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Identification of 3-Phenylaminoquinolinium and 3-Phenylaminopyridinium salts as New Agents against Opportunistic Fungal Pathogens

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Abstract

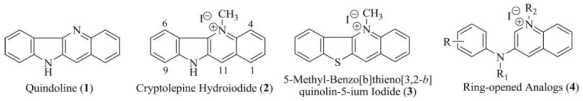
Previous studies on the indoloquinoline alkaloid, cryptolepine (**2**), revealed that it has antiinfective properties among other activities. Using Structure Activity Relationship (SAR) techniques, several ring-opened analogs of cryptolepine (3-phenylaminopyridinium and 3-phenylaminoquinolinium derivatives) were designed to improve the potency and lower the cytotoxicity shown by several of the precursor agents. Results indicate that these ring-opened analogs constitute new anti-infective agents with over a 100-fold potency and several fold lower cytotoxicity than cryptolepine from which they are derived.

Introduction

Opportunistic infections (OIs) may be caused by fungi, bacteria, viruses, or parasites. Symptoms may vary according to the microorganisms involved and the extent of their involvement. Conditions leading to immunodeficiency, including malnutrition, recurrent infections, immunosuppressive agents for organ transplant recipients, chemotherapy for cancer, acquired immunodeficiency syndrome (AIDS), genetic predisposition, antibiotic treatment, medical procedures and pregnancy among others expose these individuals to the risk of OIs.1–3 The most prevalent of these conditions is AIDS and since the discovery of the virus that causes AIDs (HIV) in 1981, a steady increase in the number of people worldwide living with HIV has been reported. This number, according to the WHO, has risen from around 8 million in 1990 to about 33 million and is still growing.3 Since 1981, more than 25 million people have died of AIDS and a large percentage of these individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system.3 In addition to immune-suppressed patients, there are indications in the literature that even immunocompetent individuals may be at risk for some opportunistic infections.4 *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* are three of the most common fungal pathogens responsible for opportunistic infections in humans. The current treatment options for the cure of these fungal pathogens are limited by toxicity, resistance development and drug interactions among others.[5,6]

Consequently, new drug entities especially those with new mechanisms of action are of great need.

Indolo[3,2-b]quinoline ring or quindoline (**1**) constitutes an important lead scaffold in the quest to find new agents against cancer, opportunistic infections, malaria, diabetes and several other diseases.[6] While the natural product cryptolepine (**2**) provided the impetus for the interest in this lead scaffold, several lines of evidence suggest caution about the long-term prospects of using cryptolepine.[7] Armed with this information, we have explored the possibility of modifying the structure to limit the cytotoxicity associated with the pharmacology of cryptolepine. Earlier on, we reported that the antiinfective properties of cryptolepine can be modified by substituting at various positions on the quindoline structure including isosteric replacement of the N-10 atom with sulfur.[8] Furthermore, it was observed that opening the ring at the benzothiophene moiety produced analogs of *N*-substituted benzothieno [3, 2-b] quinolines with potent antimicrobial activity. As a result, we opine that substitution could be used to obtain selective agents and curtail the cytotoxicity associated with cryptolepine. We further surmised that since cryptolepine intercalates into DNA in a unique fashion as a result of its flat and rigid tetracyclic structure[10,11] and since some of its toxicity may be related to this intercalation, opening the ring to allow for flexibility may decrease intercalation into DNA and hence reduce cytotoxicity.



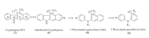
Hence, in this paper, we report the synthesis and evaluation of cryptolepine analogs substituted at all synthetically accessible positions on the indole and quinoline moieties with a chlorine atom previously shown to enhance potency and the exploration of the possibility that ring-opened analogs might offer opportunity for increased potency and lower cytotoxicity.

Design of Target Compounds

Substitution around the quindoline ring

In a previous publication[8] we have identified the electron withdrawing and hydrophobic chlorine atom to enhance antifungal potency when substituted at the 2-position of cryptolepine. To obtain the optimal position for substitution on the indoloquinoline ring, we proposed substitution of chlorine at all synthetically accessible positions on the quindoline ring. Subsequent evaluation of the antifungal properties of the synthetic compounds would lead to the identification of the optimal position with respect to electron withdrawing and hydrophobic groups.

For the ring-opened systems (see below), substituent selections will be guided by a 2D Craig plot[9] in order to use the minimum number of compounds to explore the electronic and hydrophobic space around the ring systems.



Opening of ring B and Excision of Ring D

Exploitation of a new agent's conformational flexibility (such as torsional flexibility about strategically located chemical bonds) is a powerful element of drug design.[12] The flat tetracyclic structure of cryptolepine enables it to intercalate into DNA in a previously unknown fashion.10b,13 In addition to DNA intercalation, cryptolepine is also known to interfere with topoisomerase II.14 Thus, we hypothesize that by opening ring B in

cryptolepine, the flat topography of cryptolepine would be lost and consequently its unique binding to DNA would be curtailed and thus the potential to decrease toxicity brought on by intercalation into DNA. This hypothesis will be tested by synthesizing ring opened analogs, **4**. In addition, the corresponding ring opened analogs of δ-carbolinium compounds would also be synthesized as the δ-carboline moiety was proposed as a pharmacophoric group in cryptolepine.[15]

N-5 and N-10 Alkylations

Evidence suggests that the active form of cryptolepine consists of the flat tetracyclic aromatic ring with a positive charge on the N-5 atom brought about by the N-methyl substituent.[16] It was also observed that changes in the electron density around the N-5 atom do not appear to affect activity significantly.[17] Through systematic modification of the N-5 alkyl groups, ω-cyclohexylpentyl group has been identified with an enhanced antiinfective potency. Thus, compounds proposed in this manuscript are adorned with the ω-cyclohexylpentyl group on the N-atom.

Synthesis

Literature methods were employed in the synthesis of these analogs.[18] As previously reported, substituted cryptolepine derivatives **11a–d** were obtained by a series of reactions beginning with treating the commercially available 3-aminoquinoline, **8** with substituted phenyl boronic acid **7** in the presence of copper acetate and triethylamine. This reaction gave N-phenyl quinoline-3-amines **9** in good yield (80–90%). The intermediate **9** was then heated in trifluoroacetic acid in the presence of palladium acetate to obtain the cyclized product, **10**. Methylation of the N-5 (**10a–d**) yielded the target compounds, **11a–d** (Scheme I).

