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In Vitro Antifungal Evaluation and Structure–Activity Relationships of a New Series of Chalcone Derivatives and Synthetic Analogues, with Inhibitory Properties Against Polymers of the Fungal Cell Wall

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Abstract—Here we report the synthesis, in vitro antifungal evaluation and SAR study of 41 chalcones and analogues. In addition, all active structures were tested for their capacity of inhibiting *Saccharomyces cerevisiae* β(1,3)-glucan synthase and chitin synthase, enzymes that catalyze the synthesis of the major polymers of the fungal cell wall.

Introduction

Modern medicine has greatly increased the number of immunocompromised patients. Chemotherapy, parenteral nutrition, transplantation surgery and the use of broad-spectrum antimicrobial agents, added to the occurrence of the acquired immunodeficiency syndrome (AIDS), render true 'living Petri dishes' individuals, who are highly susceptible to opportunistic infections.[1] Systemic and dermic fungal infections are the cause of great morbi-mortality in this type of patients, being dermatomycoses a serious problem for children of the Third World Nations as a consequence of poor sanitation care, too.[2,3]

Since many of the currently available drugs have undesirable side effects, are ineffective against new or re-emerging fungi, or develop a rapid resistance,[4] there is an urgent need of a next generation of new antifungal agents, which overcome the above disadvantages. The search is oriented to find new antifungal drugs, which may selectively attack the fungi without inhibiting any biochemical system of the host. Since fungal but not mammalian cells possess a cell wall, its inhibition represents an ideal mode of action for antifungal drugs.[5–8]

During the course of our screening program aimed to detect selective antifungal compounds,[9–13] we report here the antifungal activities, structure–activity relationships (SARs) and capacity of inhibiting the in vitro synthesis of the major fungal cell wall polymers, for a series of chalcone derivatives, most of them not described to date in the literature.

Previous studies on the antifungal properties for these compounds, have shown[14,15] that some phenolic synthetic chalcones, possess moderate antifungal properties. Conversely, two phenolic chalcones isolated from *Myrica serrata*[16] did not display any activity against

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Candida albicans. In addition, a short series of chalcone derivatives showed marginal antifungal activities not improved with structural modifications.[17]

Additional study of the antifungal properties of chalcone derivatives using a large number of compounds seems to be necessary, and will provide information on the SARs of this kind of compounds. Forty-one samples (**1**–**41**, Tables 1–4) were tested against a panel of human opportunistic pathogenic fungi, using the agar dilution method. Based on the results obtained in this screen, a SAR study including conformational analysis, was performed with the aim of explaining the influence of chalcone structural changes on the antifungal activity.

In addition, the mechanism of action of most active chalcones was assayed for their capacity of inhibiting in vitro β(1,3)-glucan and chitin synthases, enzymes that catalyze the biosynthesis of β(1,3)-glucan and chitin polymers of the fungal cell wall,[18,19] respectively.

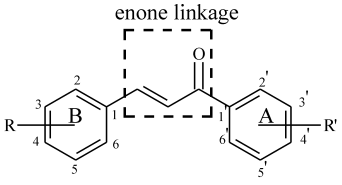
Results and Discussion

Chemistry

We prepared a number of known and novel chalcone derivatives by a Claisen–Schmidt condensation[20] of the appropriate aldehyde and acetophenone derivatives (Schemes 1–3). After purification, chalcones were obtained in 60–95%. Ketones **23**–**33** (Table 3) were prepared in turn, by means of the 1,4 addition of an anion enolate to the conjugated system as it is shown in Scheme 3. This method allows us to prepare a wide variety of substituted compounds on one or both rings. Some of them had been reported in the literature.[21–28] Their structures were established using IR, NMR spectroscopy and mass spectrometry. NMR analysis of all the chalcones obtained, showed that the *trans* isomer was formed.[29]

All the starting materials are commercially available except 2-chloro-3-formyl-6,7-dimethoxyquinoline. It was

Table 1. MIC values of chalcone derivatives acting against dermatophytes



Compd	R	R′	Mp (°C)	MIC (μg/mL)				
				M. c.[i]	*M. g.*[j]	*T. m.*[k]	*T. r.*[l]	*E. f.*[m]
1	H	H	55–57[a]	1.5	1.5	1.5	3	0.75
2	4-NO_2	H	165–166[b]	0.75	1.5	1.5	0.75	0.25
3	2-NO_2	H	111–112	12.5	6.25	6.25	12.5	3
4	2,4-Cl_2	H	210	12.5	>50	50	12.5	12.5
5	3-CH_3O	H	54–56	3	3	3	6.25	1.5
6	4-CH_3O	H	115–116[c]	3	12.5	3	12.5	1.5
7	4-CH_3	H	84–86	3	1.5	1.5	6.25	0.75
8	3,4-$(CH_3O)_2$	H	85–87[d]	12.5	12.5	3	3	3
9	2,4-$(CH_3O)_2$	H	71–72	3	6.25	3	6.25	3
10	2,3-$(CH_3O)_2$	H	Oil[e]	6.25	6.25	6.25	6.25	6.25
11	2,4-Cl_2	2′,4′-Cl_2	215–216	>50	>50	>50	>50	>50
12	4-CH_3	4′-CH_3	95–96	>50	12.5	>50	>50	12.5
13	3,4-$(CH_3O)_2$	3′,4′-$(CH_3O)_2$	102–104	25	>50	50	50	25
14	3,4,5-$(CH_3O)_3$	3′,4′,5′-$(CH_3O)_3$	130–131[f]	>50	>50	>50	>50	>50
15	4-CH_3O	4′-CH_3	93–94[g]	3	50	25	50	0.5
16	2,3-$(CH_3O)_2$	4′-Br	110–112	25	25	25	3	3
17	4-N(CH3)$_2$	3′,4′-$(CH_3O)_2$	83–85[h]	50	50	25	50	12.5
18	3-CH_3O	4′-Br	84–85	25	1.5	3	3	0.5
Amp[n]				>50	6.25	6.25	25	0.3
Ket[o]				15	6.25	12.5	15	25

[a]Lit. 56–57.[3]
[b]Lit. 164–165.[4]
[c]Lit. 114–116.[4]
[d]Lit. 88.[5]
[e].
[f]Lit. oil.[6]
[g].[7]
[h]Lit. 83–84.[8]
[i]*Microsporum canis* C 112.
[j]*Microsporum gypseum* C 115.
[k]*Trichophyton mentagrophytes* ATCC 9972.
[l]*Trichophyton rubrum* C 113.
[m]*Epidermophyton floccosum* C 114.
[n]Amp, Amphotericin B.
[o]Ket, ketoconazole.

obtained by the acetylation of 3,4-dimethoxyaniline in pyridine with acetyl chloride at room temperature, to give 3,4-dimethoxyanilide, which in turn was made to react with the Vilsmeier reagent (Scheme 2).[21]

Antifungal assays

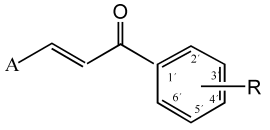
To carry out the antifungal evaluation, concentrations of chalcones up to 50 μg/mL were incorporated to growth media according with reported procedures.[10] The agar dilution method showed than none of the compounds tested was active against the yeasts *C. albicans*, *Saccharomyces cerevisiae* or *Cryptococcus neoformans* nor against the filamentous fungi *Aspergillus niger*, *A. fumigatus* or *A. flavus* (results not shown). In contrast, strong antifungal effects were obtained for the compounds of the series against dermatophytes. These results are shown in Tables 1 and 2. In particular compounds **1**–**3**, **5**–**10**, and **18**, showed strong antifungal activities comparable to those of Amphotericin B and Ketoconazole.

As main observations, it can be stated that all the tested dermatophytes were inhibited at 50 μg/mL and some of them at lower concentrations. The most sensitive species was *Epidermophyton floccosum*. Since dermatophytes are a group of fungi which characteristically infect the keratinized areas of the body and dermatomycoses are very difficult to eradicate, it is very interesting to note that chalcone derivatives showed activity against dermatophytes and not against another type of fungi.

To evaluate the SARs, the effects of structural changes in three regions of the molecule of the non-substituted chalcone **1**, were considered: variation in the A and B rings, and in the enone linkage (see general structure in Table 1).

Regarding the influence of the substituents on the B ring, an interesting structure–activity correlation can be observed: electron-donating groups tended to weaken the antifungal activity (compare MICs of compounds **5**, **6**, **8**–**10** with MICs of chalcone **1**). Electron-withdrawing groups in the para-position increased the potency: a nitro substituent (compound **2**) enhance 2–4 times the activities in three of the fungi tested (compare results for compounds **2** and **1** in *Microsporum canis*, *Trichophyton rubrum* and *E. floccosum*). Nevertheless, when the NO_2 group is in position 2 (chalcone **3**), a decrease of activity is observed with respect to compounds **1** and **2** (4–8 and 4–16 times, respectively), suggesting that the presence of a group in the *o*-position of the B ring could introduce important steric effects. The low activity showed by compound **4** is in agreement with this assumption. However, when an OCH_3 is added to C-2 of compound **6** (→**9**), the activity did not change significatively (compare MICs of compounds **6** and **9**). Molecular modelling studies performed with these compounds, are described in the section below and provide some explanations for these last findings.

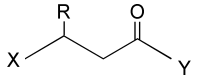
Table 2. MICs values of analogues of chalcone derivatives acting against dermatophytes



Compd	A	R	MIC (μg/mL)				
			M.c.[a]	*M.g.*[b]	*T.m.*[c]	*T.r.*[d]	*E.f.*[e]
19		2′,4′-Cl	12.5	12.5	12.5	6.25	12.5
20		H	> 50	> 50	> 50	> 50	12.5
21		2′,4′-OCH_3	> 50	> 50	> 50	> 50	> 50
22		3′-OCH_3	> 50	> 50	> 50	> 50	> 50

[a]*Microsporum canis* C 112.
[b]*M. gypseum* C 115.
[c]*Trichophyton mentagrophytes* ATCC 9972.
[d]*T. rubrum* C 113.
[e]*Epidermophyton floccosum* C 114.

Table 3. Ketones tested in vitro for antifungal properties



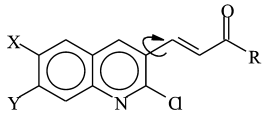
Compd[a]	X	Y	R	mp (°C)
23	C_6H_5	C_6H_5	$CH(CO_2CH_3)_2$	95–96
24	2,3-$(OCH_3)_2$ C_6H_3	C_6H_5	$CH(CO_2CH_3)_2$	135–136
25	Naphthyl	C_6H_5	$CH(CO_2CH_3)_2$	129–130
26	2,3-$(OCH_3)_2$ C_6H_3	C_6H_5	$–CH(CN)_2$	75–77
27	2,4-$(Cl)_2C_6H_3$	C_6H_5	$–CH(CN)_2$	133–135
28	2-Naphthyl	C_6H_5	$–CH(CN)_2$	154–156
29	2-Naphthyl	3′,4′-$(OCH_3)_2C_6H_3$	$–CH(CN)_2$	130–131
30	2-Naphthyl	2′,4′-$(OCH_3)_2C_6H_3$	$–CH(CN)_2$	125–127
31	2-Chloro-3-quinolinyl	C_6H_5	$–CH(CN)_2$	115–116
32	2-Chloro-3-quinolinyl	3′,4′-$(OCH_3)_2C_6H_3$	$–CH(CN)_2$	128–130
33	3,4,5-$(OCH_3)_3C_6H_2$	3′,4′,5′-$(OCH_3)_3C_6H_2$	$–CH(CN)_2$	110–112

[a]Compounds **23**–**33** inactive in all of the fungi tested up to 50 μg/mL.

