity. The analogues vary at five positions of the griseofulvin structure, namely the 4, 5, 2′, 3′, and 4′ positions. All analogues described in this paper have been prepared from commercially available griseofulvin in one to four synthetic steps.

Chemistry

The phenol **4** was synthesized by treatment of **1** with MgI_2 in diethyl ether and toluene.[15] Alkylation of **4** with either ethyl or benzyl bromide using NaH as base afforded elongated analogues **5** and **6** (Scheme 1). Nitration[16] of **1** gave **7**, which could be reduced to the amine **8** with iron powder.[17] Reductive amination with benzaldehyde yielded analogue **9** (Scheme 1).

Two synthetic routes have been utilized for the preparation of the 2′ enol ether analogues of **1**. Direct camphorsulfonic acid (CSA) mediated solvolysis of griseofulvic acid (**2**)[18] afforded

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[a] Abbreviations: CSA, camphorsulphonic acid, DBU, diaza(1,3)bicyclo-[5.4.0]undecane; NIS, *N*-iodosuccinimide; NOE, nuclear Overhauser effect; SAR, structure−activity relationship; TESOTf, triethylsilyl trifluoromethanesulfonate.

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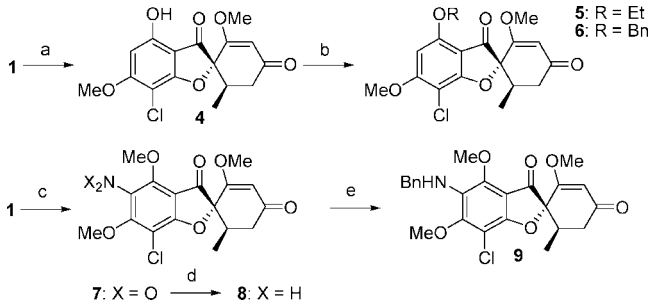
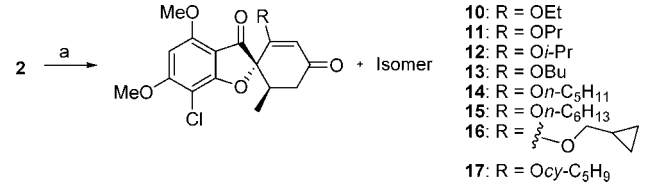
Scheme 1[a]



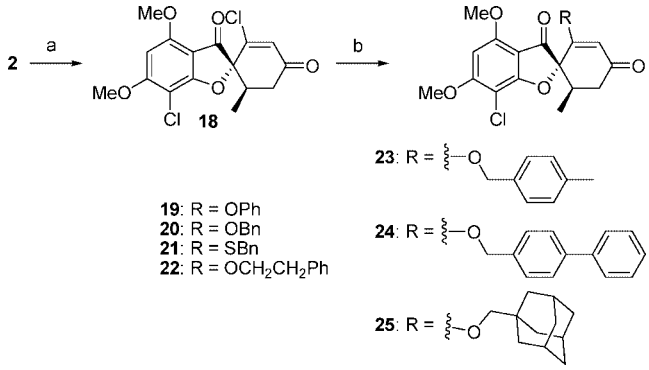
[a] (a) MgI_2, toluene, Et_2O; (b) RBr, tetrabutylammonium iodide, NaH, DMF; (c) HNO_3, Ac_2O; (d) Fe, HOAc; (e) PhCHO, $NaBH(OAc)_3$, CH_2Cl_2.

Scheme 2[a]



[a] (a) ROH, CSA, dioxane (**16** and **17**).

Scheme 3[a]



[a] (a) LiCl, $POCl_3$, dioxane; (b) ROH, DBU, THF, or dioxane.

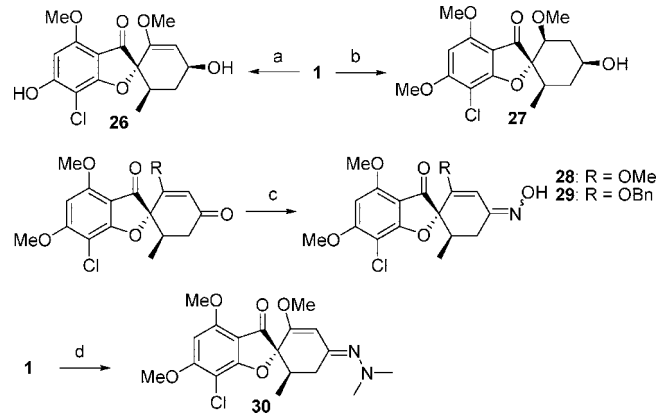
compounds **10**−**15** plus their 4′ enol ether isomers. Dioxane was used as cosolvent for alcohols with higher boiling points, leading to **16** and **17**. This method was generally applicable to the synthesis of analogues with straight chain vinyl ethers in yields of 14−22% (Scheme 2).

