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Substituted Indoloquinolines as New Antifungal Agents

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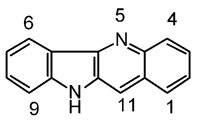
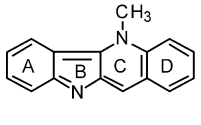
Abstract—Cryptolepine (**2**) possesses desirable properties to serve as a lead in developing new antifungal agents. Using SAR techniques, several analogues of cryptolepine were designed to increase potency and to broaden the antifungal spectrum over several opportunistic microorganisms. A number of 2-substituted indoloquinolines have been synthesized and evaluated in antifungal screens and several have been shown to increase potency and expand the antifungal spectrum of cryptolepine. Comparison of MICs of a number of these analogues with standard antifungal agents, shows them to be comparable to Amphotericin B and Ketoconazole.

Although the AIDS epidemic appears to have stabilized in the developed countries, there is continuing interest in new antifungal agents partially because of the chronic nature of the disease and the continuing dramatic rise in AIDS cases in the developing countries.[1] In sub-Saharan Africa, for example, it is estimated that 25.3 million people were living with AIDS by the close of last year. There are several additional reasons,[2] such as, toxicity and the emergence of fungi resistant to currently available antifungal agents that has kept the need to develop new agents at the forefront of current research.

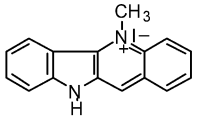
The indoloquinoline alkaloid, Cryptolepine (**2**) and its analogues have been the subject of several recent publications.[3–6] Although Cryptolepine (isolated from *Cryptolepis sanguinolenta*)[7] has been shown to have antibacterial,[8] antihypertensive,[9] anti-aggregatory,[10] anticancer,[4,11] and other properties, it was only more recently that we have documented its antifungal activity.[12] As part of a SAR study, we have shown that, N-5 alkylation of the tetracyclic structure, quindoline, **1**, is essential for antifungal activity[13] and ω-cycloalkylpentyl substituents on the N-5 atom has a broad-spectrum inhibition of several opportunistic infections.[14] Apparently, the tetracyclic structure of cryptolepine is not necessary for its antifungal activity and this has led us to propose the δ-carboline moiety as a pharmacophore for antifungal activity.[14] Cryptolepine has interesting properties to serve as a lead compound in antifungal drug development. For example, its zone of inhibition against *Candida albicans*, *Cryptococcus neoformans Aspergillus niger* are wider than those of Amphotericin B in agar well assays, it is fairly water soluble (~1.2 mg/mL), it has relatively low toxicity[15] and a broad spectrum of activity against opportunistic fungal and bacterial infections associated with AIDS.[16] The purpose of this study was to investigate the ability of substituents on the tetracyclic moiety to extend the antifungal spectrum and increase potency.



1, Quindoline



2, Free base form



2, Salt form

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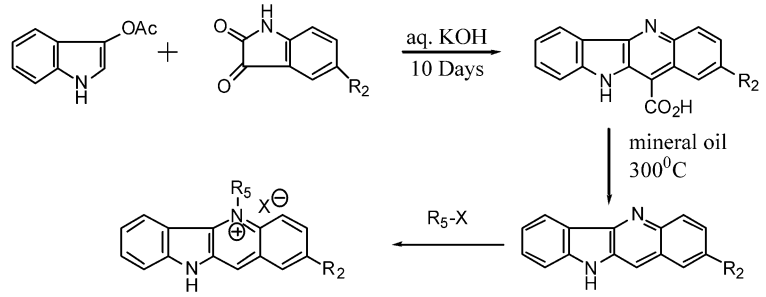
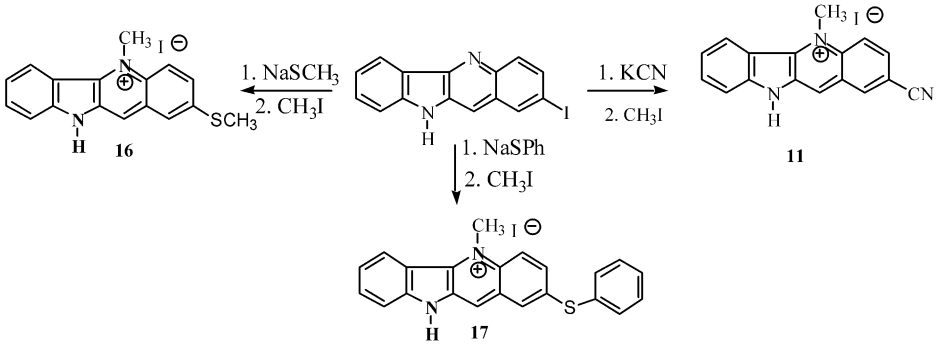


Scheme 1. Holt method of the synthesis of quindolines.



Scheme 2. Nucleophilic substitution of 2-iodoquinoline to other designed analogues.

Chemistry

Several synthetic methods leading to the construction of the tetracyclic indoloquinoline structure, have been described recently.[3,5,17–19] Because the 2-position of quindoline is *para* to the N-5 atom and because substitution on N-5 modulates antifungal potency, we selected the 2-position for exploration. Thus, in this study, methods were chosen that would enable the introduction of substituents at the 2-position of the quindoline nucleus.

Although it has a 10-day waiting period to complete, the Holt method[20] provided a first entry into the 2-substituted compounds (Scheme 1). Thus, stirring a solution of 5-substituted-isatin and 3-acetylindoxyl, under basic conditions, 11-carboxy-2-substituted quindoline was obtained in excellent yields. Decarboxylation in mineral oil and subsequent N-5 alkylation were achieved as previously reported. Compounds **3**–**6** and **13** were synthesized by this method. This approach was, however, limited by the availability of starting materials and the failure of isatins with strongly electron-withdrawing groups to undergo the condensation reaction under the stated conditions.

An alternative pathway to the 2-substituted quindolines involves obtaining 2-iodoquindoline by the Holt method and converting to the desired target compound by nucleophilic substitution (Scheme 2). Compounds **11**, **16**, and **17** and intermediates leading to **7**–**10** utilized this approach.

