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Synthesis and antifungal activity of diverse C-2 pyridinyl and pyridinylvinyl substituted quinolines

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ABSTRACT

Diverse 2-pyridinyl quinolines **6–12** and 2-pyridinilvinyl quinolines **13–17** were prepared using a straightforward synthesis based on the $BiCl_3$-catalyzed multicomponent imino Diels–Alder (imino DA) reaction or a novel tandem imino DA/catalytic tetrahydroquinoline ring oxidation/Perkin condensation sequential process. All members of the series showed activities against dermatophytes and some of them possessed a broad spectrum of action. 2-(Pyridin-4-yl)quinoline **9** and 2-(2-pyridin-4-yl)vinyl)quinoline **16** showed the best MIC_{80} and MIC_{50} against the clinically important fungi *Candida albicans* and *non-albicans Candida* species. In turn, 6-ethyl-2-(pyridin-2-yl)quinoline **6** showed the best properties against standardized as well as clinical strains of *Cryptococcus neoformans*.

1. Introduction

In the last years, fungi have emerged as major cause of human infections especially among immunocompromised hosts having an enormous impact on morbidity and mortality.[1] Although there are diverse available drugs for the treatment of systemic and superficial mycoses, they are not completely effective for their eradication.[2,3] In addition, they all possess a certain degree of toxicity and quickly develop resistance due to the large-scale use.[4] There is, therefore, an urgent need for new antifungal chemical structures alternatives to the existing ones.[5]

Compounds with a quinoline skeleton are considered attractive scaffolds to develop new antifungal agents. So, 8-hydroxyquinolines, 2-aryl- or styryl- quinolines showed potent antifungal activity.[6–9] These quinoline derivatives are low molecular weight heterocyclic aromatic molecules easily obtained by straightforward syntheses. The course of our ongoing screening program for new biologically important N-heterocycles is based on the "one-pot" imino Diels–Alder reaction, using commercially available arylamines and aldehydes as principal starting materials, that allows high degrees of structural diversification, in the construction of diverse molecular quinoline library.[10]

Herein, we report the synthesis of a new series of diverse 2-pyridinyl and pyridinylvinyl quinoline derivatives related to quinolines that have previously showed interesting biological activities.[11–16] These C2-substituted quinolines were tested for antifungal properties against standardized as well clinically important fungi including *Cryptococcus neoformans*, several species of *Candida* and *Aspergillus* genus, and dermatophytes.

2. Results and discussion

2.1. Chemistry

2-Pyridinyl quinolines **6–12** were prepared using a straightforward synthesis based on the $BiCl_3$-catalyzed multicomponent imino Diels–Alder (imino DA) reaction among substituted anilines **1**, ethyl vinyl ether **2** and isomeric α- and β- or γ-pyridinecarboxaldehydes **3** in refluxing MeCN. These quinolines were obtained as stable solids in 35–47% yield after their chromatography purification (Scheme 1, Route a).

The syntheses of the 2-(pyridinylvinyl)quinoline analogs **13–17** were accomplished through a novel tandem imino DA/catalytic tetrahydroquinoline ring oxidation/Perkin condensation sequential process (Scheme 1, Route b), which starts with a cycloaddition reaction between substituted anilines **1** and ethyl vinyl ether **2**. This first step was produced by the *o*-phthalic acid-catalyzed

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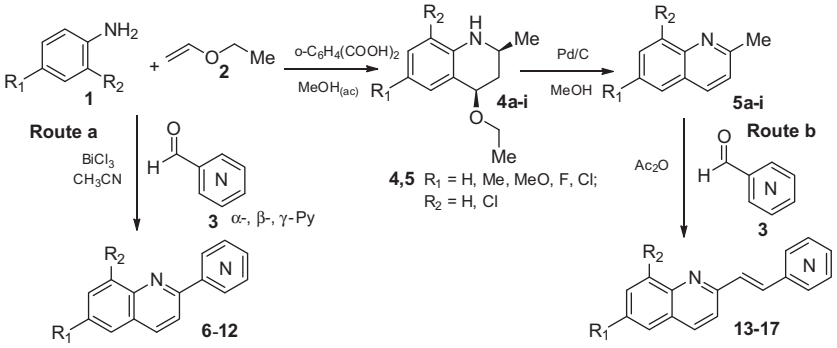


Scheme 1.

Table 1
Physico-chemical data of 2-pyridinylquinolines **6–12** and (*E*)-2-(pyridinylvinyl)quinolines **13–17**

Compd	Group	R_1	R_2	N	MF	MW	Mp (°C)	Yield (%)
6	**A**	C_2H_5	H	α	$C_{16}H_{14}N_2$	234.30	148–150	42
7	**A**	Cl	H	β	$C_{14}H_9ClN_2$	240.69	150–152	36
8	**A**	F	H	β	$C_{14}H_9FN_2$	224.23	162–164	38
9	**A**	H	H	γ	$C_{14}H_{10}N_2$	206.24	148–150	40
10	**A**	CH_3	H	γ	$C_{15}H_{12}N_2$	220.27	82–84	47
11	**A**	Cl	H	γ	$C_{14}H_9ClN_2$	240.69	138–140	39
12	**A**	H	Cl	γ	$C_{14}H_9ClN_2$	240.69	176–178	35
13	**B**	F	H	α	$C_{16}H_{11}FN_2$	250.27	oil	47
14	**B**	H	H	β	$C_{16}H_{12}N_2$	232.28	173–175	45
15	**B**	Cl	H	β	$C_{16}H_{11}ClN_2$	266.72	177–179	40
16	**B**	H	H	γ	$C_{16}H_{12}N_2$	232.28	169–172	43
17	**B**	Cl	H	γ	$C_{16}H_{11}ClN_2$	266.72	180–181	41

MF: Molecular formula; MW: Molecular weight; Mp: Melting point.

domino Povarov reaction in aqueous methanol that conducted to the formation of the 4-ethyloxy-2-methyl-1,2,3,4-tetrahydroquinoline derivatives **4a–i**. Their catalytic aromatization (10% Pd/C)[11] allowed to prepare the corresponding 2-methylquinolines **5** substituted at the arene part of quinoline ring. The last step of the synthetic scheme to 2-pyridinylvinyl quinolines **13–17** was a Perkin condensation between compound **5** and pyridinecarboxaldehydes **3** (reflux in Ac_2O). After removing the solvent by simple distillation, the crude products were subjected to chromatographic procedures, yielding pure (*E*)-2-(pyridinylvinyl)quinolines **13–17** in 40–47%.