Bulkier alcohols like benzyl alcohol and cyclopentanol led to unsatisfactory yields of 2−4%. An attractive alternative was the known[19] route through the 2′ vinyl chloride **18**, which relies on a base mediated addition of the alcohol followed by elimination of chloride (Scheme 3). Vinyl chloride **18** is accessible by treatment of **2** with lithium chloride in phosphoryl chloride. We found using dioxane as cosolvent convenient because this allowed us to reduce the amount of phosphoryl chloride to 5 equiv, greatly facilitating the workup procedure and the scale-up to multigram amounts.

The addition of alcohols to **18** performed well in DMF, THF, and dioxane with comparable yields and THF or dioxane was used as solvent for all subsequent experiments. NaH and Cs_2CO_3 were tested as bases, but in the end, diaza(1,3)bicyclo[5.4.0]undecane (DBU) turned out to be the most efficient base and it was used for preparation of analogues **19**−**25** in yields of 2−93% (Scheme 3).

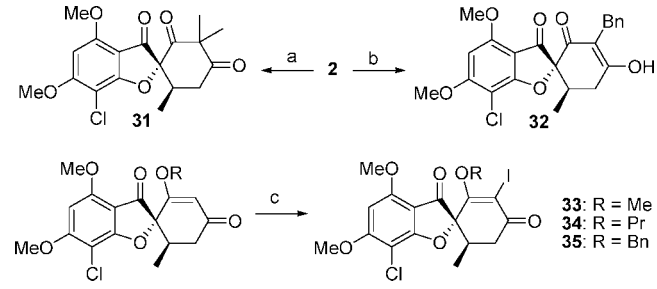
The 4′ alcohol **26** was derived from **1** using a slight modification of a known method[20] (Scheme 4). Analogue **27**

Scheme 4[a]



[a] (a) $NaBH_4$, $CeCl_3$, MeOH; (b) H_2, Pt/C, EtOAc; (c) hydroxylamine, NaOAc, EtOH, DMSO; (d) Me_2NNH_2, HOAc, toluene.

Scheme 5[a]



[a] (a) MeI, K_2CO_3, THF; (b) NaI, KOH, BnBr, H_2O; (c) NIS, TESOTf, CH_2Cl_2.

was available by reduction of **1** with hydrogen catalyzed by platinum on charcoal, an improvement of a published procedure.[21] The oximes **28** and **29** were derived from the parent ketones by treatment with hydroxylamine hydrochloride in ethanol and DMSO, a slight modification of the method of Delgado et al.[17] and isolated as inseparable 1:1 mixtures of geometrical isomers. In a similar fashion, the hydrazine **30** was synthesized by heating **1** with *N*,*N*-dimethyl hydrazine and acetic acid in toluene. The major product was the *E* analogue **30** with the geometry distinguished by NOE in proton NMR. The $H^{3'}$ proton of the two isomers showed two distinct signals in NMR. No dipolar coupling between a hydrazine methyl group and $H^{3'}$ could be observed for the pure analogue **30**, but a strong NOE was detected for the isomer.

3′,3′-Dimethyl griseofulvic acid (**31**) was derived from **2** treated with K_2CO_3 and MeI[22] (Scheme 5). Analogue **32** was synthesized from **2** by reaction with benzyl bromide, NaI, and KOH.[22] Treatment of **1** with *N*-iodosuccinimide (NIS) and triethylsilyl triflate (TESOTf) afforded **33**, and the 3′-iodo analogues **34** and **35** were obtained using the same convenient conditions. This type of compounds have previously been prepared by treating **2** with iodine monochloride and 2 equiv of KOAc,[23] followed by reacting the intermediate with an excess of an appropriate diazo alkane.[24]

Results and Discussion

Compounds **1**−**35** were evaluated for their ability to inhibit centrosomal clustering in our cellular assay[8] (Table 1). The percentages of cells with multipolar spindles were measured in triplicate at 14 different concentrations (100 μM to 12 nM), and IC_{50} values were calculated with an IC_{50} of 24 μM for griseofulvin (**1**). Analogues with IC_{50} values higher than 100 μM are regarded as inactive.

Table 1. Compounds Included in the Article and Their IC_{50} Values within a 95% Confidence Interval

Analogue	IC_{50}(μM)	Analogue	IC_{50}(μM)	Analogue	IC_{50}(μM)	Analogue	IC_{50}(μM)
1	24 ± 2	**10**	6.6 ± 0.5	**19**	1.5 ± 0.1	**28**	17 ± 1
2	>100	**11**	6.6 ± 0.5	**20**	0.9 ± 0.1	**29**	0.9 ± 0.1
3	>100	**12**	6.8 ± 0.5	**21**	1.3 ± 0.1	**30**	27 ± 2
4	>100	**13**	2.5 ± 0.2	**22**	15 ± 2	**31**	>100
5	>100	**14**	2.8 ± 0.2	**23**	0.9 ± 0.1	**32**	>100
6	>100	**15**	4.0 ± 0.2	**24**	2.9 ± 0.2	**33**	>100
7	>100	**16**	1.8 ± 0.1	**25**	3.7 ± 0.2	**34**	2.0 ± 0.1
8	>100	**17**	1.3 ± 0.1	**26**	>100	**35**	>100
9	>100	**18**	-	**27**	>100		

