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Inhibitors of the Fungal Cell Wall. Synthesis of 4-Aryl-4-*N*-arylamine-1-butenes and Related Compounds with Inhibitory Activities on β(1–3) Glucan and Chitin Synthases

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Abstract—As part of our project devoted to the search for antifungal agents, which act via a selective mode of action, we synthesized a series of new 4-aryl- or 4-alkyl-*N*-arylamine-1-butenes and transformed some of them into 2-substituted 4-methyl-tetrahydroquinolines and quinolines by using a novel three-step synthesis. Results obtained in agar dilution assays have shown that 4-aryl homoallylamines not possessing halogen in their structures, tetrahydroquinolines and quinolines, display a range of antifungal properties in particular against *Epidermophyton floccosum* and *Microsporum canis*. Regarding the mode of action, all active compounds showed in vitro inhibitory activities against β(1–3) glucan-synthase and mainly against chitin-synthase. These enzymes catalyze the synthesis of β(1–3) glucan and chitin, respectively, major polymers of the fungal cell wall. Since fungal but not mammalian cells are encased in a cell wall, its inhibition may represent a useful mode of action for these antifungal compounds.

Introduction

The incidence of fungal infections especially involving immunocompromised patients has dramatically increased during the last years.[1] Systemic mycoses and some forms of dermatomycoses are very difficult to eradicate and they are the cause of a great mortality in patients receiving antineoplasic chemotherapy, organ transplants or suffering AIDS.[2]

The mode of action of most known antifungal drugs in use today, is the inhibition of some of the steps of ergosterol biosynthesis, which are common to human cholesterol and sexual hormones biosynthesis. As a consequence, many adverse effects have been reported for them[3] and new and safe antifungal agents are still needed.

Since fungal, but not mammalian cells, are encased in a carbohydrate-containing wall, required for the growth and viability of fungi, the fungal cell wall has emerged as a major target for antifungal drugs. As part of a program aimed to identify novel antifungal agents, we focused on developing antifungal compounds that would selectively inhibit the synthesis of the fungal cell wall.[4–7]

Considering that certain 4-arylsubstituted 4-*N*-arylamine-1-butenes (homoallylamines) have showed in vitro antifungal properties against a panel of phytopathogenic fungi[8] and on the basis of our previous experience in synthesizing this type of compound,[8–11] we prepared a series of homoallylamines from the aldimines **1**–**11** (Table 1) in order to evaluate their antifungal properties

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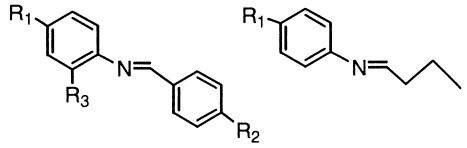
Table 1. Synthetic aldimines used as starter compounds for preparing homoallylamines



A B

Compd	Type	R_1	R_2	R_3
1	A	H	H	H
2	A	CH_3	H	H
3	A	OCH_3	H	H
4	A	F	H	H
5	A	Cl	H	H
6	A	Br	H	H
7	A	H	OCH_3	H
8	A	Cl	$N(CH_3)_2$	H
9	A	H	H	CH_3
10	B	H	—	—
11	B	CH_3	—	—

against human pathogenic fungi and to study the structural requirements for the antifungal activity.

In addition, we demonstrate here, by using a novel synthetic pathway, that homoallylamines could serve as versatile precursors to obtain 2-substituted 4-methyl quinolines via tetrahydroquinolines; both type of compounds with important biological properties.[8,12–15] The possibility of being useful intermediates for synthesizing tetrahydroquinolines and quinolines add interest to homoallylamines which, in contrast with homoallylic alcohols, have not practically been explored in heterocycle synthesis.

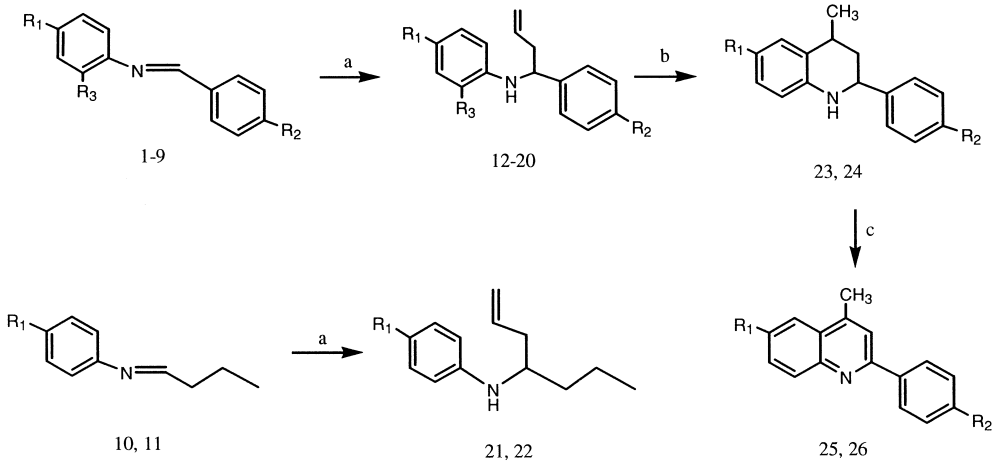
Homoallylamines **12**–**22**, tetrahydroquinolines **23** and **24** and quinolines **25** and **26**, were tested for antifungal properties with the agar dilution method against a panel of standardized dermatophytes and other yeasts and filamentous fungi. Then, to gain insight into the capacity of active compounds to interfere with the biosynthesis of fungal cell wall, these compounds were tested for their inhibitory activity on β(1–3) glucan-synthase and chitin-synthase, enzymes that catalyze the synthesis of β(1–3) glucan and chitin, two major polymers of the fungal cell wall.[16,17]