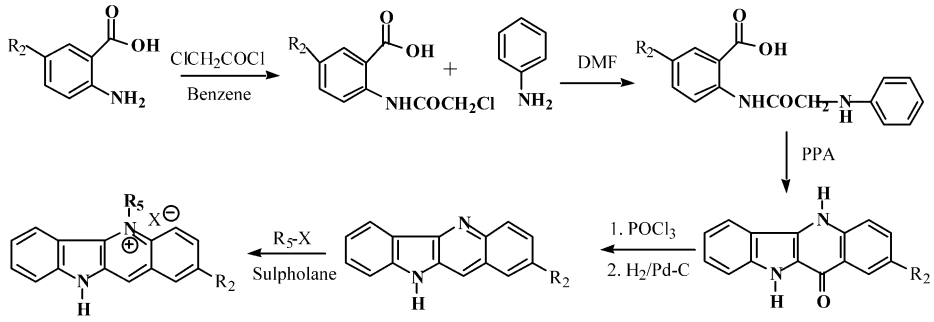
A third pathway to 2-substituted quindolines was much broader in scope and amenable to other substitutions and was therefore preferred.[21] To obtain 2-substituted quindolines by this method, 5-substituted anthranilic acids were required and were available from commercial sources. Thus, 5-substituted anthranilic acid was acylated with 2-chloroacetyl chloride and utilized in alkylating aniline to yield the desired intermediate (Scheme 3). In a double intramolecular cyclization reaction with polyphosphoric acid (PPA), the alkylated intermediate was converted to 2-substituted-11-indoloquinolone, which was subsequently converted to 2-substituted quindoline by chlorination with phosphorous oxychloride ($POCl_3$) and hydrogenation on Pd/C to remove the chlorine. The desired product (**14**) was then obtained by alkylation in the usual way. Compound **15**, was obtained from **14** by *O*-demethylation using pyridine hydrochloride.[22]

A fourth method was previously reported[23] and is shown in Scheme 4. In this method, 3-bromo-6-nitroquinoline is aminated, then phenylated using triphenylbismuth diacetate in the presence of metallic copper to afford 3-anilino-6-nitroquinoline. Subsequent cyclization was effected by palladium(II) acetate in refluxing trifluoroacetic acid to afford 2-nitroquindoline.

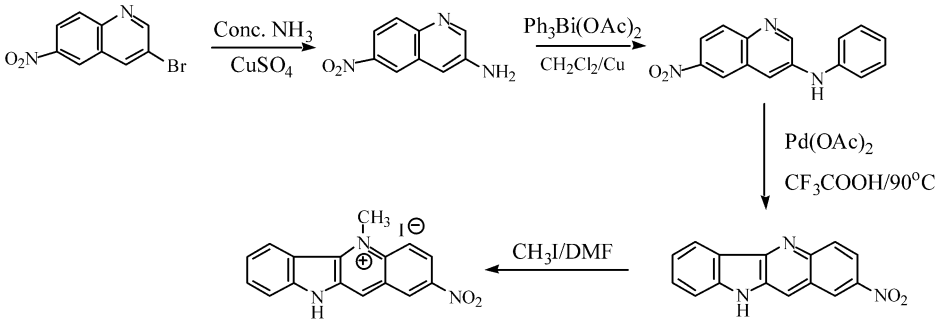
Most of the compounds reported herein were, however, obtained from 2-iodoquindoline by nucleophilic substitution with the appropriate substituents. The sulfoxides (**7** and **9**) were obtained from the corresponding sulfides by oxidation with one equivalent of mCPBA and the sulfones (**8** and **10**) by using two equivalents of mCPBA.

Results and Discussion

We have previously shown that alkylation of the tetracyclic 10*H*-indolo[3.2-*b*]quinolines confers antifungal



Scheme 3. Alternative synthesis of 2-substituted quindolines.

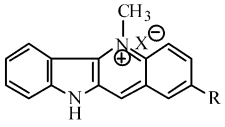


Scheme 4. Synthesis of 2-nitroquindoline.

properties to the structure.[13,14] In addition, ω-cyclohexylpentyl and ω-phenylpentyl moieties were identified as optimal N-5 substituents. Although antifungal activity increased substantially, it was hypothesized that higher activities might result from modifying the electron density around the N-5 atom through substitution on the quindoline moiety. Position 2 was selected for exploration because it was directly *para* to the N-5 atom whose substitution led to increase in anticryptococcal activity.[13] In addition, position 2 was easily accessible synthetically and substituents at this position can influence the electron density around the N-5 atom through resonance effect.

To test this hypothesis, we investigated the effect of electron withdrawing groups at position 2 and the results are reported in Table 1. While the previously reported N-5 alkylated compounds[13,14] showed activity primarily against *C. neoformans*, the 2-substituted electron-withdrawing analogues of cryptolepine showed activity against both *C. neoformans* and *C. albicans*, two important opportunistic fungi associated with AIDS. In particular, all the 2-halogenated analogues (**3**–**6**) showed higher potencies than Cryptolepine. In addition, 2-chlorocryptolepine (**4**) was even more potent against *Aspergillus flavus* (MIC = 0.2 μg/mL). Interestingly, while the two analogues, 2-cyano (**11**) and 2-nitro (**12**), with

Table 1. The effect of electron withdrawing substituents on antifungal activity



Compd	Structure	Minimum inhibition concentration (MIC, μg/mL)		
	R	*C. neoformans*	*C. albicans*	*A. flavus*
2	H	12.5–15.6	250	
3	F	7.8	15.6	
4	Cl	2.0	5.5	0.2
5[b]	Br	≤1.9	3.9	
6	I	7.8	3.9	
7	CH_3SO	50	50	
8	CH_3SO_2	>50	35[a]	
9	PhSO	>50	40[a]	
10	$PhSO_2$	>50	35[a]	
11	CN	20[a]	0.2[a]	
12	NO_2	0.3	20	
	Amphotericin B	0.39	0.39	

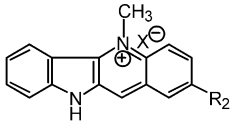
[a]IC_{50} values.
[b]While this manuscript was in review, we became aware of the article by Wright et al.[24] on the antisplasmodial action of 2-bromocryptolepine.

strong resonance electron withdrawing capacity were quite active, the sulfoxides (**7** and **9**) and sulfones (**8** and **10**) possessing equally strong resonance electron withdrawing capacity, were much less active against both fungi. These observations do not appear to support the notion that there exists a correlation between electron-withdrawing effect (at position 2) and antifungal potency.

In Table 2, we report the effect of electron donating groups on antifungal activity. In general, the analogues with electron-donating groups are more active than cryptolepine and are potent against both *C. neoformans* and *C. albicans*. Based on a comparison of compounds **13**, **16**, and **17** (with hydrophobic substituents), with analogues, **12** and **15** (possessing hydrophilic substituents) it is suggested that hydrophobicity per se does not appear to play a major role in the increase in antifungal activity. The thiomethoxy analogue, **16**, was further tested against *A. flavus* and was found to be active (MIC = 7.8 μg/mL). Clearly, substitution at the 2-position not only increases potency, but also extends or broadens the antifungal spectrum of cryptolepine. With this limited data, it is tempting to suggest that the increased potency of the compounds are due to steric factors at the 2-position, since electron-withdrawing and electron-donating as well as substituents with hydrophobic and hydrophilic properties appear to increase potency. However, the fact that compounds **7**–**10** (sulfoxide and sulfone analogues) have reduced activity makes the steric hypothesis less tenable.