At ring A, the 4 and 5 positions were altered. Elongation at the 4 position resulted in no activity for either the ethyl (**5**) or benzyl (**6**) ethers. Introduction of a nitro (**7**), amine (**8**), or benzyl amine (**9**) group at the 5 position negates all activity, indicating that bulkier groups at these two positions are not tolerated. Compound **6** has previously been tested for growth inhibition against dermatophytes (*Epidermophyton floccosum*, *Trichophyton interdigitale*, *T. persicolor*, *T. mentagrophytes*, *T. rubrum*, and *Microsporum canis*), and **7** and **8** have been tested against the latter three in addition to *T. terrestre* and all three were found to be less active than **1**.[17,25]

The 2′, 3′, and 4′ positions of the C ring have been altered in this study. Solvolysis of **2** with a given alcohol affords two isomers with the enol ether positioned at either the 2′ or 4′ position. We have tested altogether 11 analogues with a 4′ enol ether as in isogriseofulvin (**3**), including the isomers of **10**−**17** and **20**, and all have been inactive (data not shown). This strongly indicates that active analogues should have the enol ether at the 2′ position. The activity of the 4′ enol ether isomers of **3**, **10**, **13**, and **19** toward a range of dermatophytes (*Trichophyton mentagrophytes*, *T. interdigitale*, *T. rubrum*, *T. persicolor*, *Microsporum canis* and *Epidermophyton floccosum*) have been published and all showed lower activity than **1**.[25]

Several analogues with modifications at the 2′ position have been prepared, and elongation of the methoxy group in **1** (analogues **10**−**15**) increases the activity, with a maximum around 4 carbon atoms.

The activity can be further increased by the introduction of bulkier groups. The cyclopropylmethoxy (**16**) and cyclopentoxy (**17**) analogues have IC_{50} values of 1.8 and 1.3 μM, comparable to the phenoxy (**19**) and benzylthio (**21**) analogues with 1.5 and 1.3 μM. The most active 2′ analogues are the benzyloxy (**20**) and 4-methylbenzyloxy (**23**) compounds, both with an IC_{50} value of 0.9 μM. While there is little difference between **19** and **20**, the phenylethoxy analogue **22** is significantly less potent with an IC_{50} value of 15 μM. The introduction of even bulkier groups such as 4-biphenylmethoxy (**24**) and adamantylmethoxy (**25**) affords IC_{50} values of 2.9 and 3.7 μM, higher than the most active compounds but significantly more potent than **1**. Because our assay is whole-cell based, transport over the cell membrane potentially also influences the observed activity. The higher lipophilicity of **24** and **25** could render this transport easier, which would contribute to the observed increase in activity.

Growth inhibition data for the dermatophytes *Trichophyton mentagrophytes*, *T. interdigitale*, *T. rubrum*, *T. persicolor*, *Microsporum canis*, and *Epidermophyton floccosum* has been published for compounds **10**, **11**, **13**, **15**, **19**, and **20**.[25] The activities for **10** and **11** were equal to or lower than **1**, while **13** was more active against two strains (*T. interdigitale*, *T. persicolor*) and similar or less toward the remaining strains. Compound **15** showed increased activity against a single strain (*T. interdigitale*), and **19** and **20** were inactive.

A different phenotype was seen for **18**. Usually, cells are attached to the dish by focal adhesions. Treatment with **18** led to a detachment of the cell monolayer from the dish, but, interestingly, the cell−cell contacts were not affected. Because of this phenotype, induction of multipolar spindle formation could not be analyzed.

The 4′ alcohol, griseofulvol (**26**), has no activity and neither has the 2′-methoxy 4′-alcohol analogue (**27**). The 4′ position of **1** was altered to an oxime (**28**), giving rise to an increase in activity with an IC_{50} of 17 μM. To check for a possible synergistic effect, the 2′ benzyloxy analogue **20** was also fitted with an oxime affording **29**, but the activity did not exceed that of **20** as the IC_{50} value remained at 0.9 μM. Introduction of a hydrazine group at the 4′ position (**30**) led to a slight decrease in activity with an IC_{50} of 27 μM. No activity was reported for **26** toward dermatophytes *Trichophyton metagrophytes* or *T. rubrum*, but for **28**, the activity toward those two strains and *T. terrestre* and *Microsporum canis* was comparable to griseofulvin.[17]

The results from the 4′ analogues indicate that this position is important for the activity, as removal of the ketone renders the compounds (**26** and **27**) inactive. Taken together with the increase in activity for **28** and **29**, it suggests that the 4′ position should be sp^2 hybridized.

