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Identification of bis-quindolines as new antiinfective agents

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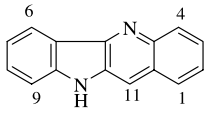
Abstract—Several N-substituted quindolines were made to further evaluate the role of N-alkylation on the activity of indoloquinolines as antifungal agents. While N-5 substitution is required for these activities, N-10 alkylation alone leads to inactive products but is tolerated in the presence of N-5 alkyl groups. It was also discovered that bis-quindolines appear to have a more expanded antimicrobial spectrum and lower cytotoxicity than their monomeric counterparts.

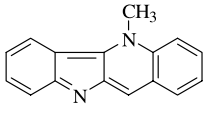
1. Introduction

The tetracyclic structure of indolo[3,2-*b*]quinoline, also referred to as quindoline (**1**) constitutes an important structural moiety in the literature because of its effect on numerous biological functions.[1] For example, cryptolepine (**2a** and **b**) and several of its analogs display antiaggregatory,[2] antifungal,[3] antihypertensive,[4] antihyperglycemic,[5] antibacterial,[6] anticancer,[7] antimalarial,[8] activities among others. A unified mechanism by which the drug produces the different biological activities has not been elucidated. However, cryptolepine has been shown to bind to DNA fragments[9] in a rather unique fashion. Furthermore, it intercalates DNA and stimulates topoisomerase II mediated cutting of DNA.[7,10] More recently, cryptolepine has been identified as a potential inhibitor of telomerase and a G-quadruplex DNA stabilizing agent.[11]

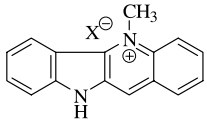
The unique structural arrangement of the quindolines has inspired our curiosity in seeking to understand the contributions of the various components. Consequently, we have shown that alkylation of the N-5 atom in quindoline is necessary for several of the activities.[12,13] In particular, we have reported that ω-phenylpentyl and ω-cyclohexylpentyl moieties on the N-5 atom of the quindoline ring produce a high antifungal potency and broaden the spectrum of activities.[12] It is interesting to note that N-5 alkylation produces an anhydronium base in which the N-5 nitrogen becomes positively charged under acidic conditions, that is, aromatic quaternary nitrogen (**2b**), but reverts to sp^3 type nitrogen under basic conditions (**2a**). This physical characteristic of cryptolepine is also accompanied by a color change from pink in a basic to orange in an acidic environment; suggesting that a significant change in electron distribution has occurred. This unique behavior may allow for



Quindoline (**1**)



Cryptolepine, **2a** (Basic form)



Cryptolepine, **2b** (Salt form)

Keywords: Cryptolepine; Indoloquinoline; Quindoline; Quaternary amines; Indole bis-quindolines; Antifungal; Antiinfective; Antiparasitic; Antimalarial.
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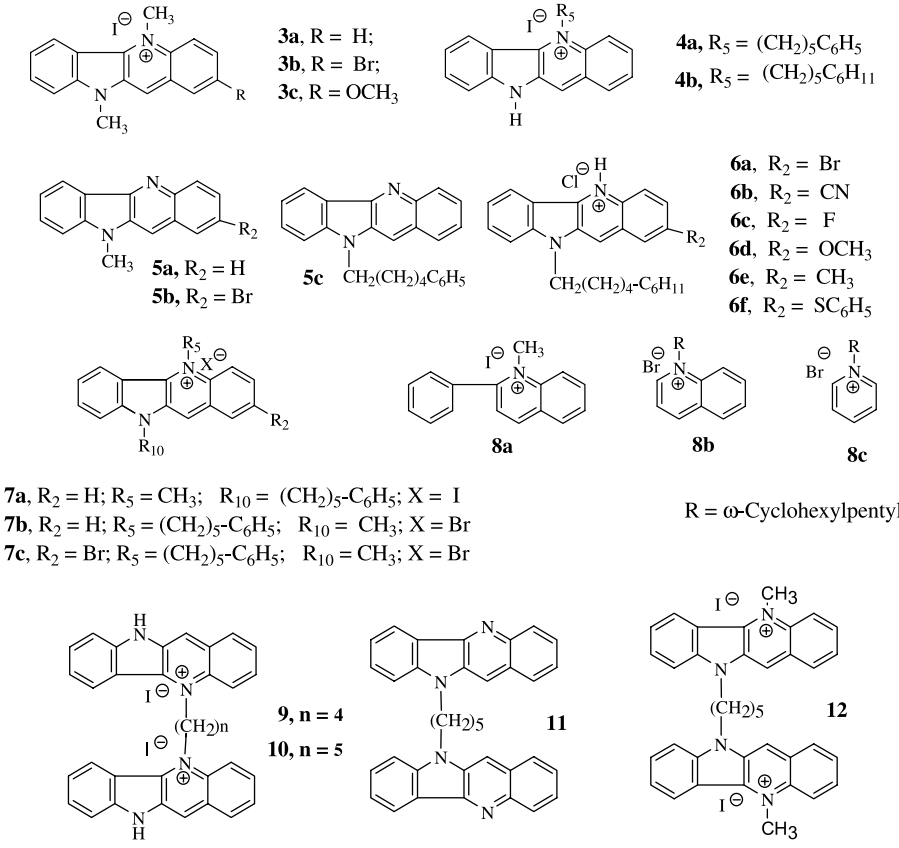


Chart 1. Compounds synthesized and tested for biological activity.

