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Probing the N-5 Region of the Indoloquinoline Alkaloid, Cryptolepine for Anticryptococcal Activity

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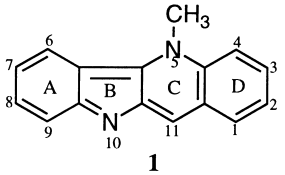
Abstract—N-5 Alkylated analogues of cryptolepine were synthesized and tested for anticryptococcal activity. Evidence provided in this study suggests that the active form of cryptolepine consists of the flat tetracyclic aromatic ring with the methyl group on the N-5 atom. It was also found that changes in the electronic density around the N-5 atom do not appear to affect activity. Steric hindrance of the N-5 substituents seems to decrease activity. Through systematic modification of the N-5 alkyl groups, ω-phenylpentyl group was shown to possess the highest potency thus far.

Introduction

Cryptococcus neoformans is a yeast-like fungus that is pathogenic to both animals and humans. Under normal conditions, infection by *C. neoformans* (cryptococcosis) is rarely fatal. Apparently, this is because cell-mediated immunity, the major defence against cryptococcosis, is efficient in protecting the host.[1,2] However, in patients with compromised cell-mediated immunity such as AIDS, lymphoma and leukemia, cryptococcosis is a cause for serious concern. Indeed, cryptococcosis is considered a major life threatening mycosis in patients with AIDS. In African patients with AIDS, for example, cryptococcal meningitis is the major opportunistic infection.[3–5]

The preferred treatment for the various forms of cryptococcosis including the most morbid form, cryptococcal meningitis, is the use of the drug amphotericin B in combination with flucytosine.[1,3,6–9] Despite their success, however, serious drawbacks exist. Relapse often occurs after a primary course of antifungal therapy, which requires that a maintenance dose be instituted. Both drugs are toxic. For example, some type of nephrotoxicity is observed in about 80% of patients receiving amphotericin B.[10] In addition, amphotericin B produces leukopenia, thrombocytopenia, febrile reactions, and other side effects. Treatment with amphotericin B also requires hospitalization or frequent hospital visits of patients to receive intravenous infusion, essentially, because of its lack of oral efficacy. Although the overall safety profile has improved by the recent use of liposome formulated amphotericin B, the associated side-effects observed in AIDS patients with cryptococcal meningitis remained the same.[1] Thus, the need for novel systemic antifungal drugs for cryptococcosis and other opportunistic infections (such as those caused by *Candida albicans*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae* and *Mycobacterium intracellulare*) is apparent in light of the significant problems associated with current drugs. Newer agents such as ketoconazole, fluconazole and others from the azole class have been introduced as possible replacements for amphotericin B. However, recent evidence suggests that azole drug resistant fungi have developed,[1,11,12] making the development of new drug entities all the more urgent.

As part of a screening programme to identify new antibacterial and systemic antifungal agents, the indoloquinoline alkaloid cryptolepine (**1**) was reisolated from *Cryptolepis sanguinolenta* and was subsequently identified in preliminary screening as a potent antibacterial and antifungal agent.[13–18]



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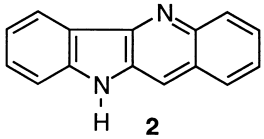

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Key words: Cryptolepine; indoloquinolines; antifungal agents; opportunistic infections; anticryptococcal agents.
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Since cryptolepine has lower toxicity {(LD_{50} = 146 mg/kg body weight in mice)[19] compared with amphotericin B (LD_{50} = 2.3 mg/kg body weight in mice)[20]}, is more water soluble (1.2 mg/mL versus 0.1 mg/mL of amphotericin B)[21] and using agar well diffusion assay cryptolepine was shown to have higher potency than amphotericin B,[16] we chose cryptolepine as the lead compound for further development. Interestingly, the *N*-demethylated analogue of cryptolepine, i.e. quindoline (**2**) shows no antifungal activities; suggesting that the N-5 methylation of cryptolepine may be a requirement for its antifungal activity as observed previously.[18] In this paper, we explore the hypothesis that modifications of the N-5 alkyl substituent on the quindoline nucleus would result in the identification of an optimal substituent for activity. Furthermore, because N-5 substitution introduces significant changes in the electronic nature of the ring and causes buckling[18] in the flat aromatic ring system (in the anhydronium base form) while retaining the flat aromatic ring system in the ionized form, we have also sought in this paper to identify the active form of cryptolepine.

Chemistry

Preparations of cryptolepine **1** and its N-5 alkyl analogues required quindoline **2**. Currently, there are a number of synthetic routes leading to quindoline.[22–28] Among them, the Holt and Petrow procedure was chosen owing to its brevity and high yields. The other synthetic methods are longer and yields are significantly lower. However, the Holt synthesis is thwarted by a 10-day condensation step required to synthesize quindoline (Scheme 1).

To overcome this problem, we recently developed a new synthetic procedure (Scheme 2)[28] which can be completed in one day. This procedure involved arylation of 3-aminoquinoline with triphenylbismuth diacetate to form the intermediate 3-anilinoquinoline. Subsequent oxidative cyclization of 3-anilinoquinoline with palladium (II) acetate in trifluoroacetic acid produces the desired product in an overall yield of 22%.