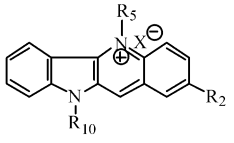
In Table 3, an attempt is made to combine 2-substitution with N-5 alkylation in order to optimize potency. Compound **19** is the ω-cyclohexylpentyl analogue of **18**[13] and shows increased potency against *C. albicans*. It was also of interest to incorporate our finding that N-10 alkylation, which introduces a permanent quaternary pyridinium nitrogen at N-5, retains or improves potency. Thus, compound **20**, an N-10 methylated analogue of **18,** has an extended antifungal spectrum to include both *C. neoformans* and *C. albicans*. However, N-10 methylation of 2-bromocryptolepine (**5**) to obtain

Table 2. The effect of electron donating substituents on antifungal activity



Compd	Structure	Minimum inhibition concentration (MIC, μg/mL)		
	R_2	*C. neoformans*	*C. albicans*	*A. flavus*
2	H	12.5–15.6	250	
13	CH_3	31.2	15.6	
14	OCH_3	≤1.9	≤1.9	
15	OH	15.6	3.9	
16	CH_3S	3.12	1.56	7.8
17	PhS	6.25	50	
	Amphotericin B	0.39	0.39	

Table 3. Effect of multi-substitution on antifungal activity

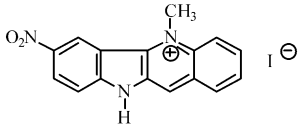


Compd	Structure[x]			Minimum inhibition concentration (MIC, μg/mL)		
	R_2	R_5	R_{10}	*C. neoformans*	*C. albicans*	*A. flavus*
2	H	CH_3	H	12.5–15.6	250	
18[a]	H	$Ph(CH_2)_5$—	H	≤1.9	125	
19	H	$C_6H_{11}(CH_2)_5$	H	≤1.9	≤1.9	
20	H	$Ph(CH_2)_5$—	CH_3	6.25	6.25	
21	Br	CH_3—	CH_3	3.12	3.12	
22	Br	$Ph(CH_2)_5$—	H	62.5	125	
23	Br	$Ph(CH_2)_5$—	CH_3	≤1.9	62.5	
24	Br	$C_6H_{11}(CH_2)_5$	H	≤1.9	≤1.9	
25	OCH_3	$Ph(CH_2)_5$—	H	≤1.9	125	
26	CH_3O	CH_3	CH_3	6.25	3.12	
27	CH_3O	$Ph(CH_2)_5$—	CH_3	12.5	3.12	
28	CN	$C_6H_{11}(CH_2)_5$	H	12.5	12.5	
		Amphotericin B		0.39	0.39	

[a]Previously reported in ref 13.

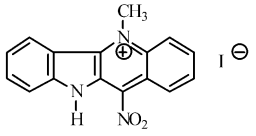
21 did not result in further increase in potency and replacing the N-5 methyl group with the optimum substituents (**22** and **23**) has a mixed effect. The brominated analogue of **19**, that is, **24** retained activity (MIC = 1.9 mg/mL for both Cn and Ca). Similar effects were obtained on the 2-methoxy analogues (**25**–**27**). Replacing N-5 methyl group on **11** with the optimum ω-cyclohexylpentyl group to form **28** also resulted in mixed effect, as there is an increase in *C. neoformans* potency but a decrease in *C. albicans* activity.

Finally, because 2-nitrocryptolepine (**12**) has a high potency against *C. neoformans*, we investigated the effect of placing the nitro group at other synthetically feasible positions on the quindoline nucleus. Thus, the 7- and 11-nitro analogues (**29** and **30**) were synthesized and subsequently evaluated against *C. neoformans* and *A. flavus*. Both compounds showed similar high potency (MIC for Cn = 0.25 and for Af = 0.39 μg/mL) but were not available in sufficient quantities for a full spectrum evaluation.



Compound **29**

Cn; 0.25: A. flavus; 0.39: Ca; NT



Compound **30**

Cn; 0.25: A. flavus; 0.39: Ca; NT

Clearly, the SAR of these substituents appears to be complex at best and we speculate that multiple mechanisms of fungal inhibition may be involved. This speculation is supported by the report[11] that cryptolepine interferes with topoisomerase II and intercalates with DNA. Whether these events occur in these opportunistic fungi evaluated in this study is unknown at this time. Toxicity studies along with the identification of the mechanism of action of this group of compounds should follow shortly.

Conclusion

This study confirms our previous report that N-5 alkylation with an appropriate group is a requirement for antifungal activity and ω-cycloalkylpentyl substitution at the 5-position enhances potency and appears to confer an optimum activity.[13,14] Substitution at the 2-position of the quindoline ring increases potency and broadens the antifungal spectrum of this group of compounds. However, combining an optimum substituent at the 5-position with 2-substituents did not result in further increases in potency. Similarly, N-10 alkylation of 2-substituted compounds resulted in minimum changes in the activity of these compounds. A cursory look at the types of substituents at the 2-position reveals that the effect of electron-donating and-withdrawing groups at the 2-position appears to be complex, as both types of substituents enhance activity. In addition, hydrophobicity does not appear to have any obvious effect on the SAR of these compounds. Further investigation of the SAR is warranted and is currently underway in our laboratories.

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 Atlantic Microlab, Inc., Norcross, GA, USA 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 stored over 4 Å molecular sieves. Sulpholane was dried over 4 Å molecular sieves. 5-Cyclohexylpentyl bromide and 5-phenylpentyl bromide were prepared by treatment of the corresponding alcohols with PBr_3.[13,14] The remaining alkyl halides were obtained from either Sigma-Aldrich Chemicals or Fisher Scientific and were used without further purification.

General procedure for synthesizing 2-substituted quindolines: method A

2-Fluoro-10*H*-indolo[3,2-*b*]quinoline (2-fluoroquindoline). A mixture of 3-indolyl acetate (3.504 g, 20 mmol), 5-fluoroisatin (3.305 g, 20 mmol) and KOH (9.60 g) in 130 mL of water was stirred for 10 days under N_2 and at room temperature. The mixture was then diluted with water. To this mixture was added concd HCl until a permanent faint yellow precipitate was observed. It was then filtered and washed with water. The filtrate was collected, an equal volume of EtOH was added and the resulting solution was acidified with concd HCl until a lot of yellow precipitate was formed (pH 3–4). It was then filtered, washed with water and EtOH, and dried at 120 °C to afford 2-fluoro-10*H*-indolo[3,2-*b*]quindoline-11-carboxylic acid as a yellow solid (3.70 g). A mixture of 2-fluoro-11-carboxylquindoline (3.0 g) in 100 mL of mineral oil was stirred at 300 °C. After 30 min, the mixture was cooled to room temperature, petroleum ether (100 mL) was added, the mixture was filtered and the solid was washed with petroleum ether. The resulting solid was chromatographed to give the decarboxylated compound, which was recrystallized from benzene to give 2.13 g of crystals: mp 254–256 °C.