We investigated the role played by ring B in the antifungal activity of chalcone **1** by replacing benzene with alternative ring systems. We found that the replacement of benzene in compound **1** by naphtalene (→**20**) and by substituted quinolines (→**34** and **37**) resulted in an almost complete loss of activity.

Several compounds were prepared in which both the B and A rings of chalcone **1** were altered. From the variation of the A ring in compounds **5** (→**18**), **6** (→**15**), **7** (→**12**), **8** (→**13**), and **10** (→**16**), we can state that: electron-donating groups on ring B such as $-OCH_3$ or-CH_3 diminished the activity (compare MICs of compounds **7** and **12**, **8** and **13**); the same tendence is also observed for compound **15** (compare its MICs with those of chalcone **6** for *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *T. rubrum*). In addition, structure **17** possessed very low activity and compound **14** was devoid of antifungal properties. The introduction of an electron-withdrawing group on ring A (compounds **5**→**18** and **10**→**16**) does not introduce clear changes in the antifungal effects, although they still possess significant antifungal activities.

Table 4. Analogues of chalcone derivatives tested for antifungal properties



Compd[a]	X	Y	R	mp (°C)
34	H	H	C_6H_5	200–201
35	H	H	2′,4′-$(OCH_3)_2C_6H_3$	163–165[b]
36	H	H	2′,4′-Dimethyl-furyl	181–182[c]
37	OCH_3	OCH_3	C_6H_5	129–131[d]
38	OCH_3	OCH_3	4′-BrC_6H_4	
39	OCH_3	OCH_3	4′-′ClC_6H_4	
40	OCH_3	OCH_3	3′,4′-$(OCH_3)_2C_6H_3$	214–215
41	OCH_3	OCH_3	2′,5′-$(OCH_3)_2C_6H_3$	> 150 desc.

[a]Compounds **34**–**41** inactive in all of the fungi tested at 50 μg/mL.
[b]Lit. 163–165.[9]
[c]Lit. 181–182.[9]
[d]Lit. 129–131.[9]

The lack of activity of compound **11**, possessing Cl in the ortho positions in both A and B rings (compare with compound **4**), suggested that the steric factors play a crucial role in the antifungal properties of substituted chalcones.

It is interesting to note that compound **14** do not inhibit the fungal cell growth up to 50 μg/mL, while other trimethoxy A substituted ring chalcones were reported as inhibitors of the human cell growth in vitro.[30]

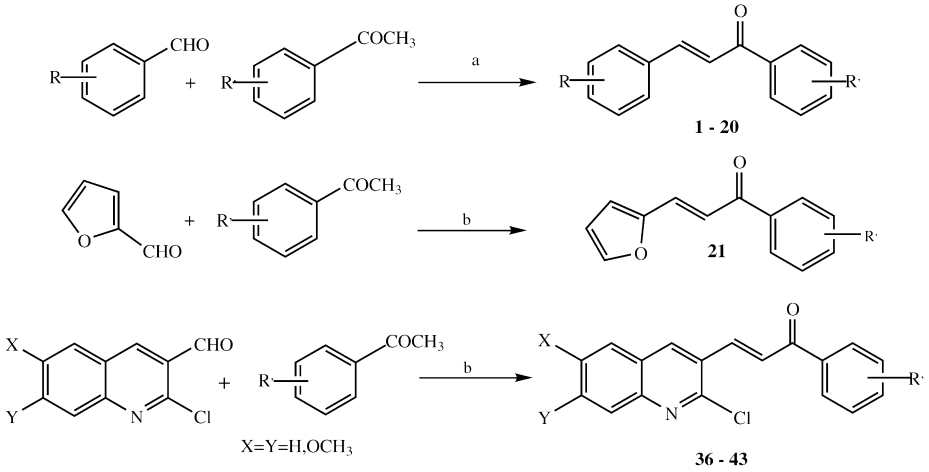
New analogues with furane, naphtalene and quinoline as ring B (compounds **19**, **21**, **22**, **35**, **36**, and **38**–**41**) were tested in order to add more information to the structural requirements for activities. All of them were inactive except compound **19**. This structure possessing a furane ring, showed a moderate but significant antifungal activity against dermatophytes with MICs between 6.25 and 12.5 μg/mL.

To gain insight into the importance of the enone linkage for the antifungal activity, we broke the double bond α, β to the carbonyl group, obtaining compounds **23**–**33** (Table 3). Since these ketones are devoid of antifungal properties, the enone appears to be required for activity. However, the lack of activity obtained for compounds **11**, **14**, **20**–**22**, **34**–**41**, clearly indicates that the presence of a enone linkage would be a structural requirement necessary but not by itself sufficient for the antifungal activity.

Conformational and electronic study

In order to understand the experimental results obtained in agar dilution assays, a computer-assisted conformational study were made on some selected chalcones (compounds **1**–**3**, **4**, and **11**). Calculations reveal that the energies of rotamers depend essentially on the torsional angles ϕ and θ (Fig. 1).

Energy profiles of chalcone **1** in two different levels of theory (AM1 and RHF/3-21G) are given in Figures 2 and 3. They show the influence of ring orientations on the potential energy of the rotamers. Figure 2 shows



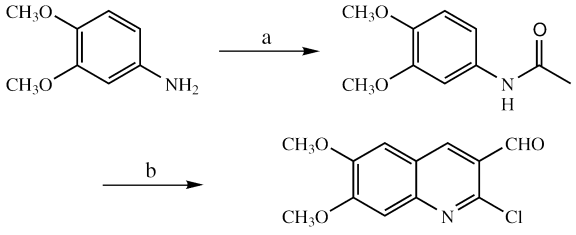
Scheme 1. Synthesis of chalcones and related compounds: (a) NaOH, MeOH, rt; (b) MeONa, MeOH, rt.

that for ϕ, both levels of theory predict two preferred conformations. Those with ϕ≅0 and 180° are the lowest in energy, confirming that planar forms are favoured in comparison with other conformations. It is clear that the energy barriers between the conformers, are completely different in function of the levels of theory used. When the minima obtained by the scans, were optimized at three different levels of theory (AM1, RHF/3-21G and RHF-6-31G), AM1 calculations predict ϕ = 18.65; RHF/3-21G: ϕ = 0.07 and RHF-6-31G: ϕ = 6.99, respectively (Table 5). This difference is even more noticeable when observing the results obtained for the torsional angle θ (Fig. 3 and Table 5). Semi-empirical calculations indicate that the rotation of the A ring is poorly restricted (low energy barrier).

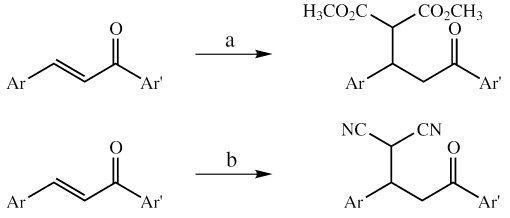
In contrast RHF/3-21G calculations predict a very high energy barrier suggesting that the planar forms are highly preferred (θ = 0.02°). RHF/6-31G calculations in turn, predicts θ = 19.99°. From the above results with all levels of theory, it is evident that we are dealing with a π electron delocalized system, therefore with a highly conjugated molecule, and hence with planar forms. Nevertheless, it seems that while AM1 calculations undervalue the single-double character of several C–C bonds in chalcones, RHF/3-21G calculations overstimate the delocalization in this structure. In turn, RHF/6-31G calculations which give intermediate values (Table 5), are in complete agreement with experimental X-ray results[31] reporting a planar conformation too, with ϕ and θ in ≅7 and ≅20°, respectively.

Different AM1 energy profiles obtained by rotating angles ϕ or θ in compounds **1**–**3**, **4**, and **11** are described in the following paragraph. In order to obtain a better degree of accuracy, the minima obtained for each compound which the AM1 scan were optimized at RHF/3-21G and RHF/6-31G levels of theory.

Figure 4 compares the different scans of compounds **1**–**4** when the B ring is rotated (modification of ϕ). Herein,



Scheme 2. Synthesis of 2-chloro-3-formyl-6,7-dimethoxyquinoline: (a) Py, AcCl, rt; (b) Vilsmeier reaction.



Scheme 3. Preparation of ketones: (a) $CH_2(CO_2CH_3)_2$, MeONa, MeOH, rt; (b) $CH_2(CN)_2$, MeONa, MeOH, rt.

the most noticeable feature is the different conformational behavior obtained for compounds possessing an *ortho*-substituent of significant size on ring B (compound **3**) with respect to that with the same substituent in the para position (chalcone **2**) and without substituents (compound **1**). The presence of the nitro substituent in the ortho position, introduces an important steric hindrance (see the high energetic barrier at 180° for compound **3**), suggesting a non-planar structure

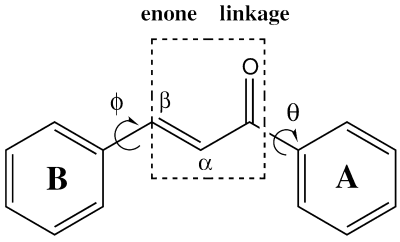


Figure 1. General structure of the chalcones showing the torsional angles ϕ and θ.

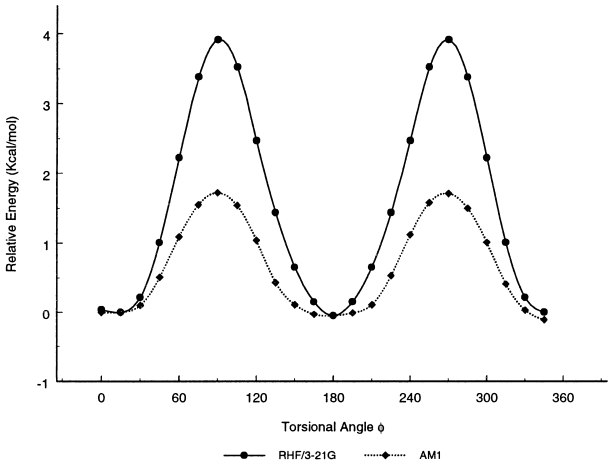


Figure 2. Conformation potential energy curves of compound **1** obtained from AM1 and RHF/3-21G relaxed single scan computations. Torsional angle ϕ measures the rotation about the B-ring.

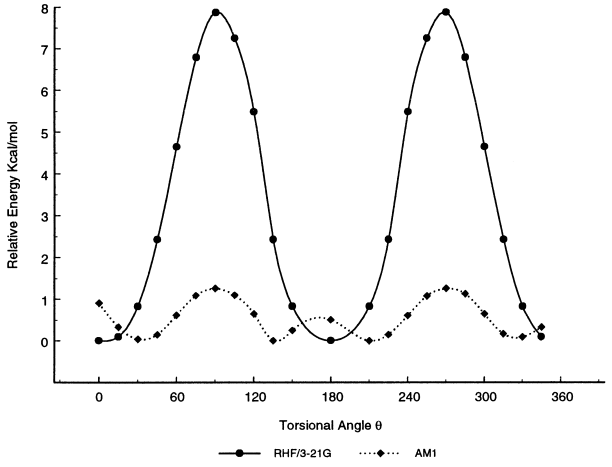


Figure 3. Conformation potential energy curves of compound **1** obtained from AM1 and RHF/3-21G relaxed single scan computations. Torsional angle θ measures the rotation about the A-ring.

which would account for its lower antifungal activity respective of chalcone **1**. Compound **4**, with a chloro atom in the *o*-position show the same profile as compound **3** confirming the steric hindrance produced by a bulky substituent. Nevertheless, the presence of a methoxy group in position 2 of ring B (compounds **9** and **10** energy profiles not shown) do not show a steric hindrance but a closely related conformational behavior to those of compounds **1** and **2** (Table 5), indicating that planar forms are the lowest energy conformations for chalcones **9** and **10** in contrast to compound **3**. This can be explained by the fact that the methoxy group possesses a very high molecular flexibility and therefore it can adopt an adequate spatial ordering without losing the planarity.