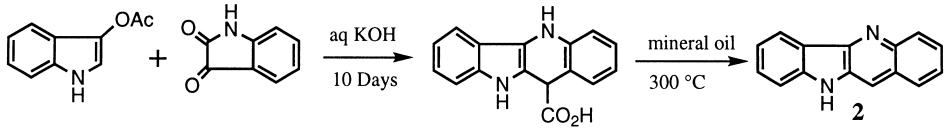
N-5 alkylation was expected to show product selectivity due to the basicity difference between the quinoline N-5 and the indole N-10 nitrogen atoms. This difference is observed experimentally in the increased rate of N-5 alkylation over that of N-10 (Table 1, **3a–3i, 3l–3n,** and **3p–3q**). However, for several alkylating agents, especially the α-carbonyl halides, N-5 and N-10 double alkylation products were obtained in addition to the desired N-5 alkylation products (**3j, 3k, 3o,** and **3r**). The formation of the double alkylation product often caused separation difficulties.

Part of this problem has recently been alleviated by conducting the alkylation in sulpholane, whereby yields were increased and double alkylation products were suppressed in favor of N-5 alkylation.[28] In all, three alkylation conditions were used to prepare cryptolepine analogues in this paper. Heating quindoline with an appropriate halide in sulpholane constitutes condition A and served as our standard condition. Condition B was by heating quindoline with neat alkyl halides in the presence of a small amount of *N,N*-dimethylformamide in order to dissolve quindoline. Condition C involved conducting the reaction in benzene at room temperature, a special condition for a highly reactive alkyl halide (**3q**). Condition A is the preferred general alkylation condition and is crucial for the synthesis of compounds **3j, 3k, 3o,** and **3r**.

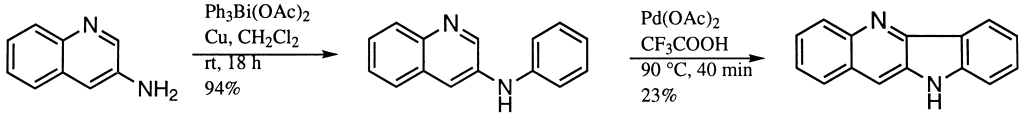
Results and Discussion

Identification of the active form of cryptolepine

Quindoline is a flat tetracyclic aromatic compound based on molecular simulations.[18] Methylation to form cryptolepine anhydronium base results in distortion in the flat shape of the tetracyclic moiety. Conversion of the anhydronium base to its salt restores the flat shape to the tetracyclic aromatic ring system. Each of these changes is accompanied by significantly large changes in the electronic nature of the structure. For example, the C-13 chemical shift value of the C9a atom in quindoline is 141.1, while those of cryptolepine and its salt are 161.0 and 145.6, respectively. These variations in the structure of cryptolepine raises the question of what the active form of the drug might be, i.e. is buckling of the structure a requirement for the antifungal activity? To answer this question, we needed to know the extent

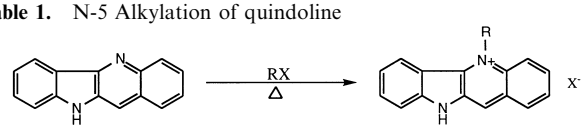


Scheme 1.



Scheme 2.

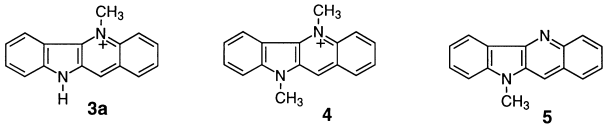
Table 1. N-5 Alkylation of quindoline



RX	Reaction temperature (°C)	Alkylation condition[a]	Product	Recrystallization solvent	Yield (%)[b]
CH_3I	50	A	**3a**	MeOH	73[c]
CH_3CH_2I	90	A	**3b**	MeOH	66
$CH_3CH_2CH_2I$	120	B	**3c**	MeOH	56
$CH_3(CH_2)_4I$	110	A	**3d**	MeOH	74
$CH_2=CHCH_2Br$	90	A	**3e**	EtOAc–MeOH	80
Cyclopropylmethyl bromide	105	B	**3f**	MeOH	57
Cyclobutylmethyl bromide	105	B	**3g**[d]	—	16
Cyclopentylmethyl bromide	115	A	**3h**	MeOH–ether	8
Cyclohexylmethyl bromide	120	A	**3i**	EtOH–ether	7
$PhCH_2Br$	90	A	**3j**	MeOH–ether	54
$PhCH_2CH_2Br$	105	A	**3k**	EtOH–ether	30
$Ph(CH_2)_3Br$	110	A	**3l**	MeOH–ether	63
$Ph(CH_2)_5Br$	115	A	**3m**	MeOH–ether	27
$Ph(CH_2)_8Br$	110	A	**3n**[d]	—	21
EtO_2CCH_2Br	85	A	**3o**[d]	—	53
$NCCH_2Br$	80	B	**3p**	EtOH	72
CH_3OCH_2I	[rt]	C	**3q**	MeOH	60[c]
$PhCOCH_2Br$	85	A	**3r**[d]	—	28