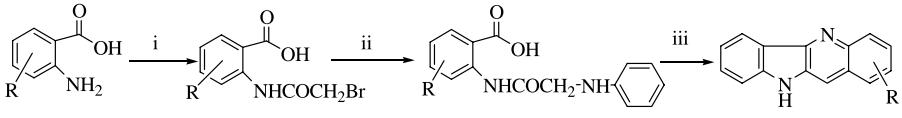
easy entry into cells in the basic form and also for the production of its pharmacological effect in the salt form.

The hypothesis that a charged N-5 atom is necessary for biological activity was tested by alkylating the N-10 atom of cryptolepine to prevent anhydronium base formation and to produce a compound with a permanently charged N-5 atom (**3a**). Because compound **3a** showed antifungal activity, we reported that the active form of the indoloquinoline ring system is the salt form in which N-5 is positively charged.[14] This view is consistent with the binding mode of cryptolepine to DNA fragments reported by Aymami and co-workers.[9] on the basis of their X-ray crystallographic work. Thus, the purpose of this study was to investigate the effect of various combinations of arylalkyl substituents (on N-5 and N-10 atoms) on the antiinfective properties of these agents (Chart 1).

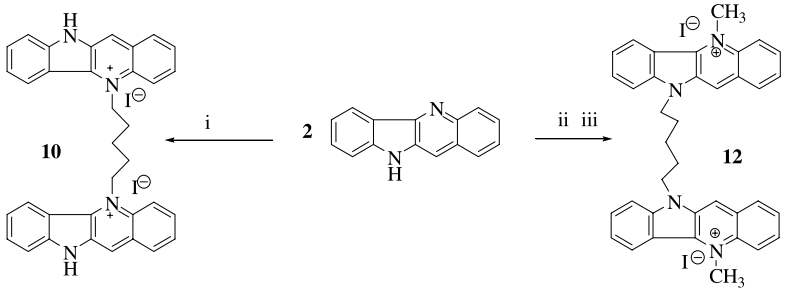
2. Chemistry

The construction of the tetracyclic structure of quindoline has been widely reported.[15–21] In this paper, we used a previously reported method[15] to construct the quindoline unit. Briefly, a substituted or unsubstituted anthranilic acid was acylated with 2-bromoacetyl bromide and the resulting alkyl halide was used to alkylate aniline. The alkylated aniline underwent a double cyclization reaction in the presence of polyphosphoric acid (PPA) to yield a quindolone, which was chlorinated with phosphorous oxychloride ($POCl_3$). The resulting chloride was dechlorinated with hydrogen on palladium to obtain the desired quindoline (Scheme 1). For compound **6f**, 2-iodoquindoline was converted to 2-thiophenylquindoline as previously reported.[14]

Specific alkylation of either the N-5 or N-10 atoms was accomplished using the methods we previously



Scheme 1. The general synthetic method for substituted quindolines. Reagents: (i) $BrCH_2COBr$, NaOH; (ii) DMF, $PhNH_2$; (iii) (a) PPA, 130 °C; (b) $POCl_3$; (c) H_2/10% Pd–C.



Scheme 2. Synthesis of bis-alkylated quindolines. Reagents: (i) trimethylene sulfone (TMS), $I(CH_2)_5I$; (ii) NaH, $I(CH_2)_5I$; (iii) CH_3I, TMS.

reported.[16] The synthesis of bis-quindolines linked through their N-5 atoms was accomplished by heating quindoline with 1,4-diiodobutane (**9**) or the corresponding 1,5-diiodopentane (**10**). The formation of bis-quindolines, joined through the N-10 atoms required a strongly basic medium and was achieved by the introduction of sodium hydride. Bis-alkylation at N-10 was then followed by bis-methylation of N-5 (Scheme 2).

3. Results

Re-evaluation of N-10 methylated cryptolepines (**3a–c**), confirmed that substitution at the 2-position with an electron withdrawing bromine while enhancing anticryptococcal activity, had little or no effect on anticandida action.[14] Similarly, the electron-donating methoxy group has little or no effect on either activity when compared to **3a**. We have previously shown that alkylation of N-5 in quindoline with ω-phenylpentyl or ω-cyclohexylpentyl moieties as in **4a** and **4b** enhances antifungal activity (Table 1). In this form, the positively charged N-5 atom is retained. Thus, it was of interest to investigate the contribution of the positively charged N-5 atom.

One way a non-N-5 alkylated quindoline can produce a positively charged N-5 atom is to form the salt. Hence, we synthesized two N-10 methylated quindolines with