Neither griseofulvic acid **2**, the 3′,3′-dimethyl analogue **31**, nor 3′-benzyl griseofulvic acid (**32**) showed activity in our assay. Introduction of iodine at the 3′ position had equivocal effects. 3′-Iodo griseofulvin (**33**) and 3′-iodo-2′-benzyloxy analogue **35** were inactive. For the 2′-propoxy-3′-iodo analogue **34**, the IC_{50} found was 2.0 μM, only a factor two higher than the most active compounds and lower than the 2′-propoxy analogue **11** itself. In antifungal screens, **33** shows lower activity toward *Trichophyton mentagrophytes*, *T. interdigitale*, *T. rubrum*, *T. persicolor*, *Microsporum canis*, and *Epidermophyton floccosum*,

where **34** has a lower activity against all save *T. interdigitale*, where the activity is the same as **1**.[25]

Conclusion

We report here for the first time a collection of griseofulvin analogues tested for inhibition of centrosomal clustering in cancer cells. Of the 34 analogues tested, 13 were inactive, two had decreased activity, and 18 displayed increased activity as compared to griseofulvin, while one analogue showed a different phenotype characterized by detachment of the cell monolayer from the dish.

All modifications of the 4 and 5 positions resulted in no activity, and thus these positions should not be altered. It appears as if the 4′ position needs to be sp^2 hybridized as reduction of the ketone results in no activity, whereas introduction of either an oxime (**28** and **29**) or a hydrazine (**30**) group leads to more active analogues. A total of 11 4′ enol ether analogues have been tested, and all were inactive. The two inactive analogues **31** and **32** also indicate that modifications must preserve the conjugated system of **1** to be beneficial.

The 2′ position has been explored the most, and elongation at this position increases the activity while introducing bulkier groups do so to an even larger extent. The optimal substituent seems to be a benzyloxy group (**20**) with the possibility of substitution on the aromatic ring. The phenylethoxy analogue **22** is 15 times less active than **20**, and a longer tether is thus not a viable option for increased activity.

The three iodo substituted analogues do not give a clear picture of the effect of substitution at the 3′ position as the activity varies. We speculate that the iodine changes the conformation of the 2′ substituent and that the benzyloxy group is shifted to a less desirable position, whereas the propyloxy group is moved to a more favorable position.

While comparing the SAR data obtained in this work for inhibition of centrosomal clustering with the growth inhibitory effect against seven dermatophytic fungi, some similarities are seen but also notable differences. Modification of the 4 and 5 positions in the A ring renders the analogues inactive with regard to both activities. For the C ring modifications, the 4′ enol ether analogues displayed reduced or no activity in both types of assay. When modifying the 2′ position, the activity toward dermatophytes is retained for the ethyl and propyl analogues. The butyl and hexyl analogues show increased activity toward some fungal strains and lower toward others. The inhibition of centrosomal clustering is increased for all straight chained 2′ analogues, compared to griseofulvin. With bulkier substituents (**19** and **20**), lower activity is seen toward all dermatophyte strains, while the inhibition of centrosomal clustering is enhanced further.

Reduction of the 4′ ketone makes the analogue inactive both toward dermatophytes and cancer. An oxime on the 4′ position retains activity toward dermatophytes but increases the centrosomal clustering inhibition. Compound **33** with substitution in the 3′ position is less active in our assay as well as against dermatophytes, while **34** has diminished antifungal activity but is 12 times more potent than **1** in our assay.

The results described herein demonstrate that the activity of griseofulvin as an inhibitor of centrosomal clustering can be enhanced by structural modifications, which renders this compound class interesting as potential anticancer agents. Furthermore, we have demonstrated that previously published data on the antifungal activity of griseofulvin analogues does not correlate directly to their activity in mammalian cells.

Experimental Section

Starting materials, reagents, and solvents were purchased from Sigma-Aldrich Chemical Co. and used without further purification. Reactions involving air or moisture sensitive reagents were carried out under N_2, and flasks were dried by flame heating under reduced pressure. DMF, CH_2Cl_2, dioxane, and toluene were dried over 3 Å molecular sieves. Evaporation of solvents was done under reduced pressure (in vacuo). Purity of all compounds was found to be greater than 95% by LC-DAD-MS (see below).

NMR spectra were recorded using either a Varian Unity Inova 500 MHz spectrometer or a Varian Mercury 300 MHz spectrometer. ^{13}C NMR spectra were recorded using either a Bruker AC 200 MHz or a Varian Mercury 300 MHz. Chemical shifts were measured in ppm and coupling constants in Hz, the field is indicated in each case. When $CDCl_3$ was used as solvent, the residual peak was used as internal reference at δ 7.27 for ^1H NMR and δ 77.00 for ^{13}C NMR spectra. When DMSO-d_6 was used, the values were δ 2.50 for ^1H NMR and δ 39.43 for ^{13}C NMR spectra.