All prepared quinoline derivatives are low molecular weight rigid and stable molecules, easily soluble in organic solvents (Table 1). The 12 compounds have been grouped according to the C-2 substituent in group **A** which contains 2-pyridinyl quinolines **6–12** and group **B** which includes 2-pyridinylvinyl quinolines **13–17**.

2.2. Antifungal assays

Quinolines **6–17** were submitted to antifungal assays (Table 2). To carry out the antifungal evaluation, concentrations of compounds up to 250 μg/mL were incorporated to growth media according to the CLSI standardized procedures.[17,18] Amphotericin B, terbinafine, and ketoconazole were used as positive controls. Table 2 summarizes the minimum concentration of compounds that completely inhibited the growth (MIC_{100}) of nine opportunistic pathogenic fungi including yeasts (*Candida albicans, C. neoformans, Saccharomyces cerevisiae*), hialohyphomycetes (*Aspergillus* spp.) as well as dermatophytes (*Microsporum* and *Trichophyton* spp.). In addition, the minimum concentration of compound that kills the fungi (Minimum Fungicide Concentration, MFC) was recorded in Table 2. The structure of each quinoline derivative was included in Table 2 too, for the sake of an easier analysis of results.

From results of Table 2, it is clearly observed that seven compounds**6, 9, 10, 13, 14, 16** and **17** show the broadest spectrum of action inhibiting the whole fungal panel. Although the activity is moderate compared to the standard drugs, it is important to highlight that they inhibit (and in some cases kill) strains of the clinically important fungi *C. neoformans* and *C. albicans*.

Regarding *C. neoformans* this fungus remains an important life-threatening complication for immunocompromised hosts, particularly for patients who have undergone transplantation of solid organs. Therefore, new compounds acting against this fungus are highly welcome.[19,20]

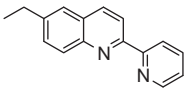
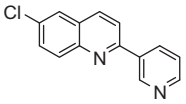
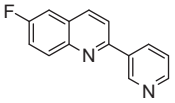
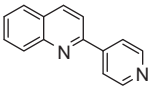
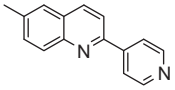
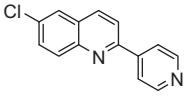
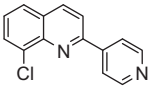
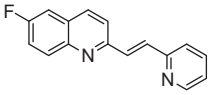
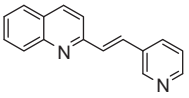
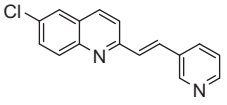
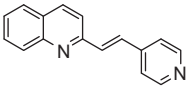
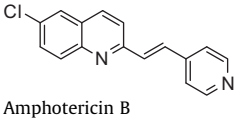
Respecting species of the *Candida* genus, it is known that they are among the leading causes of nosocomial blood stream infections worldwide and, although *C. albicans* was in the past the usual species associated to invasive infections, at present *non-albicans Candida* spp. such as *Candida tropicalis*, *Candida glabrata*, *Candida parapsilopsis*, *Candida krusei* and others, comprise more than half of the isolates of candidiasis in human beings.[21]

Compounds**6, 9, 10, 13, 14, 16** and **17** showed moderate to low activities against all fungi tested (Table 2). However, it is important to take into account that these MIC values refer to the minimum concentration necessary to inhibit 100% of the fungal growth. If, as recommended by CLSI, less stringent end-points such as MIC_{80} or MIC_{50} (the minimum concentration of compounds that inhibit 80 and 50% of growth, respectively) are determined, lower minimum inhibitory concentrations for each compound would be obtained opening the possibilities of detecting interesting hits. In addition, the application of less stringent end-points has been showed to consistently represent the in vitro activity of compounds and many times provide a better correlation with other measurements of antifungal activity such as the Minimum Fungicide Concentration (MFC).[17,18]

So, quinoline molecules **6, 9, 10, 13, 14, 16** and **17** were tested again, against *C. albicans* ATCC 10231 and *C. neoformans* ATCC 32264 by using a similar microplate design than that used for determining MIC_{100}. The inhibition percentages displayed by each compound at the different concentrations were calculated with the aid of a microplate reader, as explained in material and methods. These results were plotted in Figures. 1 and 2.