[a]Condition A: in sulpholane; condition B: in neat alkylating agents with drops of DMF; condition C: in benzene at rt.
[b]After chromatography.
[c]Not chromatographed.
[d]No recrystallization attempted.

of ionization at the physiological pH of 7.4. The pK_a of cryptolepine was estimated at 11.2 using an UV spectrophotometric method.[29] Although this value appears rather high for an aromatic nitrogen atom, it should be remembered that ionization also results in complete aromatization of the quinoline moiety and thus may supply the driving force for ionization and hence the relatively high pK_a. The high pK_a of cryptolepine can also explain its relatively high solubility in an aqueous environment.[21] Using the Henderson equation, the % ionization is calculated to be over 99.9% under physiological conditions. Thus, cryptolepine exists essentially in the salt form at pH of 7.4; suggesting that the salt form may be regarded as the active form of cryptolepine. Although this seems reasonable, the salt form has the same flat structure as quindoline which is completely devoid of antifungal activity, therefore additional evidence is required. Thus, to test this hypothesis further, we synthesized 5,10-dimethylquindoline (**4**) and its N-5 demethylated analogue, **5**. Compound **4** provides us with a permanently charged N-5 atom identical to that found in cryptolepine salt. It is also of interest to investigate the contributions of the N-10 methyl group to the activity of **4**. Since compound **4** is active (MIC = 6.25 μg/mL), and cryptolepine salt (**3a**) is also active (MIC = 12.5 μg/mL) but compound **5** is only marginally active (MIC ≥ 62.5 μg/mL), these results taken together provide additional evidence that the ionized form of cryptolepine is active and that buckling is not a necessary requirement for anticryptococcal activity.



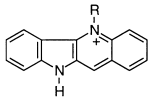
Homologation at the N-5 position

The fact that N-5 methylation of quindoline was required for its antifungal activity also raises the question of whether alkylation with other substituents can modulate activity and lead to the identification of an optimum substituent at the N-5 position. In this regard, compounds **3a–3e** were synthesized to evaluate their anticryptococcal activity. Table 2 shows the results of the antifungal activity testing. Clearly, homologation of the methyl to pentyl group has no effect on activity. While lipophilicity increased during homologation, the electron density around the N-5 atom remained essentially the same. All the compounds tested in this group showed no activity against *C. albicans*. Did anticryptococcal activity remain the same because of the constancy of electron density around N-5?

Variation of the electron density around the N-5 atom

In order to explore the possibility that variations in the electron density around the N-5 atom might explain changes in the anticryptococcal activity from **2** to **3**, we synthesized compounds **3c, 3e, 3p, 3q, 3j,** and **3r** where the *N*-alkylated moiety is N-CH_2-A, and A is a group with varying electron donating or withdrawing capability. The effect of substituents on electron density around N-5 is measured by the proton chemical shift of the methylene group directly attached to the N-5 atom (N^+-CH_2-A). Thus, the more electron withdrawing group A, the higher the chemical shift value. Applied to the compounds in Table 3, there is little or no effect on the anticryptococcal activity by varying the electron density on the N-5 atom through the alkyl substituent. Similarly, no improvements were noted in the antifungal activity against *C. albicans* except for compound **3r**.

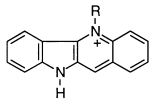
Table 2. The effect of homologation on antifungal activity



Structure[a]		Minimum inhibition concentration (MIC, μG/MI)	
Compd	R,	*C. neoformans*	*C. albicans*
2	R = H	> 250	> 250
3a	$R=CH_3$	12.5–15.6	250
3b	$R=CH_2CH_3$	31.2	250
3c	$R=CH_2CH_2CH_3$	31.2	250
3d	$R=CH_2(CH_2)_3CH_3$	31.2	250
3e	$R=CH_2CH=CH_2$	31.2	250
Amphotericin B		0.39	0.39

[a]Compounds **3a–3d** were prepared in their HI salt form while **3e** was prepared in the HBr salt form.

Table 3. The effect of variation of the electron density of the methylene group (N^+-CH_2-A) attached to the N-5 atom in quindoline



Structure		Minimum inhibition concentration (MIC, μg/mL)	
Compd	R, proton chemical shift	*C. neoformans*	*C. albicans*
3c	$CH_2CH_2CH_3$, δ = 5.50	31.2	250
3e	$CH_2CH{=}CH_2$, δ = 6.18	31.2	250
3p	CH_2 CN, δ = 6.80	31.2	250
3q	CH_2OCH_3, δ = 6.86	31.2	250
3j	CH_2Ph, δ = 6.93	> 250	> 250
3r	CH_2COPh, δ = 7.45	62.5	31.2