IR spectra were recorded using a Perkin-Elmer 1600 series FTIR. All melting points are uncorrected. TLC was performed on aluminum sheets precoated with silica gel 60 F254 (Merck 1.05554.0001). Compounds were visualized by charring after dipping in a solution of 1% $KMnO_4$, 6.7% K_2CO_3, and 0.08% NaOH in water. UV visualization was done using a model UVGL-25 Mineralight lamp.

EIMS were recorded by direct inlet to a GCMS-QP5000 gas chromatograph mass spectrometer from Shimadzu. High-resolution LC-DAD-MS was performed on an Agilent 1100 system equipped with a photodiode array detector (DAD) and coupled to a LCT orthogonal time-of-flight mass spectrometer (Waters-Micromass) with a *Z*-spray electrospray ionization (ESI) source and a LockSpray probe (M + H 556.2771) and controlled by MassLynx 4.0 software. LC-MS calibration from *m/z* 100−900 was done with a PEG mixture. Standard separation involved a LUNA 2 column with an acetonitrile (50 ppm TFA) in water gradient starting from 15% to 100% over 25 min with a flow rate of 0.3 mL/min. Microanalyses were obtained from H. Kolbe, Mikroanalytisches Laboratorium, Mülheim/Ruhr, Germany.

For the determination of IC_{50} values, we tested the compounds in our assay[8] for centrosomal clustering based on SSC114 cells, an oral squamous cell carcinoma cell line characteristic for this phenotype. Despite the presence of supernumerary centrosomes in 64.5% of SCC114 cells, only 3.6% of the cells in mitosis harbor multipolar spindles. The rest of the cell population cluster their centrosomes and undergo bipolar mitoses, making this cell line an ideal model system. SCC114 cells were grown in 96-well plates to near confluence and treated for 24 h with different griseofulvin analogues. The compounds were tested in triplicate at 14 different concentrations (100, 50, 25, 12.5, 6.3, 3.1, 2, 1, 0.4, 0.2, 0.1, 0.05, 0.02, and 0.01 μM). Cells were then fixed and examined by fluorescence microscopy. Then 100 mitotic cells per well were analyzed, with the percentage of mitotic cells with multipolar spindles being the read-out. The relationship between readout and the dose was described by a logistic model (eq 1):

$$p = (1 + \exp(-\alpha - \beta x))^{-1} \quad (1)$$

where p is the percentage of mitotic cells with multipolar spindles and x is the dose scaled according to $x = \ln(\text{dose} + 1)$. The dose axis was scaled to spread out the low concentration data. The IC_{50} values and the corresponding 95% confidence intervals were determined from the fitted dose−response curves. The calculations were performed in Matlab 7.0.4.365 (The Mathworks) using the *glmfit* and *glmval* functions.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-5-benzylamino-benzofuran-3-on)-2-spiro-1′-(2′-methoxy-6′-methyl-cyclohex-2′-ene-4′-one) (9). To a solution of 5-amino griseofulvin **8** (120 mg, 0.327 mmol) and

benzaldehyde (0.07 mL, 0.654 mmol) in CH_2Cl_2 (4 mL) was added a solution of sodium triacetoxyborohydride (104 mg, 0.490 mmol) in CH_2Cl_2 (4 mL) at 0 °C under nitrogen atmosphere. Acetic acid (90% aq, 0.03 mL, 0.654 mmol) was added and the mixture was stirred at 20 °C for 48 h. The mixture was washed with sat. aq $NaHCO_3$ (15 mL) and water (15 mL). The combined aqueous phases were extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (toluene:CH_2Cl_2:EtOAc 30:30:1) to afford the product **9**. Yield: 26 mg (17%) (yellow needles); R_f (EtOAc:heptane 5:2): 0.54; mp: 79−80 °C. IR (KBr, cm^{-1}) 1709, 1664. ^1H NMR ($CDCl_3$) δ 7.31−7.18 (5H, m), 5.52 (1H, s), 4.30 (2H, s), 3.90 (3H, s), 3.86 (3H, s), 3.60 (3H, s), 2.93 (1H, dd, J = 16.1, 13.3 Hz), 2.85−2.75 (1H, m), 2.40 (1H, dd, J = 16.1, 4.1 Hz), 0.89 (3H, d, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 196.8, 194.1, 171.1, 163.9, 156.8, 146.6, 140.2, 128.8 (2C), 128.2 (2C), 127.6, 110.2, 106.2, 105.2, 105.1, 90.1, 62.6, 60.5, 57.0, 51.5, 40.2, 36.8, 14.5. HRMS (ESI^+) calcd for $[C_{24}H_{25}ClNO_6]^+$ 458.1370, found 458.1371.