2- Substituted cryptolepine derivatives, **12** were obtained as previously reported[8] and compounds **16a–d** were obtained as detailed in scheme II below.[17]

Compound **19**, 5-iodopentylcyclohexane was obtained by the reaction of cyclohexyl magnesium chloride, **17** with 1, 5-dibromopentane in the presence of lithium tetrachloro cuprate in THF to yield the intermediate 5-bromopentylcyclohexane **18**, which was then converted to the more reactive 5-iodopentylcyclohexane, **19** by the reaction of **18** with sodium iodide in acetone (Scheme III).

To prepare 3-phenylaminoquinolinium analogs **21a–m**, intermediate **9** was converted to **20** by deprotonation of the secondary aromatic amine with sodium hydride and subsequently alkylated with 5-iodopentylcyclohexane to obtain **19**. The target compounds, **21a–m**, were obtained by treating **20** with iodomethane, 5-phenylpentyl iodide or **19** in toluene (Scheme IV).

Similarly, the first step in the synthesis of 3-phenylaminopyridinium analogs, **26a–t**, involved coupling the available substituted phenyl boronic acid or halide **22**, with 3-aminopyridine **23** to give substituted phenylaminopyridine, **24**. The conversion of **24** to **25** required selective alkylation of the secondary diarylamine which was accomplished by deprotonation with sodium hydride followed by alkylation with the appropriate alkyl halide. The target compounds were then obtained by methylation of the pyridine nitrogen, **25** to form the desired target compounds, **26a–t** (Scheme V).

Results & Discussions

Biological evaluation of all analogs was performed against three common opportunistic fungal pathogens *Candida albicans* (*Ca*), *Cryptococcus neoformans* (Cn) and *Aspergillus fumigatus* (*Af*). Evaluation of chlorine-substituted cryptolepine analogs (**11a–d** and **16a–d**)

was intended to identify the optimum location on the indoloquinoline moiety. There was an increase in potency against *C. albicans* and *C. neoformans* for all chloro-substituents when compared to the unsubstituted cryptolepine except for the 6- and 9-chloro substituents which were inactive. Position 4 was not investigated because all attempts to alkylate the N-5 atom of the quinoline with chlorine at the 4-position were unsuccessful. Apparently, the chlorine atom sterically and electronically hindered the introduction of the methyl group onto the N-atom. Replacement of the iodide with trifluoromethanesulfonate (triflate) co-ion in **16a** & **16b** did not have any significant change on antifungal activity of these analogs. Overall, all chloro-substituted cryptolepine analogs were inactive against *A. fumigatus* and showed considerable cytoxicity against Vero cell lines, an indication of their possible toxicity in humans (Table 1). The exception to this observation was when N-10 is alkylated with ω-cyclohexylpentyl moiety (**16d**).

We have previously shown that substitution of ω-cyclohexylpentyl group on the N-5 atom of ring C enhanced potency against fungi[17] However, cytotoxicity against Vero cell lines was increased as well. Alkylation of 2-chlorocryptolepine's N-10 atom with ω-cyclohexylpentyl (**16d**) was investigated to find if potency would be retained and if cytotoxicity would be attenuated. While potency was enhanced against all three fungal pathogens, cytotoxicity was increased as well (TC_{50} = 1.4 μg/mL). It became clear that the tetracyclic structure per se may be responsible for the cytotoxicity and we needed to deviate from the linear and flat tetracycle.

One approach to modify the linear flat topography of cryptolepine is to open the ring system. The pyrrole ring of the indole moiety offers a synthetically attractive target for opening and was thus selected for investigation, while maintaining the chlorine atom at the *o*, *m*- and *p*-positions of the phenyl ring. Compounds **21a–d**, the N-methylated analogs showed no activity at 20 μg/mL (Table 2). Next, quinoline N-substituted ω-phenyllpentyl analog, **21e** were synthesized and evaluated. These showed improved activity and in addition produced no cytotoxicity at 10 μg/mL. Replacing the ω-phenylpentyl group with ω-cyclohexylpentyl group, **21f–h**, led to further improvements in activity. Finally, substituting the ω-cyclohexylpentyl group on the diarylamino group while retaining the necessary N-methyl group on the quinoline, produced the most potent analogs among the series with no cytotoxicity at 10 μg/mL. While the presence of the chlorine atom improved activity against *Ca* and *Af*, it appears to have no effect in the case of *Cn* (cf **21i–k** vs **21*l***). Substitution of the chlorine atom at the *para* position of the phenyl A ring appears to provide the optimum effect for activity, Thus, 3-phenylaminoquinolinium derivatives, with an appropriately substituted ω-cyclohexylpentyl group, **21f–*l***, offer a new promising scaffold for further investigation.

We have also previously reported that alkylating cryptolepine with an ω-cyclohexylpentyl group on the diarylamino function showed potent antifungal activity but significant cytotoxicity.[17] Since ring opening resulted in attenuating cytotoxicity in cryptolepine, we hypothesized that using a similar strategy for the δ-carboline system might lead to improvements in cytotoxicity. With no substitution on the diarylamino function (**26a**), there was no activity against any of the fungi. Introduction of the ω-cyclohexylpentyl group on the diarylamino N-atom with the pyridine methyl group in place (**26e**) led to significant improvement in antifungal activity and in particular no cytotoxicity was observed at 10 μg/mL. It was of interest at this point to probe the electronic effect and lipophilicity on the phenyl ring as this would affect the electron density at the diaryl nitrogen. The *para* position was first selected for exploration as it was the optimum position for substitution in the quinolinium series (Table 2). The presence of a fluorine atom (**26d**) at the *para* position has no effect on activity but a 4-chloro (**26b**) and the more potent electron withdrawing trifluoromethyl group (**26c**) both increased activity significantly against all three pathogens

and showed no cytotoxicity at 10 μg/mL against Vero cells. Similarly, increase in potency was observed for the electron donating and lipophilic alkyl substituents (**26f & 26g**) and the thiomethyl group (**26i**) at the 4-position. However, activity decreased somewhat for the weakly hydrophilic methoxy (**26h**) along with the even more hydrophilic cyano substituent (**26j**).