Results and Discussion

Chemistry

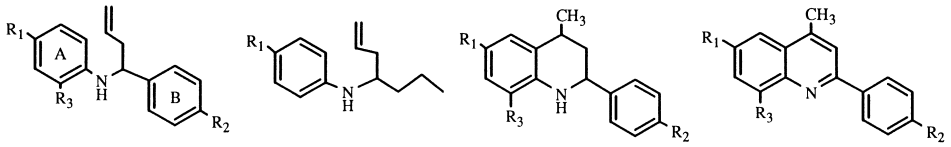
The synthesis of 4-methyl-(4,6-dimethyl)-2-phenyl-quinolines was performed from readily accesible aldimines,[18,19] following Scheme 1. The starting compounds (**1**–**11**; Table 1) were readily prepared in high yield from *p*-substituted anilines and benzaldehydes in following a known procedure.[11] The 4-aryl- or 4-alkyl-4-*N*-arylamino-1-butenes **12**–**22** (Table 2) were obtained (Scheme 1) by addition of allylmagnesium bromide to aromatic aldimines **1**–**11** using the Grignard procedure. Allylmagnesium bromide was prepared from allyl bromide and magnesium in anhydrous Et_2O. To the best of our knowledge, homoallylamines **12**–**22** have not been reported previously. They were isolated as stable yellow oils in 53–98% after distillation under reduced pressure followed by chromatographic technics. Their structure was established using IR, NMR spectroscopy and mass spectrometry. Their IR spectra showed the bands of the amine group in the region of 3401–3416 cm^{-1}. In the ^1H NMR spectra of these compounds the allyl radical protons generated three groups of signals: the multiplet signal of HC= appeared in a zone 5.77 ppm, the protons of $=CH_2$ groups observed in 5.18 ppm as triple-doublets, and the protons of CH_2-group resonated in the region 2.61 ppm as two multiplets (H_A and H_B are diastereotopic).

Then, we performed electrophilic cyclization of selected homoallylamines (**12** and **13**) under acidic conditions (H_2SO_4) and obtained the 4-methyl-(4,6-dimethyl)-2-phenyl-1,2,3,4-tetrahydroquinolines (**23** and **24**). These tetrahydroquinolines were prepared in 58–66% yields as a mixture of *cis*/*trans* isomers[9] and then oxidized



Scheme 1. Preparation of homoallylamines, 4-methyl-1,2,3,4-tetrahydroquinolines and 4-methylquinolines. (a) Allylbromide + Mg/Et_2O, 10 °C; (b) H_2SO_4 75% W/V; (c) DDQ/Bz, 35 °C.

Table 2. MIC values (μg/mL) of homoallylamines, tetrahydroquinolines and quinolines acting against dermatophytes



C D E F

Compd	Type	R_1	R_2	R_3	*M. c*[a]	*M. g*[b]	*T. m*[c]	*T. r*[d]	*E. f*[e]
12	C	H	H	H	30	30	30	30	12.5
13	C	CH_3	H	H	30	>50	>50	>50	3.12
14	C	OCH_3	H	H	30	>50	30	30	3.12
15	C	F	H	H	>50	>50	>50	>50	30
16	C	Cl	H	H	>50	>50	>50	>50	30
17	C	Br	H	H	>50	>50	>50	>50	30
18	C	H	OCH_3	H	30	>50	>50	>50	3.12
19	C	Cl	$N(CH_3)_2$	H	>50	>50	>50	>50	>50
20	C	H	H	CH_3	30	>50	>50	>50	3.12
21	D	H	—	—	>50	>50	>50	>50	>50
22	D	CH_3	—	—	>50	>50	>50	>50	>50
23	E	H	H	H	50	25	25	25	12.5
24	E	CH_3	H	H	50	25	25	25	12.5
25	F	H	H	H	25	12.5	12.5	25	12.5
26	F	CH_3	H	H	0.75	12.5	25	12.5	12.5
Amphotericin B					>50	6.25	6.25	25	0.3
Ketoconazole					15	6.25	12.5	15	25

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

(DDQ, benzene, 35 °C) to known quinolines (**25** and **26**)[20] as outlined in Scheme 1.

Antifungal assays

Homoallylamines **12**–**22**, tetrahydroquinolines **23** and **24**, and quinolines **25** and **26** were evaluated for antifungal properties with the agar dilution method[21] against a panel of human pathogenic dermatophytes and other fungi and yeasts.