Table 1. Physicochemical data and antifungal activities of synthetic compounds

Comp	Solv[a]	% Yield[b]	MP (°C)[c]	Empirical[d] formula	IC_{50}(μg/mL)	
					Cn	Ca
2b[e]	A	73	265–268	$C_{16}H_{13}N_2I$	15.6	180
3a[e]	A	100	304–306	$C_{17}H_{15}N_2I$	6.3	3.1[f]
3b[e]	A	57	285–288	$C_{17}H_{14}N_2BrI$	0.4	3.1[f]
3c[e]	A	27	262–264	$C_{18}H_{17}N_2OI{\cdot}1.0MeOH$	4.3	2.1
4a[e]	A	67	218–219	$C_{26}H_{25}N_2Br$	1.3	80
4b[e]	A	34	262–264	$C_{26}H_{31}N_2Br$	0.3	⩽1.3
5a	B	94	110–112	$C_{16}H_{12}N_2{\cdot}0.3H_2O$	62.5[f]	125[f]
5b	B	62	160–162	$C_{16}H_{11}N_2Br$	11	43
5c	A	54	223–224	$C_{26}H_{25}N_2Cl{\cdot}HCl$	9.0	18.0
6a	A	54	223–224	$C_{26}H_{29}N_2Br{\cdot}HCl$	>50	>50
6b	A	73	229–231	$C_{27}H_{29}N_3{\cdot}HCl$	>50	>50
6c	A	64	231–233	$C_{26}H_{29}N_2F{\cdot}HCl$	>50	>50
6d	A	71	232–234	$C_{27}H_{32}N_2O{\cdot}HCl$	>50	>50
6e	A	88	231–233	$C_{27}H_{32}N_2{\cdot}HCl$	>50	>50
6f	A	43	221–223	$C_{32}H_{34}N_2S{\cdot}HCl$	>50	>50
7a	A	67	215–217	$C_{27}H_{27}N_2I$	15	>20
7b[e]	A	30	203–204	$C_{27}H_{27}N_2Br{\cdot}1.4MeOH$	4.3	4.3
7c[e,g]	A	100	217–220	$C_{27}H_{26}N_2Br_2$	1.3	43
8a	C	22	196–198	$C_{16}H_{14}NI$	43	180
8b	C	65	79–83	$C_{20}H_{28}NBr{\cdot}0.8H_2O$	20	80
8c	A	45	86–89	$C_{16}H_{26}NBr{\cdot}1.4H_2O$	3.5	20
9	D	78	252–254	$C_{34}H_{30}N_4I_2{\cdot}0.5H_2O$	4.0	>50
10	A	76	256–258	$C_{35}H_{30}N_4I_2{\cdot}3.5H_2O$	1.5	>50
11	A	87	Oily	$C_{35}H_{28}N_4{\cdot}0.6H_2O$	NA	NA
12	A	75	238–240	$C_{37}H_{34}N_4I_2{\cdot}0.5H_2O$	2.0	2.0
Amphotericin B					0.6	0.1

Abbreviations in the table are as follows: Ca = *Candida albicans*, Cn = *Cryptococcus neoformans*. NA = not active at 180 μg/mL.

[a] Recrystallization solvents are: A = MeOH–Et_2O, B = hexane–EtOAc, C = EtOH–Et_2O, D = MeOH–CH_2Cl_2.

[b] Yields were not optimized.

[c] Melting points were uncorrected.

[d] All compounds were subjected to CHN analysis and each passed within 0.4% of the theoretical value.

[e] Compounds were previously reported.[12,14]

[f] MIC values in microgram per milliliter.

[g] Compound crystallized with $0.5H_2O$ and 1.0MeOH.

N-5 atoms, which should be converted to their salt form in aqueous solution (**5a** and **5b**), for evaluation. The results showed that these compounds were only weakly active against *Cryptococcus neoformans* and *Candida albicans*. Compound **5c** was synthesized to explore the possibility that antifungal potency might be enhanced in the same way that ω-phenylpentyl groups at N-5 enhanced the potency of quindoline. Indeed, a moderate increase in potency (~10-fold) over **5a** was observed. The enhanced potency of compound **4b** over cryptolepine and the increased potency of 2-substituted analogs[14] also led to the synthesis of several analogs (**6a–f**) with ω-cyclohexylpentyl substituent at N-10. The N-10 alkylated analogs were also of interest because N-10 alkylation enables both the free base form and the salt form to co-exist in an aqueous medium, allowing the free base form of the drug to penetrate fungal cell membranes while the presumed active protonated form interferes with cell reproduction. The results indicate that N-10 alkylated analogs of quindoline have little or no activity as antifungal agents. Since quindoline salts have no antifungal activity, but N-5 alkylated quindolines do have activity, it appears that a positive N-5 atom along with a hydrophobic interaction with the N-5 alkyl groups may be important for the actions of these alkylated indoloquinolines.

Since alkylation of both N-5 and N-10 appears to be beneficial (**3a–c**), it became of interest to examine the position for optimum placement of the alkyl groups. Thus, compounds **7a–c** were prepared and evaluated for their antifungal properties and the results are recorded in Table 1. These results indicate that when N-5 is substituted with the longer ω-phenylpentyl moiety and N-10 is methylated (**7b**), this combination is more potent than the reverse (**7a**) in *C. neoformans* and *C. albicans*. However, there appears to be no significant overall improvement in potency compared to the doubly methylated compounds, **3a–c**.

The importance of the quaternary N-5 atom in these indoloquinolines also led us to investigate compounds **8a–c** in order to dispel the notion that the antifungal activity of these compounds was intricately associated with any functionality with a quaternary N+ atom and an alkyl function such as the ω-cyclohexylpentyl moiety. As shown in Table 1, there was little antifungal activity associated with **8a–c** suggesting that the A and B rings constitute important structural features for the activities observed in N-alkyl substituted quindolines.

Since quindolines with phenyl and cyclohexyl moieties placed five carbon atoms away from N^+-5 display high antifungal potency, we investigated the possibility that bis-quindolines five carbons from each other might similarly show enhanced antifungal potency perhaps by interacting with two adjoining DNA molecules. Compounds **10**, in which the pyridine N-atoms are connected, and **11** and **12**, where the tetracycles are joined by the indole N-atoms, were thus synthesized and evaluated for biological activity. The results show that the positively charged quaternary atom is required for activity even in the bis-quindolines; compounds **10** and **12** possess potent activity while compound **11** (the non-alkylated compound) displays no activity. In addition, consistent with our previous observation that an ω-cycloalkylpentyl substituent on N-5 displays an enhanced potency, a bis-quindoline separated by a 5-carbon chain (compound **10**) was more potent than that separated by a 4-carbon chain (compound **9**). Although alkylation on the indole N-atom did not result in activity (compound **11**), methylation of the N-5 atoms (compound **12**) resulted in potent activity and there was little difference in the antifungal activities of **10** and **12** when compared together.