These results would appear to point to the importance of lipophilicity and not electronic effects on activity. The 3-position of the phenyl ring was also evaluated using the chloro (2**6k**), trifluoromethyl (**26l**) and methyl (**26m**) groups. These analogs also showed significant potency albeit generally lower than the substituents at the 4-position. Finally, the same substituents were evaluated at the 2-position (**26p–t**). Overall, activities of the 2-substituents were similar to those of the 3-substituents.

Conclusion

Using the method previously reported by our labs, we were able to synthesize the target compounds in relatively good yield. Biological results indicated that substituted cryptolepine analogs, **11a–d** and **16a–d** are active but continue to show significant toxicity. However, the corresponding ring-opened analogs, 3-phenylaminoquinolinium **21a–l** and 3-phenylaminopyridinium derivatives **26a–t** now constitute novel anti-infective agents with high potency and low cytotoxicity. On average, these analogs are over 100-fold (Cn) and over 250-fold (Ca) more potent than cryptolepine, the original natural product. Substitution on the phenyl ring of ring-opened analogs with electron withdrawing and hydrophobic groups (such as Cl, CF_3) tends to enhance activity. The *in vitro* cytotoxicity assay against mammalian kidney fibroblast (Vero) cells indicates that opening cryptolepine's indole ring and introducing the ω-cyclohexylpentyl group on N-atoms resulted in a decrease in toxicity. Finally, 3-phenylaminopyridinium and 3-phenylamino-quinolinium derivatives are not only very potent against the selected microorganisms but they also have lower toxicity than cryptolepine.

Experimental

Reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich, Alfa Aesar and VWR) and except for tetrahydrofuran (THF) which was dried over sodium metal, all other reagents and solvents were used without further purification. Flash Chromatography was performed with Selecto Scientific silica gel (63–200 mesh). Analytical TLC was performed on pre-coated Whatman Ltd aluminium plates (Silica gel 60 Å). NMR spectra were obtained on Varian 300-MHZ NMR spectrometer at FAMU. Melting points were determined on Gallenkamp (UK) apparatus. CHN analyses, performed with a Perkin Elmer 2400 CHN elemental analyzer, were carried out by Atlantic Microlab, Inc., Norcross, GA and are within 0.4% of theoretical values unless otherwise noted.

a) General Procedure for the synthesis of N-phenylquinolin-3-amine derivatives (9) & N-phenylpyridin-3-amine derivatives (24)

To a solution of 3-aminoquinoline or 3-aminopyridine (1 g) in dichloromethane (30 mL) was added portion-wise phenyl boronic acid (1.63g, 13.36 mmol), triethylamine (1.5g, 10.41 mmol), copper acetate (1.90g, 10.41 mmol) and molecular sieves (2g) under stirring. The reaction mixture was stirred at room temperature for 12–24 h and monitored by TLC. Once reaction is completed, the reaction was quenched by the drop wise addition of aqueous ammonia (15 ml). The resulting mixture was extracted with dichloromethane, washed with brine then with water, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue purified by column chromatography using ethyl acetate and hexanes as eluent.

b) General Procedure for the synthesis of 10*H*-indolo[3,2-*b*]quinoline derivatives (10)

To a solution of phenyl-quinolin-3-yl-amine derivatives **9** (400 mg) in CF_3COOH (8 ml), was added $Pd(OAc)_2$ (300 mg, 1.34 mmol) and the reaction mixture was refluxed for 6 h at 72°C. The reaction mixture was allowed to cool to room temperature, poured on ice cold water (15 ml), neutralized with aqueous NH_3 and extracted with EtOAc (3× 50 mL), washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified on column chromatography using hexane: EtOAc; 2:3.

c) General Procedure for the synthesis of 10H-indolo[3,2-b]quinoline-11-carboxylic acid derivatives (13)

A mixture of *1H*-indol-3-yl acetate (5g, 29 mmol), isatin derivative (5.2g) and KOH (22g) in water (200 mL) was stirred for 10 days at rt, filtered and the filtrate was acidified to pH 1 with concentrated HCl. The precipitate of quindoline-11-carboxylic acid derivative **13** was collected, washed with water, dried, and used for the next step without further purification.

d) General Procedure for the synthesis of 10H-indolo[3,2-b]quinoline derivatives (14)

A mixture of quindoline-11-carboxylic acid derivative **13** (3.2g) and parafin oil (30 mL) was heated at 300 °C for 3 h, allowed to cool to rt and diluted in hexanes (100 ml), filtered and washed with hexanes (3 × 50 ml). The crude product was purified by column chromatography (hexane: EtOAc 1:1) to yield **14**.

e) Synthesis of 5-Bromopentylcyclohexane (18)

To a solution of 1, 5-dibromopentane (16g, 70 mmol) in THF (20 ml) was added Li_2CuCl_4 (12.7g, 62 mmol) under N_2 at 5–10 °C. The resulting mixture was stirred for 25 min and then cyclohexyl magnesium bromide (10g, 70 mmol) was added drop-wise over 30 min. The reaction continued to stir at 0°C for another 1 h and then at rt for 12 hr. The reaction mixture was cooled to 0°C, saturated NH_4Cl solution (20 ml) added and then EtOAc (100 mL). The organic layer was separated, pooled, washed with brine and then dried over anhydrous Na_2SO_4. The filtrate was subjected to rotary evaporation and the crude product was purified by column chromatography using hexanes as eluent to yield **18** as an oily liquid (75% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.8 (t, 2H, *J* = 10.2 Hz), 1.00–1.38 (m, 9H), 1.52–168 (m, 6H), 1.70–1.80 (m, 2H), 3.38 (t, 2H, *J* = 7.2 Hz).

f) Synthesis of 5-Iodopentylcyclohexane (19)