Concentrations up to 50 μg/mL of each compound, were incorporated into growth media according to reported procedures.[4]

Results showed that none of the compounds tested displayed any activity against the yeasts *Candida albicans*, *Candida tropicalis*, *Saccharomyces cerevisiae* or *Cryptococcocus neoformans* nor against the filamentous fungi *Aspergillus niger*, *Aspergillus fumigatus* or *Aspergillus flavus* (results not shown). In contrast, different results were obtained for the compounds of the series against dermatophytes. These results are shown in Table 2.

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 *E. floccosum*.

Concerning the spectra of action of homoallylamines tested, only the non-substituted structure **12** displayed significant activity (MICs <50 μg/mL) against all the tested dermatophytes. Compound **14**, with an OCH_3 on ring A, showed broad activity too, inhibiting all fungi except *Microsporum gypseum*. Nevertheless, it is interesting to note that when OCH_3 changes from ring A to B (Compounds **14**→**18**) the spectrum of action narrows down, being compound **18** active only against *M. canis* and *E. floccossum*. This same range of inhibition was observed with compounds **13** and **20**, both having methyl groups on different positions of ring A.

Within the activity shown by homoallylamines against the most sensitive fungus *E. floccosum,* variations in the electronic properties appear to affect the antifungal activities. Structures containing halogen atoms (compounds **15**–**17** and **19**) were drastically less active than the non-substituted analogue (compound **12**). The presence of methyl or methoxyl groups bound to benzene rings (A or B) enhance up to four times the activity of these compounds relative to the non-substituted analogue (compare activities of compounds **13**, **14**, **18**, **20** (MICs = 3.12 μg/mL) and **12** (MIC = 12.5 μg/mL). Position changes of the methyl substituent within the same ring (compounds **13** and **20**), do not produce changes in the fungistatic action (MICs = 3.12 μg/mL). In addition, structures containing an aliphatic chain instead of the benzene ring B, do not show activity (compounds **21** and **22**) suggesting that the phenyl group in C-4 is necessary for activity in homoallylamines.

In order to add more information about activities of homoallylamines, we cycled compounds **12** and **13** into

tetrahydroquinolines **23** and **24** and then oxidized them to quinolines **25** and **26**.

Results obtained in the agar dilution assays showed that cyclic compounds **23** and **25** displayed the same activity as non-substituted compound **12** against *E. floccosum*. However, the cyclization of the methyl derivative **13** (MIC = 3.12 μg/mL) diminished four times the inhibitory activity (MICs = 12.5 μg/mL for compounds **24** and **26**). Analyzing the results obtained in the remaining fungi tested, the most striking result was that with compound **26**. It displays an activity 66-fold higher than its non-aromatic analogue **24** (MICs = 0.75 and 50 μg/mL, respectively) and 40-fold higher than homoallylamine **13**. In addition, compound **26** was active only against *M. canis*, which was scarcely inhibited by all the other compounds tested.

To gain insight into the mode of action of the most active antifungal compounds, we tested them for their capacity of inhibiting in vitro either β(1–3) glucan synthase[5,17] or chitin synthase 1 activities.[22] Results of the in vitro assays are listed in Table 3. Regarding activity against β(1–3) glucan synthase, all homoallylamines tested (compounds **12**–**14**, **18** and **20**) exhibited a moderate inhibitory activity at 20 μg/assay (% of inhibition ranging from 25 to 50%). Their cyclic derivatives, tetrahydroquinoline **23** and quinoline **25** showed inhibitory activities between 42 and 60%.

Since compounds **12**, **13**, **20**, **23** and **25** displayed a β(1–3) glucan synthase inhibition ⩾50%, five serial dilutions of them (1–20 μg) were tested for enzyme inhibition and their average inhibitory concentration calculated (Fig. 1). Among them, homoallylamine **12** and its derivatives **23** and **25** showed the most potent effects with similar IC_{50} values (0.25, 0.23 and 0.23 μg/μL, respectively). These values are lower than the well-known, β(1–3) glucan synthase inhibitor Aculeacin A (IC_{50} ⩾ 0.5 μg/μL).

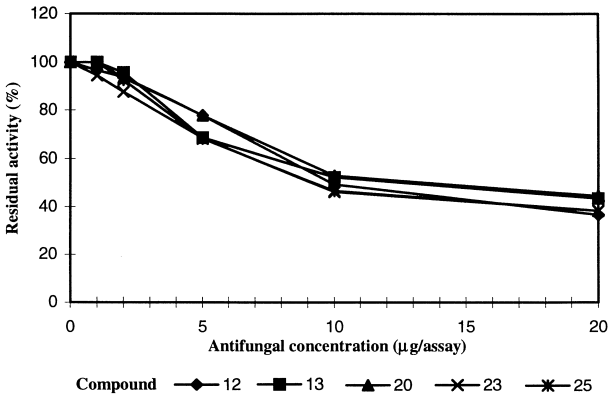


Figure 1. Effect of different concentrations of antifungal homoallylamines, 4-methyl 1,2,3,4-tetrahydroquinolines and 4-methylquinolines on in vitro incorporation of [^{14}C]-glucose into insoluble β(1—3) glucan expressed as residual activity of the enzyme (% incorporation compared to the incorporation in the absence of antifungal compounds). Points are the mean of duplicate tests.