General Procedure for the Synthesis of Enol Ethers by Solvolysis (16 and 17). CSA (0.1 mmol, 0.1 equiv) was added to a solution of griseofulvic acid (0.6 mmol, 1 equiv), the appropriate alcohol (3 mmol, 5 equiv), and 1,4-dioxane (3 mL). The mixture was stirred at 100 °C for 6 h and then cooled to 20 °C. EtOAc (20 mL) was added to the solution, and the mixture was washed with sat. NaH_2PO_4 (20 mL) and then water (20 mL). The combined aqueous phases were extracted with EtOAc (3 × 20 mL), dried ($MgSO_4$), and then concentrated. The residue was purified by column chromatography (toluene:CH_2Cl_2:EtOAc 7:7:1) to afford the desired product and the isomer. When possible the product was recrystallized from EtOAc/Heptane.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-on)-2-spiro-1′-(2′-cyclopropylmethoxy-6′-methyl-cyclohex-2′-en-4′-one) (16). Yield: 86 mg (4%) (white crystals); R_f (EtOAc:heptane 5:1): 0.51; mp: 190−191 °C. IR (KBr, cm^{-1}) 1704, 1659, 1608. ^1H NMR(500 MHz, $CDCl_3$) δ 6.13 (1H, s), 5.47 (1H, s), 4.03 (3H, s), 3.98 (3H, s), 3.65 (2H, d, J = 6.5 Hz), 3.03 (1H, dd, J = 16.7, 13.5 Hz), 2.83 (1H, ddq, J = 13.5, 4.7, 6.6 Hz), 2.41 (1H, dd, J = 16.7, 4.7 Hz), 1.05−0.98 (1H, m), 0.96 (3H, d, J = 6.6 Hz), 0.50−0.43 (2H, m), 0.22−0.13 (2H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.0, 192.5, 169.9, 169.6, 164.4, 157.6, 105.0 (2C), 97.1, 90.8, 89.3, 73.2, 56.9, 56.3, 39.9, 36.2, 14.2, 9.0, 2.7 (2C). HRMS (ESI^+) calcd for $[C_{20}H_{22}ClO_6]^+$ 393.1105, found 393.1108. Anal. ($C_{20}H_{21}ClO_6$): C, H.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-on)-2-spiro-1′-(2′-cyclopentoxy-6′-methyl-cyclohex-2′-en-4′-one) (17). Yield: 50 mg (4%) (yellow oil); R_f (EtOAc:heptane 5:1): 0.50. IR (KBr, cm^{-1}) 1705, 1652, 1615. ^1H NMR (500 MHz, $CDCl_3$) δ 6.11 (1H, s), 5.49 (1H, s), 4.56−4.51 (1H, m), 4.03 (3H, s), 3.97 (3H, s), 3.03 (1H, dd, J = 16.7, 13.5 Hz), 2.82 (1H, ddq, J = 13.5, 4.8, 6.7 Hz), 2.40 (1H, dd, J = 16.7, 4.8 Hz), 1.79−1.72 (2H, m), 1.72−1.64 (2H, m), 1.58−1.44 (4H, m), 0.95 (3H, d, J = 6.7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.1, 192.6, 169.6, 169.0, 164.3, 157.5, 105.8, 105.1, 97.0, 90.9, 89.2, 81.6, 56.8, 56.3, 39.8, 36.1, 32.1 (2C), 23.7 (2C) 14.2. HRMS (ESI^+) calcd for $[C_{21}H_{24}ClO_6]^+$ 407.1261, found 407.1262.