A mixture of (5-bromopentyl)cyclohexane **18** (2 g, 8.6 mmol) in acetone (20 mL) and NaI (2.57g, 17.2 mmol) was refluxed for 12 h and then allowed to cool to rt. The solvent was removed in vacuum; the residue was diluted with H_2O (50 ml) and extracted with EtOAc (30 ml). The organic layer was washed with brine (30 ml), dried over anhydrous Na_2SO_4, solvent removed under vacuum and the crude product purified on column chromatography using hexanes as eluent to yield the pure product as an oily liquid (90 % yield, Boiling point 291°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.8 (t, 2H, *J* = 10.2 Hz), 1.00–1.38 (m, 9H), 1.52–168 (m, 6H), 1.70–1.80 (m, 2H), 3.10 (t, 2H, *J* = 6.9 Hz).

g) General Procedure for the synthesis of 20 & 25 derivatives

To a solution of **9** or **24** (100 mg) in 1, 2-dimethoxyethane (5 ml), NaH (20 mg, 0.83 mmol) and 5-iodo-pentylcyclohexane (636 mg, 2.3 mmol) were added in that order. The mixture was stirred at rt for 12 h, solvent was evaporated and the residue partitioned between H_2O (10 mL) and EtOAc (2 × 30 mL). The pooled organic phase was washed with brine and dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude product was purified on column chromatography (hexane: EtOAc, 4:1).

h) General procedure for synthesis of 5-methyl-10H-indolo[3,2-b] quinolin-5-ium triflate derivatives 16a–b

To a solution of **14** (100 mg) in toluene (3 ml), in a sealed pressure tube, methyl triflate (0.3 g, 1.8 mmol) was added. The reaction mixture was stirred at rt for 24 h, then diluted with Et_2O (15 mL), the precipitate was filtered and washed with Et_2O (3 × 20 ml). The crude product was recrystallized from MeOH.

1-Chloro-5-methyl-10H-indolo[3,2-b]quinolin-5-ium triflate (16a)

Yellow solid (71% yield), mp: 289–291°C; ^{1}H NMR (300 MHz, CD_3OD): δ 5.14 (s, 3H), 7.57–7.63 (m, 1H), 7.85 (d, 1H, *J* = 8.4 Hz), 7.97–8.02 (m, 1H), 8.08–8.14 (m, 2H), 8.64–8.68 (m, 1H), 8.76 (d, 1H, *J* = 8.7 Hz), 9.41 (s, 1H). Anal. Calcd for $C_{17}H_{12}ClF_3IN_2O_3S.0.5H_2O$: C, 47.95; H, 2.84; N, 6.58. Found: C, 47.93; H, 2.81; N, 6.58.

3-Chloro-5-methyl-10H-indolo[3,2-b]quinolin-5-ium triflate (16b)

Yellow solid (82% yield), mp: 226–228°C; ^{1}H NMR (300 MHz, CD_3OD): δ 5.06 (s, 3H), 7.54–7.59 (m, 1H), 7.81 (d, 1H, *J* = 8.7 Hz), 7.90–7.98 (m, 2H), 8.46 (d, 1H, *J* = 8.7 Hz), 8.72–8.76 (m, 2H), 9.13 (s, 1H). Anal. Calcd for $C_{17}H_{12}ClF_3IN_2O_3S.0.5H_2O$: C, 47.95; H, 2.84; N, 6.58. Found: C, 47.88; H, 2.84; N, 6.54.

i) General Procedure for the synthesis of 11a–d, 16c–d, 21a–l & 26a–t derivatives

To a solution of **10, 15, 20 or 25** (100 mg) in toluene (3 ml), in a sealed pressure tube, CH_3I (0.3 g, 2.1 mmol) was added. The mixture was stirred at 110 °C for 24 h, allowed to cool to rt, diluted with Et_2O (15 mL), the precipitate filtered and washed with Et_2O (3 × 20 ml). The crude product was recrystallized from MeOH.

6-Chloro-5-methyl-10H-indolo[3,2-b]quinolin-5-ium Iodide (11a)

Yellow solid (67% yield), mp: 211–212°C; ^{1}H NMR (300 MHz, DMSO-d_6): 13.28 (s, 1H), 9.37 (s, 1H), 8.63 (d, 1H, *J* = 9.0 Hz), 8.59 (d, 1H, *J* = 9.0 Hz), 8.21 (t, 1H, *J* = 8.1 Hz), 7.97 (t, 1H, *J* = 7.5 Hz), 7.88 (m, 2H), 7.60 (d, 1H, *J* = 7.1 Hz), 5.08 (s, 3H). Anal. Calcd for $C_{16}H_{12}ClIN_2$: C, 48.70; H, 3.06; N, 7.10; Found: C, 48.49; H, 3.05; N, 6.97.

7-Chloro-5-methyl-10H-indolo[3,2-b]quinolin-5-ium iodide (11b)

Yellow solid (45% yield), mp: 206–207°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.78 (d, 1H, *J* = 1.8 Hz), 8.68 (d, 1H, *J* = 9.0 Hz), 8.50 (d, 1H, *J* = 7.5 Hz), 8.24–8.18 (m, 1H), 7.96 (t, 1H, *J* = 6.3 Hz), 7.90 (d, 1H, *J* = 2.1 Hz), 7.82 (d, 1H, *J* = 9.0 Hz), 5.08 (s, 3H). Anal. Calcd for $C_{16}H_{12}ClIN_2$: C, 48.70; H, 3.06; N, 7.10. Found: C, 48.70; H, 3.06; N, 6.99.

7-Bromo-5-methyl-10H-indolo[3,2-b]quinolin-5-ium Iodide (11c)

Yellow solid (74% yield), mp: 260–261°C; ^{1}H NMR (300 MHz, DMSO-d_6): 13.04 (s, 1H), 9.36 (s, 1H), 9.00 (s, 1H), 8.10 (d, 1H, *J* = 8.8 Hz), 8.59 (d, 1H, *J* = 8.1 Hz), 8.19 (t, 1H, *J* = 8.8 Hz), 8.06 (d, 1H, *J* = 8.4 Hz), 7.98 (t, 1H, *J* = 7.2 Hz), 7.83 (d, 1H, *J* = 8.8 Hz), 5.03 (s, 3H). Anal. Calcd for $C_{16}H_{12}BrIN_2$: C, 43.77; H, 2.75; N, 6.38. Found: C, 44.05; H, 2.81; N, 6.20.