Regarding the behavior against *C. neoformans* (Fig. 1), all compounds but **14** inhibited 50% of fungal growth at concentrations

Table 2
Minimum inhibitory concentrations and minimum fungicide concentration (MIC and MFC in μg/mL, showed as MIC/MFC) of C-2 pyridinyl and pyridinylvinyl substituted quinolines **6**–**17**

Compd	Structure	*Ca*	*Sc*	*Cn*	*Afl*	*Afu*	*Ani*	*Mg*	*Tr*	*Tm*
6		62/250	62/250	31/125	62/125	62/125	250/>250	62/62	62/62	62/62
7		>250	>250	62/125	>250	>250	>250	31/125	31/125	31/125
8		>250	>250	62/250	125/250	125/250	250/>250	62/62	62/62	62/62
9		62/250	62/250	62/125	62/125	62/125	62/125	62/62	62/62	62/62
10		125/250	62/125	62/125	62/125	62/125	125/250	62/62	62/62	62/62
11		>250	>250	125/250	>250	>250	>250	125/125	125/125	125/125
12		>250	>250	125/250	>250	>250	>250	31/62	31/62	62/125
13		125/>250	250/>250	125/>250	250/>250	250/>250	>250	125/>250	125/>250	125/>250
14		250/>250	250/>250	125/250	125/>250	125/>250	125/>250	62/250	62/250	62/250
15		>250	>250	>250	250/>250	250/>250	>250	250/>250	250/>250	250/>250
16		62/250	62/250	62/125	125/250	125/250	125/>250	31/125	31/125	31/125
17		125/>250	125/>250	125/250	250/>250	250/>250	250/>250	62/125	62/125	62/125
	Amphotericin B	0.98	0.49	0.25	0.49	0.49	0.49	0.12	0.06	0.06
	Terbinafine	1.95	3.90	0.49	0.98	0.98	1.96	0.04	0.01	0.03
	Ketoconazole	0.49	0.49	0.25	0.12	0.49	0.25	0.06	0.03	0.03

Ca: *Candida albicans* ATCC 10231, *Sc*: *Saccharomyces cerevisiae* ATCC 9763, *Cn*: *Cryptococcus neoformans* ATCC 32264, *Afu*: *Aspergillus fumigatus* ATCC 26934, *Afl*: *Aspergillus flavus* ATCC 9170, *Ani*: *Aspergillus niger* ATCC 9029, *Mg*: *Microsporum gypseum* CCC 115, *Tr*: *Trichophyton rubrum* CCC 113, *Tm*: *T. mentagrophytes* ATCC 9972. CCC = CEREMIC (Centro de Referencia en Micologia). ATCC: American Type Culture Collection. MIC or MFC >250 μg/mL is indicative that the compound is inactive.

lower than 20 μg/mL and six compounds of them (**6, 9, 10, 13** and **16**) inhibited 80% of the same fungus at concentrations lower than 31.2 μg/mL. Quinoline derivatives **6** and **9** showed striking results, the first one inhibiting 50% and 80% of *C. neoformans* at 3.9 and 15.6 μg/mL respectively, and the second one inhibiting 80% of the same fungus at 15.6 μg/mL.

As it can be observed in Figure 2, all compounds but **14** inhibited 50% of *C. albicans* growth, at concentrations lower than 20 μg/mL, and five of them (**6, 9, 13, 16** and **17**) inhibited 80% of the same fungus at concentrations lower than 31.2 μg/mL. Interesting enough, compounds **6, 9, 16** showed 50% inhibition at 7.8 μg/mL, and 80% inhibition at 15.6 μg/mL against *C. albicans*.

Based on the previous results, all compounds but **14** were tested against six clinical isolates of *C. albicans* and four non-*albicans Candida* spp., all of them provided by CEREMIC (see Section 4) and seven strains of *C. neoformans* provided by Malbrán Institute (Buenos Aires). Results showed (Table 3) that C. *albicans*, non-*albicans Candida* spp. and *C. neoformans* possess different sensitivities to the selected compounds. The whole panel of clinical strains of *C. albicans* are more sensitive to compounds **9** and **16** (MIC_{80} = 15.6–31.2 μg/mL, MIC_{50} = 7.8–15.6 μg/mL), which both possess a γ-pyridinyl group and no substituents on the quinoline ring.

In turn, clinical isolates of *C. neoformans* are also very sensitive to **9** (MIC_{80} = 15.6–31.2 μg/mL, MIC_{50} = 7.8–31.2 μg/mL) and **16**

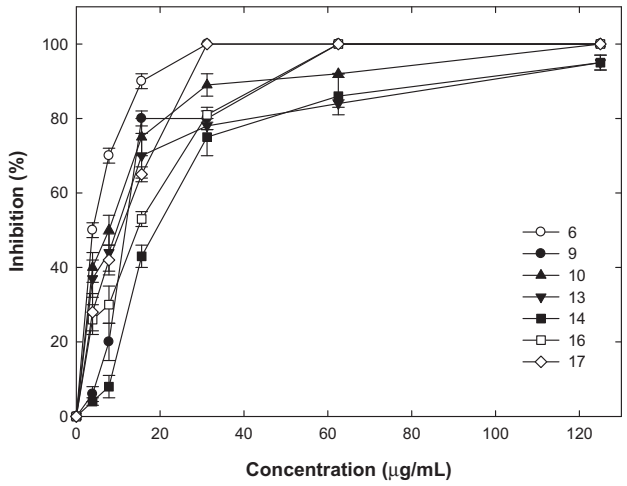


Figure 1. Percentages of inhibition of *Cryptococcus neoformans* ATCC 32264, produced by C-2 pyridinyl and pyridinyl-vinyl substituted quinolines **6**, **9**, **10**, **13**, **14**, **16** and **17** at different concentrations.

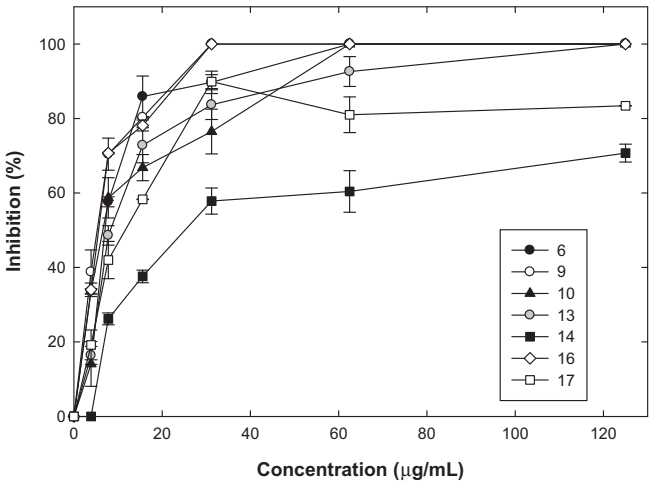


Figure 2. Percentages of inhibition of *Candida albicans* ATCC 10231, produced by C-2 pyridinyl and pyridinyl-vinyl substituted quinolines **6**, **9**, **10**, **13**, **14**, **16** and **17** at different concentrations.