General Procedure for the Synthesis of Enol Ethers by Addition−Elimination (19−25). To a solution of **18** (0.65 mmol, 1 equiv) in 1,4-dioxane (3 mL, 0.2 M) was added the desired alcohol (1.30 mmol, 2 equiv) and DBU (1.63 mmol, 2.5 equiv). The mixture was heated to 100 °C and stirred for 12 h. The mixture was then cooled to 20 °C, and excess reagent was quenched with sat. aq NH_4Cl (30 mL). The aqueous phase was extracted with EtOAc (3 × 30 mL) and the combined organic phases were dried ($MgSO_4$) and then concentrated. The residue was purified by column chromatography (heptane:EtOAc 3:2) affording the product. When possible the product was recrystallized from EtOAc/heptane.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-on)-2-spiro-1′-(6′-methyl-2′-(2-phenylethoxy)-cyclohex-2′-en-4′-one) (22). Yield: 199 mg (86%); R_f (toluene:CH_2Cl_2:EtOAc 1:1:1): 0.50. ^1H NMR (300 MHz, $CDCl_3$) δ 7.20−7.12 (3H, m), 7.03−6.95 (2H, m), 6.13 (1H, s), 5.49 (1H, s), 4.06 (3H, s), 3.97 (3H, s), 3.95−3.84 (2H, m), 3.02 (1H, dd, J = 16.5, 13.5 Hz), 2.89−2.75 (3H, m), 2.40 (1H, dd, J = 16.5, 4.6 Hz), 0.94 (3H, d, J = 6.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) 197.0, 192.5, 169.8, 169.5, 164.5, 157.6, 137.2, 128.8 (2C), 128.3 (2C), 126.4, 105.1, 105.0, 97.1, 90.7, 89.4, 69.9, 57.0, 56.3, 39.9, 36.1, 34.7 14.1. HRMS (ESI^+) calcd for $[C_{24}H_{24}ClO_6]$ 443.1261, found 443.1264.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-one)-2-spiro-1′-(6′-methyl-2′-(4-methylbenzyloxy)-cyclohex-2′-ene-4′-one) (23). Yield: 201 mg (54%) (white crystals); R_f (EtOAc:heptane 5:1): 0.45; mp: 176−178 °C. IR (KBr, cm^{-1}) 1709, 1664. ^1H NMR (300 MHz, $CDCl_3$) δ 7.11−7.04 (4H, m), 6.09 (1H, s), 5.58 (1H, s), 4.87 (1H, d, J = 12.2 Hz), 4.76 (1H, d, J = 12.2 Hz), 4.01 (3H, s), 3.95 (3H, s), 3.04 (1H, dd, J = 16.5, 13.4 Hz), 2.85 (1H, ddq, J = 13.2, 4.5, 6.6 Hz), 2.41 (1H, dd, J = 16.4, 4.4 Hz), 2.30 (3H, s), 0.97 (3H, d, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1, 192.7, 169.9, 169.8, 164.7, 157.9, 138.2, 131.8, 129.5 (2C), 127.0 (2C), 106.2, 105.8, 97.1, 91.0, 89.7, 71.0, 57.2, 56.6, 40.2, 36.7, 21.4, 14.5. HRMS (ESI^+) calcd for $[C_{24}H_{24}ClO_6]^+$ 443.1261, found 443.1273.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-one)-2-spiro-1′-(2′-(4-biphenylmethoxy)-6′-methyl-cyclohex-2′-ene-4′-one) (24). Yield: 54 mg (19%); R_f (toluene:CH_2Cl_2:heptane 2:2:1): 0.32. IR (KBr, cm^{-1}) 1704, 1662. ^1H NMR (300 MHz, $CDCl_3$) δ 7.58−7.50 (4H, m), 7.46−7.40 (2H, m), 7.37−7.31 (1H, m), 7.28−7.23 (2H, m), 6.10 (1H, s), 5.63 (1H, s), 4.97 (1H, d, J = 12.4 Hz), 4.85 (1H, d, J = 12.4 Hz), 4.01 (3H, s), 3.96 (3H, s), 3.07 (1H, dd, J = 16.5, 13.4 Hz), 2.88 (1H, ddq, J = 13.4, 4.6, 6.6 Hz), 2.45 (1H, dd, J = 16.5, 4.6 Hz), 1.00 (3H, d, J = 6.6 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.0, 192.4, 169.5 (2C), 164.5, 157.7, 141.0, 140.4, 133.6, 128.7 (4C), 127.2, 127.0 (4C), 105.9, 105.3, 97.2, 90.7, 89.4, 70.4, 56.9, 56.3, 40.0, 36.3, 14.2. HRMS (ESI^+) calcd for $[C_{29}H_{26}ClO_6]^+$ 505.1418, found 505.1421.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-one)-2-spiro-1′-(2′-(1-adamantylmethoxy)-6′-methyl-cyclohex-2′-ene-4′-one) (25). Yield: 5 mg (2%); R_f (toluene:CH_2Cl_2:EtOAc 3:3:1): 0.16. ^1H NMR (300 MHz, $CDCl_3$) δ 6.05 (1H, s), 5.37 (1H, s), 3.96 (3H, s), 3.91 (3H, s), 3.26 (1H, d, J = 9.2 Hz), 3.13 (1H, d, J = 9.2 Hz), 3.03 (1H, dd, J = 16.6, 13.6 Hz), 2.87−2.71 (1H, m), 2.36 (1H, dd, J = 16.6, 4.8 Hz), 1.83−175 (3H, m), 1.63−1.50 (4H, m), 1.45−1.28 (6H, m), 1.20−1.13 (2H, m), 0.95 (3H, d, J = 6.7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.1, 192.8, 170.3 (2C), 164.5, 157.7, 104.2 (2C), 97.4, 91.3, 89.3, 78.6, 57.0, 56.4, 40.3, 38.8 (3C), 36.8 (3C), 35.7, 33.5, 27.9 (3C), 14.3. HRMS (ESI^+) calcd for $[C_{27}H_{32}ClO_6]^+$ 487.1887, found 487.1888.