General procedure for synthesizing 2-substituted quindolines: method B

2-Methoxy-10*H*-indolo[3,2-b]quinoline (2-methoxyquindoline). A mixture of 5-methoxy-anthranilic acid (10.0 g, 60 mmol), and chloroacetyl chloride (4.8.0 mL, 60 mmol) in benzene (150 mL) was heated at 100 °C for 5 h

and then allowed to cool to room temperature. The solvent was evaporated and the crude residue was taken up in DMF (30 mL). Aniline (20 mL, 219 mmol) was added to the residue and then heated at 90 °C for 5 h. The mixture was allowed to cool to room temperature, H_2O (50 mL) was added and the resulting mixture was extracted exhaustively with $CHCl_3$. The pooled $CHCl_3$ extracts were washed with H_2O (50 mL), dried (Na_2SO_4) and the solvent was evaporated to give a residue, which was recrystallized from EtOAc to give a solid (7.8 g).

A mixture of the above product (7.8 g) and polyphosphoric acid (PPA, 100g) was heated at 130 °C for 3 h. After cooling, the mixture was poured onto ice and neutralized with a saturated solution of $NaHCO_3$ before filtration of the solid. The solid was washed several times with H_2O and oven-dried at 115 °C for 1 h. A solution of the crude 2-methoxy-11-quindolone in $POCl_3$ (150 g) was heated at 125 °C for 2 h, at which point $POCl_3$ was evaporated and a saturated solution of $NaHCO_3$ was added. The solution was extracted with EtOAc (3×100 mL), dried (Na_2SO_4) and solvent was evaporated to yield crude 2-methoxy-11-chloroquindoline, (1.4 g). A portion of this material (1.1 g), NaOAc (4.5 g) and 10% Pd/C (0.5 g) in AcOH (100 mL) was hydrogenated at 60 psi for 2 h. The solution was filtered to remove Pd/C, solvent evaporated to a small volume, basified with ice-cold saturated solution of $NaHCO_3$ and extracted with EtOAc (3×100 mL). The pooled solution was washed with H_2O (50 mL), dried (Na_2SO_4) and solvent was removed under reduced pressure to yield 2-methoxyquindoline (**1g**). ^{1}H NMR ($CDCl_3$) δ 8.43 (1H, d, J=8.1Hz), 8.15 (1H, d, J=10.5Hz), 7.90 (1H, s, br), 7.83 (1H, s), 7.50 (1H, dd, J=8.1, 2.5Hz), 7.35 (1H, d, J=10.5Hz), 7.25 (2H, m), 7.10 (1H, d, J=3.5Hz), 3.90 (3H, s).

General procedure for synthesizing 2-substituted quindolines: method C

2-Nitro-10*H*-indolo[3,2-*b*]quinoline (2-nitroquindoline). A mixture of 3-bromo-6-nitroquinoline (2.5 g), concd NH_3 (14 mL) and $CuSO_4$ (0.3 g) was heated in a sealed tube at 145–150 °C for 17 h and allowed to cool to room temperature. Water (200 mL) was added and resulting mixture was extracted exhaustively with EtOAc. Solvent was removed under reduced pressure and the residue chromatographed over silica gel to yield a yellow solid (0.6 g): mp 255–257 °C. A mixture of the solid (0.5 g, 2.6 mmol), $Ph_3Bi(OAc)_2$ (2.3 g, 3.1 mmol) and Cu (0.3 g) in CH_2Cl_2 (14 mL) was stirred under N_2 at room temperature for 48 h. The mixture was then quenched with H_2O, basified with NH_4OH and extracted with EtOAc (4×50 mL). The pooled EtOAc extract was dried (Na_2SO_4) and solvent removed under reduced pressure to yield a residue, which was purified by chromatography on silica gel to yield deep orange solid. 6-Nitro-3-anilinoquinoline (0.3 g, 1.13 mmol) and $Pd(OAc)_2$ (0.38 g, 1.13 mmol) in CF_3COOH (5 mL) was heated to 90 °C for 30 min. The mixture was cooled to room temperature and poured into 5% ammonia (50 mL) and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation, to yield a residue. The residue was chromatographed on silica gel, with EtOAc/hexanes (30–60%), to obtain a yellow solid (24 mg): mp 344–346 °C. ^{1}H NMR (DMSO-d_6) δ 11.73 (1H, s), 9.21 (1H, s), 8.63 (1H, s), 8.38 (1H, d, J=8.1 Hz), 8.32 (1H, d, J=1.2 Hz), 8.31 (1H, d, J=1.2 Hz), 7.69 (1H, dt, J=8.1, 1.2 Hz), 7.61 (1H, d, J=8.1 Hz), 7.33 (1H, dt, J=8.1, 1.2 Hz).

General procedure for quindoline alkylation: method D

A mixture of alkyl halide (0.8 mL), 2-substituted quindoline (0.100 g) and sulpholane (2.0 mL) was heated in a sealed 10 mL round-bottomed flask overnight. After cooling to room temperature, the mixture was directly chromatographed on silica gel with 5–20% MeOH/CH_2Cl_2 (gradient elution) to give a yellow solid which was usually recrystallized from an appropriate solvent.

General procedure for quindoline alkylation: method E

A mixture of quindoline or 2-substituted quindoline (0.100 g), alkyl halide (2.0 mL) and a few drops of DMF was refluxed in a sealed 10 mL round-bottomed flask overnight. The mixture was allowed to cool to room temperature and then precipitated with Et_2O–MeOH. Flash chromatography with 5–20% MeOH/CH_2Cl_2 (gradient elution) yielded a yellow solid, which was recrystallized from an appropriate solvent.

2-Fluoro-5-methylquindolinium iodide (3). Method D was applied to 2-fluoroquindoline, obtained above, to produce the desired product, **3** which was recrystallized from MeOH to yield yellow crystals (0.16 g): mp 251–253 °C. ^{1}H NMR (DMSO-d_6) δ 12.85 (1H, s, br), 9.21 (1H, s), 8.87 (1H, dd, J=8.1, 3.2 Hz), 8.82 (1H, d, J=8.1 Hz), 8.43 (1H, dd, J=5.4, 2.7 Hz), 8.11 (1H, dt, J=5.4, 2.7 Hz), 7.95 (1H, t, J=5.4 Hz), 7.87 (1H, d, J=5.4 Hz), 7.53 (1H, d, J=5.4 Hz), 5.06 (3H, s). Anal. calcd for $C_{16}H_{12}FIN_2$: C, 50.82; H, 3.20; N, 7.41; found: C, 50.74; H, 3.19; N, 7.35.