Table 3
80% and 50% Minimum Inhibitory Concentrations (MIC_{80} and MIC_{50}) of quinoline derivatives **6**, **9**, **10**, **13**, **16** and **17** against six *C. albicans*, four *non-albicans Candida* and six *Cryptococcus neoformans* clinical isolated strains

		6		**9**		**10**		**13**		**16**		**17**		Amph. B
Strain	Voucher specimen	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}
C. albicans	ATCC 10231	15.6	7.8	15.6	7.8	31.25	15.6	31.2	15.6	15.6	7.8	31.2	15.6	1.00
C. albicans	CCC 125	125	62.5	15.6	15.6	125	31.2	31.25	15.6	15.6	7.8	125	31.2	0.78
C. albicans	CCC 126	31.2	15.6	31.2	15.6	62.5	31.2	62.5	62.5	31.2	15.6	62.5	31.2	1.56
C. albicans	CCC 127	62.5	62.5	15.6	7.8	125	31.2	62.5	31.2	15.6	15.6	62.5	31.2	0.78
C. albicans	CCC 128	62.5	31.2	15.6	7.8	125	31.2	62.5	31.2	15.6	7.8	125	62.5	1.56
C. albicans	CCC 129	62.5	31.2	15.6	7.8	125	62.5	62.5	31.2	31.2	15.6	62.5	31.2	0.78
C. albicans	CCC 130	62.5	31.2	31.2	15.6	125	62.5	31.25	15.6	15.6	7.8	62.5	31.2	0.50
C. glabrata	CCC 115	62.5	62.5	15.6	15.6	15.6	7.8	31.2	15.6	62.5	31.2	>250	>250	0.39
C. parapsilopsis	CCC 124	31.2	15.6	31.2	15.6	31.2	7.8	15.6	7.8	31.2	7.8	>250	>250	0.78
C. krusei	CCC 117	62.5	31.2	62.5	31.2	62.5	31.2	31.2	15.6	31.2	15.6	>250	>250	0.39
C. tropicalis	CCC 131	31.2	15.6	31.2	15.6	125	15.6	125	62.5	62.5	31.2	125	31.2	0.50
C. neoformans	ATCC 32264	15.6	3.9	15.6	15.6	31.2	7.8	31.2	15.6	31.2	15.6	31.2	15.6	0.25
C. neoformans	IM 983040	7.8	3.9	31.2	15.6	31.2	7.8	62.5	31.2	31.2	7.8	62.5	15.6	0.13
C. neoformans	IM 972724	7.8	3.9	31.2	31.2	31.2	15.6	62.5	31.2	31.2	7.8	62.5	31.2	0.06
C. neoformans	IM 042074	7.8	3.9	31.2	15.6	31.2	15.6	62.5	15.6	31.2	3.9	62.5	15.6	0.25
C. neoformans	IM 983036	7.8	3.9	31.2	7.8	62.5	31.2	62.5	31.2	31.2	7.8	62.5	7.8	0.13
C. neoformans	IM 00319	15.6	7.8	31.2	31.2	31.2	15.6	62.5	15.6	31.2	7.8	62.5	31.2	0.25
C. neoformans	IM 972751	15.6	3.9	31.2	7.8	31.2	7.8	31.2	15.6	31.2	3.9	31.2	15.6	0.25

MIC_{80} and MIC_{50}: concentration of a compound that induced 80% or 50% reduction of the growth control respectively. ATCC = American Type Culture Collection (Illinois, USA); CCC = Center of Mycological Reference (Rosario, Argentina), IM = Malbran Institute (Buenos Aires, Argentina). *C. albicans* = *Candida albicans*; *C. glabrata* = *Candida glabrata; C. parapsilopsis* = *Candida parapsilopsis; C. krusei* = *Candida krusei; C. tropicalis* = *Candida tropicalis; C. neoformans* = *Cryptococcus neoformans.* Amph B = Amphotericin B.
For the sake of comparison, MIC_{80} and MIC_{50} of all compounds against an ATCC standardized strain of *C. albicans* and *C. neoformans* are included.

(MIC_{80} = 31.2 μg/mL, MIC_{50} = 3.9–15.6 μg/mL) but they are more sensitive to **6** (MIC_{80} = 7.8–15.6 μg/mL, MIC_{50} = 3.9–7.8 μg/mL), which possesses two important differences with **9** and **16**: it possesses a α-pyridinyl moiety on C-2 and an ethyl substituent on the quinoline ring.

These results are not uncommon since some antifungal drugs have demonstrated selectivity for one type of fungi, such as the case of terbinafine which has activity mainly on dermatophytes, which are mainly used to treat esophageal candidiasis and invasive aspergilloses, refractory to other antifungal drugs.

3. Conclusion

We report here the synthesis and antifungal properties of a series C-2 pyridinyl and pyridinylvinyl substituted quinolines acting as antifungal agents. All members of the series showed activities against dermatophytes and some of them possessed a broad spectrum of action. 2-(Pyridin-4-yl)quinoline **9** and 2-(2-pyridin-4-yl) vinyl)quinoline **16** showed the best MIC_{80} and MIC_{50} against the clinically important fungi *C. albicans* and *non-albicans Candida* species. In turn, 6-ethyl-2-(pyridin-2-yl)quinoline **6** showed the best properties against standardized as well as clinical strains of *C. neoformans.*

It is important to take into account that one of the strategies for avoiding antifungal resistance is the treatment of fungal infections with the appropriate antifungal agent when the ethiological agent is known. As a consequence, our findings could open an avenue for the development of antifungal agents which inhibits either *C. albicans* or *C. neoformans* which, as stated above, are clinically important fungi for which new inhibitory structures are welcome.