Figure 5 compares the AM1 scans obtained for compounds **4** and **11** when A ring is rotated. While the conformational behavior of compound **4** (without substituents on ring A) is very similar to that of chalcone **1** (Fig. 3) when the angle θ is modified, the conformations of compound **11** (possessing a chloro atom in the *o*-position of ring A) at θ≅0 and 180° are very high in energy, suggesting the loss of planarity.

Regarding the correlation of the antifungal activity of compounds **1**–**3**, **4**, and **11** with the planarity of their molecules, a decrease of activity was observed for these compounds with a bulky *ortho* substituent on ring B (compounds **3** and **4**) respective to the non-substituted chalcone **1**. The additional presence of *ortho* substituents on ring A, which affect the planarity too, conducts to a total loss of activity (compare MICs of chalcones **1**, **4**, and **11** against the fungi tested). The lack of antifungal activity of compound **11** add new evidence to the importance of planar forms as a conformational requeriment of chalcones for their antifungal effects.

To rationalize a possible site of action at the electronic level for these chalcones, we have performed an electronic analysis. We used the Lowest Unoccupied Molecular Orbital (LUMO) and Molecular Electrostatic Potential (MEP) which delineate electron deficient areas which could be critical to the possible mechanism of action.

On the map in Figure 6, regions with the highest absolute value of LUMO are indicated in 'blue', while regions with the lowest values are indicated in 'red'. Our results indicate that the derivatives taken as models for this study (chalcones **1**, **2**, and **6**) have two highly positive blue areas (β- and carbonyl carbons), which provide rich sites for nucleophilic attacks.

The MEP energy isosurfaces of derivatives **1**, **2**, and **6** are shown color-coded in the range from −60 (deepest red) to 40 kcal/mol (deepest blue) in Figure 7. The highest electrostatic potentials also reveal that the most positive centers (blue areas) lie near to the β- and carbonyl carbons. The decrease or enhancement of antifungal activity of chalcones when they possess donating or attracting groups respectively, are in agreement with these electronic studies.

The general picture emerging from the data reported here, is that planar forms in chalcones, are the most adequate spatial ordering needed to produce the antifungal response, being β- and carbonyl carbons the most susceptible sites toward a possible nucleophilic attack of the enzyme or receptor.

Table 5. Torsional angles obtained for compounds **1**–**4**, **9**, **11** by using different levels of theory

Compd	AM1		HF/3-21 G		HF/6-31 G	
	Torsional angles (°)		Torsional angles (°)		Torsional angles (°)	
	ϕ	θ	ϕ	θ	ϕ	θ
1	18.65	33.49	0.07	0.02	6.99	19.99
2	0.39	0.43	0.46	0.75	16.21	18.34
3	43.74	26.85	47.13	7.67	42.11	18.80
4	34.54	31.46	34.01	6.19	33.30	8.63
9	17.54	33.97	10.28	0.10	17.24	20.33
11	31.98	119.03	31.06	138.9	30.7	132.89

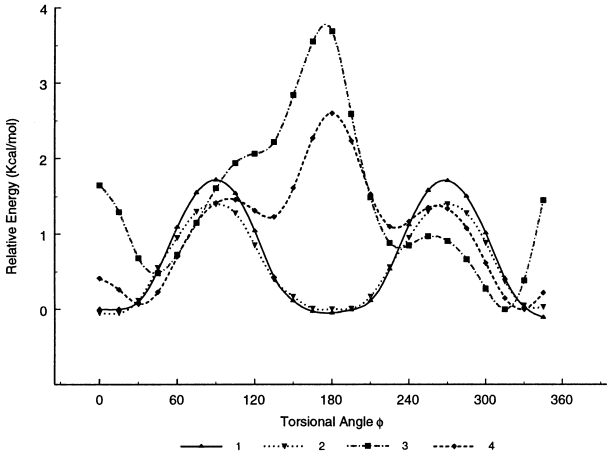


Figure 4. A comparison of potential energy curves (PEC) for the torsional angle ϕ of compounds **1**, **2**, **3**, and **4** each calculated from AM1 calculations.

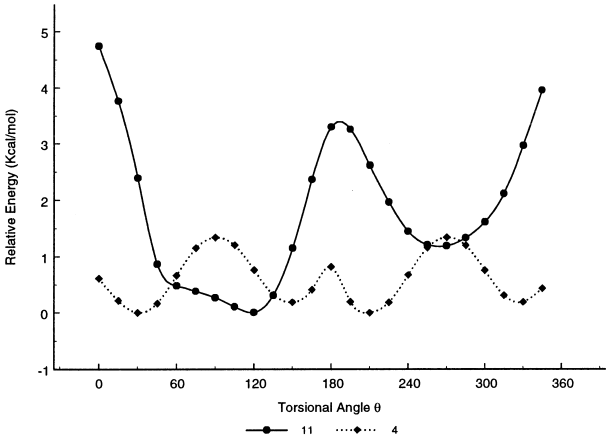


Figure 5. A comparison of potential energy curves (PEC) for the torsional angle ϕ of compounds **4** and **11** each calculated from AM1 calculations.

Mode of action studies

To gain insight into the mode of action of chalcone derivatives, we tested the most active antifungal compounds (structures **1**–**3**, **5**–**10**, **18** and **19**) for their capacity of inhibiting in vitro the *S. cerevisiae* β(1,3)-glucan synthase[32] or chitin synthase,[33] enzymes that catalyzes the synthesis of the major polymers of the fungal cell-wall, β(1,3)-glucan and chitin respectively. Results on the in vitro assays are listed in Table 6.

Regarding activity against chitin synthase-1, all the compounds tested exhibited inhibitory activities at 20 μg/assay (% of inhibition ranging from 15 to 95%). Since compounds **1**, **5**–**10**, **18**, and **19** showed inhibitions $\geqslant 50\%$, serial dilutions of them (1, 2, 5, 10, and 20 μg/assay) were tested for enzyme inhibition and their average inhibitory effect was calculated (Figs 8 and 9). The IC_{50} values were all $\leqslant 0.22\ \mu g/\mu L$, being compound **1** the most active one with 94% inhibition at 20 μg ($IC_{50} = 0.02\ \mu g/\mu L$).

Results obtained from glucan synthase assays, showed that most of chalcone derivatives (compounds **2**, **3**, **5**–**9**, and **18**) are very weak inhibitors of this enzyme ($IC_{50} > 0.5\ \mu g/\mu L$). Nevertheless, chalcones **1** and **10** and furane derivative **19**, showed interesting inhibitory activities ($0.14 \leqslant IC_{50} \leqslant 0.44\ \mu g/\mu L$), being chalcone **1** ($IC_{50} = 0.14\ \mu g/\mu L$) a strong inhibitor of β(1,3)-glucan synthase, comparable to the known β(1,3)-glucan synthase inhibitors Papulacandin B ($IC_{50} = 0.10\ \mu g/\mu L$) and Aculeacin A ($IC_{50} > 0.50\ \mu g/\mu L$).

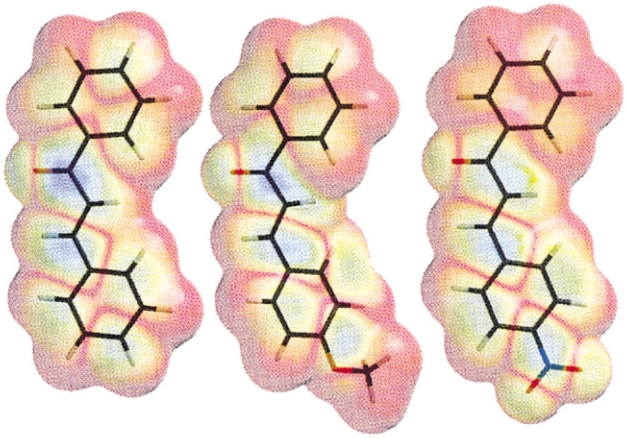


Figure 6. The calculated LUMO for compounds **1**, **6**, and **2**.

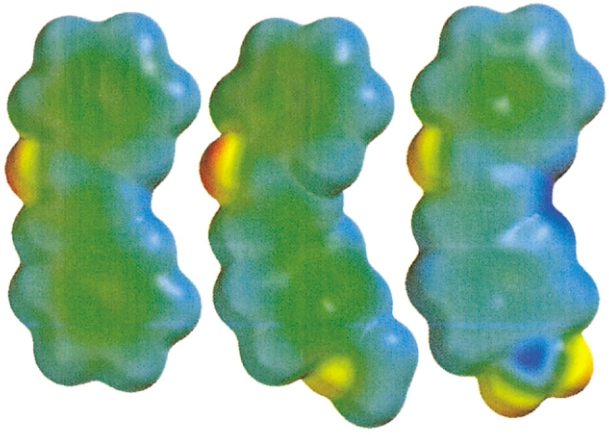


Figure 7. Molecular ectrostatic potential (MEP) maps obtained for compounds **1**, **6**, and **2**. The MEP were calculated from AM1 wave function.

Figure 10 shows the comparative inhibitory activities of the most active compounds against β(1,3)-glucan and chitin synthases as a measure of percent of residual activities for each enzyme. An analysis of this graphic clearly shows that chalcones are much better inhibitors of chitin synthase than of glucan synthase, suggesting that they could be selective inhibitors of that enzyme. This could be a very interesting feature in the possible development of these compounds as antifungal agents.

Although very active in cellular assays, chalcones **2** and **3** seem not to act by inhibiting the synthesis of the major polymers of the fungal cell wall (Table 6 and Fig. 9).

Alternatively, since the crude enzymes were prepared from *S. cerevisiae*, the species difference could account for a lack of a strict correlation between the activities shown by the compounds in the cellular and enzymatic assays.

Conclusions

In an attempt to generate novel antifungal structures with significant activities against human opportunistic fungi but less toxic than those in use nowadays, we have tested a series of chalcone derivatives and analogues, most of them new compounds, with agar dilution methods.

According with the results obtained in these assays, chalcones with different substituents on rings A and B, showed to be active against dermatophytes and not against another group of fungi. This type of fungi are responsible for dermatomycoses, a type of infections very difficult to eradicate.

A detailed SAR study supported by theoretical calculations, aided to identify and understand the structural requirements for the antifungal action of chalcones and its derivatives.

Regarding their mode of action, active compounds were assayed for their in vitro capacity of inhibiting β(1,3)-glucan and chitin synthases, enzymes that catalyze the synthesis of the two major polymers of the fungal cell wall, β(1,3)-glucan and chitin. Results reported here allow us to infer that some of the assayed chalcone derivatives and analogues, might act at least in part, by inhibiting the biosynthesis of one or both polymers of the fungal cell wall.