Results obtained in the chitin synthase-1 assay, showed that 20 μg of antifungal compounds **12**–**14**, **18**, **20**, **23**–**26** possess a strong inhibitory effect on enzyme activity (% of inhibition from 50 to 88% (Table 3)). All homoallylamines tested displayed IC_{50} <0.20 μg/μL, with quinolines **25** and **26** as the most active inhibitors (IC_{50} = 0.02 and 0.04 μg/μL, respectively (Fig. 2)).

The results here reported allow us to infer that the assayed homoallylamines, tetrahydroquinolines and quinolines would act by inhibiting the biosynthesis of the two major polymers of fungal wall as one of their possible mode of action.

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

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

	Glucan synthase assay		Chitin synthase assay	
Compd	% I[a]	IC_{50}[b]	% I[c]	IC_{50}[b]
12	60.10 ± 4.36	0.25	87.07 ± 1.04	0.09
13	53.35 ± 3.27	0.32	56.36 ± 2.57	0.19
14	25.34 ± 5.78	>0.50	68.42 ± 1.02	0.15
18	36.02 ± 4.63	>0.50	75.26 ± 0.65	0.07
20	51.67 ± 3.94	0.33	70.01 ± 1.77	0.17
23	53.48 ± 4.90	0.23	73.41 ± 0.10	0.10
24	41.64 ± 7.16	>0.50	49.75 ± 0.28	0.40
25	59.46 ± 9.20	0.23	87.90 ± 0.88	0.02
26	34.32 ± 3.08	>0.50	84.11 ± 0.50	0.04
Papulacandin B		0.10		
Aculeacin A		>0.50		
Nikkomicin				0.0006[d]

[a]% of inhibition at 20 μg/assay (total volume: 40 μL): mean ± SEM.
[b]Concentration (μg/μL) that produces 50% of inhibition.
[c]% of inhibition at 20 μg/assay (total volume: 50 μL): mean ± SEM.
[d]Value obtained from ref 29.

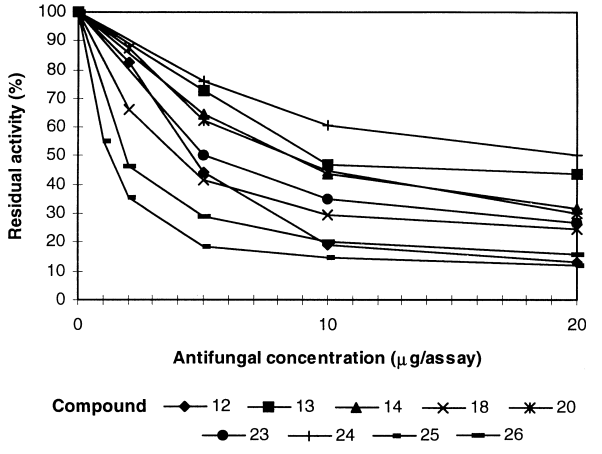


Figure 2. Effect of different concentrations of antifungal homoallylamines, 4-methyl-1,2,3,4-tetrahydroquinolines and 4-methylquinolines on the in vitro chitin synthase 1 activity, expressed as residual activity of the enzyme (see legend of Fig. 1 for details).

Conclusions

In an attempt to generate lead structures that might have selective antifungal activities, we have synthesized a series of aryl and alkyl homoallylamines from aldimines **1**–**11**. Then, we transformed some of them into 4-methylquinolines **25**–**26** via the 4-methyl-1,2,3,4-tetrahydroquinolines **23** and **24** by using a novel synthetic pathway, showing that homoallylamines could serve as useful precursors for this type of biologically important compounds. Agar dilution assays with human pathogenic fungi have shown that structures **12**–**14**, **18** and **20** possess potent antifungal properties in particular against *E. floccosum*. Tetrahydroquinolines **23** and **24** and quinolines **25** and **26** also display antifungal activities; compound **26** being particularly active against *M. canis*. Since dermatophytes are a group of highly specialized fungi which characteristically infect the keratinized areas of the body and dermatophytoses are extremely common and very difficult to eradicate, it is interesting to note that 4-aryl homoallylamines and their derivatives, display similar or stronger antifungal activities than Amphotericin B or Ketoconazole on the species tested in our experiments.

Regarding the mode of action, active compounds **12**–**14**, **18**, **20** and **23**–**26** showed inhibition against β(1–3) glucan synthase and chitin synthase activities. The fact that these compounds inhibit the synthesis of both, glucan and chitin at the same time is particularly attractive. We should keep in mind that combinations of chitin synthase and glucan synthase inhibitors are usually required.[23]

The antifungal activity of homoallylamines, tetrahydroquinolines and quinolines against some dermatophytes, combined with their selective mode of action, make these compounds attractive leaders for the development of most potent and mainly safer antifungal drugs.