4. Experimental

4.1. Chemistry

The melting points (uncorrected) were determined on a Fisher–Johns melting point apparatus. The IR spectra were recorded on a Lumex infralum FT-02 spectrophotometer in KBr. ^{1}H NMR and

^{13}CNMR spectra were recorded on Bruker AC-200 or Bruker AC-400 spectrometers. Chemical shifts are reported in ppm (d) relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at 7.24 ppm for protons). Signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; t, triplet; td, triplet of doublets; q, quartet; m, multiplet; br., broad. A Hewlett–Packard 5890a series II Gas Chromatograph interfaced to an HP 5972 mass selective detector (MSD) with an HP MS Chemstation Data system was used for MS identification at 70 eV using a 60 m capillary column coated with HP-5 [5%-phenyl-poly(dimethyl-siloxane)]. Elemental analyses were performed on a Perkin–Elmer 2400 Series II analyzer, and were within ±0.4 of theoretical values. The reaction progress was monitored using thin layer chromatography on a silufol UV254 TLC aluminum sheet.

4.1.1. General procedure for synthesis of 2-pyridinylquinolines 6–12

To a solution of the appropriate aniline (1.00 mmol) and pyridinecarboxaldehyde (1 mmol) in anhydrous CH_3CN (15 mL) under N_2, 20 mol % $BiCl_3$ was added, and to the resulting mixture was added ethyl vinyl ether (4.0 mmol). The reaction mixture was stirred at gentle reflux for 6 h and then quenched with a solution of Na_2CO_3. The organic layer was separated, and dried with Na_2SO_4. The organic solvent was removed in vacuo, the crude which were separated by chromatography column to afford the respective 2-pyridinylquinolines **3–10**.

4.1.1.1. 6-Ethyl-2-(pyridin-2-yl)quinoline (6). Brown solid. mp 148–150 °C; Yield 32%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.72 (1H, d, J = 4.6 Hz, 3′-H_{Py}), 8.64–8.60 (1H, m, 4′-H_{Py}), 8.51 (1H, d, J = 8.6 Hz, 3H), 8.21 (1H, d, J = 8.6 Hz, 8H), 8.09 (1H, d, J = 8.6 Hz, 4H), 7.83 (1H, ddd, J = 7.8, 7.8, 1.7 Hz, 5′-H_{py}), 7.62 (1H, br s, 5H), 7.60 (1H, dd, J = 7.9, 2.2 Hz, 7H), 7.26–7.32 (1H, m, 6′-H_{Py}), 2.84 (2H, q, J = 7.8 Hz, 6-CH_2CH_3), 1.34 (3H, t, J = 7.5 Hz, 6-CH_2CH_3); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 142.9, 136.9, 136.4, 133.7, 130.9, 129.6, 128.4, 128.0, 125.2, 123.9, 122.3, 121.8, 120.4, 118.9, 34.9, 15.4; GC–MS: t_R: 22.71 min, m/z: 234 ($M^{+\cdot}$). Anal. Calcd for $C_{16}H_{14}N_2$: C, 82.02; H, 6.02; N, 11.96. Found: C, 82.03; H, 6.00; N, 11.94.

4.1.1.2. 6-Chloro-2-(pyridin-3-yl)quinoline (7). Brown solid; Yield 36%; mp 150-152 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.34 (1H, d, J = 2.5 Hz, 2′-H_{Py}), 8.70 (1H, dd, J = 4.8, 1.4 Hz, 4′-H_{Py}), 8.48 (1H, dt, J = 7.8, 1.8 Hz, 5′-H_{Py}), 8.18 (1H, d, J = 8.6 Hz, 3-H), 8.10 (1H, d, J = 8.9 Hz, 8-H), 7.90 (1H, d, J = 8.2 Hz, 4-H), 7.83 (1H, d, J = 2.1 Hz, 5-H), 7.68 (1H, dd, J = 8.9, 2.5 Hz, 7-H), 7.46 (1H, ddd, J = 7.9, 4.6, 0.7 Hz, 6′-H_{Py}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 154.9, 150.5, 148.8, 146.8, 136.3, 134.9, 134.7, 132.6, 131.4, 131.0, 127.9, 126.3, 123.8, 119.4; GC–MS: t_R: 27.03 min, m/z: 240 ($M^{+\cdot}$). Anal. Calcd for $C_{14}H_9ClN_2$: C, 69.86; H, 3.77; N, 11.64. Found: C, 69.89; H, 3.75; N, 11.65.

4.1.1.3. 6-Fluoro-2-(pyridin-3-yl)quinoline (8). Brown solid; Yield 23%; mp 162–164 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.32 (1H, d, J = 2.5 Hz, 2′-H_{Py}), 8.69 (1H, dd, J = 4.6, 1.7 Hz, 4′-H_{Py}), 8.47 (1H, dt, J = 7.8, 1.8 Hz, 5′-H_{Py}), 8.19 (1H, d, J = 8.6 Hz, 3-H), 8.15 (1H, dd, J = 9.3, 5.3 Hz, 5-H), 7.52 (1H, dd, J = 8.6, 2.0 Hz, 8-H), 7.88 (1H, d, J = 8.2 Hz, 4-H), 7.47-7.41 (1H, m, 7-H), 7.47-7.41 (1H, m, 6′-H_{Py}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (d, $^1J_{C,F}$ = −248.1 Hz), 154.0, 150.5, 150.3, 148.7, 145.5, 136.5, 134.8, 132.3 (d, $^3J_{C,F}$ = 9.0 Hz), 128.0, 123.7, 120.3 (d, $^2J_{C,F}$ = 25.7 Hz), 119.2, 110.6 (d, $^2J_{C,F}$ = 23.0 Hz); GC–MS: t_R: 22.68 min, m/z: 224 ($M^{+\cdot}$). Anal. Calcd for $C_{14}H_9FN_2$: C, 74.99; H, 4.05; F, 8.47; N, 12.49. Found: $C_{14}H_9FN_2$: C, 74.96; H, 4.03; N, 12.48.