(2*S*,6′*R*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-one)-2-spiro-1′-(2′-benzyloxy-6′-methylcyclohex-2′-ene-4′-one-4′-oxime) (29). To a solution of **20** (0.20 mmol, 1.0 equiv) in EtOH (5 mL, 0.03M) and DMSO (2.5 mL, 0.03M) was added hydroxylamine hydrochloride (0.70 mmol, 3.5 equiv) and sodium acetate (0.86 mmol, 4.3 equiv). The mixture was stirred at 75 °C for 24 h, allowed to reach 20 °C, and diluted with CH_2Cl_2 (20 mL). The mixture was washed with distilled water (2 × 15 mL) and then brine (15 mL). The organic phase was dried ($MgSO_4$) and concentrated. The crude mixture was purified by column chromatography (toluene:CH_2Cl_2:EtOAc 2:2:1) to afford the desired product.

Yield: 167 mg (82%) (white needles); R_f (EtOAc:heptane 5:1): 0.39 and 0.36; mp: 139−141 °C. IR (KBr, cm^{-1}) 1706, 1614. ^1H NMR (300 MHz, $CDCl_3$) δ 8.63 (1H, s), 7.30−7.13 (5H, m), 6.36 (0.5H, s), 6.07 (1H, s), 6.68 (0.5H, s), 4.99−4.71 (2H, m), 3.99 (3H, s), 3.93 (3H, s), 3.14 (0.5H, dd, J = 16.6, 4.7 Hz), 3.04 (0.5H, dd, J = 15.0, 13.2 Hz), 2.73 (0.5H, dd, J = 16.6, 13.0 Hz), 2.69−2.51 (1H, m), 2.42 (0.5H, dd, J = 15.0, 4.2 Hz), 0.98 (1.5H, d, J = 6.6 Hz), 0.97 (1.5H, d, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.0 (0.5C), 193.8 (0.5C), 169.5, 164.2, 159.8 (0.5C), 157.4, 157.2 (0.5C), 155.1 (0.5C), 151.8 (0.5C), 135.7 (0.5C), 135.5 (0.5C), 128.3 (2C), 127.7 (0.5C), 127.6 (0.5C), 126.6, 126.5, 105.7, 100.3 (0.5C), 97.0, 93.7 (0.5C), 91.5 (0.5C), 91.4 (0.5C), 89.1, 70.0 (0.5C), 69.7 (0.5C), 56.9, 56.2, 36.4 (0.5C), 35.2 (0.5C), 30.9 (0.5C), 25.5 (0.5C), 14.4 (0.5C), 14.3 (0.5C). HRMS (ESI^+) calcd for $[C_{23}H_{23}ClNO_6]^+$ 444.1214, found 444.1204.

(2*S*,6′*R*,*E*)-(7-Chloro-4,6-dimethoxy-benzofuran-3-one)-2-spiro-1′-(2′-methoxy-6′-methyl-cyclohex-2′-ene-4′-one-4′-dimethylhydrazine) (30). To a solution of griseofulvin (1.0 g, 2.83 mmol) in toluene (28.3 mL) was added *N*,*N*-dimethylhydrazine (0.9 mL, 11.32 mmol) and 90% aq acetic acid (0.5 mL, 8.66 mmol). The mixture was heated to 50 °C for 24 h and cooled to 20 °C. The mixture was diluted with diethyl ether (100 mL), washed with sat. aq $NaHCO_3$ (50 mL) and brine (50 mL). The combined aqueous phases were extracted with diethyl ether (50 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (toluene:CH_2Cl_2:EtOAc 1:1:2) to yield compound **30**. Yield: 208 mg (19%) (orange needles); R_f (EtOAc:heptane 5:1): 0.22; mp: 118−120 °C. IR (KBr, cm^{-1}) 1708, 1613. ^1H NMR (300 MHz, $CDCl_3$) δ 6.11 (1H, s), 5.67 (1H, s), 4.02 (3H, s), 3.97 (3H, s), 3.56 (3H, s), 3.13 (1H, dd, J = 16.2, 4.5 Hz), 2.77 (1H, dd, J = 16.2, 12.9 Hz), 2.60 (1H, ddq, J = 12.8, 4.7, 6.6 Hz), 2.53 (6H, s), 0.94 (3H, d, J = 6.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.4, 169.7, 164.5, 162.7, 160.0, 157.7, 105.8, 103.3, 97.3, 91.9, 89.4, 57.2, 56.5, 56.2, 47.5 (2C), 36.1, 29.4, 14.6. HRMS (ESI^+) calcd for $[C_{19}H_{24}ClN_2O_5]^+$ 395.1374, found 395.1374.

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Supporting Information Available: Full experimental data for compounds **2**, **4**−**8**, **10**−**15**, **18**−**21**, **26**−**28**,and **31**−**35** as well as copies of NMR spectra for compounds **2** and **4**−**35**. This material is available free of charge via the Internet at http://pubs.acs.org.

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