2-Chloro-5-methylquindolinium iodide (4). Methods A and D were used: mp 287–289 °C. ^{1}H NMR (DMSO-d_6) δ 12.95 (1H, s), 9.22 (1H, s), 8.23 (1H, d, J=5.4 Hz), 8.77 (1H, dd, J=5.4, 1.2 Hz), 7.96 (1H, d, J=8.1 Hz), 7.87 (1H, t, J=5.4 Hz), 7.52 (1H, t, J=5.4 Hz), 5.03 (3H, s). Anal. calcd for $C_{16}H_{12}ClIN_2$: C, 48.70; H, 3.06; N, 7.10; found: C, 48.53; H, 3.14; N, 7.00.

2-Bromo-5-methylquindolinium iodide (5). Methods A and D were used: mp 294–297 °C. ^{1}H NMR (DMSO-d_6) δ 12.93 (1H, s, br), 9.21 (1H, s), 8.91 (1H, d, J=2.7 Hz), 8.83 (1H, d, J=8.1 Hz), 8.74 (1H, d, J=8.1 Hz), 8.26 (1H, dd, J=5.4, 2.7 Hz), 7.95 (1H, t, J=5.4 Hz), 7.87 (1H, d, J=5.4 Hz), 7.54 (1H, t, J=5.4 Hz), 5.03 (3H, s). Anal. calcd for $C_{16}H_{12}BrIN_2$: C, 43.77; H, 2.75; N, 6.83; found: C, 43.89; H, 2.80; N, 6.32.

2-Iodo-5-methylquindolinium iodide (6). Methods A and D were used: mp 312–314 °C. ^{1}H NMR (DMSO-d_6): δ 9.17 (1H, s), 9.05 (1H, d, J=2.7), 8.79 (1H, d, J=8.1),

8.55 (1H, d, $J=8.1$), 8.36 (1H, dd, $J=8.1$, 2.7), 7.95 (1H, t, $J=8.1$), 7.83 (1H, d, $J=8.1$), 7.52 (1H, t, $J=8.1$), 5.01 (3H, s). Anal. calcd for $C_{16}H_{12}I_2N_2$: C, 39.54; H, 2.49; N, 5.76; found: C, 39.58; H, 2.44; N, 5.69.

5-Methyl-2-phenylsulfenylquindolinium Iodide (9). To a solution of 2-iodoquindoline (0.1 g, 0.29 mmol), obtained by method A, and CuI (10.0 mg) in HMPA (2.0 mL) was added NaSPh (0.2 g, 1.5 mmol) and the resulting mixture was stirred at 130 °C overnight. The reaction was quenched with water and the mixture was extracted with ETOAc (3×25 mL). The combined extracts was washed with brine, dried over (Na_2SO_4), concentrated in vacuo and chromatographed on silica gel to give the desired sulfide, a yellow solid (70 mg): mp 216–217 °C. ^{1}H NMR (DMSO-d_6): δ 11.46 (1H, s), 8.33 (1H, d, $J=8.1$), 8.24 (1H, s), 8.17 (1H, d, $J=8.1$), 8.15 (1H, d, $J=2.7$), 7.60 (2H, m), 7.49 (1H, dd, $J=8.1$, 2.7), 7.35 (6H, m). Anal. calcd for $C_{21}H_{14}N_2S$: C, 77.27; H, 4.32; N, 8.58; found: C, 77.16; H, 4.42; N, 8.54.

To a solution of the above sulfide (0.13 g, 0.4 mmol) in CH_2Cl_2 (3.0 mL) and MeOH (3.0 mL) was added mCPBA (90 mg, 0.52 mmol) at 0 °C and the resulting mixture was stirred for 1.5 h at room temperature. The mixture was chromatographed directly to give 2-phenylsulfenylquindoline, as a bright yellow solid (80 mg): mp 281–282 °C. ^{1}H NMR (DMSO-d_6): δ 11.60 (1H, s), 8.64 (1H, d, $J=1.8$), 8.49 (1H, s), 8.35 (1H, d, $J=8.1$), 8.26 (2H, d, $J=8.1$), 7.79 (2H, dd, $J=5.4$, 1.8), 7.71 (1H, dd, $J=5.4$, 1.8), 7.55 (5H, m), 7.30 (1H, dt, $J=5.4$, 1.8). Anal. calcd for $C_{21}H_{14}N_2OS{\cdot}1.1H_2O$: C, 69.63; H, 4.51; N, 7.73; found: C, 69.79; H, 4.70; N, 7.30.

Using method D, the above sulfoxide (50 mg) was converted to the desired product, **9**, as a yellow solid. Mp 245–246 °C. ^{1}H NMR (DMSO-d_6): δ 9.48 (1H, s), 8.97 (1H, d, $J=1.8$), 8.70 (2H, d, $J=8.1$), 8.22 (1H, dd, $J=8.1$, 1.8), 7.99 (1H, dt, $J=8.1$, 1.8), 7.85 (3H, m), 7.36 (4H, m), 5.10 (3H, s). Anal. calcd for $C_{22}H_{17}IN_2OS{\cdot}1.1H_2O$: C, 52.41; H, 3.84; N, 5.52; found: C, 52.35; H, 3.62; N, 5.52.

5-Methyl-2-methylsulfenylquindolinium iodide (7). Same procedure as for compound **9**: mp 245–246 °C. ^{1}H NMR (DMSO-d_6): δ 13.14 (1H, s), 9.46 (1H, s), 8.95 (1H, d, $J=8.1$), 8.93 (2H, s), 8.84 (1H, d, $J=8.1$), 8.36 (1H, dd, $J=8.1$, 1.8), 7.99 (1H, t, $J=5.4$), 7.87 (1H, d, $J=5.4$), 7.55 (1H, dt, $J=5.4$, 1.8), 5.10 (3H, s), 2.95 (3H, s). Anal. calcd for $C_{17}H_{15}IN_2OS$: C, 48.35; H, 3.58; N, 6.63; found: C, 48.42; H, 3.53; N, 6.58.

5-Methyl-2-phenylsulfonylquindolinium Iodide (10). To a solution of 2-methylthio-quindoline (0.13 g, 0.4 mmol) in CH_2Cl_2 (4.0 mL) and MeOH (4.0 mL) was added mCPBA (0.17 g, 1.0 mmol) and the resulting mixture was stirred at room temperature for 1 h. After the mixture was basified with NH_4OH and chromatographed to give the desired sulfone, as a bright yellow solid (72 mg): mp 275–278 °C. ^{1}H NMR (DMSO-d_6) δ 11.72 (1H, s), 8.95 (1H, d, $J=1.8$ Hz), 8.6 (1H, s), 8.38 (1H, d, $J=8.1$ Hz), 8.32 (2H, d, $J=8.1$ Hz), 8.05 (2H, dd, $J=5.4$, 1.8 Hz), 7.95 (1H, dd, $J=5.4$, 1.8 Hz), 7.63 (5H, m), 7.32 (1H, t, $J=5.4$ Hz). Anal. calcd for $C_{21}H_{14}N_2O_2S$: C, 70.37; H3.94; N, 7.82; found: C, 70.20; H, 4.02; N, 7.71.