4.1.1.4. 2-(Pyridin-4-yl)quinoline (9) [7]. Brown solid; Yield 22%; mp 148–150 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.75 (2H, each dd, J = 6.1, 1.0 Hz, 3′-H_{Py} and 5′-H_{Py}), 8.22 (1H, d, J = 8.6 Hz, 3-H), 8.15 (1H, d, J = 8.6 Hz, 8-H), 8.05 (2H, each dd, J = 6.4, 1.0 Hz, 2′-H_{Py} and 6′-H_{Py}), 7.85 (1H, d, J = 7.0 Hz, 4-H), 7.81 (1H, d, J = 8.2 Hz, 5-H), 7.74 (1H, ddd, J = 7.8, 7.8, 1.4 Hz, 6-H), 7.54 (1H, ddd, J = 7.2, 7.2, 2.1 Hz, 7-H); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 154.4, 150.5 (2C), 148.3, 146.6, 137.3, 130.1, 129.9, 127.7, 127.6, 127.3, 121.6 (2C), 118.4; GC–MS: t_R: 21.26 min, m/z: 206 ($M^{+\cdot}$). Anal. Calcd for $C_{14}H_{10}N_2$: C, 81.53; H, 4.89; N, 13.58. Found: C, 81.54; H, 4.87; N, 13.59.

4.1.1.5. 6-Methyl-2-(pyridin-4-yl)quinoline (10). Brown solid; Yield 42%; mp 82–84 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.69 (2H, each dd, J = 6.1, 1.0 Hz, 3′-H_{Py} and 5′-H_{Py}), 8.07 (1H, d, J = 8.2 Hz, 3-H), 8.00 (1H, d, J = 7.0 Hz, 8-H), 7.98 (2H, each dd, J = 6.1, 1.1 Hz, 2′-H_{Py} and 6′-H_{Py}), 7.74 (1H, d, J = 8.2 Hz, 4-H), 7.52 (1H, d, J = 6.5 Hz, 7-H), 7.51 (1H, br s, 5-H), 2.49 (3-H, s, 6-CH_3); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 153.4, 150.4 (2C), 146.8, 146.7, 137.3, 136.6, 132.4, 129.6, 127.9, 126.4, 121.5 (2C), 118.4, 21.7. Anal. Calcd for $C_{15}H_{12}N_2$: C, 81.79; H, 5.49; N, 12.72. Found C, 81.78; H, 5.87; N, 12.73.

4.1.1.6. 6-Chloro-2-(pyridin-4-yl)quinoline (11). Brown solid; Yield 33%, mp 138–140 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.77 (2H, each dd, J = 6.1, 1.8 Hz, 3′-H_{Py} and 5′-H_{Py}), 8.19 (1H, d, J = 8.6 Hz, 3-H), 8.11 (1H, d, J = 7.1 Hz, 8-H), 8.05 (2H, each dd, J = 4.3, 1.4 Hz, 2′-H_{Py} and 6′-H_{Py}), 7.91 (1H, d, J = 8.6 Hz, 4-H), 7.83 (1H, d, J = 2.2 Hz, 5-H), 7.68 (1H, dd, J = 8.9, 2.2 Hz, 7-H); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 154.7, 150.6 (2C), 148.0, 146.2, 136.4, 133.0, 131.6, 131.1, 128.4, 126.3, 121.6 (2C), 119.3; GC–MS: t_R: 23.28 min, m/z: 240 ($M^{+\cdot}$). Anal. Calcd for $C_{14}H_9ClN_2$: C, 69.86; H, 3.77; N, 11.64. Found: C, 69.85; H, 3.77; N, 11.64.

4.1.1.7. 8-Chloro-2-(pyridin-4-yl)quinoline (12). Brown solid; Yield 25%; mp 176–178 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.86 (1H, d, J = 6.1 Hz, 3-H), 8.75 (2H, each dd, J = 4.3, 1.4 Hz, 3′-H_{Py} and 5′-H_{Py}), 7.76 (2H, each dd, J = 4.3, 2.2 Hz, 2′-H_{Py} and 6′-H_{Py}), 7.68 (1H, d, J = 6.1 Hz, 4-H), 7.44 (1H, dd, J = 7.5, 1.4 Hz, 7-H), 7.23 (1H, dd, J = 9.0, 7.2 Hz, 6-H), 7.00 (1H, dd, J = 7.5, 1.7 Hz, 5-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.9, 151.5 (2C), 150.0, 140.0, 130.5, 130.0, 127.8, 127.5, 126.4, 125.9, 123.2, 120.1 (2C); GC–MS: t_R: 19.75 min, m/z: 216 ($M^{+\cdot}$). Anal. Calcd for $C_{14}H_9ClN_2$: C, 69.86; H, 3.77; N, 11.64. Found: C, 69.87; H, 3.76; N, 11.64.