The antifungal activity of these compounds against dermatophytes, combined with their useful mode of action, make these compounds attractive leaders for the development of more potent and mainly safer antifungal drugs.

Experimental

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL GSX (270 MHz) spectrometer. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). NMR values are given in δ units relative at $CDCl_3$. Mass spectrometry (MS) spectra were obtained using a Hewlett-Packard HP 5971A Mass Selective Detector with EI (70 eV). IR spectra were recorded as KBr pellets using a Shimadzu model 470 spectrometer. The uncorrected melting points were determined on a Thomas micro hot stage apparatus. Column chromatography was performed on silica gel (Macherey–Nagel, 0.063–0.2 mm) using a mixture of hexane and ethyl acetate as eluent. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA (USA); results were within ±0.4% of predicted values for all compounds.

Preparation of the following compounds were reported previously: chalcone (**1**),[22,23] 4-nitrochalcone (**2**),[23] 4-methoxychalcone (**6**),[23] 3,4-dimethoxychalcone (**8**),[24] 2,3-dimethoxychalcone (**10**),[25] 4-methoxy-4′-methylchalcone (**15**),[26] 1-phenyl-3-(2-chloro-3-quinolinyl)-2-propenone (**35**),[28] 1-(3′,5′-dimethyl-2-furyl)-3-[3-(2-chloroquinolinyl)]-2-propenone (**36**),[28] 1-phenyl-3-(2-chloro-6,7-dimethoxy-3-quinolinyl)-2-propenone (**37**).[28]

Chalcones **1**–**18** were prepared using a sodium hydroxide catalyzed condensation, whereas the remaining compounds **19**–**22** and **34**–**41** were prepared using sodium methoxide.

Table 6. Capacity of inhibiting β(1,3)-glucan and chitin synthases expressed in% of inhibition and IC_{50} values (μg/μL)

Compd	β(1,3)-Glucan synthase assay		Chitin synthase assay	
	% I[a]	IC_{50}[b]	% I[c]	IC_{50}[b]
1	69.78±0.52	0.14	93.70±0.09	0.02
2	14.17±1.39	>0.50	15.47±0.66	>0.50
3	20.62±0.68	>0.50	32.74±0.11	>0.50
5	32.54±3.57	>0.50	67.38±0.92	0.13
6	14.09±0.64	>0.50	61.71±0.46	0.07
7	8.72±1.94	>0.50	57.31±0.62	0.22
8	42.06±0.15	>0.50	70.12±1.11	0.10
9	39.81±1.87	>0.50	78.78±0.02	0.10
10	54.15±0.91	0.44	76.28±1.79	0.08
18	14.55±0.45	>0.50	52.94±0.04	0.06
19	24.58±0.67	>0.50	57.45±0.93	0.22
21	54.20±1.21	0.33	94.64±0.12	0.03
Pap		0.10		
Ac		>0.50		
Nik				0.0006[d]

[a]Percent of inhibition at 20 μg/assay (total volume: 40 μL): mean ±SEM.
[b]Concentration (μg/μL) that produces 50% of Inhibition.
[c]Percent of inhibition at 20 μg/assay (total volume: 50 μL): mean ±SEM.
[d]Value obtained from ref 29; Pap, papulacandin B; Ac, Aculeacin A; Nik, Nikkomicin.

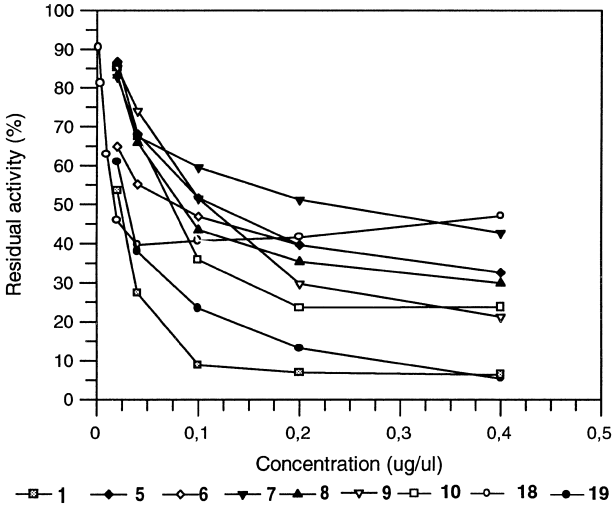


Figure 8. Effect of different concentrations of chalcones and a related compound on the in vitro incorporation of [^{14}C] *N*-acetylglucosamine into insoluble chitin, expressed as residual activity of the enzyme chitin synthase-1.

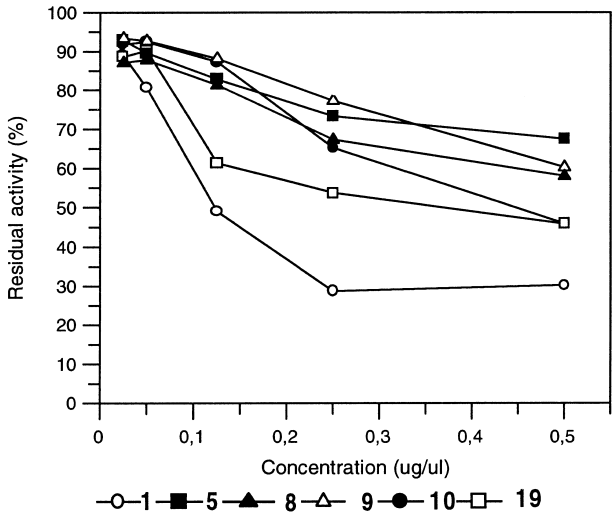


Figure 9. Effect of different concentrations of chalcones and a related compound on the in vitro incorporation of [^{14}C] glucose into insoluble β(1,3)-glucan expressed as residual activity of the enzyme β(1,3)-glucan synthase.

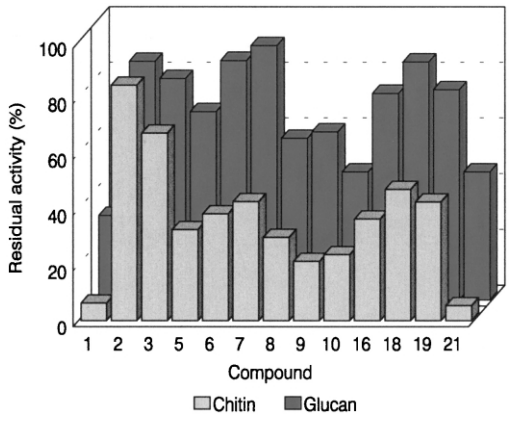


Figure 10. Comparative values of the inhibitory activities of antifungal chalcones and a related compound, against β(1,3)-glucan and chitin synthase-1, expressed as percent of residual activity.

General procedures to prepare different substituted chalcone derivatives

A solution of an appropriately substituted methyl ketone (5 mmol), an adecuated substituted aldehyde (5 mmol), NaOH (0.5 g, 12.5 mmol) or NaOMe (5.4 mg, 0.1 mmol) in MeOH or EtOH (10 mL) was stirred at room temperature for 1–24 h. Formation of a precipitate, generally accompanied by a color change in the reaction mixture, is indicative for product formation. The solid product was recrystallized from a minimum amount of solvent (EtOH/H_2O, 1:1 v/v). Other cases, the reaction were monitored for TLC. The solvent was evaporated under reduced pressure. Water was added and the product was extracted four times with dichloromethane. The organic extract was concentrated in vacuum, and the residue was purified by column chromatography to give the expected compounds as white or yellow solids. On the other hand, if the reaction yielded an oil instead of a solid, column chromatography was used to purify the product.

2-Nitrochalcone (3). This was synthesized using 2-nitrobenzaldehyde (0.14 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) as starting materials: white solid (0.89 g, 70%). IR (KBr): 1654 (C=O), 1592 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.99–7.98 (m, 2H, H2′, H6′), 7.56–7.53 (m, 3H, H3′, H4′, H5′), 7.52 (d, 1H, $J=8.4$ Hz, H6), 7.51 (m, 3H, H3, H4, H5), 6.89 (d, 1H, $J=15.38$ Hz, Hβ), 6.38 (d, 1H, $J=15.38$ Hz, Hα); ^{13}C NMR ($CDCl_3$) δ 189.9.0 (C=O), 132.7 (C1), 149.4 (C2), 132.5 (C4), 148.3 (C3), 129.3 (C5), 132.3 (C6), 141.7 (Cβ), 123.3 (Cα), 135.9 (C1′), 128.7 (C2′, C3′, C5′, C6′), 132.9 (C4′). MS m/z $[M^+]$ 253, $[M-1]^+$ 252, $[M-29]^+$ 224. Anal. ($C_{15}H_{11}NO_3$) C, H, N; C: calcd 71.14; found, 71.19; H: calcd, 4.38; found, 4.39; N: calcd 5.53; found 5.54.

2,4-Dichlorochalcone (4). This was synthesized using 2,4-dichlorobenzaldehyde (0.18 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) as starting materials: beige solid (1.20 g, 87%); IR (KBr) 1658 (C=O), 1596 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.51 (s, 1H, H3), 8.16 (dd, 1H, $J=7.6$, 1.99 Hz, H5), 8.02 (d, 1H, H $J=8.4$ Hz, H6), 7.65 (d, 1H, $J=15.83$ Hz Hα), 7.60–7.50 (m, 5H, H1′–H6′), 7.80 (d, 1H, $J=15.83$ Hz, Hβ), 7.6–7.70 (m, 5H, H2′–H6′); ^{13}C NMR ($CDCl_3$) δ 189.9 (C=O), 137.9 (C1′), 136.9 (Cβ), 134.8 (C2, C4), 133.0 (C1, C4′), 128.0 (C2′, C3′, C5′, C5, C6), 123.3 (Cα). MS m/z $[M^+]$ 277, $[M-1]^+$ 276, $[M-29]^+$ 248. Anal. ($C_{15}H_{10}Cl_2O$) C, H, Cl; C: calcd 65.21; found, 65.23; Cl: calcd 25.34; found 25.35; H: calcd, 3.65; found, 3.64.

3-Methoxychalcone (5). This was synthesized using 3-methoxybenzaldehyde (0.14 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) as starting materials: yellow crystals (1.13 g, 95%). IR (KBr): 1665 (C=O), 1595 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.18 (dd, 1H, $J=8.9$, 1.9 Hz, H4), 7.98 (dd, 2H, $J=9.0$, 2.2 Hz, H2′, H6′), 7.43 (d, 1H, $J=1.9$ Hz, H2), 7.43 (dd, 1H $J=8.2$, 2.2 Hz, H6), 7.82 (dd, 1H, $J=8.9$, 6.5 Hz, H5), 7.56–7.53 (m, 3H, H3′, H4′, H5′). 7.40 (d, 1H, $J=15.38$ Hz, Hβ), 7.27 (d, 1H, $J=15.38$ Hz, Hα); ^{13}C NMR ($CDCl_3$): 190.2 (C=O), 161.9 (C3), 144.6 (Cβ), 138.1 (C1′), 136.1 (C1), 132.7 (C4′), 129.8 (C5, C2′; C3′; C5′; C6′), 122.3 (Cα), 121.0 (C6), 116.3 (C2), 113 (C4), 55.7 (CH_3). MS m/z $[M^+]$ 238, $[M-1]^+$ 277, $[M-29]^+$ 209. Anal. ($C_{16}H_{14}O_2$) C, H; C: calcd, 80.64; found, 80.63; H: calcd, 5.93; found, 5.94.