Using method D, the above sulfone (40 mg), was converted to **10**, as an orange solid: mp 250–251 °C. ^{1}H NMR (DMSO-d_6) δ 9.53 (1H, s), 9.40 (1H, d, $J=1.2$ Hz), 8.93 (1H, d, $J=8.1$ Hz), 8.84 (1H, d, $J=8.1$ Hz), 8.46 (1H, dd, $J=5.4$, 1.2 Hz), 8.12 (2H, dd, $J=5.4$, 1.2 Hz), 7.99 (1H, t, $J=5.4$ Hz), 7.88 (1H, d, $J=5.4$ Hz), 7.70 (3H, m), 7.55 (1H, t, $J=5.4$ Hz), 5.03 (3H, s). Anal. calcd for $C_{22}H_{17}IN_2O_2S{\cdot}H_2O$: C, 50.98; H, 3.69; N, 5.40; found: C, 51.04; H, 3.63; N, 5.37.

5-Methyl-2-methylsulfonylquindolinium iodide (8). Same procedure as **10**, mp 244–246 °C. ^{1}H NMR (DMSO-d_6) δ 13.18 (1H, s), 9.65 (1H, s), 9.28 (1H, d, $J=1.2$ Hz), 9.03 (1H, d, $J=8.1$ Hz), 8.78 (1H, d, $J=8.1$ Hz), 8.55 (1H, dd, $J=5.4$, 1.2 Hz), 8.02 (1H, t, $J=5.4$ Hz), 7.89 (1H, d, $J=5.4$ Hz), 7.58 (1H, t, $J=5.4$ Hz), 5.10 (3H, s), 3.45 (3H, s). Anal. calcd for $C_{17}H_{15}IN_2O_2S$: C, 46.59; H, 3.45; N, 6.39; found: C, 46.71; H, 3.55; N, 6.33.

2-Cyano-5-methylquindolinium iodide (11). A mixture of 2-iodoquindoline (34 mg, 0.1 mmol), $Pd(PPh_3)_4$ (6 mg, 0.005 mmol) and KCN (13 mg, 0.2 mmol) in THF (2 mL) was heated under N_2 overnight at 70 °C. The resulting mixture was poured into ammonia solution and extracted exhaustively with EtOAc. The pooled extracts was dried (Na_2SO_4) and solvent was removed under reduced pressure to yield a residue, which was purified by chromatography over silica gel. Recrystallization was achieved from Toluene to afford a greenish-yellow solid (19 mg): mp 287–288 °C.

Method D was used to convert the solid to the desired product, **11**: mp 257–258 °C. ^{1}H NMR (DMSO-d_6) δ 9.35 (1H, s), 9.23 (1H, d, $J=1.8$ Hz), 8.95 (1H, d, $J=8.1$ Hz), 8.84 (1H, d, $J=8.1$ Hz), 8.46 (1H, dd, $J=8.1$, 1.8 Hz), 8.01 (1H, dt, $J=8.1$, 1.8 Hz), 7.91 (1H, d, $J=8.1$ Hz), 8.57 (1H, $J=8.1$, 1.8 Hz), 5.06 (3H, s). Anal. calcd for $C_{17}H_{12}IN_3$: C, 53.01; H, 3.14; N, 10.91; found: C, 53.14; H, 3.18; N, 10.85.

2,5-Dimethylquindolinium iodide (13). Method A was used with 5-methylisatin as starting material to obtain 2-methylquindoline, which was methylated with iodomethane (method D): mp 283–286 °C. ^{1}H NMR (DMSO-d_6) δ 12.73 (1H, s, br), 9.15 (1H, s), 8.78 (1H, d, $J=8.1$ Hz), 8.56 (1H, d, $J=8.1$ Hz), 8.42 (1H, s), 8.01 (1H, d, $J=5.4$ Hz), 7.92 (1H, t, $J=5.4$ Hz), 7.83 (1H, d, $J=5.4$ Hz), 7.51 (1H, t, $J=5.4$ Hz), 5.01 (3H, s), 2.63 (3H, s). Anal. calcd for $C_{17}H_{15}IN_2O$: C, 54.56; H, 4.04; N, 7.49; found: C, 54.51; H, 3.96; N, 7.41.

2-Methoxy-5-methylquindolininium iodide (14). Using method D, 2-methoxyquindoline (15 mg) was converted to **14**: mp 286–288 °C. ^{1}H NMR (DMSO-d_6) δ 12.70 (1H, s, br), 9.09 (1H, s), 8.74 (1H, d, $J=8.1$ Hz), 8.67 (1H, d, $J=8.1$ Hz), 7.95 (1H, d, $J=2.7$ Hz), 7.90 (1H, t, $J=5.4$ Hz), 7.78 (2H, m), 7.50 (1H, t, $J=5.4$ Hz), 5.02 (3H, s), 4.03 (3H, s). Anal. calcd for $C_{17}H_{15}IN_2O$: C, 52.33; H, 3.87; N, 7.18; found: C, 52.27; H, 3.82; N, 7.10.

2-Hydroxy-5-methylquindolinium iodide (15). A mixture of 2-methoxyquindoline (15 mg) and pyridine hydrochloride (1 g) was heated under N_2 at 175 °C for 2 h and then neutralized by $NaHCO_3$ solution. The solution was then extracted with $CHCl_3$ (4×50 mL), dried (Na_2SO_4) and solvent was removed by evaporation, under reduced pressure, to give the product (6 mg). Subsequently, additional product (35 mg) was obtained, then methylated to form a yellow solid, **15**, (50 mg) using method D: mp 312–314 °C. ^{1}H NMR (DMSO-d_6) δ 9.03 (1H, s), 8.72 (1H, d, J = 8.1 Hz), 8.61 (1H, d, J = 8.1 Hz), 7.88 (1H, t, J = 4.1 Hz), 7.79 (1H, d, J = 4.1 Hz), 7.70 (1H, s), 7.68 (1H, d, J = 4.1 Hz), 7.74 (1H, t, J = 4.1 Hz), 4.98 (3H, s). Anal. calcd for $C_{17}H_{15}IN_2O$: C, 52.33; H, 3.87; N, 7.18; found: C, 52.27; H, 3.82; N, 7.10.