An interesting observation in Table 3 is the lack of activity of compound **3j** (i.e. the benzyl analogue of cryptolepine). In an attempt to explain the lack of activity of **3j** we hypothesized that there is a steric requirement for substituents accommodated close to the N-5 atom. Alternatively, the high electron density in the region occupied by the phenyl ring in **3j** may be responsible for this lack of activity. To further explore these issues, we designed and synthesized compounds **3e–3i** and evaluated their anticryptococcal activity (Table 4). Reduction of the benzene ring of compound **3j** to obtain compound **3i** restored activity (MIC of **3i** = 62.5 μg/mL). Reduction in the size of the six-membered ring to five (**3h**, MIC = 62.5 μg/mL) has no further effect on activity. Further reduction in the size of the ring to cyclobutyl methyl (**3g**, MIC = 15.6 μg/mL), and cyclopropylmethyl (**3f**, MIC = 31.2 μg/mL) led to only minimal increase in activity. The allyl substituent which often mimics the cyclopropylmethyl group resulted in the same activity profile. In summary, the flat aromatic ring is disfavored around the N-5 region of quindoline while smaller and flexible ring substituents appear to be tolerated.

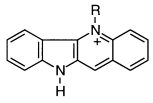
Distance of the phenyl ring from the N-5 atom

The fact that the benzyl group is not tolerated on the N-5 atom of quindoline raises the question of whether the disfavored region around the N-5 atom is limited in space. We therefore synthesized compounds **3k–3n** extending the phenyl ring away from the N-5 atom according to the Fibbonacci series (Table 5). Thus, anticryptococcal activity was restored for the phenylethyl analogue (**3k**, MIC = 31.2 μg/mL), phenylpropyl analogue (**3l**, MIC = 15.6 μg/mL) and phenypentyl analogue (**3m**, MIC = 1.9 μg/mL). In fact, compound **3m** is about eight times more potent than cryptolepine and constitutes the most active anticryptococcal agent in this series. Further extension of the phenyl ring to obtain the phenyloctyl analogue of cryptolepine (**3n**, MIC = 10 μg/mL) shows it to be as active as cryptolepine but is about five times less active than **3m**. These results suggest that a phenylpentyl substituent at N-5 constitutes the optimum substituent for anticryptococcal activity.

Summary

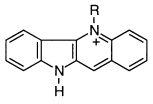
In conclusion, while quindoline is without anticryptococcal activity, its N-5 methylated analogue, cryptolepine, possesses activity. Since cryptolepine is flat in the salt form but is buckled at the N-5 atom position in the anhydronium base form, it was also of interest to investigate the active form of the drug. This work further suggests that the protonated quaternary form is the active form. Changes in the electron density around the N-5 quaternary atom, brought about by changing the N-5 substituents, do not appear to have any effect on antifungal activity. There appears, however, to be a steric requirement close to the N-5 atom and in fact extending steric bulk away from the N-5 atom has resulted in the most potent compound to date. Another interesting observation in this study relates to the selectivity of the compounds against *C. neoformans* and not against *C. albicans*. These results, along with the fact that cryptolepine shows a higher activity against *C. neoformans* than amphotericin B when tested in agar well diffusion assays, warrant an in depth evaluation of the anticryptococcal activity and toxicity of cryptolepine and its analogues. These studies are currently underway.

Table 4. The effect of variation of ring type and size on antifungal activity of the indoloquinolines



Structure		Minimum inhibition concentration (MIC, μg/mL)	
Number	R	*C. neoformans*	*C. albicans*
3j	CH_2Ph	> 250	> 250
3i	CH_2-cyclohexyl	62.5	62.5
3h	CH_2-cyclopentyl	62.5	250
3g	CH_2-cyclobutyl,	15.6	125
3f	CH_2-cyclopropyl,	31.2	250
3e	$CH_2CH{=}CH_2$	31.2	250

Table 5. The effect of the distance of the phenyl ring from the N-5 atom on antifungal activity of the indoloquinolines



Structure		Minimum inhibition concentration (MIC, μg/mL)	
Compd	R	*C. neoformans*	*C. albicans*
3j	CH_2Ph	> 250	> 250
3k	CH_2CH_2Ph	31.2	250
3l	$CH_2CH_2CH_2Ph$	15.6	31.2
3m	$CH_2(CH_2)_3CH_2Ph$	1.9	125
3n	$CH_2(CH_2)_6CH_2Ph$	10	10
Amph B.		0.39	0.39

Experimental

Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. NMR spectra were obtained on a Bruker 270 MHz NMR spectrometer at Florida State University. Elemental analyses were carried out by the Atlantic Microlab, Inc., Norcross, GA 30091, and are within 0.4% of theory unless otherwise noted. Flash chromatography was performed with Davisil grade 634 silica gel.

N,*N*-Dimethylformamide was distilled from $CaSO_4$ and then stored over 4 Å molecular sieves. Sulpholane was dried over 4 Å molecular sieves. Cyclopentylmethyl bromide, 5-phenylpentyl bromide and 8-phenyloctyl bromide were prepared by treatment of the corresponding alcohols with PBr_3.[30] The remaining alkyl halides were obtained from either Aldrich Chemicals or Fisher Scientific and were used without further purification.