4-Methylchalcone (7). This was synthesized using 4-methylbenzaldehyde (0.12 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) as starting materials: yellow solid (1.05 g, 95%). IR (KBr): 1666 (C=O), 1596 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.03 (dd, 2H, H2′, 6′ $J=8.50$, $J=1.90$ Hz), 7.79 (d, 1H, Hβ $J=15.83$ Hz), 7.45–7.57 (m, 7H, Ar, Hα), 7.21 (d, 1H, H4′ $J=9.95$, $J=2.01$ Hz), 2.38 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 191.4 (C=O), 144.7 (Cβ), 140.9 (C4), 138.3 (C1′), 132.5 (C4′), 132.1 (C1), 129.6 C3; C5, C2; C6, C2′, C3′, C5′; C6′), 121.0 (Cα), 21.5 (–CH_3). MS m/z $[M^+]$ 222, $[M-1]^+$ 221, $[M-29]^+$ 194. Anal. ($C_{16}H_{14}O$) C, H; C: calcd, 86.45; found, 86.42; H: calcd, 6.35; found, 6.36.

2,4-Dimethoxychalcone (9). This was synthesized using 2,4-dimethoxybenzaldehyde (0.17 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) as starting materials: yellow solid (1, 14 g, 85%). IR (KBr) 1654 (C=O), 1595 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.98 (dd, 2H, Ar, $J=8.41$, $J=1.48$ Hz), 7, 54 (d, 1H, Hβ $J=15.89$ Hz), 7.38 (dd, 1H, H6 $J=1.98$, $J=8.16$ Hz), 7.15 (d, 1H, H2 $J=1.98$ Hz), 6.89 (d, 1H, H5 $J=8.16$ Hz); 3.94 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 289.90 (C=O), 162.75 (C2), 160.16 (C4), 139.67 (Cβ), 138.1 (C1′), 132.68 (C4′), 130.79 (C6), 128.60 (C2′, C6′, C3′, C5′). 120.61 (Cα), 105.20 (C5), 98.32 (C3), 55.60 (OCH_3), 55.33 (OCH_3). MS m/z $[M-31]^+$ 237, $[M-62]^+$ 206, $[M-63]^+$ 205, $[M-92]$ 176. Anal. ($C_{17}H_{16}O_3$) C, H; C: calcd 76.09; found 76.08; H: calcd, 6.01; found, 6.02.

2,4-Dichloro-2′,4′-dichlorochalcone (11). This was synthesized using 2,4-dichlorobenzaldehyde (0.18 g, 1 mmol) and 2′,4′-dichloroacetophenone (0.19 g, 1 mmol) as starting materials: white solid (0.33 g, 95%). IR (KBr) 1680 (C=O), 1592 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.87 (d, 1H, $J=1.50$ Hz, H6′), 7.61 (d, 1H, $J=15.83$ Hz, Hβ), 7.52 (s, 1H, H3), 7.49 (s, 1H, H3′), 7.43 (d, 1H, $J=15.83$ Hz, Hα) 7.40 (d, 1H, $J=1.50$ Hz, H6′) 7.30 (d, 1H, $J=2.2$ Hz, H5), 6.90 (d, 1H, $J=8.20$ Hz, H6); ^{13}C NMR ($CDCl_3$) δ 187.51 (C=O), 138.51 (C4, C4′), 137.21(C2′), 136.89 (Cβ), 135.13 (C1′), 134.83 (C2), 132.77 (C1), 130.72 (C3′;C3), 129.28 (C6), 128.80 (C6′), 128.01 (C5′), 127.89 (C5), 123.93 (Cα). MS m/z $[M-1]^+$ 345, $[M-29]^+$ 317, $[346-100]^+$. Anal. ($C_{15}H_8Cl_4O$) C, H, Cl; C: calcd 52.34; found 52.22; H: calcd 2.34, found: 2.33, Cl: calcd, 40.67, found, 40.80.

4-Methyl-4′-methylchalcone (12). This was synthesized using 4-methylbenzaldehyde (0.12 g, 1 mmol) and 4′-methylacetophenone (0.134 g, 1 mmol) as starting materials: yellow solid (0.189 g, 80%). IR (KBr) 1654 (C=O), 1596 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.92 (d, 2H, $J=8.16$ Hz, H2′, H6′), 7.78 (d, 1H, $J=15.83$ Hz, Hβ), 7.54–7.45 (m, 3H, Ar, Hα), 7.38 (d, 2H, $J=7.91$, H3, H5), 7.20(d, 2H, $J=7.91$ Hz, H3, H5), 2, 42 (s, 3H,–CH_3), 2.37 (s, 3H,–CH_3); ^{13}C NMR ($CDCl_3$) δ 187.0

(C=O), 142.1 (C4′), 140.5 (Cβ), 136.9 (C4), 131.9 (C1′), 129.1, 128.5, 126.1, 121.0 (Cα), 22 (CH_3). MS *m/z* $[M-1]^+$ 235, $[M-29]^+$ 206. Anal. ($C_{17}H_{16}O$). C, H; C: calcd 86.40; found, 86.40; H: calcd, 6.83; found, 6.84.

3,4-Dimethoxy-3′,4′-dimethoxychalcone (13). This was synthesized using 3,4-dimethoxybenzaldehyde (0.17 g, 1 mmol) and 3,4-dimethoxyacetophenone (0.18 g, 1 mmol) as starting materials: yellow solid (0.30 g, 95%). IR (KBr) 1680 (C=O), 1592 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.68 (dd, 1H, *J* = 8.0, 1.9 Hz, H6′), 7.74 (d, *J* = 1.9 Hz, H2′), 7.26 (d, *J* = 15.38 Hz, Hβ), 7.25 (d, *J* = 15.38 Hz, Hα), 7.06 (dd, 1H, *J* = 8.2, 2.1 Hz, H6), 7.01 (d, 1H, *J* = 1.91 Hz, H2), 6.92 (d, 1H. *J* = 8.9 Hz, H5′), 6.70 (d, 1H, *J* = 8.2 Hz, H5), 3.88 [s, 6H, 2(OCH_3)], 3.87 [s, 3H, 2(OCH_3)]; ^{13}C NMR ($CDCl_3$) δ 194.4 (C=O), 151.3 (C4), 150.8 (C4′), 149.2 (C3′), 147.8 (C3′), 131.8 (C1′), 128.0 (Cl), 144.1 (Cβ), 122.9 (Cα), 212.3 (C6), 120.7 (C6), 114.9 (C5′, C2), 112.8 (C2′, C5), 59.2; 55.3; 55.1 and 52.0 (OCH_3). MS *m/z* $[M-31]^+$ 297, $[M-62]^+$ 266, $[M-162]^+$ 166. Anal. ($C_{19}H_{20}O_5$) C, H; C: calcd, 69.48; found, 69.50 H: calcd 6.14; found 6.16.

2,3-Dimethoxy-4′-bromochalcone (16). This was synthesized using 2,3-dimethoxybenzaldehyde (0.166 g, 1 mmol) and 4-bromoacetophenone (0.199 g, 1 mmol) as starting materials: yellow solid (0.29 g, 85%). IR (KBr) 1660 (C=O), 1594 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.08 (d, 1H, *J* = 15.83 Hz, Hβ), 7.86 (d, 1H, *J* = 8.66 Hz, H3′, H5′), 7.75 (d, 2H, *J* = 8.66 Hz, H5′, H6′), 7.52 (d, 1H, *J* = 15.83 Hz, Hα), 7.25 (d, 1H, *J* = 7.42 Hz, H2, H6), 7.08 (t, 1H, *J* = 7.91 Hz, H5), 6.96 (d, 1H, *J* = 7.17 Hz, H4); 3.87 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): 188.9 (C=O), 152.9, 147.9(C3), 139.0 (C2), 138.6 (Cβ), 136.7 (C1′), 130.5, 131, 127.7 (C4′), 124.7, 123.9, 123.0 (Cα), 114.0, 61.40 (CH_3), 56.0 (CH_3). MS *m/z* $[M-1]^+$ 346, $[M-29]^+$ 318. Anal. ($C_{17}H_{15}BrO_3$). C, H; C: calcd, 58.96; found, 58.97; H: calcd, 4.37; found 4.36, Br: calcd, 22.81; found, 22.78.

3-Methoxy-4′-bromochalcone (18). This was synthesized using 3-methoxybenzaldehyde (0.14 g, 1 mmol) and 4′-bromoacetophenone (0.20 g, 1 mmol) as starting materials: yellow solid (1.49 g, 94%) IR (KBr) 1654 (C=O), 1595 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.88 (d, 2H, *J* = 8.39 Hz, H5′), 7.76 (d, 1H, Hβ *J* = 15.35 Hz), 7.64 (d, 2H, *J* = 8.39 Hz Ar), 7.4 (d, 1H, *J* = 15.35 Hz, Hα), 7.33 (dd, 1H, *J* = 8.9 Hz, H5); 7.21 (dd, 1H, *J* = 2.02, *J* = 8.9 Hz, H4), 7.13 (d, 1H, *J* = 1.98 Hz, H2); 6.97 (dd, 1H, *J* = 1.98, *J* = 8.41 Hz, H6), 3.84 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 188.9 (C=O), 150.0 (C3), 144.6 (Cβ), 136.8 (C1′), 131.8 (C3′, C5′), 129.9 (C5), 127.8 (C4′), 122.3 (Cα), 116.3 (C2), 113.4 (C4). 55.1 (OCH_3). MS *m/z* $[M-31]^+$ 286, $[M-32]^+$ 285, $[M-61]^+$ 256. Anal. ($C_{16}H_{13}BrO_2$) C, H; C: calcd 60.76; found 60.73 H: calcd, 4.15; found, 4.15; Br: calcd, 24.97; found, 25.04.

1-(2′,4′-Dichlorophenyl)-3-(2-furyl)-2-propen-1-one (19). This was synthesized using 2-furylaldehyde (0.096 g, 1 mmol) and 2′,4′-dichloroacetophenone (0.1 g, 1 mmol) as starting materials: yellow solid (0.20 g, 75%). IR (KBr) 1683 (C=O), 1590 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.85 (d, 1H, *J* = 8.9 Hz, H6′), 7.80 (d, 1H, *J* = 1.73 Hz, H3′), 7.51 (d, 1H, *J* = 4 Hz, H5), 7.49 (d, 1H, *J* = 16.0 Hz, Hβ), 7.42 (dd, 1H, *J* = (.0, 4 Hz, H5′), 7.00 (d, 1H, *J* = 16 Hz, Hα), 6.47 (dd, 1H, *J* = 8.0, 4 Hz, H4), 6.39 (dd, 1H, *J* = 8.0, 4.0 Hz, H3); ^{13}C NMR ($CDCl_3$) δ 187.0 (C=O), 155.3 (C2), 145.0 (C5), 141.6 (Cβ), 139.6 (C4′), 135.9 (C2′), 135.3 (C1′), 131.4 (C6′), 130.6 (Cα), 129.2 (C3′, C5′). MS *m/z*: $[M-H]^+$ 266, $[M-29]^+$ 238, $[M-118]^+$ 121. Anal. ($C_{13}H_8Cl_2O_2$) C: calcd: 58.65, found: 58.71 H: calcd 3.03, found 3.04; Cl: calcd, 26.29, found, 26.24.