9-Chloro-5-methyl-10H-indolo[3,2-b]quinolin-5-ium iodide (11d)

Yellow solid (57% yield), mp: 251–253°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.70 (s, 1H), 9.80 (s, 1H), 9.26–9.18 (m, 1H), 8.90 (d, 1H, *J* = 8.4 Hz), 8.70-8.60 (m, 1H), 8.20-8.10 (m, 2H), 8.00 (d, 1H, *J* = 7.5 Hz), 7.55 (t, 1H, *J* = 7.5 Hz), 4.80 (s, 3H). Anal. Calcd for $C_{16}H_{12}ClIN_2.0.73\ H_2O$: C, 45.65; H, 3.22; N, 6.65. Found: C, 45.64; H, 3.18; N, 6.62.

3,11-Dichloro-5-methyl-10H-indolo[3,2-b]quinolin-5-ium iodide (16c)

Yellow solid (33% yield), mp: 236–238°C; ^{1}H NMR (300 MHz, CD_3OD): δ 5.00 (s, 3H), 7.55–7.61 (m, 1H), 7.89–8.01 (m, 3H), 8.62 (d, 1H, *J* = 9 Hz), 8,71 (d, 1H, *J* = 9 Hz), 8.763 (d, 1H, *J* = 1.5 Hz). Anal. Calcd for $C_{16}H_{11}Cl_2IN_2.5.5H_2O$: C, 36.39; H, 2.10; N, 5.30. Found: C, 36.32; H, 2.48; N, 5.21.

2-Chloro-10-(5-cyclohexylpentyl)-5-methyl-10H-indolo[3,2-b]quinolin-5-ium iodide (16d)

Yellow solid (72% yield), mp: 215–215°C; ^{1}H NMR (300 MHz, CD_3OD): δ 9.36 (s, 1H), 8.81 (d, 1H, *J* = 8.4 Hz), 8.71 (d, 1H, *J* = 9.6 Hz), 8.58 (d, 1H, *J* = 2.4 Hz), 8.13 (dd, 1H, *J* = 2.4, 2.1 Hz), 8.02 – 7.96 (m, 2H), 7.63 (t, 1H, *J* = 7.2), 5.12 (s, 3H), 4.72 (t, 2H, *J* = 7.2 Hz), 2.02 – 1.97 (m, 2H), 1.68 – 1.38 (m, 9H), 1.22 – 1.14 (m, 6H), 0.82 (t, 2H, *J* = 6 Hz). Anal. Calcd for $C_{27}H_{32}ClIN_2$: C, 59.29; H, 5.90; N, 5.12. Found: C, 59.15; H, 6.07; N, 5.08.

1-Methyl-3-(phenylamino)quinolinium iodide (21a)

Yellow solid (86% yield), mp: 182–183°C; ^{1}H NMR (300 MHz, CD_3OD): δ 4.6 (s, 3H), 7.18 (m, 1H), 7.36 (dd, 2H, *J* = 0.9, 7.5 Hz), 7.44 (m, 2H), 7.82 (t, 2H, *J* = 6.0 Hz), 7.94 (m, 2H), 8.10 (d, 1H, *J* = 8.4 Hz), 8.26 (d, 1H, *J* = 8.6 Hz), 8.46 (d, 1H, *J* = 2.4 Hz), 9.04 (d, 1H, *J* = 2.7 Hz). Anal. Calcd for $C_{16}H_{15}IN_2.1.3\ H_2O$: C, 46.98; H, 4.34; N, 6.85. Found: C, 46.81; H, 3.95; N, 6.54

3-(2-Chlorophenylamino)-1-methylquinolinium iodide (21b)

Yellow solid (55% yield), mp: 208–210°C; ^{1}H NMR (300 MHz, CD_3OD): δ 9.10 (d, 1H, *J* = 2.4 Hz), 8.34 (d, 2H, *J* = 9.0 Hz), 8.10 (d, 1H, *J* = 8.4 Hz), 7.96 (t, 1H, *J* = 9.0 Hz), 7.84 (t, 1H, *J* = 7.5 Hz), 7.58 (dd, 2H, *J* = 8.1, 1.5 Hz), 7.40 (t, 1H, *J* = 8.1 Hz), 7.24 (t, 1H, *J* = 7.2 Hz), 4.90 (s, 3H). Anal. Calcd for $C_{16}H_{14}ClIN_2.1.8.CH_3OH$: C, 42.30; H, 3.11; N, 6.17. Found: C, 42.49; H, 3.23; N, 6.09.

3-(3-Chlorophenylamino)-1-methylquinolinium iodide (21c)

Yellow solid (42% yield), mp: 211–213°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.42 (s, 1H), 9.24 (d, 1H, *J* = 2.4 Hz), 8.64 (d, 1H, *J* = 2.4 Hz), 8.34 (d, 1H, *J* = 9.0 Hz), 8.26 (d, 1H, *J* = 8.1 Hz), 7.98-7.92 (t, 1H, *J* = 7.5 Hz), 7.87 (t, 1H, *J* = 7.8 Hz), 7.39 (t, 1H, *J* = 8.1 Hz), 7.32 (t, 1H, *J* = 2.1 Hz), 7.24 (dd, 1H, *J* = 6.3, 1.2 Hz), 7.08 (dd, 1H, *J* = 6.0, 1.2 Hz), 4.60 (s, 3H). Anal. Calcd for $C_{16}H_{14}ClIN_2.\ 0.39H_2O$: C, 47.61; H, 3.50; N, 6.94. Found: C, 47.75; H, 3.52; N, 6.89.

3-(4-Chlorophenylamino)-1-methylquinolinium iodide (21d)

Yellow solid (45% yield), mp: 153–155°C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.03 (d, 1H, *J* = 2.4 Hz), 8.50 (d, 1H, *J* = 2.4 Hz), 8.29 (d, 1H, *J* = 8.7 Hz), 8.11 (d, 1H, *J* = 7.2 Hz), 7.96 – 7.91 (m, 1H), 7.87 – 7.81 (m, 1H), 7.43 – 7.41 (m, 2H), 7.35 - 7.31 (m, 2H), 4.63 (s, 3H). Anal. Calcd for $C_{16}H_{14}ClIN_2$: C, 48.45; H, 3.56; N, 7.06. Found: C, 49.00; H, 3.88; N, 6.70.