General procedure for quindoline alkylation. (Method A) Alkyl halide (0.8 mL) was added to a 10-mL round-bottomed flask containing quindoline (0.100 g, 0.46 mmol) and sulpholane (2.0 mL). The mixture was heated in a sealed flask overnight at the indicated temperature (Table 1). After cooling to room temperature, the mixture was directly chromatographed with 5–20% MeOH: CH_2Cl_2 (gradient elution) to give a yellow solid which was usually recrystallized from appropriate solvent (Table 1).

General procedure for quindoline alkylation. (Method B) A mixture of quindoline (0.100 g, 0.46 mmol), alkyl halide (2.0 mL) and a few drops of DMF in a 10-mL round-bottomed flask was heated in a sealed flask overnight at the indicated temperature (Table 1). The mixture was allowed to cool to room temperature and then precipitated with ether–MeOH. Flash chromatography with 5–20% MeOH:CH_2Cl_2 (gradient elution) yielded a yellow solid which was recrystallized from an appropriate solvent (Table 1).

5-Methyl-10*H*-indolo[3,2-*b*]quindolinium iodide (3a). Prepared by method A except that there was no chromatographic separation. Product **3a** was obtained as a pure yellow solid upon precipitation with MeOH–ether, followed by filtration and recrystallization from MeOH: mp 280–283 °C; ^{1}H NMR (DMSO-d_6) δ 5.04 (s, 3 H), 7.52 (ddd, 1H, J = 7.7, 7.7, 1.0 Hz), 7.86 (d, 1H, J = 8.3 Hz), 7.93 (dd, 1H, J = 7.2, 7.2 Hz), 7.96 (dd, 1H, J = 8.1, 8.1 Hz), 8.17 (ddd, 1H, J = 8.0, 8.0, 1.4 Hz), 8.58 (dd, 1H, J = 7.4, 1.0 Hz), 8.77 (d, 1H, J = 9.1 Hz), 8.81 (d, 1H, J = 8.5 Hz), 9.30 (s, 1H), 12.87 (s, 1H); Anal $C_{16}H_{13}N_2I$, C, H, N.

5-Ethyl-10*H*-indolo[3,2-*b*]quindolinium iodide (3b). Prepared by method A: mp 274–276 °C; ^{1}H NMR (DMSO-d_6) δ 1.76 (t, 3H, J = 7.2 Hz), 5.55 (q, 2H, J = 7.2 Hz), 7.56 (ddd, 1H, J = 7.6, 7.6, 1.1 Hz), 7.88 (d, 1H, J = 8.2 Hz), 7.95 (dd, 1H, J = 8.2, 8.2 Hz), 7.97 (dd, 1H, J = 7.3, 7.3 Hz), 8.18 (ddd, 1H, J = 8.4, 8.4, 1.4 Hz), 8.60 (dd, 1H, J = 8.3, 1.2 Hz), 8.66 (d, 1H, J = 8.5 Hz), 8.78 (d, 1H, J = 9.1 Hz), 9.32 (s, 1H), 12.92 (s, 1H); Anal. $C_{17}H_{15}N_2I$, C, H, N.

5-nPropyl-10*H*-indolo[3,2-*b*]quindolinium iodide (3c). Prepared by method B: mp 277–279 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 7.3 Hz), 2.15 (quint, 2H, J = 7.8 Hz), 5.47 (t, 2H, J = 8.1 Hz), 7.56 (dd, 1H, J = 7.6, 7.6 Hz), 7.88 (d, 1H, J = 7.8 Hz), 7.94 (dd, 1H, J = 6.8, 6.8 Hz), 7.97 (dd, 1H, J = 8.3, 8.3 Hz), 8.17 (ddd, 1H, J = 8.1, 8.1, 1.0 Hz), 8.53 (d, 1H, J = 8.8 Hz), 8.60 (d, 1H, J = 7.3 Hz), 8.79 (d, 1H, J = 9.3 Hz), 9.32 (s, 1H), 12.85 (s, 1H); Anal. $C_{18}H_{17}N_2I$, C, H, N.

5-nPentyl-10*H*-indolo[3,2-*b*]quindolinium iodide (3d). Prepared by method A: mp 251–252 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7.2 Hz), 1.41 (sextet, 2H, J = 7.5 Hz), 1.67 (quint, 2H, J = 7.7 Hz), 2.12 (quint, 2H, J = 7.9 Hz), 5.50 (t, 2H, J = 8.1 Hz), 7.58 (ddd, 1H, J = 7.5, 7.5, 1.0 Hz), 7.88 (d, 1H, J = 8.0 Hz), 7.95 (dd, 1H, J = 6.7 Hz), 7.98 (dd, 1H, J = 8.0 Hz), 8.19 (ddd, 1H, J = 7.7, 7.7, 1.3 Hz), 8.53 (d, 1H, J = 8.7 Hz), 8.60 (dd, 1H, J = 8.7, 1.2 Hz), 8.77 (d, 1H, J = 9.2 Hz), 9.33 (s, 1H), 12.91 (s, 1H); Anal. $C_{20}H_{21}N_2I$, C, H, N.