4.1.2. General procedure for synthesis of (*E*)-2-(2-(pyridinyl)-vinyl) quinolines 13–17

The quinaldines **5** (1.0 mmol) were solubilized in the presence of acetic anhydride were then added α,β,γ-pyridincarboxaldehyde **3** (1.00 mmol), the reaction mixture was stirred at gentle reflux for 12 h. The reaction mass was subjected to simple distillation, removing the solvent, the products obtained were purified by column chromatography on silica gel with gradual increase in polarity by using mixtures of solvents (hexane/ethyl acetate) as eluents.

4.1.2.1. (*E*)-6-Fluoro-2-(2-(pyridin-2-yl)vinyl)quinoline (13). Red oil; Yield 37%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.72 (1H, d, J = 4.6 Hz, 3′-H_{Py}), 8.60-8.64 (1H, m, 4′-H_{Py}), 8.26 (1H, ddd, J = 7.2, 7.2, 1.4 Hz, 7-H), 8.19 (1H, d, J = 8.6 Hz, 3-H), 8.15 (1H, dd, J = 9.3, 5.3 Hz, 8-H), 7.83 (1H, ddd, J = 7.8, 7.8, 1.7 Hz, 5′-H_{Py}), 7.88 (1H, d, J = 8.2 Hz, 4-H), 7.52 (1H, dd, J = 8.6, 4.7 Hz, 5-H), 7.26-7.32 (1H, m, 6'-H_{Py}), 7.23 (1H, d, J = 20.9 Hz, H_a), 7.21 (1H, d, J = 24.4 Hz, H_b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.2. (d, $^1J_{C,F}$ = 260.1 Hz), 157.0, 150.1, 149.4, 146.5, 136.4, 131.3 (2C), 130.0 (d, $^3J_{C,F}$ = 7.2 Hz), 129.3, 125.6, 123.0, 122.8, 122.5, 122.0 (d, $^2J_{C,F}$ = 28.4 Hz), 120.7 (d, $^2J_{C,F}$ = 27.0 Hz); GC–MS: t_R: 30.71 min, m/z: 250 ($M^{+\cdot}$). Anal. Calcd for $C_{16}H_{11}FN_2$: C, 76.79; H, 4.43; F, 7.59; N, 11.19. Found: C, 76.78; H, 4.42; F, 7.59; N, 11.20.

4.1.2.2. (*E*)-2-(2-(Pyridin-3-yl)vinyl)quinoline (14). Green solid; Yield 35%; mp 173–175 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.82 (1H, d, J = 2.2 Hz, 2′-H_{Py}), 8.53 (1H, dd, J = 4.6, 2.0 Hz, 4′-H_{Py}), 8.14 (1H, d, J = 8.9 Hz, 3-H), 8.10 (1H, d, J = 8.9 Hz, 4-H), 8.0 (1H, td, J = 5.4, 3.5 Hz, 5′-H_{Py}), 7.77 (1H, dd, J = 8.0, 1.4 Hz, 8-H), 7.76-7.71 (1H, m, 7-H), 7.66–7.59 (1H, m, 6′-H_{Py}), 7.50 (1H, ddd, J = 7.9, 7.9, 1.0 Hz, 6-H), 7.38 (1H, d, J = 16.0 Hz, H_a), 7.33 (1H, d, J = 7.9 Hz, 5-H), 7.30 (1H, d, J = 23.3 Hz, H_b); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 154.7, 148.7, 148.6, 147.8, 136.2, 133.2, 132.0, 130.6, 130.0, 129.6, 128.9, 127.4, 127.2, 126.2, 123.5, 119.1; GC–MS: t_R: 27.43 min, m/z: 232 ($M^{+\cdot}$). Anal. Calcd for $C_{16}H_{12}N_2$: C, 82.73; H, 5.21; N, 12.06. Found: C, 82.74; H, 5.22; N, 12.03.

4.1.2.3. (*E*)-6-Chloro-2-(2-(pyridin-3-yl)vinyl)quinoline (15). Beige solid; Yield 39%; mp 177–179 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.80 (1H, d, J = 2.2 Hz, 2′-H_{Py}), 8.52 (1H, dd, J = 5.2, 1.8 Hz, 4′-H_{Py}), 8.02 (1H, d, J = 7.9 Hz, 3-H), 7.97 (1H, d, J = 8.6 Hz, 4-H), 7.90 (1H, td, J = 7.9, 1.8 Hz, 5′-H_{Py}), 7.72 (1H, d, J = 8.2 Hz, 8-H), 7.73 (1H, bs, 5-H), 7.63–7.59 (1H, m, 7-H), 7.35 (1H, d, J = 22.2 Hz, H_a), 7.32–7.26 (1H, m, 6′-H_{Py}), 7.29 (1H, d, J = 22.3 Hz, H_b); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 159.6, 155.4, 149.6, 149.3, 147.9, 146.6, 136.2, 133.3, 132.0, 131.0, 130.9, 130.4, 128.0, 126.3, 123.7, 120.4; GC–MS: t_R: 24.88 min, m/z: 265 ($M^{+\cdot}$). Anal. Calcd for $C_{16}H_{11}ClN_2$: C, 72.05; H, 4.16; N, 10.50. Found: C, 72.06; H, 4.17; N, 10.51.

4.1.2.4. (*E*)-2-(2-(Pyridin-4-yl)vinyl)quinoline (16). Green solid; Yield 43%; mp 169–172 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.52 (2H, d, J = 6.1 Hz, 3′-H_{Py} and 5′-H_{Py}), 8.02 (2H, each d, J = 8.2 Hz, 2′-H_{Py} and 6′-H_{Py}), 7.67 (1H, d, J = 8.0 Hz, 3-H), 7.65 (1H, d, J = 7.2 Hz, 8-H), 7.61 (1H, d, J = 7.4 Hz, 5-H), 7.52 (1H, d, J = 8.2 Hz, 4-H), 7.44 (1H, d, J = 18.0 Hz, H_a), 7.42–7.37 (1H, m, 6-H), 7.42–7.37 (1H, m, 7-H), 7.41 (1H, d, J = 17.2 Hz, H_b); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 174.7, 154.4, 148.9 (2C), 147.8, 144.9, 136.9, 133.7, 131.2, 130.2, 129.0, 127.6, 126.9, 121.7 (2C), 119.4; GC–MS: t_R: 27.07 min, m/z: 249 ($M^{+\cdot}$). Anal. Calcd for: $C_{16}H_{12}N_2$: C, 82.73; H, 5.21; N, 12.06. Found C, 82.74; H, 5.20; N, 12.04.