3-(4-Chlorophenylamino)-1-(5-phenylpentyl)quinolinium iodide (21e)

Yellow solid (36% yield), mp: 182–183°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.36 (s, 1H), 9.18 (d, 1H, *J* = 2.4 Hz), 8.58 (d, 1H, *J* = 2.4 Hz), 8.38 (d, 1H, *J* = 8.7 Hz), 8.22 (d, 1H, *J* = 8.1 Hz), 7.91 (t, 1H, *J* = 7.2 Hz), 7.83(t, 1H, *J* = 7.2 Hz), 7.42 (d, 2H, *J* = 9.0 Hz), 7.30 (d, 2H, *J* = 9.0 Hz), 7.23 (t, 2H, *J* = 6.9 Hz), 7.16-7.10 (m, 3H), 4.98 (t, 2H, *J* = 7.5 Hz), 2.56 (t, 2H, *J* = 7.5 Hz), 2.02-1.90 (m, 2H), 1.66-1.56 (m, 2H), 1.46-1.36 (m, 2H). Anal. Calcd for $C_{26}H_{26}IClN_2$: C, 59.05; H, 4.96; N, 5.30. Found: C, 58.99; H, 4.99, N, 5.22.

1-(5-Cyclohexylpentyl)-3-(phenylamino)quinolinium iodide (21f)

Yellow solid (13% yield), 156–158°C ^{1}H NMR (300 MHz, CD_3OD): δ 0.80–1.00 (m, 2H), 1.00–1.38 (m, 7H), 1.40–1.58 (m, 4H), 1.60–1.80 (m, 4H), 2.00–2.20 (m, 2H), 5.00 (t, 2H, *J* = 7.2 Hz), 7.20 (t, 1H, *J* = 6.8 Hz), 7.35 (d, 2H, *J* = 8.6 Hz), 7.46 (t, 2H, *J* = 7.0 Hz), 7.80 (t, 1H, *J* = 7.6 Hz), 7.82–8.00 (m, 1H), 8.10 (d, 1H, *J* = 8.4 Hz), 8.38 (d, 1H, *J* = 9.0 Hz), 8.45 (d, 1H, *J* = 2.4 Hz), 9.0 (d, 1H, *J* = 2.7 Hz). Anal. Calcd for $C_{26}H_{33}IN_2$.1.4 H_2O: C, 56.99; H, 6.55; N, 5.08. Found: C, 56.63; H, 6.25; N, 4.77.

3-(3-Chlorophenylamino)-1-(5-cyclohexylpentyl)quinolinium iodide (21g)

Yellow solid (74% yield), mp: 128–129°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.40 (s, 1H), 9.28 (d, 1H, *J* = 2.4 Hz), 8.66 (d, 1H, *J* = 1.8 Hz), 8.42 (d, 1H, *J* = 9.0 Hz), 8.28 (d, 1H, *J* = 8.1 Hz), 7.93 (t, 1H, *J* = 7.2 Hz), 7.84(t, 1H, *J* = 7.2 Hz), 7.37 (t, 1H, *J* = 8.1 Hz), 7.32 (d, 1H, *J* = 1.5 Hz), 7.24-7.20 (dd, 1H, *J* = 6.9, 1.2 Hz), 7.08 (d, 1H, *J* = 8.1 Hz), 4.98 (t, 2H, *J* = 7.2 Hz), 1.92 (t, 2H, *J* = 6.6 Hz), 1.70-1.50 (m, 5H), 1.40-1.26 (m, 4H), 1.20-1.00 (m, 6H), 0.81 (t, 2H, *J* = 9.9 Hz). Anal. Calcd for $C_{26}H_{32}$ $ClIN_2$: C, 58.38; H, 6.03; N, 5.24. Found: C, 58.36; H, 5.99; N, 5.17.

3-(4-chlorophenylamino)-1-(5-cyclohexylpentyl)quinolinium iodide (21h)

Yellow solid (40% yield), mp: 177–178°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.38 (s, 1H), 9.18 (d, 1H, *J* = 2.4 Hz), 8.58 (d, 1H, *J* = 2.1 Hz), 8.40 (d, 1H, *J* = 8.7 Hz), 8.22 (d, 1H, *J* = 8.1 Hz), 7.90 (t, 1H, *J* = 7.2 Hz), 7.82 (t, 1H, *J* = 6.9 Hz), 7.42 (d, 2H, *J* = 9.0 Hz), 7.30 (d, 2H, *J* = 9.0 Hz), 4.96 (t, 2H, *J* = 7.5 Hz), 1.9 (t, 2H, *J* = 6.9 Hz), 1.70-1.50 (m, 5H), 1.40-1.28 (m, 4H), 1.20-1.00 (m, 6H), 0.80 (t, 2H, *J* = 9.6 Hz). Anal. Calcd for $C_{26}H_{32}$ $ClIN_2$.0.6 H_2O: C, 57.22; H, 5.91; N, 5.13. Found: C, 57.26; H, 5.86; N, 5.14.

3-((2-Chlorophenyl)(5-cyclohexylpentyl)amino)-1-methylquinolinium iodide (21i)

Yellow solid (29% yield), mp:131–132°C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.87 (d, 1H, J = 2.7 Hz), 8.25 (d, 1H, J = 9.0 Hz), 8.16 (dd, 1H, *J* = 1.5, 7.2 Hz), 8.04 (d, 1H, *J* = 2.4 Hz), 7.90 – 7.78 (m, 2H), 7.57 – 7.748 (m, 3H), 4.57 (s, 3H), 3.81 (t, 2H, *J* = 7.2 Hz), 1.63 – 1.59 (m, 7H), 1.35 – 1.28 (m, 4H), 1.21 – 1.05 (m, 6H), 0.81 (t, 2H, *J* = 9.0 Hz). Anal. Calcd for $C_{27}H_{34}ClIN_2$. 0.8 H_2O: C, 57.57; H, 6.08; N, 4.97, Found: C, 57.56; H, 6.27; N, 4.96.