2-Methylthio-5-methylquindolinium iodide (16). Method D was used with 2-methylthio-quindoline as starting material: mp 274–277 °C. ^{1}H NMR (DMSO-d_6) δ 12.81 (1H, s, br), 9.13 (1H, s), 8.76 (1H, d, J = 8.1), 8.65 (1H, d, J = 8.1), 8.30 (1H, s), 8.01 (1H, d, J = 5.4), 7.91 (1H, t, J = 5.4), 7.85 (1H, d, J = 5.4), 7.51 (1H, t, J = 5.4), 5.01 (3H, s), 2.70 (3H, s). Anal. calcd for $C_{17}H_{15}IN_2S$: C, 50.26; H, 3.72; N, 6.89; found: C, 50.07; H, 3.71; N, 6.77.

2-Phenylthio-5-methylquindolinium iodide (17). Method D was used with 2-phenylthio-quindoline as starting material: mp 253–256 °C. ^{1}H NMR (DMSO-d_6) δ 12.86 (1H, s, br), 9.20 (1H, s), 8.79 (1H, d, J = 8.1 Hz), 8.72 (1H, d, J = 8.1 Hz), 8.43 (1H, d, 1.8), 7.90 (4H, m), 7.51 (6H, m), 5.01 (3H, s). Anal. calcd for $C_{22}H_{17}IN_2S$: C, 56.42; H, 3.66; N, 5.98; found: C, 56.36; H, 3.69; N, 5.93.

5-(5-Phenylpentyl)quindolinium bromide (18). Method D was used to obtain the desired product: mp 218–219 °C. ^{1}H NMR (DMSO-d_6) δ 12.95 (1H, s, br), 9.33 (1H, s), 8.76 (1H, d, J = 5.4 Hz), 8.61 (1H, d, J = 5.4 Hz), 8.52 (1H, d, J = 5.4 Hz), 8.18 (1H, t, J = 5.4 Hz), 7.95 (3H, m), 7.56 (1H, t, J = 5.4 Hz), 7.20 (5H, m), 5.51 (2H, t, J = 5.4 Hz), 2.58 (2H, s, br), 2.15 (2H, s, br), 1.70 (4H, s, br). Anal. calcd for $C_{26}H_{25}BrN_2$: C, 70.11; H, 5.66; N, 6.29; found: C, 69.71; H, 5.74; N, 6.23.

10-Methyl-5-(5-phenylpentyl)quindolinium bromide (20). Method F. A mixture of quindoline (0.15 g), 5-phenylpentylbromide[##] (1.0 mL) and sulpholane (2.0 mL) was heated to 95 °C for 24 h. After cooling to room temperature, the mixture was chromatographed with 5–20% methanol in CH_2Cl_2 (gradient elution) to yield a yellow solid (60 mg) which was converted to the free base with ammonia solution. The solution was extracted with CH_2Cl_2 (4×25 mL), pooled and dried (Na_2SO_4). The solvent was removed and the residue (45 mg) was taken up in acetone (50 mL). Iodomethane (2 mL) was added and the mixture was refluxed for 24 h. After cooling to room temperature, the solvent was removed and the residue was purified by chromatography over silica gel (5–20% MeOH in CH_2Cl_2) to give (15 mg): mp 203–204 °C. ^{1}H NMR (DMSO-d_6) δ 9.38 (1H, s), 8.65 (1H, d, J = 5.4 Hz), 8.52 (2H, t, J = 5.4 Hz), 8.21 (1H, dt, J = 5.4, 1.8 Hz), 7.98 (3H, m), 7.63 (1H, dt, J = 5.4, 1.8 Hz), 7.16 (5H, m), 5.55 (2H, t, J = 5.4 Hz), 4.22 (3H, s), 2.67 (2H, t, J = 4.1 Hz), 2.28 (2H, m), 1.80 (4H, m). Anal. calcd for $C_{27}H_{27}BrN_2.1.5H_2O$) C, 64.31; H, 5.40; N, 5.55; found: C, 64.36; H, 5.44; N, 5.54.

2-Bromo-5-(5-phenylpentyl)quindolinium bromide (22). Method D was used with 2-bromoquindoline as starting material: mp 254–255 °C. ^{1}H NMR (DMSO-d_6) δ 13.16 (1H, s, br), 9.27 (1H, s), 8.94 (1H, d, J = 2.7 Hz), 8.73 (1H, d, J = 8.1 Hz), 8.52 (1H, d, J = 8.1 Hz), 8.25 (1H, dd, J = 5.4, 2.7 Hz), 7.97 (1H, t, J = 5.4 Hz), 7.90 (1H, d, J = 5.4 Hz), 7.58 (1H, t, J = 5.4 Hz), 7.20 (5H, m), 5.50 (2H, t, J = 5.4 Hz), 2.57 (2H, t, J = 4.1 Hz), 2.13 (2H, br), 1.65 (4H, br). Anal. calcd for $C_{26}H_{24}Br_2N_2$: C, 59.56; H, 4.61; N, 5.34; found: C, 59.66; H, 4.67; N, 5.42.

2-Bromo - 10 - methyl - 5 - (- 5 - phenylpentyl)quindolinium iodide (23). Method F was used with 2-bromoquindoline as starting material: mp 217–220 °C. ^{1}H NMR (DMSO-d_6) δ 9.46 (1H, s), 9.01 (1H, d, J = 2.7 Hz), 8.76 (1H, d, J = 10.8 Hz), 8.57 (1H, d, J = 10.8 Hz), 8.28 (1H, dd, J = 10.8, 2.7 Hz), 8.08 (1H, d, J = 2.7 Hz), 8.06 (1H, s), 7.63 (1H, m), 7.19 (5H, m), 5.52 (2H, t, J = 8.1 Hz), 4.16 (3H, s), 2.58 (2H, t, J = 5.4 Hz), 2.11 (2H, br), 1.67 (4H, br). Anal. calcd for $C_{27}H_{26}Br_1N_2{\cdot}1.0$ MeOH: C, 55.97; H, 4.70; N, 4.84; found: C, 56.05; H, 4.60; N, 4.77.

2-Bromo - 5 - (5 - cyclohexylpentyl)quindolinium bromide (24). Method D was used with 2-bromoquindoline as starting material: mp 256–258 °C. ^{1}H NMR (DMSO-d_6) δ 9.25 (1H, s), 8.91 (1H, s), 8.72 (1H, d, J = 8.1 Hz), 8.51 (1H, d, J = 8.1 Hz), 8.25 (1H, d, J = 5.4 Hz), 7.98 (1H, d, J = 5.4 Hz), 7.91 (1H, t, J = 5.4 Hz), 7.56 (1H, t, J = 5.4 Hz), 6.42 (1H, s), 5.48 (2H, t, J = 4.1 Hz), 2.08 (4H, m), 1.63 (8H, m), 1.35 (1H, m), 1.15 (4H, m), 0.82 (2H, m). Anal. calcd for $C_{26}H_{30}Br_2N_2$: C, 58.88; H, 5.70; N, 5.28; found: C, 61.69; H, 6.08; N, 5.55.