5-Allyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3e). Prepared by method A: mp 255–257 °C; ^{1}H NMR (DMSO-d_6) δ 4.81 (d, 1H, J = 16.2 Hz), 5.29 (d, 1H, J = 10.5 Hz), 6.18 (s, 2H, broad), 6.47 (m, 1H), 7.51 (dd, 1H, J = 6.8, 6.8 Hz), 7.91 (m, 3H), 8.16 (dd, 1H, J = 6.8, 6.8 Hz), 8.52 (d, 1H, J = 9.0 Hz), 8.59 (dd, 1H, J = 9.0, 9.0 Hz), 8.68 (dd, 1H, J = 9.0 Hz), 9.41 (s, 1H), 13.05 (s, 1H); Anal. $C_{18}H_{15}N_2Br$, C, H, N.

5-Cyclopropylmethyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3f). Prepared by method B: mp 264–265 °C; ^{1}H NMR (DMSO-d_6) δ 0.62 (m, 2H), 0.77 (m, 2H), 1.58 (m, 1H), 5.58 (d, 2H, J = 5.9 Hz), 7.57 (dd, 1H, J = 6.8, 6.8 Hz), 7.89 (dd, 1H, J = 8.1, 8.1 Hz), 7.94 (dd, 1H, J = 7.3, 7.3 Hz), 7.97 (dd, 1H, J = 7.5, 7.5 Hz), 8.17 (ddd, 1H, J = 7.1, 7.1, 1.1 Hz), 8.61 (d, 1H, J = 7.3 Hz), 8.69 (d, 1H, J = 7.6 Hz), 8.81 (d, 1H, J = 8.2 Hz), 9.36 (s, 1H), 13.03 (s, 1H); Anal. $C_{19}H_{17}N_2Br$, C, H, N.

5-Cyclobutylmethyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3g). Prepared by method B: mp 262–263 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (m, 2H), 1.95 (m, 5H), 5.60 (d, 2H, J = 6.9 Hz), 7.48 (ddd, 1H, J = 7.6, 7.6, 1.3 Hz), 7.81 (d, 1H, J = 7.9 Hz), 7.86 (dd, 1H, J = 6.9, 6.9 Hz), 7.89 (dd, 1H, J = 7.6 Hz), 8.09 (ddd, 1H, J = 8.1, 8.1, 1.4 Hz), 8.49 (d, 1H, J = 8.9 Hz), 8.52 (d, 1H, J = 8.9 Hz), 8.80 (d, 1H, J = 9.2 Hz), 9.27 (s, 1H); Anal. $C_{20}H_{19}N_2Br{\cdot}0.3\ H_2O$, C, H, N.

5-Cyclopentylmethyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3h). Prepared by method A: mp 280–283 °C; ^{1}H NMR (DMSO-d_6) δ 0.75–1.80 (m, 9H), 5.63 (d, 1H, J = 7.9 Hz), 7.55 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz), 7.92 (m, 3H), 8.15 (ddd, 1H, J = 8.1, 8.1, 1.2 Hz), 8.60 (m, 2H), 8.88 (d, 1H, J = 9.5 Hz), 9.35 (s, 1H); Anal. $C_{21}H_{21}N_2Br$, C, H, N.

5-Cyclohexylmethyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3i). Prepared by method A: mp 270–271 °C; ^{1}H

NMR (DMSO-d_6) δ 1.00–1.70 (m, 11H), 5.48 (s, 2H, broad), 7.57 (ddd, 1H, J = 7.9, 7.9, 1.0 Hz), 7.88 (d, 1H, J = 8.0 Hz), 7.93 (dd, 1H, J = 7.1, 7.1 Hz), 7.97 (dd, 1H, J = 8.0 Hz), 8.15 (ddd, 1H, J = 8.1, 8.1, 1.6 Hz), 8.59 (d, 1H, J = 9.0 Hz), 8.60 (d, 1H, J = 7.9 Hz), 8.85 (d, 1H, J = 9.4 Hz), 9.35 (s, 1H), 13.05 (s, 1H); Anal. $C_{22}H_{23}N_2Br{\cdot}0.5\ H_2O$, C, H, N.

5-Benzyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3j). Prepared by method A: mp 245–246 °C; ^{1}H NMR (CH_3OH-d_4) δ 5.48 (s, 2H), 6.85 (s, 2H), 7.21 (m, 2H), 7.38 (m, 4H), 7.90 (m, 3H), 8.10 (ddd, 1H, J = 7.9, 7.9, 1.5 Hz), 8.28 (d, 1H, J = 8.5 Hz), 8.45 (d, 1H, J = 9.1 Hz), 8.57 (dd, 1H, J = 7.1, 1.2 Hz), 9.31 (s, 1H); Anal. $C_{22}H_{17}N_2Br$, C, H, N.