A selected number of the compounds (**7a**, **9**, **10**, and **12**) based on their novelty, was further evaluated in additional assays available in our laboratories with cryptolepine as the benchmark, to explore their antimicrobial spectrum. Their cytotoxicities to a mammalian cell were also determined. The results are reported in Tables 2 and 3. Evaluation of these results shows that compounds **7a**, **10**, and **12** have more expansive antimicrobial/antiparasitic spectra than cryptolepine. All three compounds also display activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Similarly, all four compounds displayed significant activity against *Mycobacterium intracellulare* (Mi). Among the four compounds tested however, only **7a** showed significant potency against *Aspergillus fumigatus* (Af), *Candida krusei* (Ck), and *Plasmodium falciparum* (Pf) and only compound **10** displayed activity against *Pseudomonas aeruginosa* (Pa). Thus, among the bis-quindolines, joining the pyridine nitrogens (compounds **9** and **10**) by an alkyl chain appears to be more effective than through

Table 2. The effect of N-alkylation on the antimicrobial activity of selected quindolines

Compound	IC_{50} (μg/mL)					
	Af	Ck	MRSA	Mi	Pa	Sa
Cryptolepine	>20	20	20	15.0	>20	10.0
7a	2.5	2.0	2.0	1.0	>20	0.60
9	>20	>20	>20	5.5	>20	>20
10	20	>20	3.0	7.0	1.5	3.0
12	20	15	6.0	15.0	>20	4.0
Amphotericin B	0.31	0.60	NT	NT	NT	NT
Ciprofloxacin	NT	NT	0.15	0.20	0.06	0.10

Abbreviations: NT = not tested. *Aspergillus fumigatus* (Af), *Candida krusei* (Ck), methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium intracellulare* (Mi); *Pseudomonas aeruginosa* (Pa); *Staphylococcus aureus* (Sa).

Table 3. Antimalarial activity and cytotoxicity of selected quindolines

Compound	*Plasmodium falciparum* (IC_{50}, ng/mL)				Cytotoxicity (Vero) (TC_{50}, ng/mL)
	Pf (D6)	SI	Pf (W2)	SI	
Cryptolepine	180	23.3	420	10	4200
7a	62	145	28	321	9000
9	>528		NA		NC
10	120	>198	88	>270	>23,800
12	260	>91.5	140	>170	>23,800
Chloroquine	14.5	>1400	75	>317	>23,800
Artemisinin	11	>2800	6	>7677	>23,800
Amphotericin B	NT		NT		6500

Abbreviations: NC = no cytotoxicity observed up to 5 μg/mL; NT = not tested. NA = not active up to 20 μg/mL; SI = IC_{50} (vero cells)/IC_{50} (Pf). D6 = D6 Clone; W2 = W2 Clone.

the indole nitrogen (compound **11**). In addition, the 5-chain compound (**10**) was more potent than the 4-chain structure (**9**). Interestingly, cryptolepine was more toxic than any of the four selected compounds. It is important to note that these compounds may not be acting through their monomeric units since that would have resulted in similar or higher toxicity and decreased potency per unit weight of compound. The fact that compounds **10** and **12** displayed no cytotoxicity up to 23.8 μg/mL and are potent against a wide spectrum of microorganisms, suggest that these bis-quindolines may have therapeutic advantage over their monomeric counterparts as new potential antiinfectives.

4. Conclusion

Evaluation of the indoloquinolines prepared in this article confirms the importance of N-5 alkylation. The basicity of this nitrogen and consequently the formation of the positive charge appear to be important. On the other hand, alkylation of the non-basic indole nitrogen does not lead to activity. Despite this observation, simultaneous alkylation of both nitrogen atoms results in improved potency and extension in antiinfective spectrum. Similarly, bis-quindolines obtained by double alkylation of the pyridine N-5 atoms produce a broad spectrum antimicrobial activity while connection through the indole N-10 atoms led to compounds without activity. Subsequent alkylation of the N-5 in this type of bis-quindolines however, resulted in similar antiinfective properties. The broad spectrum of antimicrobial actions and the lower cytotoxicity displayed by the bis-quindolines indicate this group may be acting through a different mechanism of action from that of their monomeric counterparts. These results suggest a more systematic study of the doubly alkylated compounds, including the bis-quindolines, in these and other antiinfective assays, is warranted.

5. Experimental

Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. NMR spectra were obtained on a Varian 300 MHz Mercury or a Bruker 270 MHz NMR Spectrometer. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA 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. Sulfolane 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. The remaining alkyl halides were obtained from either Sigma–Aldrich Chemicals or Fisher Scientific and were used without further purification. Quindoline (10*H*-indolo[3,2-*b*]quinoline), the starting material in several of the syntheses below was obtained as previously reported.[12]

5.1. General method for the synthesis of N-10 alkylated quindolines

5.1.1. 10-Methylquindoline (5a). A mixture of quindoline (400 mg, 1.83 mmol), KOH (0.2 g), acetone (20 mL), and iodomethane (0.5 mL) was stirred at room temperature for 30 min. A second portion of an equal amount of KOH and iodomethane was added. The mixture was stirred for another 2 h, acetone was removed by rotary evaporation and the residue chromatographed using 20:30% EtOAc–hexanes (gradient elution) to yield a yellow solid (0.42 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. Calcd for: $C_{16}H_{12}N_2 \cdot 0.3H_2O$, C, 80.85; H, 5.34; N, 11.79. Found: C, 80.60; H, 5.52; N, 11.64.