1-(Phenyl)-3-(2-napthyl)-2-propen-1-one (20). This was synthesized using 2-naphtylaldehyde (0.16 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) as starting materials: yellow solid (0.21 g, 80%). IR (KBr) 1681 (C=O), 1592 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.11–8.09 (m, 2H, H2, H3), 8.04 (d, 2H, *J* = 8.0 Hz, H7, H8), 8.03–7.98 (m, 5H, Ar), 7.76 (d, 1H, *J* = 15.38 Hz, Hb), 7.75–7.43 (m, 3H, Ar), 7.34 (d, 1H, *J* = 15.38 Hz, Hα); ^{13}C NMR ($CDCl_3$) δ 188.9 (C=O), 140.5 (Cβ), 137.4 (C1′), 133.5 (C9), 132.9 (C4′), 132.8 (C5), 132.3 (C2), 128.4 (C2′, C3′, C5′, C6′), 128.0 (C3, C6), 127.9 (C4), 126.6 (Cα), 125.9 (C7, C8, C1). $[M]^+$ 258, $[M-1]^+$ 257, $[M-29]^+$ 229. Anal. ($C_{19}H_{14}O$): C: calcd, 88.34, found, 88.35 H: calcd, 5.47, found, 5.46.

1-(2′,4′-Dimethoxyphenyl)-3-(2-napthyl)-2-propen-1-one (21). This was synthesized using 2-naphtylaldehyde (0.14 g, 1 mmol) and 2′,4′-dimethoxyacetophenone (0.18 g, 1 mmol) as starting materials: yellow solid (0.22 g, 85%). IR (KBr) 1680 (C=O), 1590 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.01 (d, 1H, *J* = 8.0 Hz, H4), 8.00 (d, 1H, *J* = 8.0 Hz, H8), 7.85 (d, 1H, 8.02 Hz, H3), 7.84 (d, 1H, *J* = 8.0 Hz, H5), 7.75 (s, 1H, H1), 7.74 (d, 1H, *J* = 8.0 Hz, H6′), 7.51–7.49 (m, 2H, H6, H7), 7.44 (d, 1H, *J* = 16.0 Hz, Hβ), 7.34 (d, 1H, *J* = 16.0, Hα), 6.53 (dd, 1H, *J* = 8.0, 4.0 Hz, H5′), 6.17 (d, 1H, *J* = 4.0 Hz, H3′), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 188.9 (C=O), 167.4 (C4′), 163.1 (C2′), 140.5 (Cβ), 133.5 (C3, C4), 132.6 (C1), 130.6 (C6′), 127.9 (C5), 127.9 (C5), 126.6 (Cα), 125.7 (C2), 106.3 (C5′), 56.0 (2 OCH_3). $[M-1]^+$ 317, $[M-29]^+$ 289, $[M-32]^+$ 286, $[M-137]^+$ 181. Anal. ($C_{21}H_{18}O_3$): C: calcd 79.21, found 79.19, H: calcd 5.70, found 5.71.

1-(3′-Methoxyphenyl)-3-(3-methoxy-2-napthyl)-2-propenone (22). This was synthesized using 3-methoxy-2-naphtylaldehyde (0.19 g, 1 mmol) and 3′-methoxyacetophenone (0.12 g, 1 mmol) as starting materials: orange solid (0.19 g, 68%). 1662 (C=O), 1590 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.12 (d, 1H, *J* = 16.0 Hz, Hβ), 8.01 (d, 1H, *J* = 8.2 Hz, H8), 7.99 (s, 1H, H4), 7.97 (d, 1H, *J* = 8.1 Hz, H4′), 7.77 (s, 1H, H1), 7.85 (d, 1H, *J* = 8.1 Hz, H5), 7.51–7.49 (m, 4H, H6, H7, H5′, H6′), 7.45 (d, 1H, *J* = 16.0 Hz, Hα), 7.42 (d, 1H, 1.89 Hz, H2′), 8.83 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 187.0 (C=O), 161.9 (C3′), 155.4 (C3), 140.5 (Cβ), 138.4 (C1′), 129.4 (C5′, C8a), 127.2 (C1, C8), 126.4 (Cα, C5, C6), 125.2 (C2), 123.7 (C7), 120.9 (C6′),

118.5 (C4′), 114.2 (C2′), 56.0 (2 OCH_3). MS m/z $[M]^+$ 318 (30), $[M-1]^+$ 317(90), $[M-29]^+$ 289 (100). Anal. ($C_{21}H_{18}O_3$): C: calcd 79.21, found 79.21 H: calcd 5.70, found 5.69.

General procedure for preparation of ketones (23–26)

To a solution of dimethylmalonate (0.5 mmol) and a chalcone derivative (0.5 mmol) in methanol (5 mL), sodium methoxide (0.05 mmol) was added and warmed until complete solubility. The mixture was stirred at room temperature between 4 and 6 h or until a solid appeared, indicating the formation of the product. The products were filtered and recristallized in EtOH–H_2O.

1,3-Diphenyl-3-methylmalonyl-propanone (23). This was synthesized using compound **13** (104 mg, 0.5 mmol), dimethyl malonate (66 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: white solid (0.162 g, 95%), (MeOH–H_2O). IR (KBr) 1731.2 (C=O, ester), 1664.0 (C=O), cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.99–796 (m, 2H, Ar), 7.50–7.48 (m, 3H, Ar), 7.42 (m, 2H, Ar), 7.38–7.34 (m, 3H, Ar), 4, 27 (d, 1H, $J=4$ Hz), 3.83 (m, 1H), 3.75 (s, 6H, 2OCH_3), 3.62–3.27 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 197.6 (C=O), 174.5 (C10a, C10b), 139.4 (C1), 137.2 (C1′), 132.7 (C4′), 128.6 (C2, C3, C5, C6, C2′, C3′, C5′, C6′), 125.7 (C4), 52.9 (C10), 50.4 (2 OCH_3), 46.7 (C8), 24.6 (C9). MS m/z $[M-132]^+$ 258, [258−H]+ 257 $[257-CO]^+$ 229, $[M-73]^+$ 258. Anal. ($C_{20}H_{20}O_5$), C: calcd 70.56, found 70.58, H: calcd 5.93, found 5.94.

1-(2,4-Dimethoxyphenyl)-3-(phenyl)-3-methylmalonyl-propanone (24). This was synthesized using compound **9** (0.14 g, 0.5 mmol), dimethylmalonate (66 mg, 0.5 mmol and sodium methoxide (0.27 mg, 0.05 mmol) in methanol (5 mL). Beige solid, (0.06 g, 60%). IR (KBr): 1731 (C=O, esther), 1664 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.99 (dd, 2H, $J=8.0$, 2.1 Hz, H2′, H6′), 7.50 (dd, 2H, $J=8.0$, 4.0 Hz, H4′, H5′), 7.31 (dd, 1H, $J=8.0$, 4.0 Hz, H5), 7.49 (m, 1H, H3′), 7.05 (d, $J=8.2$ Hz, H6), 6.81 (d, 1H $J=8.2$ Hz, H4), 4.48–4.43 (m, 2H), 3.79 (s, 6H, 2OCH_3), 3.75 (s, 6H, 2CO_2CH_3), 3.68–3.33 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 197.6 (C=O), 174.5 (2CO_2CH_3), 147.4 (C2, C3), 137.4 (C1′), 132.9 (C4′), 126.0 (C1), 128.6 (C2′, C3′, C5′, C6′), 121.6 (C5, C6), 56.6 (2 OCH_3), 53.2 (CH), 50.4 (2 CO_2CH_3), 47.0 (CH_2), 19.0 (CH). MS m/z $[M-132]^+$ 268, $[268-H]^+$ 267. Anal. ($C_{22}H_{24}O_7$), C: calcd 65.97, found 65.96, H: 6.04, found 6.02.

1-(2-Napthyl)-3-(phenyl)-3-methylmalonyl-propanone (25). This was synthesized using compound **22** (129 mg, 0.5 mmol), dimethyl malonate (66 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: white solid (0.27 g, 68%). IR (KBr) 1731 (COOR), 1660 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.98 (dd, 2H $J=8.0$, 2.1 Hz, H2′, H6′), 7.49 (m, 3H, H3′, H4′, H5′), 7.45–7.33 (m, 7H, H5, H6, H7, H8, H9), 4.45–4.43 (m, 2H), 3.75 (s, 6H, OCH_3), 3, 65–3.30 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 197.6 (C=O, ketone), 174.5 (2 C=O, esther), 137.4 (C1′), 135.2 (C2), 133.5 (C8a), 132.9 (C4′), 131.6 (C4a), 128.4 (C2′, C3′, C5′, C6′), 127.5 (C3, C4, C5, C8), 126.7 (C1), 125.7 (C7), 124.8 (C6), 52, 9 (CH), 50.4 (2 OCH_3), 46.7 (CH2), 24.6 (CH). MS m/z $[M-132]^+$ 258, $[258-H]^+$ 257, $[257-CO]^+$ 229. Anal. ($C_{24}H_{22}O_5$) C: calcd 73.82, found 73.85, H: calcd 5.68, found 5.68.

1-(Phenyl)-3-(2,3-dimethoxyphenyl)-3-malonomethyl-propanone (26). This was synthesized using compound **10** (74 mg, 0.5 mmol), dimethyl malonate (66 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: pale-yellow solid (0.18 g, 70%). IR (KBr) 1740 (COOR), 1660 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.11 (dd, 2H, $J=8.1$, 1.98 Hz, H2′, H6′), 7.49 (m, 3H, H3′, H4′, H5′), 7.37 (d, 1H, $J=8.0$ Hz, H6), 7.35–6.8 (m, 2H, H4, H5), 4.73 (d, 1H, $J=8.0$ Hz, CH), 4.41 (d, 1H, $J=8$ Hz, CH), 3.82 (t, 1H, $J=16.0$ Hz), 3.46 (t, 1H, $J=16.0$), 3.79 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 197.6 (C=O), 147.4 (C2, C3), 137.9 (C1′), 132.9 (C4′), 128.4 (C2′, C3′, C5′, C6′), 126.0 (C1), 121.6 (C4, C5, C6), 107.2 (2 C≡N), 56.6 (2 OCH_3), 46.0 (CH_2), 35.1 (2 CH). MS m/z $[M-CH_3OH]^+$ 368, $[M-CH_2(CO_2CH_3)_2]^+$ 268. Anal. ($C_{20}H_{18}N_2O_3$) C: calcd 71.83, found 71.89, H: calcd 5.43, found 5.43, N: calcd 8.38, found 8.36.

1-(Phenyl)-3-(2,4-dichlorophenyl)-1,1-dicyane-methyl-propanone (27). This was synthesized using compound **5** (139 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: beige solid IR (KBr) 2208 (CN), 1680 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.08 (m, 2H, Ar), 7.60 (d, 1H, $J=8.2$ Hz, H6), 7.59–7.56 (m, 3H, Ar), 7.52 (d, 1H, $J=1.9$ Hz, H3), 7, 35 (d, 1H, $J=8.2$ Hz, H5), 4.44 (d, 1H, $J=8.0$ Hz), 3.72 (t, 1H, $J=16.0$ Hz), 3.37 (t, 1H, $J=16.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 196.5 (C=O), 137.3 (C1, C1′), 135.2 (C2), 134.9 (C6), 133.4 (C4′, C6), 132.4 (C2′, C6′), 129.6 (C3), 126.8 (C3′, C5), 126.2 (C5), 107.0 (2 C≡N), 45.0 (CH_2), 35.10 (2 CH). MS m/z $[M-C_3H_2N_2]^+$ 277, $[277-H]^+$ 276. Anal. ($C_{18}H_{12}Cl_2N_2O$) C: calcd 63.15, found 63.10, H: calcd 3.54, found 3.53, N: calcd 8.19, found 8.19, Cl: calcd, 20.45, found, 20.51.