Experimental

Chemistry

All reagents were purchased from Aldrich, commercial grade. IR spectra were recorded on a Perkin–Elmer 599B-FT spectrometer. ^{1}H NMR spectra were determined on a Jeol 400 MHz in $CDCl_3$ with tetramethylsilane (TMS, δ 0 ppm) as internal standard. ^{13}C NMR spectra were recorded with $CDCl_3$ as an internal standard at δ = 77.0 at 100 MHz on a Jeol spectrometer. MS spectra were obtained with an HP 5890 series II gas chromatograph interfaced to an HP 5972 mass selective detector that used electron impact ionization (70 eV). Elemental analyses were performed on a Leco CHN-600 analyzer. Column chromatography was carried out on columns packed with SiO_2.

The aldimines were prepared according to the literature.[18] The solid products were obtained with nearly quantitative yield and confirmed by IR spectra.

4-Aryl or 4-alkyl-4-*N*-arylamino-1-butenes (12–22). General procedure. The aldimines (**1**–**11**) (32 mmol) dissolved in 25 mL of Et_2O were added slowly to a magnetically stirred suspension of allyl magnesium bromide prepared from allyl bromide (95 mmol, 11.45 g) and magnesium (158 mmol, 3.84 g) in 100 mL Et_2O, at 10 °C. The mixture was heated to 30–35 °C during 4 h, cooled to 0 °C and treated with a NH_4Cl solution (pH 8–9). Two liquid–liquid extractions with Et_2O (50 mL each) were performed. The organic layers were combined and dried over anhydrous $MgSO_4$. The residue from ether evaporation was purified by column chromatography over silica to give products **12**–**22**, which were obtained as yellow oils as follows:

12. Yield 58%. IR (film): ν 3413 cm^{-1} (s, NH); ^{1}H NMR ($CDCl_3$): δ 2.51 (1H, m, H_2C–C=), 2.63 (1H, m, H_2C–C=), 4.16 (1H, br. s, H–N), 4.42 (1H, dd, H–C–Ar), 5.18 (2H, td, H_2C=), 5.77 (1H, m, H–C=), 6.52 (2H, d, *o*-Ph–N), 6.67 (1H, t, *p*-Ph-N), 7.10 (2H, t, *m*-Ph–N), 7.26 (1H, t, *p*-Ph–C), 7.35 (2H, t, *m*-Ph–C), 7.36 (2H, d, *o*-Ph-C); ^{13}C NMR ($CDCl_3$): δ 43.40, 57.23, 113.57, 117.46, 118.39, 126.39, 126.92, 128.67, 129.16, 134.74, 143.64, 147.43; MS *m*/*z* (EI): 223 (M+, 3%), 182 (M−C_3H_5, 100%). Found: C, 86.00; H, 7.81; N, 6.19; calcd for $C_{16}H_{17}N$: C, 86.05; H, 7.67; N, 6.27.

13. Yield 53%. IR (film): ν 3411 cm^{-1} (s, NH); ^{1}H NMR ($CDCl_3$): δ 2.55 (1H, m, H_2C–C), 2.62 (1H, m, H_2C–C=), 3.95 (1H, br. s, H–N), 4.36 (1H, dd, H–C–Ar), 5.17 (2H, td, H_2C=), 5.76 (1H, m, H–C=), 6.42 (2H, d, *o*-Ph–N), 6.90 (2H, d, *m*-Ph–N), 7.23 (1H, t, *p*-Ph–C), 7.35 (2H, t, *m*-Ph–C), 7.36 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ 20.44, 43.44, 57.45, 113.66, 118.32, 126.37, 126.58, 126.99, 128.64, 129.86, 134.85, 143.84, 145.0; MS *m*/*z* (EI): 237 (M+, 4%), 196 (M−C_3H_5, 100%). Found: C, 85.90; H, 8.18; N, 5.82; calcd for $C_{17}H_{19}N$: C, 86.03; H, 8.07; N, 5.90.

14. Yield 78%. IR (film): ν 3401 cm^{-1} (s, NH); ^{1}H NMR ($CDCl_3$): δ 2.48 (1H, m, H_2C–C=), 2.60 (1H, m, H_2C–C=), 3.94 (1H, br. s, H–N), 4.31 (1H, dd, H–C–Ar), 5.17 (2H, td, H_2C=), 5.78 (1H, m, H–C=), 6.46 (2H, d, *o*-Ph–N), 6.46 (2H, d, *m*-Ph–N), 7.25 (1H, t, *p*-Ph–C), 7.35 (2H, t, *m*-Ph–C), 7.35 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ 43.54, 55.81, 57.99, 114.72, 114.79, 118.36, 126.43, 127.03, 128.66, 134.90, 141.71, 143.93, 152.03; MS *m*/*z* (EI): 253 (M+, 7%), 212 (M−C_3H_5, 100%). Found: C, 80.53; H, 7.80; N, 5.25; calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53.