5.1.2. 2-Bromo-10-methylquindoline (5b). Compound **5b** was similarly obtained and recrystallized from hexane–EtOAc, mp 160–162 °C. ^{1}H NMR ($CDCl_3$): δ 4.82 (3H, s), 7.38 (2H, m), 7.65 (2H, m), 7.74 (1H, s), 8.05 (1H, d, J = 2.2 Hz), 8.18 (1H, d, J = 8.6 Hz), 8.51 (1H, d, J = 7.4 Hz). Anal. Calcd for $C_{16}H_{11}BrN_2$: C, 61.76; H, 3.56; N, 9.00. Found: C, 61.71; H, 3.64; N, 8.90.

5.1.3. 10-(5-Phenylpentyl)quindoline HCl (5c). Compound **5c** was similarly obtained and recrystallized from MeOH–Et_2O, mp 223–224 °C. ^{1}H NMR (DMSO-d_6): δ 1.36 (2H, m), 1.57 (2H, m), 1.90 (2H, m), 2.51 (2H, m), 4.60 (2H, m), 7.13 (5H, m), 7.46 (1H, m), 7.88 (4H, m), 8.00 (1H, t, J = 7.2 Hz), 8.35 (1H, d, J = 8.1 Hz), 8.53

(1H, d, $J = 8.8$ Hz), 8.84 (1H, d, $J = 7.8$ Hz), 9.02 (1H, s). Anal. Calcd for $C_{26}H_{25}ClN_2$: C, 77.89; H, 6.28; N, 6.99. Found: C, 77.87; H, 6.34; N, 6.95.

5.1.4. 2-Bromo-10-cyclohexylpentyl quindoline HCl (6a). A mixture of KOH (0.115 g), acetone (4 mL), 2-bromoquindoline[14] (0.12 g, 0.4 mmol), and cyclohexylpentyl bromide (1.5 mL) was refluxed with stirring at 50 °C for 1 h, after which the acetone was evaporated in vacuo. The crude product was chromatographed on a column of silica gel and eluted with gradient ethyl acetate–hexane to yield the pure free base product. The HCl salt was obtained by dissolving the product in MeOH and adding excess ethereal HCl. Recrystallization from MeOH–Et_2O afforded 2-bromo-10-cyclohexylpentyl quindoline HCl (0.105 g, 54%) mp: 223–224 °C. ^{1}H NMR ($CDCl_3$): δ 0.79 (m, 2H), 1.11 (m, 6H), 1.29 (s, br, 6H), 1.60 (m, 6H), 1.83 (s, br, 2H), 4.51 (t, 1H, $J = 5.9$ Hz), 7.24 (s, 1H), 7.31 (t, 2H, $J = 8.8$ Hz), 7.68 (t, 2H, $J = 8.8$ Hz), 8.18 (s, 1H), 8.53 (s, 1H), 8.68 (d, 1H, $J = 8.8$ Hz), 9.13 (d, 1H, $J = 7.8$ Hz). Anal. Calcd for $C_{26}H_{30}BrClN_2$: C, 64.27; H, 6.22; N, 5.77. Found: C, 64.19; H, 6.25; N, 5.64.

5.1.5. 2-Cyano-10-cyclohexylpentyl quindoline HCl (6b). This compound was prepared from 2-cyanoquindoline[14] and cyclohexylpentyl bromide according to the procedure described above. Recrystallization in MeOH–Et_2O afforded 2-cyano-10-cyclohexylpentyl quindoline HCl (0.13 g, 73%) mp: 229–231 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (m, 2H), 1.35 (m, 11H), 1.65 (m, 4H), 2.0 (m, 2H), 4.68 (t, 2H, $J = 7.4$ Hz), 7.58 (t, 1H, $J = 7.8$ Hz), 7.93 (d, 1H, $J = 8.8$ Hz), 8.02 (m, 1H), 8.22 (dd, 1H, $J = 1.4$, 7.3 Hz), 8.50 (d, 1H, $J = 8.8$ Hz), 8.65 (d, 1H, $J = 8.3$ Hz), 8.9 (s, 1H), 9.3 (s, 1H). Anal. Calcd for $C_{27}H_{30}ClN_3$: C, 75.07; H, 7.00; N, 9.73. Found: C, 75.20; H, 7.18; N, 9.72.

5.1.6. 2-Fluoro-10-cyclohexylpentyl quindoline HCl (6c). This compound was prepared from 2-fluoroquindoline[14] and cyclohexylpentyl bromide according to the procedure described above. Recrystallization in MeOH–Et_2O afforded 2-fluoro-10-cyclohexylpentyl quindoline HCl (0.11 g, 64%) mp: 231–233 °C. ^{1}H NMR ($CDCl_3$): δ 0.80 (s, br, 2H), 1.1 (s, br, 6H), 1.31 (s, br, 3H), 1.62 (s, br, 6H), 1.86 (s, br, 1H), 4.48 (t, 2H $J = 6.8$ Hz), 7.34 (s, 1H), 7.37 (s, 1H), 7.5 (m, 1H), 7.7 (m, 2H), 8.5 (s, 1H), 8.95 (dd, 1H, $J = 4.9$, 9.2 Hz), 9.2 (d, 1H, $J = 7.8$ Hz). Anal. Calcd for $C_{26}H_{30}ClFN_2$: C, 73.48; H, 7.12; N, 6.59. Found: C, 73.38; H, 7.03; N, 6.49.