1-(Phenyl)-3-(2-napthyl)-1,1-dicyane-methyl)-1-propanone (28). This was synthesized using compound **22** (129 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: orange solid (0.24 g, 75%). IR (KBr) 2208 (CN), 1680 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.10 (dd, 2H, $J=7.5$, 1.3 Hz, H2′, H6′), 8.03 (d, 1H, $J=9.0$ Hz, H4), 7.99 (m, 2H, Ar), 7.77 (dd, 1H, $J=7.6$, 1.3 Hz, H5), 7.63 (dd, 1H, $J=9.0$, 2.0 = Hz, H3), 7.49 (m, 3H, Ar, 7.43 (m, 2H, Ar), 4.43 (d, $J=8.0$ Hz, $CH(CN)_2$), 4.16 (m, 1H,–CH–), 3.73 (dd, $J=12$, 8 Hz,–**H**CH–), 3.38 (dd, 12, 8 Hz, –HC**H**–); ^{13}C NMR ($CDCl_3$) δ 196.5 (C=O), 137.5 (C1′), 137.1 (C8a), 135.2 (C2), 133.4 (C4′), 132.4 (C2′, C6′), 128.3 (C6), 127.6 (C4), 126.8 (C3′, C5′), 126.0 (C5), 125.0 (C7), 107.0 (2 C≡N), 44.8 (CH_2), 37.8 (CH), 33.9 (CH). MS m/z $[M-C_3H_2N_2]^+$ 258, $[258-H]^+$ 257, $[257-CO]^+$ 229. Anal. ($C_{22}H_{16}N_2O$) C: calcd 81.45, found 81.49, H: calcd 4.97, found 4.96, N: calcd, 8.64, found, 8.63.

1-(3′,4′-Dimethoxylphenyl)-3-(2-napthyl)-1,1-dicyane-methyl-1-propanone (29). This was synthesized using **24** (159 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol)

and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: yellow solid (227 mg, 70%). IR (KBr) 2208 (CN), 1660 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.03 (d, 1H, $J=8.1$ Hz, H4), 7.99 (m, 2H, Ar), 7.78 (d, 1H, $J=8.1$ Hz, H5), 7.77 (dd, 1H, $J=8.0$, 2.1 Hz; H6′), 7.70 (d, 1H, $J=2.1$ Hz, H1′), 7.43 (m, 2H, Ar), 6.88 (dd, 1H, $J=8.0$, 2.1 Hz, H3), 4.20–4.13 (m, 2H, $CH(CN)_2$,–CH–), 3.90 (s, 6H, OCH_3), 3.76 (m, 1H,–**H**CH–), 3.40 (m, 1H, –HC**H**–); ^{13}C NMR ($CDCl_3$) δ 195.6 (C=O), 148.8 (C4′), 147.3 (C3′), 137.0 (C8a, C8), 127.6 (C4, C3), 126.1 (C5), 125.2 (C5′), 114.5 (C5′), 113.5 (C2′), 107.0 (2 C≡N), 55.9 (OCH_3), 55.7 (OCH_3), 44.8 (CH_2), 37.8 (CH), 33.9 (CH). MS *m/z* $[M-C_3H_2N_2]^+$ 318, $[318-H]^+$ 317 $[317-CO]^+$ 289. Anal. ($C_{24}H_{20}N_2O_3$) C: calcd 74.97, found 74.95, H: calcd 5.25, found 5.24, N: calcd 7.29, found 7.30.

1-(2′,4′-Dimethoxyylphenyl)-3-(2-napthyl)-1,1-dicyane-methyl-1-propanone (30). This was synthesized using compound **23** (159 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: orange solid (230 mg, 60%). IR (KBr) 2208 (CN), 1680 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.06 (d, 1H $J=7.8$ Hz, H4), 8.00 (m, 2H, Ar), 7.78 (d, 1H, $J=7.9$ Hz, H5), 7.76 (d, 1H, $J=8.0$ Hz, H6′), 7.63 (d, 1H, $J=7.8$ Hz, H3), 7.43 (m, 2H, Ar), 6.49–6.48 (m, 2H, Ar), 4.24–4.13 (m, 2H, $CH(CN)_2$,–CH–), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.82–3.46 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 196.0 (C=O), 165.3 (C2′), 161.7 (C4′), 137.0 (C8a, C8), 127.6 (C4, C3), 126.1 (C5), 117.8 (C1′), 107.0 (2 C≡N), 114.5 (C5′), 111.0 (C5′), 99.6 (C3′, C6′), 55.9 (2 OCH_3), 44.8 (CH_2), 37.8 (CH), 33.9 (CH). MS *m/z*: $[M-32]^+$ 353 $[M-C_3H_2N_2]^+$ 318. Anal. ($C_{24}H_{20}N_2O_3$) C: calcd 74.97, found 74.99 H: calcd 5.25, found 5.24, N: calcd 7.29, found 7.30.

1-(Phenyl)-3-(2-chloro-3-quinolinyl)-1,1-dicyane-methyl-1-propanone (31). This was synthesized using compound **36** (147 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: orange solid (225 mg, 60%). IR (KBr) 2208 (CN), 1660 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.00 (s, 1H, H2), 8.16–8.14 (m, 4H, H4, H5, H2′, H6′), 8.03–7.69 (m, 3H, H6, H7, H8), 7.49–7.50 (m, 3H, H3′, H4′, H5′), 4.44 (d, 1H, $J=8$ Hz, $CH(CN)_2$), 4.16 (m, 1H, CH), 3.73–3.37 (m, 1H, CH_2); ^{13}C NMR ($CDCl_3$) δ 180.0 (C=O), 150.8 (C2), 147.4 (C8a), 137.4 (C1′), 136.9 (C4), 132.9 (C4′), 128.6 (C2′, C3′, C5′, C6′), 128.1 (C6, C7), 127.9 (C4α), 127.0 (C5), 119.2 (CN), 119.0 (CN), 45.7 (CH_2), 24.8 (CH), 22.8 (CH). MS *m/z* $[M-HCl]^+$ 338, $[M-C_3H_2N_2]^+$ 309. Anal. ($C_{21}H_{14}ClN_3O$) C: calcd, 70.18, found, 70.16 H: calcd, 3.93, found, 3.93, N: calcd, 11.70, found, 11.71, Cl: calcd, 9.74, found, 9.76.

1-(3′,4′-Dimethoxyphenyl)-3-(2-chloro-3-quinolinyl)-1,1-dicyane-methyl)-propanone (32). This was synthesized using 3-(2-chloro-3-quinolinyl)-1-(3′,4′-dimethoxyphenyl)-propenone (177 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: yellow solid (269 mg, 75%). IR (KBr) 2208 (CN), 1660 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.28 (s, 1H, H3), 8.23 (d, 1H, $J=8.0$ Hz, H4), 8.05–7.78 (m, 3H, Ar), 7.76 (dd, 1H, $J=8.0$, 2.0 Hz, H6′), 7.70 (d, 1H, $J=1$, 9 Hz, H1′), 6.89 (d, 1H, $J=7.0$ Hz, H5′), 4.6–4.3 (m, 2H), 3.90 (s, 6H, OCH_3), 3.67–3.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 195.6 (C=O), 148.6 (C4′), 147.3 (C3′), 145.3 (C2), 141.9 (C8a), 134.1 (C4), 132.8 (C1), 130.6 (C7), 127.8 (C8), 126.5 (C6), 122.1 (C6′), 116.6 (C3), 113.5 (C2′), 107.0 (CN), 107.5 (CN), 55.9 (OCH_3), 55.7 (OCH_3), 45.1 (CH_2), 35.2 (CH), 35.1 (CH). MS *m/z* $[M-31]^+$ 388, $[M-72]^+$ 357, $[M-C_3H_2N_2]^+$ 353. Anal. ($C_{23}H_{18}ClN_3O_3$) C: calcd 65.85, found 65.96 H: calcd 4.33, found 4.31, N: calcd 10.02, found 9.98, Cl: calcd 8.34, found 8.32.

1-(3′,4′,5′-Trimethoxyphenyl)-3-(3,4,5-trimethoxiphenyl)-1,1-dicyane-methyl-1-propanone (33). This was synthesized using compound **14** (194 mg, 0.5 mmol), malononitrile (33 mg, 0.5 mmol) and sodium methoxide (0.27 mg, 0.05 mmol) as starting materials: yellow solid (295 mg, 65%). IR (KBr) 2208 (CN), 1660 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.20 (s, 2H, H2′, H6′), 5.92 (s, 2H, H2, H6), 4.37–4.23 (m, 2H), 3.98 (s, 6H, OCH_3), 3.90 (s, 6H OCH_3), 3.79–3.40 (m, 2H,–CH_2–); ^{13}C NMR ($CDCl_3$) δ 157.9 (C3, C5), 153.6 (C3′, C5′), 140.08 (C4′, C4), 131.0 (C1, C1′), 113.4 (C2′, C6′), 105.9 (C2, C6), 104.9 (2 CN), 61.1 (4 OCH_3), 56.5 (OCH_3), 56.4 (OCH_3), 54.6 (CH_2). MS *m/z* $[M-66]^+$ 388, $[388-H]^+$ 387, $[387-CO]^+$ 283. Anal. ($C_{24}H_{26}N_2O_7$) C: calcd, 63.41, found, 63.36 H: calcd, 5.77, found, 5.78, N: calcd, 6.17, found, 6.16.

General procedure to prepare 2-chloro-6,7-dimethoxy-quinolynilchalcones (34–41)

A solution of the acetophenone respective (1 mmol), 2-chloro-6,7-dimethoxy-3-formyl (1 mmol) and sodium methoxide (0.01 mmol) in methanol (5 mL) were stirred at room temperature. After 24 h, a yellow solid was isolated by filtration.

1-Phenyl-3-(2-chloro-3-quinolinyl)-2-propen-1-one (34). (0.25 g, 85%). IR (KBr) 1651 (C=O), 1595 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.31 (d, 1H, $J=8.30$ Hz, H5), 8.25 (s, 1H, H4), 8.11 (d, 1H, $J=8.41$ Hz, H8), 7.98 (d, 2H, 8.91 Hz, H2′, H6′), 7.74 (d, 1H, $J=15.83$ Hz, Hβ), 7.63–7.53 (m, 4H, H5′, H4′, H3′, H7), 7.34 (d, 1H, $J=15.83$ Hz); ^{13}C NMR ($CDCl_3$) δ 190 (C=O), 144.1 (C2), 139.9 (C8a), 139.6 (C1′), 136.9 (Cβ), 135.5 (C4), 132.7 (C4′), 131.1 (C4a), 130.9 (C5), 129.7 (C6, C7), 128.6 (C2′, C3′, C5′, C6′). 129.5 (C7), 125.8 (C3), 121.7 (Cα). MS *m/z* $[M]^+$ 293, $[M-1]^+$, $[M-36]^+$ 257, $[M-28]^+$ 265, $[M-77]^+$ 216. Anal. ($C_{18}H_{12}ClNO$). C: calcd 73.70; found 73.72; H: calcd 4.13; found 4.14; N: calcd 4.78, found 4.77 Cl: calcd 11.93 found 11.92.