15. Yield 74%. IR (film): ν 3414 cm^{-1} (s, NH); ^{1}H NMR ($CDCl_3$): δ 2.50 (1H, m, H_2C–C=), 2.63 (1H, m, H_2C–C=), 4.12 (1H, br. s, H–N), 4.35 (1H, dd, H–C–Ar), 5.22 (2H, td, H_2C=), 5.80 (1H, m, H–C=), 6.46 (2H, dd, *o*-Ph–N), 6.82 (2H, t, *m*-Ph–N), 7.24 (1H, dd, *p*-Ph–C), 7.36 (2H, t, *m*-Ph–C), 7.38 (2H, d, *o*-Ph-C); ^{13}C NMR ($CDCl_3$): δ 43.51, 57.77, 114.4, 115.5, 118.57, 126.41, 127.22, 128.95, 134.73, 144.0, 148.0, 160.3; MS *m*/*z* (EI): 241 (M+, 4%), 200 (M−C_3H_5, 100%). Found: C, 79.50; H, 6.95; N, 5.65; calcd for $C_{16}H_{16}FN$: C, 79.64; H, 6.68; N, 5.80.

16. Yield 72%. IR (film): ν 3416 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 2.50 (1H, m, H_2C–C=), 2.63 (1H, m, H_2C–C=), 4.19 (1H, br. s, H–N), 4.35 (1H, dd, H–C–Ar), 5.20 (2H, td, H_2C=), 5.76 (1H, m, H–C=), 6.45 (2H, d, *o*-Ph–N), 7.05 (2H, d, *m*-Ph–N), 7.20 (1H, d, *p*-Ph–C), 7.38 (2H, dd, *m*-Ph–C), 7.52 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ 43.15, 57.05, 114.48, 118.47, 126.15, 126.3, 127.17, 128.60, 128.77, 134.33, 142.91, 145.74; MS *m*/*z* (EI): 257 (M+, 4%), 216 (M−C_3H_5, 100%). Found: C, 74.80; H, 6.10; N, 5.30; calcd for $C_{16}H_{16}ClN$: C, 74.55; H, 6.26; N, 5.43.

17. Yield 60%. IR (film): ν 3414 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 2.55 (1H, m, H_2C–C=), 2.69 (1H, m, H_2C–C=), 4.29 (1H, br. s, H–N), 4.42 (1H, dd, H–C–Ar), 5.25 (2H, td, H_2C=), 5.83 (1H, m, H–C=), 6.45 (2H, d, *o*-Ph–N), 7.23 (2H, d, *m*-Ph–N), 7.32 (1H, t, *p*-Ph–C), 7.33 (2H, t, *m*-Ph–C), 7.41 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ = 43.38, 57.22, 109.19, 115.29, 118.77, 126.43, 127.37, 128.89, 131.94, 134.57, 143.08, 146.43; MS *m*/*z* (EI): 301 (M+, 5%), 260 (M−C_3H_5, 100%). Found: C, 63.08; H, 5.60; N, 4.51; calcd for $C_{16}H_{16}BrN$: C, 63.59; H, 5.34; N, 4.63.

18. Yield 85%. IR (film): ν 3411 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 2.54 (1H, m, H_2C–C=), 2.64 (1H, m, H_2C–C=), 4.19 (1H, br. s, H–N), 4.41 (1H, dd, H–C–Ar), 5.23 (2H, td, H_2C=), 5.83 (1H, m, H–C=), 6.56 (2H, d, *o*-Ph–N), 6.71 (1H, t, *p*-Ph–N), 6.92 (2H, d, *m*-Ph–C), 7.15 (2H, t, *m*-Ph–N), 7.35 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ 43.54, 55.35, 56.69, 113.64, 114.12, 117.45, 118.31, 127.48, 129.21, 134.93, 135.66, 147.56, 158.69; MS *m*/*z* (EI): 253 (M+, 2%), 212 (M−C_3H_5, 100%). Found: C, 80.52; H, 7.70; N, 5.28; calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53.

19. Yield 77%. IR (film): ν 3415 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 2.60 (1H, m, H_2C–C=), 2.70 (1H, m, H_2C–C=), 4.26 (1H, br. s, H-N), 4.40 (1H, t, H–C–Ar), 5.28 (2H, t, H_2C=), 5.90 (1H, m, H–C=), 6.54 (2H, d, *m*-Ph–C), 6.83 (2H, d, *o*-Ph–N), 7.15 (2H, d, *m*-Ph–N), 7.32 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ 40.79, 43.48, 56.88, 112.95, 114.84, 118.25, 121.82, 127.21, 129.06, 130.76, 135.15, 146.96, 149.96; MS *m*/*z* (EI): 259 (M−C_3H_5, 100%). Found: C, 71.60; H, 7.25; N, 9.44; calcd for $C_{18}H_{21}ClN_2$: C, 71.87; H, 7.04; N, 9.31.