5-(2-Phenylethyl)-10*H*-indolo[3,2-*b*]quindolinium bromide (3k). Prepared by method A. Two products were obtained after chromatography. The more polar product was found to be the desired product **3k**: mp 256–257 °C; ^{1}H NMR (DMSO-d_6) δ 3.47 (t, 2H, J = 6.8 Hz), 5.81 (t, 2H, J = 6.7 Hz), 7.21 (m, 5H), 7.54 (ddd, 1H, J = 6.9, 6.9, 1.1 Hz), 7.87 (d, 1H, J = 7.5 Hz), 7.92 (m, 2H), 8.06 (ddd, 1H, J = 7.2, 7.2, 1.4 Hz), 8.58 (m, 3H), 9.35 (s, 1H), 13.01 (s, 1H); Anal. $C_{23}H_{19}N_2Br$, C, H, N.

5-(3-Phenylpropyl)-10*H*-indolo[3,2-*b*]quindolinium bromide (3l). Prepared by method A: mp 230–232 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (m, 2H, J = 7.8 Hz), 3.08 (t, 2H, J = 7.3 Hz), 5.49 (t, 2H, J = 8.2 Hz), 7.35 (m, 6H), 7.92 (m, 4H), 8.17 (ddd, 1H, J = 8.0, 8.0, 1.4 Hz), 8.60 (dd, 1H, J = 8.5, 1.3 Hz), 8.73 (d, 1H, J = 9.0 Hz), 9.31 (s, 1H), 12.2 (s, 1H); Anal. $C_{24}H_{21}N_2Br{\cdot}0.4\ (C_2H_5)_2O$, C, H, N.

5-(5-Phenylpentyl)-10*H*-indolo[3,2-*b*]quindolinium bromide (3m). Prepared by method A: mp 218–219 °C; ^{1}H NMR (DMSO-d_6) δ 1.70 (m, 4H), 2.15 (m, 2H), 2.58 (t, 2H, J = 7.0 Hz), 5.50 (t, 2H, J = 8.0 Hz), 7.15 (m, 3H), 7.22 (m, 2H), 7.55 (ddd, 1H, J = 7.4, 7.4, 1.3 Hz), 7.89 (d, 1H, J = 8.3 Hz), 7.95 (dd, 1H, J = 6.4, 6.4 Hz), 7.97 (dd, 1H, J = 6.4, 6.4 Hz), 8.18 (ddd, 1H, J = 8.0, 8.0, 1.2 Hz), 8.51 (d, 1H, J = 8.9 Hz), 8.60 (dd, 1H, J = 8.5, 1.0 Hz), 8.76 (d, 1H, J = 9.3 Hz), 9.33 (s, 1H), 12.98 (s, 1H). Anal; $C_{26}H_{25}N_2Br$, C, H, N.

5-(8-Phenyloctyl)-10*H*-indolo[3,2-*b*]quindolinium bromide (3n). Prepared by method A: mp 245–247 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (m, 6H), 1.53 (quint, 2H, J = 7.0 Hz), 1.67 (quint, 2H, J = 7.2 Hz), 2.09 (m, 2H, J = 7.1 Hz), 2.53 (t, 2H, J = 7.5 Hz), 5.49 (t, 2H, J = 7.9 Hz), 7.14 (m, 3H), 7.24 (m, 2H), 7.55 (ddd, 1H, J = 7.1, 7.1, 1.0 Hz), 7.88 (d, 1H, J = 7.8 Hz), 7.94 (dd, 1H, J = 7.0, 7.0 Hz), 7.97 (dd, 1H, J = 8.0, 8.0 Hz), 8.17 (ddd, 1H, J = 8.8, 8.8, 1.3 Hz), 8.51 (d, 1H, J = 8.5 Hz), 8.60 (dd, 1H, J = 8.3, 1.0 Hz), 8.76 (d, 1H, J = 9.1 Hz), 9.33 (s, 1H), 12.99 (s, 1H); Anal. $C_{29}H_{31}N_2Br$, C, H, N.

5-Carbethoxymethyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3o). Prepared by method A. Repeated chromatography afforded two products. The more polar product was found to be the desired product **3o**: mp 196–197 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 3H, J = 6.4 Hz), 4.30 (q, 2H, J = 6.4 Hz), 6.55 (s, 2H, broad), 7.51 (ddd, 1H, J = 6.9, 6.9, 1.2 Hz), 7.89 (d, 1H, J = 7.8 Hz), 7.93 (dd, 1H, J = 7.0, 7.0 Hz), 7.98 (dd, 1H, J = 7.9, 7.9 Hz), 8.18 (ddd, 1H, J = 6.6, 6.6, 1.3 Hz), 8.39 (d, 1H, J = 7.6 Hz), 8.64 (dd, 1H, J = 7.2, 1.2 Hz), 8.72 (d, 1H, J = 9.1 Hz), 9.45 (s, 1H); Anal. $C_{19}H_{17}N_2O_2Br{\cdot}0.5\ H_2O$, C, H, N.