3-((3-Chlorophenyl)(5-cyclohexylpentyl)amino)-1-methylquinolinium iodide (21j)

Yellow solid (74% yield), mp: 85–87°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (d, 1H, *J* = 2.7 Hz), 8.57 (d, 1H, *J* = 2.4 Hz), 8.30 (d, 1H, *J* = 8.7 Hz), 8.23 (d, 1H, *J* = 9.3 Hz), 7.99-7.93 (m, 1H), 7.87 (t, 1H, *J* = 7.5 Hz), 7.43 (t, 1H, *J* = 8.4 Hz), 7.38 (t, 1H, *J* = 2.1 Hz), 7.27-7.22 (m, 2H), 4.53 (s, 3H), 3.88 (t, 2H, *J* = 7.2 Hz), 1.63-1.59 (m, 6H), 1.35-1.26 (m, 5H), 1.15-1.08 (m, 6H), 0.81 (t, 2H, *J* = 9.6 Hz). Anal. Calcd for $C_{27}H_{34}ClIN_2$: C, 59.08; H, 6.24; N, 5.10. Found: C, 59.25; H, 6.26; N, 5.21.

3-((4-Chlorophenyl)(5-cyclohexylpentyl)amino)-1-methylquinolinium iodide (21k)

Yellow solid (79% yield), mp: 176–177°C; ^{1}H NMR (300 MHz, (DMSO-d_6): δ 9.10 (d, 1H, *J* = 2.7 Hz), 8.46 (d, 1H, *J* = 2.4 Hz), 8.26 (d, 1H, *J* = 8.7 Hz), 8.20-8.18 (dd, 1H, *J* = 7.2, 1.2 Hz), 7.96-7.90 (m, 1H), 7.84 (t, 1H, *J* = 7.8 Hz), 7.50 (d, 2H, *J* = 9.0 Hz), 7.36 (d, 2H, *J* = 9.0 Hz), 4.54 (s, 3H), 3.86 (t, 2H, *J* = 7.5 Hz), 1.70-1.50 (m, 7H), 1.40-1.24 (m, 4H), 1.20-1.04 (m, 6H), 0.79 (t, 2H, *J* = 10.2 Hz). Anal. Calcd for $C_{27}H_{34}ClIN_2$: C, 59.08; H, 6.24; N, 5.10. Found: C, 59.25; H, 6.26; N, 5.21.

3-((5-Cyclohexylpentyl)(phenyl)amino)-1-methylquinolinium iodide (21l)

Yellow solid (74% yield), mp: 156–158°C; ^{1}H NMR (300 MHz, (DMSO-d_6): δ 9.06 (d, 1H, *J* = 2.4 Hz), 8.36 (d, 1H, *J* = 2.4 Hz), 8.24 (d, 1H, *J* = 9.0 Hz), 8.16 (d, 1H, *J* = 8.4 Hz), 7.90-7.78 (m, 2H), 7.46 (t, 2H, *J* = 7.8 Hz), 7.34 (d, 2H, *J* = 7.8 Hz), 7.26 (t, 1H, *J* = 7.5 Hz), 4.50 (s, 3H), 3.86 (t, 2H, *J* = 7.5 Hz), 1.70-1.50 (m, 7H), 1.40-1.20 (m, 4H), 1.20-1.00 (m, 6H), 0.79 (t, 2H, *J* = 10.2 Hz). Anal. Calcd for $C_{27}H_{35}IN_2$: C, 63.03; H, 6.86; N, 5.44. Found: C, 62.86; H, 6.88; N, 5.28.

1-Methyl-3-(phenylamino)pyridinium iodide (26a)

Yellow solid (55 % yield), mp: 161–162°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ9.30 (s, 1H), 8.42 (s, 1H), 8.28 (d, 1H, *J* = 6.0), 7.96-7.90 (dd, 1H, *J* = 6.6, 2.1 Hz), 7.81 (dd, 1H, *J* = 8.7, 3.3 Hz), 7.42-7.38 (m, 2H), 7.28-7.24 (m, 2H), 7.10 (t, 1H, *J* = 7.2 Hz), 4.20 (s, 3H). Anal. Calcd for $C_{27}H_{35}IN_2$. 1.5 H_2O: C, 42.50; H, 3.86; N, 8.26, Found: C, 42.66; H, 3.79; N, 8.18.

3-((4-Chlorophenyl)(5-cyclohexylpentyl)amino)-1-methylpyridinium iodide (26b)

Yellow solid (75 % yield), mp: 134–135°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 (d, 2H, *J* = 1.8 Hz), 7.67 (t, 2H, *J* = 6.3 Hz), 7.57 - 7.54 (m, 2H), 7.34 - 7.31 (m, 2H), 4.3 (s, 3H), 3.78 (t, 2H, *J* = 7.8 Hz), 3.04 – 2.99 (m, 2H), 2.22 - 2.17 (m, 2H), 1,70 - 1.62 (m, 6H), 1.35-1.16 (m, 9H). Anal. Calcd for $C_{23}H_{32}ClIN_2$.0.05H_2O: C, 55.37; H, 6.47; N, 5.62. Found: C, 55.28; H, 6.45; N, 5.61.

3-((5-Cyclohexylpentyl)(4-(trifluoromethyl)phenyl)amino)-1-methylpyridinium iodide (26c)

Yellow solid (48 % yield), mp: 130–131°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51(s,1H), 8.23(d, 1H, *J* = 5.7Hz), 7.79 (d, 2H, *J* = 8.4 Hz), 7.65 - 7.60 (m, 1H), 7.45 (d,3H, *J* = 8.1), 4.60 (s, 3H), 3.95(t, 2H, *J* =7.2Hz), 1.69 - 1.61 (m,10H), 1.17-1.13 (m, 9H). Anal. Calcd for $C_{24}H_{32}F_3IN_2$: C, 54.14; H, 6.06; N, 5.26. Found: C, 53.91; H, 6.04; N, 5.17.

3-((5-Cyclohexylpentyl)(4-fluorophenyl)amino)-1-methylpyridinium iodide (26d)

Yellow solid (66 % yield), mp: 129–130°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.46 (s, 1H), 8.23 (d, 1H, *J* = 6Hz), 7.63-7.48 (m, 2H), 7.40 - 7.36 (m, 1H), 7.15 - 7.09 (m,2H), 6.99 - 6.95 (m, 1H), 4.59(s, 3H), 3.91 (t, 2H, *J* = 7.8 Hz), 1.68 - 1.64 (m, 11H), 1.30 -1.14 (m, 8H). Anal. Calcd for $C_{23}H_{32}FIN_2$: C, 57.26; H, 6.69; N, 5.81. Found: C, 57.28; H, 6.61; N, 5.73.