5.1.7. 2-Methoxy-10-cyclohexylpentyl quindoline HCl (6d). This compound was prepared from 2-methoxyquindoline[14] and cyclohexylpentyl bromide according to the procedure described above. Recrystallization in MeOH–Et_2O afforded 2-methoxy-10-cyclohexylpentyl quindoline HCl (0.13 g, 71%) mp: 232–234 °C. ^{1}H NMR ($CDCl_3$): δ 0.8 (m, 2H), 1.10 (m, 6H), 1.28 (m, 3H), 1.62 (m, 6H), 1.81 (m, 2H), 3.98 (s, 3H), 4.42 (t, 2H, $J = 6.8$ Hz), 7.18 (s, 1H), 7.27 (m, 3H), 7.64 (t, 1H, $J = 8.3$ Hz), 8.39 (s, 1H), 8.65 (d, 1H, $J = 9.8$ Hz), 9.15 (d, 1H, $J = 7.8$ Hz). Anal. Calcd for $C_{27}H_{33}ClN_2O$: C, 70.05; H, 7.18; N, 6.05. Found: C, 70.18; H, 7.36; N, 6.04.

5.1.8. 2-Methyl-10-cyclohexylpentyl quindoline HCl (6e). This compound was prepared from 2-methylquindoline[14] and cyclohexylpentyl bromide according to the procedure described above. Recrystallization in MeOH–Et_2O afforded 2-methyl-10-cyclohexylpentyl quindoline HCl (0.15 g, 88%) mp: 231–233 °C. ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 2H), 1.1 (m, 6H), 1.29 (s, br, 3H), 1.62 (m, 6H), 1.84 (s, br, 2H), 2.47 (s, 3H), 4.46 (s, br, 2H), 7.30 (m, 2H), 7.47 (d, 1H, $J = 8.8$ Hz), 7.61 (t, 1H, $J = 7.3$ Hz), 7.76 (s, 1H), 8.45 (s, 1H), 8.70 (d, 1H, $J = 8.8$ Hz), 9.10 (d, 1H, $J = 7.8$ Hz). Anal. Calcd for $C_{27}H_{33}ClN_2$: C, 77.03; H, 7.90; N, 6.65. Found: C, 76.86; H, 7.76; N, 6.56.

5.1.9. 2-Thiophenyl-10-cyclohexylpentyl quindoline HCl (6f). This compound was prepared from 2-thiophenylquindoline[14] and cyclohexylpentyl bromide according to the procedure described above. Recrystallization in MeOH–Et_2O afforded 2-thiophenyl-10-cyclohexylpentyl quindoline HCl (0.1 g, 43%) mp: 221–223 °C. ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 2H), 1.10 (s, br, 6H), 1.52 (s, br, 3H), 1.60 (s, br, 6H), 1.82 (s, br, 2H), 4.42 (t, 2H, $J = 7.3$ Hz), 7.32 (m, 2H), 7.43 (m, 3H), 7.59 (m, 4H), 7.70 (s, 1H), 8.3 (s, 1H), 8.82 (d, 1H, $J = 9.3$ Hz), 9.20 (d, 1H, $J = 7.8$ Hz). Anal. Calcd for $C_{32}H_{35}ClN_2S$: C, 71.52; H, 6.56; N, 5.21. Found: C, 71.63; H, 6.72; N, 5.24.

5.1.10. Synthesis of 5-methyl-10-(5-phenylpentyl)quindolinium iodide (7a). A mixture of quindoline (420 mg, 1.93 mmol) and 5-phenyl-1-iodopentane (600 mg, 2.19 mmol) in DME (15 mL) was added with stirring at 0 °C, to NaH (100 mg, 4.15 mmol). After the addition was complete, the mixture was refluxed under N_2 for 10 h and allowed to cool to room temperature. The resulting mixture was filtered through a short pad of silica gel and the filtrate was concentrated in vacuo to dryness. The residue was purified by column chromatography to give the desired product (610 mg, 87%). A mixture of the N-5 alkylated product (320 mg, 0.88 mmol), MeI (0.2 mL), and sulfolane (2 mL) was sealed in a tube and then heated at 100 °C for 16 h. After cooling to room temperature, Et_2O (20 mL) was added to precipitate a yellow solid, which was collected by filtration. The solid was recrystallized from CH_2Cl_2–MeOH and Et_2O to give compound **7a** (360 mg, 80%); mp: 215–217 °C. ^{1}H NMR (DMSO-d_6) 1.38 (m, 2H), 1.59 (m, 2H), 1.87 (m, 2H), 2.49 (t, 2H, $J = 3.0$ Hz), 4.70 (t, 2H, $J = 7.1$ Hz), 5.05 (s, 6H), 7.12 (m, 5H), 7.57 (t, 1H, $J = 8.0$ Hz), 8.02 (m, 3H), 8.19 (t, 1H, $J = 7.5$ Hz), 8.52 (d, 1H, $J = 7.6$ Hz), 8.75 (d, 1H, $J = 9.0$ Hz), 8.85 (d, 1H, $J = 8.4$ Hz), 9.60 (s, 1H). Anal. Calcd for $C_{27}H_{27}IN_2$: C, 64.04; H, 5.37; N, 5.53. Found: C, 63.77; H, 5.40; N, 5.56.