5-Cyanomethyl-10*H*-indolo[3,2-*b*]quindolinium bromide (3p). Prepared by method B: mp 250–252 °C; ^{1}H NMR (DMSO-d_6) δ 6.79 (s, 2H), 7.61 (dd, 1H, $_J$ = 7.6, 7.6 Hz), 7.93 (d, 1H, J = 8.4 Hz), 8.01 (d, 1H, J = 8.7 Hz), 8.04 (dd, 1H, J = 8.7, 8.7 Hz), 8.30 (ddd, 1H, J = 8.1, 8.1, 1.1 Hz), 8.68 (d, 1H, J = 7.9 Hz), 8.86 (d, 1H, J = 8.9 Hz), 8.88 (d, 1H, J = 8.6 Hz), 9.49 (s, 1H), 13.17 (s, 1H); Anal. $C_{17}H_{12}N_3Br$, C, H, N.

5-Methoxymethyl-10*H*-indolo[3,2-*b*]quindolinium iodide (3q). Prepared by method C. A mixture of quindoline (0.150 g, 0.688 mmol), CH_3OCH_2I (0.60 mL), and benzene (10 mL) was stirred in a 25-mL round-bottomed flask at rt for 2 h. The mixture was diluted with EtOAc, the yellow precipitates formed were filtered and recrystallized from MeOH to give crystalline needles (0.160 g, 60%): mp 232–235 °C; ^{1}H NMR (DMSO-d_6) δ 3.54 (s, 3H), 6.87 (s, 2H), 7.53 (dd, 1H, J = 6.8, 6.8 Hz), 7.87 (d, 1H, J = 8.3 Hz), 7.96 (m, 2H), 8.20 (ddd, 1H, J = 7.1, 7.1, 1.0 Hz), 8.62 (d, 1H, J = 7.2 Hz), 8.65 (d, 1H, J = 7.4 Hz), 8.90 (d, 1H, J = 8.2 Hz), 9.44 (s, 1H), 13.02 (s, 1H); Anal. $C_{17}H_{15}N_2IO$, C, H, N.

10-Methyl-indolo[3,2-*b*]quinoline (5). A mixture of quindoline (0.400 g, 1.83 mmol), KOH (0.200 g), acetone (20 mL), and iodomethane (0.5 mL) was stirred at rt for 30 min. A second portion of an equal amount of KOH and iodomethane was added. After the mixture was stirred for another 2 h, acetone was removed by rotary evaporation. Chromatography of the concentrated material using 20–30% EtOAc:hexanes (gradient elution) yielded a yellow solid (0.420 g, 98%). Recrystallization using EtOAc:hexanes afforded the desired product: mp 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 7.33 (dd, 1H, J = 7.7, 7.7 Hz), 7.40 (d, 1H, J = 8.1 Hz), 7.52 (ddd, 1H, J = 7.1, 7.1, 1.3 Hz), 7.65 (m, 2H), 7.90 (s, 1H), 7.95 (dd, 1H, J = 8.5, 0.9 Hz), 8.33 (d, 1H, J = 8.2 Hz), 8.55 (dd, 1H, J = 7.8, 0.9 Hz). Anal. $C_{16}H_{12}N_2{\cdot}0.3\ H_2O$.

5-Benzoylmethyl-10*H*-indoloquinolium[3,2-*b*]bromide (3r). Prepared by method A to give **3r** in 28%: mp. 250.5–251.5 °C; LR-MS(CI), m/z 337 (MW-Br-); ^{1}H NMR (DMSO-d_6) δ 7.32 (m, 1H), 7.44 (s, 2H, broad), 7.76 (dd, 2H, J = 6.8, 6.8 Hz), 7.91 (m, 4H), 8.11 (m, 2H), 8.37 (m, 2H), 8.66 (m, 2H), 9.48 (s, 1H); Calculated for $C_{23}H_{17}N_{23}OBr{\cdot}0.6\ H_2O$: C, 64.53; H, 4.28; N, 6.54. Found: C, 64.30; H, 4.67; N, 7.44.

In vitro antifungal activity. Inhibitory activity against *C. albicans* B311 and *C. neoformans* (ATCC 52657) was determined in 96-well microtiter plates.[31,32] To prepare inocula, a 24 h culture of *C. albicans* was diluted to 10,000 colony-forming units/mL in Sabouraud's dextrose broth and a 72 h culture of *C. neoformans* was diluted to 200,000 CFUs/mL in mycophil broth.

To each well of a 96-well microtiter plate, an aliquot of 175 mL of inoculum was added to 25 mL of sample (serially diluted in saline) such that the greatest final concentration tested was 250 mg/mL. Each plate contained a positive drug control (amphotericin B), a positive-growth control (no drug added), and a vehicle control (DMSO, highest concentration was 2.5%). The plates were incubated at 37 °C for 24 h (*C. albicans*) or at 30 °C for 48 h (*C. neoformans*). Following incubation, the plates were sealed, vortexed, and turbidity recorded as absorbance at 630 nm measured with an EL-340 plate reader (Bio-Tek Instruments, Inc., Winooski, VT). The inhibitory activity of the sample was expressed as the minimum inhibitory concentration (MIC), the lowest concentration tested which inhibited 80% of growth compared to controls.

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