4.1.2.5. (*E*)-6-Chloro-2-(2-(pyridin-4-yl)vinyl)quinoline (17). Green solid; yield 40%; mp 175–177 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.78 (2H, each dd, J = 6.0, 4.2 Hz, H-3′$_{Py}$ and H-5′$_{Py}$), 8.65 (1H, dd, J = 6.1, 4.3 Hz, H-2′$_{py}$ and H-6′$_{py}$), 8.10 (1H, d, J = 9.3 Hz, 3-H), 8.10 (1H, d, J = 9.3 Hz, 4-H), 7.90 (1H, d, J =7.9 Hz, 8-H), 7.79 (1H, d, J = 2.5 Hz, 5-H), 7.68 (1H, dd, J = 8.3, 1.0 Hz, 7-H), 7.56 (1H, d, J = 21.5 Hz, H_a), 7.53 (1H, d, J = 16.9 Hz, H_b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.5, 150.0 (2C), 145.9, 145.4, 133.4, 133.0, 132.5, 130.0, 129.4 (2C), 126.8, 125.9, 123.0, 121.2 (2C); GC–MS: t_R: 27.09 min, m/z: 266 (M+·). Anal. Calcd for: $C_{16}H_{11}ClN_2$: C, 72.05; H, 4.16; N, 10.50. Found: C, 72.04; H, 4.17; N, 10.51.

4.2. Biology

4.2.1. Microorganisms and media

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, and CEREMIC (CCC), Centro de Referencia en Micología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina were used in a first instance of screening: *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *A. fumigatus* ATTC 26934, *A. niger* ATCC 9029, *Trichophyton rubrum* CCC 110, *T. mentagrophytes* ATCC 9972 and *M. gypseum* CCC 115.

Active compounds were tested against clinical isolates from CEREMIC and Malbrán Institute [(M), Av. Velez Sarsfield 563. Buenos Aires)]. The isolates included 10 strains of *Candida* spp. (6 of them of *C. albicans* and 4 of *C. non-albicans*) and 6 strains of *Cryptococcus neoformans*. The number of voucher specimens is showed in Table 3. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C, maintained on slopes of Sabouraud-Dextrose agar (SDA, Oxoid) and sub-cultured every 15 days to prevent pleomorphic transformations. Inocula of cell or spore suspensions were obtained according to reported procedures and adjusted to 1–5 × 10^3 cells/spores with colony forming units (CFU) /mL.[17,18]

4.2.2. Antifungal susceptibility testing

Minimum Inhibitory Concentration (MIC) of each extract or compound was determined by using broth microdilution techniques according to the guidelines of the National Committee for Clinical Laboratory Standards for yeasts (M27-A3)[17] and for filamentous fungi (M 38-A2).[18] MIC values were determined in RPMI-1640 (Sigma, St. Louis, Mo, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 30 °C for yeasts and species of *Aspergillus* and at 28–30 °C for dermatophytes in a moist, dark chamber. MICs were visually recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi.

For the assay, stock solutions of pure compounds were twofold diluted with RPMI from 250–0.98 μg/mL (final volume = 100 μl) and a final DMSO concentration ⩽1%. A volume of 100 μl of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. Amphotericin B, Terbinafine and Ketoconazole were used as positive controls.

Endpoints recorded in Table 2 were defined as the lowest concentration of drug resulting in total inhibition (MIC_{100}) of visual growth compared to the growth in the control wells containing no antifungal. Compounds with MICs >250 μg/mL were considered inactive.

4.2.3. MFC determination

The minimum fungicidal concentration (MFC) of each compound against each isolate was also determined as follows: After determining the MIC, an aliquot of 5 μL sample was withdrawn from each clear well of the microtiter tray and plated onto a 150 mm RPMI-1640 agar plate buffered with MOPS (Remel, Lenexa, Kans.). Inoculated plates were incubated at 30 °C, and MFCs were recorded after 48 h. The MFC was defined as the lowest concentration of each compound that resulted in total inhibition of visible growth.

4.2.4. Inhibition percentage determination

The test was performed in 96-well microplates. Compound test wells (CTWs) were prepared with stock solutions of each quinoline derivative in DMSO (maximum concentration ⩽1%), diluted with RPMI-1640 to final concentrations 250–0.98 μg/mL. Inoculum suspension (100 μl) was added to each well (final volume in the well = 200 μl). A growth control well (GCW) (containing medium, inoculum, the same amount of DMSO used in CTW, but compound-free) and a sterility control well (SCW) (sample, medium and sterile water instead of inoculum) were included for each fungus tested. Microtiter trays were incubated in a moist, dark chamber at 30 °C, 24 or 48 h for *C. albicans* or *C. neoformans*, respectively. Microplates were read in a VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). Amphotericin B was used as positive control.

Tests were performed in duplicate. Reduction of growth for each compound concentration was calculated as follows: % of inhibition = 100 − (OD_{405} CTW − OD_{405} SCW)/(OD_{405} GCW − OD_{405} SCW).

4.2.5. MIC_{80} and MIC_{50} determination

MIC_{80} and MIC_{50} were defined as the lowest concentration of a compound that showed 80% or 50% reduction of the growth control respectively and was determined from the results obtained in the Inhibition percentage determination.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.036.

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