2-Methoxy-5-(5-phenylpentyl)quindolinium bromide (25). Method D was used with 2-methoxyquindoline as starting material: mp 233–234 °C. ^{1}H NMR (DMSO-d_6) δ 12.86 (1H, s), 9.15 (1H, s), 8.67 (1H, d, J = 8.1 Hz), 8.46 (1H, d, J = 8.1 Hz), 8.02 (1H, d, J = 2.71 Hz), 7.92 (1H, t, J = 5.4 Hz), 7.89 (1H, d, J = 5.4 Hz), 7.80 (1H, dd, J = 5.4, 2.7 Hz), 7.53 (1H, t, J = 5.4 Hz), 7.20 (5H, m), 5.48 (2H, t, J = 5.4 Hz), 4.02 (3H, s), 2.57 (2H, t, J = 4.1 Hz), 2.11 (2H, br), 1.65 (4H, br). Anal. calcd for $C_{27}H_{27}BrN_2O$: C, 68.21; H, 5.72; N, 5.89; found: C, 68.11; H, 5.78; N, 5.94.

5,10-Dimethyl - 2 - methoxyquindolinium iodide (26). Method D was used with excess iodomethane and 2-methoxyquindoline as starting materials: mp 262–264 °C. ^{1}H NMR (CD_3COD) δ 9.18 (1H, s), 8.75 (1H, d, J = 8.1 Hz), 8.60 (1H, d, J = 8.1 Hz), 8.56 (1H, s), 7.87 (3H, m), 7.61 (1H, d, J = 4.1 Hz), 5.12 (3H, s), 4.18 (3H, s), 4.07 (3H, s). Anal. calcd for $C_{18}H_{17}IN_2O$: C, 49.55; H, 3.93; N, 6.42; found: C, 49.65; H, 4.13; N, 6.02.

2-Methoxy - 10 - methyl - 5 - (5 - phenylpentyl)quindolinium iodide (27). Method F was used with 2-methoxyquindoline as starting material: mp 237–238 °C. ^{1}H NMR (DMSO-d_6) δ 9.37 (1H, s), 8.82 (1H, d, J = 8.1 Hz), 8.51

(1H, d, $J=8.1$ Hz), 8.02 (2H, d, $J=2.7$ Hz), 7.89 (2H, d, $J=2.7$ Hz), 7.58 (1H, m), 7.19 (5H, m), 5.01 (2H, t, $J=5.4$ Hz), 4.13 (3H, s), 4.02 (3H, s), 2.57 (2H, s, br), 2.12 (2H, s, br), 1.65 (4H, s, br). Anal. calcd for $C_{28}H_{29}BrN_2O\cdot1.5$ MeOH: C, 62.57; H, 5.44; N, 5.21; found: C, 62.66; H, 5.47; N, 5.18.

2-Cyano - 5 - (5 - cyclohexylpentyl)quindolinium bromide (28). Method D was used with 2-cyanoquindoline as starting material: mp 245–248 °C. ^{1}H NMR (DMSO-d_6) δ 9.40 (1H, s), 9.28 (1H, S), 8.98 (1H, d, $J=8.1$ Hz), 8.57 (1H, d, $J=8.1$ Hz), 8.47 (1H, dd, $J=8.1$, 1.8 Hz), 8.03 (1H, t, $J=8.1$ Hz), 7.95 (1H, d, $J=8.1$ Hz), 7.62 (1H, $J=8.1$ Hz), 5.02 (2H, t, $J=5.4$ Hz), 2.10 (4H, m), 1.63 (6H, m), 1.37 (1H, m), 1.05 (6H, m), 0.84 (2H, m). Anal. calcd for $C_{27}H_{30}IN_2$: C, 68.06; H, 6.35; N, 8.82; found: C, 68.01; H, 6.42; N, 8.72.

7-Nitro-5-methylquindolinium iodide (29). To a solution of quindoline (300 mg) in AcOH (10 mL) was added fuming HNO_3 (10 mL) at 0 °C and the resulting mixture was stirred at room temperature overnight. Cold H_2O (20 mL) and NH_4OH were added until the solution became basic (pH ~11) and extracted with EtOAc. The combined extracts was washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed with 40% EtOAc in hexane, using gradient elution, to give 11-nitroquindoline (50 mg) and 7-nitroquindoline (260 mg).

A mixture of 7-nitroquindoline (260 mg), methyl iodide (2 mL) in sulpholane (2 mL) was heated at 55.0 °C overnight. The mixture was cooled to room temperature and diluted with EtOAc and the solid formed was washed with EtOAc and MeOH, and dried to give **29**, as a yellow solid (82 mg, 21%) mp 263–266 °C. ^{1}H NMR (DMSO-d_6) δ 9.57 (1H, s), 9.48 (1H, s), 8.85 (1H, d, $J=5.4$ Hz), 8.71 (1H, d, $J=5.4$ Hz), 8.65 (1H, d, $J=5.4$ Hz), 8.24 (1H, t, $J=5.4$ Hz), 8.02 (2H, m), 5.13 (3H, s). Anal. calcd for $C_{16}H_{12}IN_3O_2$: C, 47.43; H, 2.99; N, 10.37; found: C, 47.24; H, 2.89; N, 10.24.

11-Nitro-5-methylquindolinium iodide (30). Compound **30** was obtained by method D using the 11-nitro obtained above as starting material: mp 261–264 °C. ^{1}H NMR (DMSO-d_6) δ 13.45 (1H, s, br), 9.36 (2H, m), 8.89 (2H, m), 8.75 (1H, d, $J=5.4$ Hz), 8.26 (1H, t, $J=5.4$ Hz), 8.07 (1H, t, $J=5.4$ Hz), 7.72 (1H, t, $J=5.4$ Hz), 5.10 (3H, s). Anal. calcd for $C_{16}H_{12}IN_3O_2$: C, 47.43; H, 2.99; N, 10.37; found: C, 47.20; H, 3.07; N, 10.13.

Evaluation of antifungal activity

Quantitative assay. Minimum inhibitory concentration (MIC),[13] of promising compounds were determined using a 2-fold serial broth dilution assay in Sabouraud-dextrose broth (SDB) for *C. neoformans* and *A. flavus* or yeast nitrogen broth for *C. albicans*. A solution of the compound in DMSO was added to SDB and the inoculum for the MIC determination was prepared as previously described.[13,14] The MIC value was taken as the lowest of the concentration that inhibited the growth of the test organisms after 24 and 48 h of incubation at 37 °C. Amphotericin B was included as positive control in each assay.

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