5.2. General method for alkylation (compounds 8a–c)

Alkyl halide (0.8 mL) was added to a 10-mL round-bottomed flask containing quinoline (100 mg, 0.46 mmol) and sulfolane (2.0 mL). The mixture was heated at about 100 °C in a sealed flask overnight and allowed to cool to room temperature. The mixture was directly chromatographed with 5:20% MeOH–CH_2Cl_2 (gradient

elution) to give a solid, which was recrystallized from an appropriate solvent system as shown below.

5.2.1. Synthesis of 1-methyl-2-phenylquinolinium iodide (8a). Precipitated solid was recrystallized from EtOH–Et_2O to produce **8a** (65 mg, 4%); mp: 196–198 °C. ^1H NMR (DMSO-d_6) 4.37 (3H, s), 7.72 (3H, m), 7.80 (2H, m), 8.08 (1H, t, $J = 7.8$ Hz), 8.16 (1H, d, $J = 8.4$ Hz), 8.32 (1H, t, $J = 8.7$ Hz), 8.52 (1H, d, $J = 8.1$ Hz), 8.62 (1H, d, $J = 9.0$ Hz), 9.27 (1H, d, $J = 8.7$ Hz), Anal. Calcd for $C_{16}H_{14}IN$: C, 55.35; H, 4.06; N, 4.03. Found: C, 55.23; H, 4.09; N, 4.02.

5.2.2. Synthesis of 5-cyclohexylpentylquinolinium bromide (8b). Precipitated solid was recrystallized from EtOH–Et_2O to give the desired product (yield 65%), mp: 79–83 °C. ^1H NMR (DMSO-d_6) 0.83 (2H, m), 1.22 (10H, m), 1.63 (5H, m), 1.96 (2H, m), 5.05 (2H, t, $J = 7.8$ Hz), 8.05 (1H, t, $J = 7.3$ Hz), 8.19 (1H, dd, $J = 5.8$ Hz, $J = 8.8$ Hz), 8.27 (1H, m), 8.49 (1H, d, $J = 8.3$ Hz), 8.62 (1H, d, $J = 8.8$ Hz), 9.30 (1H, d, $J = 8.8$ Hz), 9.57 (1H, d, $J = 5.8$ Hz). Anal. Calcd for $C_{20}H_{28}NBr{\cdot}0.8H_2O$: C, 63.76; H, 7.49; N, 3.72. Found: C, 63.89; H, 7.71; N, 3.64.

5.2.3. Synthesis of 5-cyclohexylpentylpyridinium bromide (8c). Precipitated solid was recrystallized from EtOH–Et_2O to give the desired product (yield 45%), mp: 86–89 °C. ^1H NMR (DMSO-d_6) δ (ppm) 0.83 (2H, m), 1.20 (10H, m), 1.62 (5H, m), 1.89 (2H, m), 4.61 (2H, t, $J = 7.5$ Hz), 8.16 (2H, m), 8.61 (1H, t, $J = 7.8$ Hz), 9.15 (2H, d, $J = 5.4$ Hz). Anal. Calcd for $C_{16}H_{26}NBr{\cdot}1.4H_2O$: C, 56.94; H, 8.60; N, 4.15. Found: C, 56.60; H, 8.15; N, 4.02.

5.2.4. Synthesis of 1,4-bis(10-indolo[3,2-*b*]quinolin-5-ium)-butane diiodide (9). A mixture of 10*H*-indolo[3,2-*b*]quinoline (700 mg, 3.2 mmol), 1,4-diiodobutane (400 mg, 1.3 mmol), and sulfolane (4 mL) was sealed in a tube, and the solution was heated at 100 °C for 16 h and allowed to cool to room temperature. Et_2O (20 mL) was added to form a yellow precipitate and was collected by filtration. The solid was recrystallized from CH_2Cl_2–MeOH and Et_2O to give compound **9** (850 mg, 78%); mp: 252–254 °C. ^1H NMR (DMSO-d_6) 2.58 (br s, 4 H), 5.58 (br s, 4H), 7.42 (t, 2H, $J = 7.4$ Hz), 7.95 (m, 6H), 8.15 (t, 2H, $J = 7.6$ Hz), 8.50 (d, 2H, $J = 8.5$ Hz), 8.59 (d, 2H, $J = 7.8$ Hz), 8.80 (d, 2H, $J = 9.1$ Hz), 9.32 (s, 2H), 12.99 (br s, 2H). Anal. Calcd for $C_{34}H_{30}I_2N_4{\cdot}0.5H_2O$: C, 53.91; H, 3.99; N, 7.40. Found: C, 54.07; H, 3.64; N, 7.40.