1-(4′-Bromo-phenyl)-3-(2-chloro-6,7-dimethoxy-3-quinolinyl)-2-propen-1-one (38). (0.41 g, 95%). IR (KBr) 1638 (C=O), 1577 (C=C). ^1H NMR ($CDCl_3$) δ 8.52 (d, 2H, $J=2.1$ Hz, H2′, H6′), 8.32 (s, 1H, H4), 8.29 (d, 1H, $J=15.70$ Hz, Hβ), 7.96 (d, 2H, $J=8.42$ Hz, Ar), 7.63 (d, 2H, $J=8.42$ Hz, Ar), 7.52 (d, 1H, $J=15.70$ Hz, Hα), 7.33 (s, 1H, H8), 7.06 (s, 1H, H5), 4.02 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 189.7 (C=O), 155.2 (C6, C7), 145.9 (C2), 140.6 (C1′, C8a), 136.7 (Cβ), 130.0 (C2′, C3′, C5′, C6′, C4, C4a), 128.2 (C4′), 125.0 (C3), 122.6 (Cα),

118.0 (C8), 105.1 (C5), 56.8 (OCH_3), 56.7 (OCH_3). MS *m/z*: $[M]^+$ 353 $[M-1]^+$ 352, $[M-31]^+$ 322, $[M-29]^+$ 324, $[M-77]^+$ 276. Anal. ($C_{20}H_{15}ClBrNO_3$), C: calcd 55.69, found 55.75 H: calcd 3.51, found 3.51; N: calcd 3.25, found 3.24; Cl: calcd 8.11, found 8.09; Br: calcd 18.31, found 18.30.

1-(4′-Chloro-phenyl)-3-(2-chloro-6,7-dimethoxy-3-quinolinyl)-2-propen-1-one (39). (0.29 g, 72%). IR (KBr) 1657 (C=O), 1574 (C=C). ^1H NMR ($CDCl_3$) δ 8.55 (d, 2H, $J=2.1$ Hz, H2′, H6′), 8.36 (s, 1H, H4), 8.30 (d, 1H, $J=15.70$ Hz, Hβ), 7.90 (d, 2H, $J=8.0$ Hz, Ar), 7.59 (d, 2H, $J=8.42$ Hz, Ar), 7.55 (d, 1H, $J=15.70$ Hz, Hα), 7.35 (s, 1H, H8), 7.10 (s, 1H, H5), 3.99 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 189.7 (C=O), 156.2 (C6, C7), 145.8 (C2), 140.5 (C1′, C8a), 139.2 (C4′), 136.7 (Cβ), 129.9 (C2′, C3′, C5′, C6′, C4, C4a), 124.0 (C3), 122.9 (Cα), 118.1 (C8), 105.0 (C5), 56.9 (OCH_3), 56.8 (OCH_3). MS *m/z*: $[M-H]^+$ 387, $[M-OCH_3]^+$ 357, $[M-29]^+$ 356, $[M-77]^+$ 311. Anal. ($C_{20}H_{15}Cl_2NO_3$), C: calcd 62.01; found 62.02; H: calcd 3.91; found 3.90; N: calcd 3.62, found 3.61; Cl: calcd 18.07, found 18.12.

1-(3′,4′-Dimethoxy-phenyl)-3-(2-chloro-6,7-dimethoxy-3-quinolinyl)-2-propen-1-one (40). (042 g, 92%). IR (KBr) 1648 (C=O), 1574 (C=C). ^1H NMR ($CDCl_3$) δ 8.30 (s, 1H, H4), 8.18 (d, 1H, $J=16.0$ Hz, Hβ), 7.91 (d, 2H, $J=2.1$ Hz, H2′, H6′), 7.55 (d, 1H, $J=15.70$ Hz, Hα), 7.35 (s, 1H, H8), 7.10 (s, 1H, H5), 4.0 (s 6H, OCH_3), 3.99 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 188.5 (C=O), 153.1 (C6, C7), 147.8 (C3′), 146.0 (C2), 138.6 (C8a), 138.9 (Cβ), 133.2 (C1), 129.7 (C4′), 125.7 (Cα), 120.7 (C6′), 117.8 (C8), 114.9 (C5′), 112.9 (C2′), 104.7 (C5), 56.6 (2 OCH_3), 55.9 (OCH_3). MS *m/z*: $[M-H]^+$ 387, $[M-OCH_3]^+$ 357, $[M-29]^+$ 356, $[M-77]^+$ 311. Anal. ($C_{22}H_{20}ClNO_5$), C: calcd 63.91, found 63.89; H: calcd 4.88, found 4.90; N: calcd 3.39, found 3.35; Cl: calcd 8.46, found 8.45.

1-(2′,5′-Dimethoxyphenyl)-3-(2-chloro-6,7-dimethoxy-3-quinolinyl)-2-propen-1-one (41). (0.40 g, 90%). IR (KBr) 1651 (C=O), 1609 (C=C). ^1H NMR ($CDCl_3$) δ 8.30 (s, 1H, H4), 7.98 (d, 1H, $J=16.0$ Hz, Hβ), 7.49 (d, 1H, $J=15.70$ Hz, Hα), 7.36 (s, 1H, H8), 7.26 (s, 1H, H6′), 7.12 (s, 1H, H5), 7.03 (dd, 2H, $J=8.2$, 2.2, H3′, H4′), 4.02 (s 6H, OCH_3), 3.86 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 189.8 (C=O), 155.2 (C6, C7), 152.0 (C5′, C6), 153.2 (C2′, C7), 148.6 (C3′), 14 (C2), 138.6 (C8a), 138.9 (Cβ), 134.5 (C1), 129.0 (C4a), 125.83 (Cα), 122.8 (C6′), 119.0 (C8), 105.1 (C5), 56.8 (OCH_3), 56.3 (OCH_3), 56.2 (OCH_3), 56.0 (OCH_3). MS *m/z*: $[M-1]^+$ 413, $[M-29]^+$ 383, $[M-31]^+$ 383, $[M-138]^+$ 275. Anal. ($C_{22}H_{20}ClNO_5$), C: calcd 63.91; found 63.93; H: calcd 4.88, found 4.87; N: calcd 3.39, found 3.38; Cl: calcd 8.46, found 8.47.

Biological Evaluation

Microorganisms and media

The microorganisms used for the fungistatic evaluation were purchased from the American Type Culture Collection (Rockville, MD, USA): *C. albicans* ATCC 10231, *S. cerevisiae* ATCC 9763, *C. neoformans* ATCC 32264, *A. flavus* ATCC 9170, *A. fumigatus* ATCC 26934, *A. niger* ATCC 9029 and *T. mentagrophytes* ATCC 9972. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C. Cell suspensions in sterile distilled water were adjusted to give a final concentration of 10^6 viable yeast cells/mL.[34] Dermatophytes: *M. canis* C 112, *T. rubrum* C 113, *E. floccosum* C 114 and *M. gypseum* C 115 as well as *Candida tropicalis* C131 are clinical isolates and were kindly provided by CEREMIC, Centro de Referencia Micológica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina.

The strains were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Spore suspensions were obtained according to reported procedures[34] and adjusted to 10^6 spores with colony forming ability/mL.

Antifungal assays

The antifungal activity of chalcones was evaluated with the agar dilution method by using Sabouraud-chloramphenicol agar for both yeast and dermatophyte species as previously described.[11,12] Stock solutions of compounds (10 mg/mL in DMSO) were diluted to give serial two-fold dilutions that were added to each medium resulting in concentrations ranging from 0.10 to 50 μg/mL MIC for each compound was defined as the lowest concentration that produces no visible fungal growth after the incubation time.

Enzymatic assays

Strains and culture conditions. The *S. cerevisiae* strain used was MATa *trp1 ura3 leu2 his3 pep4::HIS3 nuc1::LEU2*. Routine yeast growth (YEPD) was as described.[35]

Enzyme preparation. Cell extracts were obtained essentially as described previously.[36] Early logarithmic phase cells grown in 100 mL YEPD medium were collected, washed once with 50 mM Tris–HCl pH 7.5, suspended in 100 μL of the same buffer and broken with glass beads in a FastPrep FP120 apparatus (Savant, BIO 101, Inc.) (once a 15 s pulse at speed of 6.0). Broken material was collected and cell debris was removed by low speed centrifugation ($5000\times g$, 10 min at 4 °C). The supernatant was centrifuged at $18{,}000\times g$ for 30 min at 4 °C and the pellet was resuspended in 50 mM Tris–HCl pH 7.5 containing 33% glycerol (at a concentration of approximately 3 mg protein per mL) and stored at −80 °C. Protein was quantified by the Bradford dye-binding procedure[37] using the Bio-Rad Protein Assay Dye Reagent and bovine serum albumin as standard.

β(1,3)-Glucan synthase assay. β(1,3)-glucan synthase assay was essentially as described previously.[36] The standard assay mixture contained 5 μL enzyme (15 μg protein), in a total volume of 40 μL. Two microliters of methanol or the corresponding compounds (kept in stock solution,

10 mg/mL in methanol at −20 °C) were added to each reaction. The reaction was incubated for 30 min at 30 °C and stopped by addition of 1 mL 10% trichloroacetic acid. All reactions were carried out in duplicate.

The drugs Papulacandin B and Aculeacin A were generous gifts from K. Scheibli and P. Traxler (Novartis, Basel, Switzerland) and K. Mizuno (Tokyo Jozo Co. Ltd., Tagatagun, Shizuoka-ken, Japan), respectively. The antibiotics were kept in stock solution (10 mg/mL in methanol) at −20 °C.

Chitin synthase-1 assay. Chitin synthase-1 assay was performed as described previously.[38] The standard assay mixture contained 10 μL enzyme (30 μg protein), in a total volume of 50 μL. Two microliters of methanol or the corresponding compounds (kept in stock solution, 10 mg/mL in methanol at −20 °C) were added to each reaction. Enzyme activation was performed by partial proteolysis of the reaction mixture with 2 μL trypsin (0.25 μg/μL) during 15 min at 30 °C and stopped by the addition of 2 μL trypsin inhibitor (0.375 μg/μL). The reaction was incubated for 90 min at 30 °C and stopped by addition of 1 mL 10% trichloroacetic acid. All reactions were carried out in duplicate.

Computational methods

Gaussian 98[39] has been used to perform RHF calculations in the compounds of interest. The calculations have been carried out at both RHF/3-21G and RHF/6-31G levels of theory.

In a first step a systematic conformational search using the GASCOS method[40–42] developed by the San Luis group was carried out. The compounds of interest were then modeled by the semi-empirical AM1 method.[43,44] After the structures were determined at RHF/3-21G level of theory, partially relaxed scan calculations were run for every 15° of rotation of the torsional angles ϕ and θ. The minima obtained by the scan for each compound were optimized at RHF/3-21G and RHF/6-31G levels of theory. Frequency calculations performed at the RHF/6-31G level ensured that the critical points given as minima by the optimizations are indeed minima on the potential energy surface.

AM1 and the RHF/3-21G optimized coordinates were imported into PC Spartan.[45] Single point calculations were performed to generate the wave functions, using PC Spartan, thus enabling the display of LUMO and MEP maps.

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