20. Yield 98%. IR (film): ν 3431 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 2.49 (1H, m, H_2C–C=), 2.61 (1H, m, H_2C–C=), 4.07 (1H, br. s, H–N), 4.39 (1H, dd, H–C–Ar), 5.18 (2H, td, H_2C=), 5.77 (1H, m, H–C=), 6.28 (2H, d, *o*-Ph–N), 6.58 (1H, t, *m*-Ph–N), 6.91 (1H, t, *p*-Ph–N), 7.02 (1H, d, *m*-Ph–N), 7.20 (2H, t, *m*-Ph–C), 7.29 (1H, t, *p*-Ph–C), 7.34 (2H, d, *o*-Ph–C); ^{13}C NMR ($CDCl_3$): δ 17.77, 43.93, 57.12, 117.15, 118.62, 122.18, 126.43, 127.19, 128.84, 130.15, 135.21, 143.92, 145.44; MS *m*/*z* (EI): 237 (M+, 5%), 196 (M−C_3H_5, 100%). Found: C, 85.85; H, 8.10; N, 5.82; calcd for $C_{17}H_{19}N$: C, 86.03; H, 8.07; N, 5.90.

21. Yield 66%. IR (film): ν 3407 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 0.98 (3H, t, -CH_3), 1.49 (4H, m, -$(CH_2)_2$-), 2.30 (2H, m, H_2C–C=), 3.49 (1H, t, HC–N), 3.57 (1H, br. s, H–N), 5.14 (2H, d, H_2C=), 5.87 (1H, m, H–C=), 6.63 (2H, d, *o*-Ph–N), 6.72 (1H, t, *p*-Ph–N), 7.21 (2H, t, *m*-Ph–N); ^{13}C NMR ($CDCl_3$): δ 14.32, 19.40, 36.79, 38.71, 52.20, 113.26, 116.95, 117.63, 129.42, 135.12, 147.92; MS *m*/*z* (EI): 189 (M+, 7%), 148 (M−C_3H_5, 100%). Found: C, 82.38; H, 10.20; N, 7.52; calcd for $C_{13}H_{19}N$: C, 82.48; H, 10.12; N, 7.40.

22. Yield 43%. IR (film): ν 3411 cm^{-1} (s, NH); ^1H NMR ($CDCl_3$): δ 0.97 (3H, t, CH_3), 1.49 (4H, m, -$(CH_2)_2$-), 2.07 (2H, m, H_2C–C=), 2.26 (3H, s, CH_3–Ph), 3.43 (1H, t, H–C–N), 3.67 (1H, br. s, H–N), 5.13 (2H, d, H_2C=), 5.82 (1H, m, H–C=), 6.55 (2H, d, *o*-Ph–N), 7.02 (2H, d, *m*-Ph–N); ^{13}C NMR ($CDCl_3$): δ 14.28, 19.38, 20.50, 36.81, 38.69, 52.54, 113.48, 117.45, 126.7, 129.89, 135.72, 145.64; MS *m*/*z* (EI): 203 (M+, 8%), 162 (M−C_3H_5, 100%). Found: C, 82.64; H, 10.46; N, 6.95; calcd for $C_{14}H_{21}N$: C, 82.70; H, 10.41; N, 6.89.

Synthesis of tetrahydroquinolines (23 and 24). General method. Sulfuric acid 75% w/v (2.0 mL) was added to 1.0 g of the allylamines (**12** and **13**) and the mixture was heated to 60 °C for 8–12 h while stirring vigorously. The reaction progress was monitored via TLC (heptane, chromatoplates of Silufol UV254). After the reaction was completed, the mixture was cooled down to room temperature and a concentrated ammonium hydroxide solution was added (pH 8–9). Two 30 mL extractions with Et_2O were performed. The organic layers were combined and dried with anhydrous Na_2SO_4. The residue after ether evaporation was purified by column chromatography over silica to give products **23** and **24** as yellow oils as follows.

23. Yield 58%. IR (film): ν 3383 cm^{-1} (s, NH); MS *m*/*z* 223 (M^+, 100%), having similar properties to those reported.[9]

24. Yield 66%. IR (film): ν 3373 cm^{-1} (s, NH); ^1H NMR: δ 1.36 (3H, d, 6.95 Hz, 4-CH_3), 1.78 (1H, dd, 3-H_{ax}), 2.12 (1H, ddd, 3-H_{eq}), 2.28 (3H, s, 6-CH_3), 3.13 (1H, m, 4-H), 3.85 (1H, s, N-H), 4.46 (1H, d, 2-H), 6.46 (1H, d, 8-H), 6.84 (1H, d, 7-H), 7.32 (1H, t, *p*-Ph), 7.38 (2H, t, *m*-Ph), 7.44 (2H, t, *o*-Ph); ^{13}C NMR ($CDCl_3$): δ 20.35, 20.78, 31.39, 41.94, 57.19, 114.37, 126.37, 127.52, 128.69, 142.52, 144.78. MS *m*/*z* (EI) 237 (M^+, 100%). Found: C, 85.85 H, 8.22; N, 5.85; calcd for $C_{17}H_{19}N$: C, 86.03; H, 8.07; N, 5.90.