5.2.5. Synthesis of 1,5-bis(10-indolo[3,2-*b*]quinolin-5-ium)-pentane diiodide (10). A mixture of 10*H*-Indolo[3,2-*b*]-quinoline (400 mg, 1.84 mmol), 1,5-diiodopentane (280 mg, 0.87 mmol), and sulfolane (4 mL) was sealed in a tube, and the solution was heated at 100 °C for 16 h and allowed to cool to room temperature. Et_2O (20 mL) was added to precipitate a yellow solid, which was collected by filtration. The solid was recrystallized from CH_2Cl_2–MeOH and Et_2O to give compound **10** (520 mg, 76%); mp: 256–258 °C. ^1H NMR (DMSO-d_6) 2.00 (m, 2H), 2.23 (m, 4H), 5.52 (m, 4H), 7.50 (dd, 2H, $J = 7.5$, 9.0 Hz), 7.88 (d, 2H, $J = 9.0$ Hz), 7.93 (d, 2H, $J = 9.5$ Hz), 7.98 (d, 2H, $J = 8.0$ Hz), 8.10 (t, 2H, $J = 8.0$ Hz), 8.54 (d, 2H, $J = 8.5$ Hz), 8.59 (d, 2H, $J = 8.5$ Hz), 8.71 (d, 2H, $J = 9.5$ Hz), 9.31 (s, 2H), 12.92 (s, 2H). Anal. Calcd for $C_{35}H_{30}I_2N_4{\cdot}3.5H_2O$: C, 51.05; H, 3.67; N, 6.80. Found: C, 51.06; H, 3.69; N, 6.80.

5.2.6. Synthesis of 1,5-bis(indolo[3,2-*b*]quindolin-10-yl)-pentane (11). A mixture of 10*H*-indolo[3,2-*b*]quinoline (860 mg, 3.9 mmol), and 1,5-diiodopentane (640 mg, 1.95 mmol) was dissolved in DME (15 mL) and NaH (200 mg, 8.3 mmol) was added with stirring at 0 °C. After the addition was completed, the mixture was refluxed under N_2 for 10 h and allowed to cool to room temperature. The mixture was then filtered through a short pad of silica gel and the filtrate was concentrated in vacuo to dryness. The residue was purified by column chromatography to give a soft solid, compound **11** (860 mg, 87.5%). ^1H NMR ($CDCl_3$) 1.43 (m, 2H), 1.89 (m, 4 H), 4.20 (t, 4H, $J = 6.8$ Hz), 7.25 (m, 4H), 7.49 (m, 4H), 7.61 (m, 2H), 7.77 (s, 2H), 7.81 (d, 2H, $J = 7.6$ Hz), 8.28 (d, 2H, $J = 8.4$ Hz), 8.50 (d, 2H, $J = 7.6$ Hz). Anal. Calcd for $C_{35}H_{28}N_4{\cdot}0.6H_2O$: C, 81.56; H, 5.48; N, 10.87. Found: C, 81.60; H, 5.35; N, 10.76.

5.2.7. Synthesis of 1,5-bis(indolo[3,2-*b*]quindolin-10-ium)-pentane dioodide (12). A mixture of bis-quindoline, **11** (300 mg, 0.69 mmol), MeI (0.2 mL), and sulfolane (2 mL) was sealed in a tube, and the solution was heated at 100 °C for 16 h and allowed to cool to room temperature. Et_2O (20 mL) was added to produce a yellow precipitate, which was collected by filtration. The resulting solid was recrystallized from CH_2Cl_2–MeOH and Et_2O to give compound **12** (410 mg, 75%); mp: 238–240 °C. ^1H NMR (DMSO-d_6) 1.43 (br s, 2H), 1.91 (br s, 4H), 4.63 (t, 4H, $J = 6.7$ Hz), 5.03 (s, 6H), 7.51 (t, 2H, $J = 7.9$ Hz), 7.91 (m, 6H), 8.18 (m, 2H), 8.36 (d, 2H, $J = 7.5$ Hz), 8.76 (d, 2H, $J = 9.3$ Hz), 8.81 (d, 2H, $J = 8.5$ Hz), 9.48 (s, 2H). Anal. Calcd for $C_{37}H_{34}I_2N_4{\cdot}0.5H_2O$: C, 55.72; H, 4.30; N, 7.03. Found: C, 55.54; H, 4.36; N, 6.94.

6. Biological testing

Compounds were evaluated in vitro against a panel of microorganisms, including *C. albicans* ATCC 90028 (*Ca*), *C. krusei* ATCC 6258 (*Ck*), *C. neoformans* ATCC 90113 (*Cn*), *Staphylococcus aureus* ATCC 29213 (*Sa*), methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *P. aeruginosa* ATCC 27853 (*Pa*), *A. fumigatus* ATCC 90906 (*Af*), and *M. intracellulare* ATCC 23068 (*Mi*) as previously reported.[22] All organisms were obtained from the American Type Culture Collection (Manassas, Va.). Susceptibility testing was performed using a modified version of the NCCLS methods[23–25] for all organisms except for *M. intracellulare*, for which the modified Alamar blue procedure described by Franzblau et al.[26] was followed. Briefly, samples (dissolved in DMSO) were serially diluted by using 0.9% saline and transferred in duplicate to 96-well microplates. Microbial inocula were prepared after comparison of the absorbance (at

630 nm) of cell suspensions to the 0.5 McFarland standard and dilution of the suspensions in broth (Sabouraud dextrose and cation-adjusted Mueller–Hinton broth [Difco] for the fungi and bacteria, respectively, and 5% Alamar blue [BioSource International] in Middlebrook 7H9 broth with oleic acid–albumin–dextrose–catalase enrichment for *M. intracellulare*) to afford recommended inoculum sizes. Microbial inocula were added to the samples to achieve a final volume of 200 μL and final sample concentrations starting with 100 μg/mL. Growth, solvent, and medium controls were included on each test plate. The plates were read at either 630 nm or excitation and emission wavelengths of 544 and 590 nm (for *Mi*) prior to and after incubation. Percent growth was calculated and plotted with the concentration tested to afford the concentration that inhibits 50% of growth (IC_{50}). Antimalarial and cytotoxicity testing were conducted in a similar manner as previously reported.[27,28]

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