Synthesis of quinolines (25 and 26). General method. To a solution of 1.0 mmol of the tetrahydroquinolines **23** or **24** dissolved in 30 mL of benzene, 2.1 mmol of DDQ dissolved in benzene were added slowly. The reaction mixture was stirred at room temperature for 4 h, monitoring progress via TLC (heptane:ethylacetate (50:1), chromatoplates of Silufol UV254). At the end of the reaction the mixture was filtered (solid product is DDHQ). The benzene was distilled; the residue was purified by column chromatography over silica (with heptane:ethyl acetate, 50:1) to yield quinolines **25** and **26** as yellow oils as follows.

25. Yield 59%. ^1H NMR: δ 2.74 (3H, s, 4-CH_3), 7.47 (1H, t, *p*-Ph), 7.53 (2H, t, *m*-Ph), 7.69 (1H, s, 3-H), 7.73

(2H, d, *o*-Ph), 7.97 (1H, d, 8-H), 8.14–8.23 (3H, m, 5–7-H); ^{13}C NMR ($CDCl_3$): δ 19.13, 119.87, 123.73, 126.12, 127.34, 127.65, 128.68, 128.89, 129.31, 129.45, 130.35, 139.91, 144.93, 148.20, 157.17. MS *m*/*z* (EI) 219 (M^+, 100%). Found: C, 87.57; H, 6.10; N, 6.27; calcd for $C_{16}H_{13}N$: C, 87.64; H, 5.98; N, 6.39.

26. Yield 34%. ^1H NMR: δ 2.57 (3H, s, 6-CH_3), 2.74 (3H, s, 4-CH_3), 7.43 (1H, t, *p*-Ph), 7.51 (2H, t, *m*-Ph), 7.52 (2H, d, *o*-Ph), 7.55 (1H, s, 5-H), 7.68 (1H, s, 3-H), 8.05 (1H, d, 7-H), 8.12 (1H, d, 8-H); MS *m*/*z* (EI) 233 (M^+, 100%). Found: C, 87.35; H, 6.70; N, 5.90; calcd for $C_{17}H_{15}N$: C, 87.52; H, 6.48; N, 6.00.

Biological Evaluation

Microorganisms and media

The following microorganisms used for the fungistatic evaluation were purchased from American Type Culture Collection (Rockville, MD): *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 *Trichophyton 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.[24] Dermatophytes: *M. canis* C 112, *Trichophyton rubrum* C 113, *E. floccosum* C 114 and *M. gypseum* C 115 are clinical isolates and were kindly provided by CEREMIC, Centro de Referencia Micológica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina.

Organisms 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[24] and adjusted to 10^6 spores with colony forming ability/mL.

Antifungal assays

The antifungal activity of homoallylamines was evaluated with the agar dilution method by using Sabouraud-chloramphenicol agar for both yeast and dermatophyte species. The assay was carried out in 96-well microtiter plates. Stock solutions of compounds in DMSO were diluted to give serial 2-fold dilutions that were added to each medium resulting in concentrations ranging from 0.10 to 50 μg/mL. The final concentration of DMSO in the assay did not exceed 2%. Five μL of yeast cell or spore suspensions were added to each Sabouraud-chloramphenicol agar well. The antifungal agents Ketoconazole (Janssen Pharmaceutica) and Amphotericin B (Sigma Chemical Co.) were included in the assay as positive controls. Drug-free solution was also used as blank control. The plates were incubated 24, 48, or 72 h at 30 °C (according to the control fungus growth) and up to 15 days for dermatophyte strains. MIC was defined as the lowest compound concentration, showing no visible fungal growth after incubation time.

Enzymatic assays

Strains and culture conditions. The *S. cerevisiae* strain used was MATa *ura3 leu2 his3 pep4::HIS3 nuc1::LEU2*. Routine yeast growth (YES)[25] was as described.

Enzyme preparation. Cell extracts were obtained essentially as described previously.[26]

Early logarithmic phase cells grown in 100 mL YES 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). Broken material was collected and cell debris was removed by low speed centrifugation (5000 *g*, 5 min at 4 °C). The supernatant was centrifuged at 48,000 *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 3 mg protein per mL) and stored at −70 °C. Protein was quantified by the Bradford dye-binding procedure[27] 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.[26] The standard assay mixture contained 5 μL enzyme (15 μg protein), in a total volume of 40 μL. Two μL of ethanol or the corresponding compounds (kept in stock solution, 10 mg/mL in ethanol at −20 °C) were added to each reaction. The reaction was incubated for 1 h 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 (Ciba-Geigy, 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.[28] The standard assay mixture contained 10 μL enzyme (30 μg protein), in a total volume of 50 μL. Two μL of ethanol or the corresponding compounds (kept in stock solution, 10 mg/mL in ethanol at −20 °C) were added to each reaction. Enzyme activation was performed by partial proteolysis of the reaction mixture with 2 μL trypsin (0.166 μ g/μL) during 15 min at 30 °C and stoped by the addition of 2 μL tripsin inhibitor (0.25 μg/μL). The reaction was incubated for 1 or 2 h at 30 °C and stopped by addition of 1 mL 10% trichloroacetic acid. All reactions were carried out in duplicate.

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