3-((5-Cyclohexylpentyl)(phenyl)amino)-1-methylpyridinium iodide (26e)

Yellow solid (62% yield), mp: 125–126°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.17 (s, 1H), 7.63 -7.26 (m, 7H), 4.62 (s, 3H), 3.95 (t, 2H, *J* = 7.5 Hz), 1.73-1.66 (m, 12H), 1.59 - 1.22 (m, 9H). Anal. Calcd for $C_{23}H_{33}IN_2$: C, 59.48; H, 7.16; N, 6.03. Found: C, 59.35; H, 7.11; N, 5.90.

3-((5-Cyclohexylpentyl)(p-tolyl)amino)-1-methylpyridinium iodide (26f)

Yellow solid (60% yield), mp: 150–151°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, 1H, *J* = 5.4 Hz), 8.19 (s, 1H), 7.60 - 7.55 (m, 1H), 7.32 (d, 3H, *J* = 7.8 Hz), 7.10 (d, 2H, *J* = 8.7 Hz), 4.53(s, 3H), 3.84 - 3.79 (m, 2H), 2.42 (s, 3H), 1.66 - 1.63 (m, 12H), 1.15 - 1.12 (m, 7H). Anal. Calcd for $C_{24}H_{35}IN_2$.0.05H_2O: C, 60.14; H, 7.36; N, 5.84. Found: C, 60.09; H, 7.31; N, 5.79.

3-((5-Cyclohexylpentyl)(4-ethylphenyl)amino)-1-methylpyridinium iodide (26g)

Yellow solid (65 % yield), mp: 159–160°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (s, 1H), 8.14 (d, 1H, *J* = 2.1Hz), 7.55 - 7.50 (m, 1H), 7.36 - 7.12 (m, 5H), 4.54 (s, 3H), 3.84 (t, 2H, *J*

= 7.8 Hz), 2.75 - 2.68 (m, 2H), 1.65 (d, 4H, *J* = 9.3 Hz), 1.52-1.29 (m, 12H), 1.27 - 1.09 (m, 6H). Anal. Calcd for $C_{25}H_{37}IN_2.0.05H_2O$: C, 60.97; H, 7.57; N, 5.69. Found: C, 60.86; H, 7.56; N, 5.68.

3-((5-Cyclohexylpentyl)(4-methoxyphenyl)amino)-1-methylpyridinium iodide (26h)

Yellow solid (50 % yield), mp: 115–116°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (d, 2H, *J* = 5.4 Hz), 7.56 - 7.54 (m, 1H), 7.32 - 7.01 (m, 5H), 4.6 (s, 3H), 3.86 - 3.77 (m, 5H), 3.51 - 3.44 (m, 9H), 1.67 - 1.61 (m, 5H), 1.23 - 1.14 (m, 5H). Anal. Calcd for $C_{24}H_{35}IN_2O.0.05H_2O$: C, 58.30; H, 7.13; N, 5.67. Found: C, 58.19; H, 7.12; N, 5.66.

3-((5-Cyclohexylpentyl)(4-(methylthio)phenyl)amino)-1-methylpyridinium iodide(26i)

Yellow solid (55 % yield), mp: 134–135°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34(s, 1H), 8.09(d, 1H, *J* = 6Hz), 7.56 - 7.51 (m, 1H), 7.39 - 7.31 (m, 1H), 7.27 - 7.16 (m, 5H), 4.55 (s, 3H), 3.85 (t, 2H, *J* = 7.8 Hz), 2.54 (s, 3H), 1.67 - 1.54 (m, 10H). Anal. Calcd for $C_{24}H_{35}IN_2S$: C, 56.46; H, 6.91; N, 5.49. Found: C, 56.59; H, 7.01; N, 5.61.

3-((4-Cyanophenyl)(5-cyclohexylpentyl)amino)-1-methylpyridinium iodide (26j)

Yellow solid (50 % yield), mp: 50–51°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (s, 1H), 8.41 (d, 1H, *J* = 6 Hz), 7.80 - 7.70 (m, 3H), 7.60 - 7.57 (m, 1H), 7.46 (d,2H, *J* = 8.4 Hz), 7.27 (s. 1H), 4.60 (s, 3H), 3.95 (t, 2H, *J* = 7.5 Hz), 1.68 - 1.65 (m, 8H), 1.29 - 1.13 (m, 9H). Anal. Calcd for $C_{24}H_{32}IN_3$. $0.05H_2O$: C, 58.90; H, 6.59; N, 8.59. Found: C, 58.79; H, 6.58; N, 8.57.

3-((3-Chlorophenyl)(5-cyclohexylpentyl)amino)-1-methylpyridinium iodide (26k)

Yellow solid (55 % yield), mp: 115–116°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.45 (s, 1H), 8.27 (d, 1H, *J* = 5.7 Hz), 7.63 -7.7.59 (m, 1H), 7.52-7.46 (m, 1H), 7.41 - 7.35 (m, 2H), 7.27 - 7.21 (m, 2H), 4.60 (s, 3H), 3.91 (d, 2H, *J* = 7.8 Hz), 1.67 - 1.59 (m, 8H), 1.39 - 1.16 (m, 10H), 0.86 (d, 1H, *J* = 9.6 Hz). Anal. Calcd for $C_{23}H_{32}ClIN_2$. $0.05H_2O$: C, 55.37; H, 6.47; N, 5.62, Found: C, 55.28; H, 6.45; N, 5.61.

3-((5-Cyclohexylpentyl)(3-(trifluoromethyl)phenyl)amino)-1-methylpyridinium iodide (26l)

Yellow solid (35 % yield), mp: 124–125°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.22 (d, 1H, *J* = 5.1 Hz), 7.62 -7.51 (m, 1H), 7.49-7.38 (m, 3H), 7.27 - 7.20 (m, 2H), 4.6 (s, 3H), 3.89 (t, 2H, *J* = 7.8 Hz), 1.67 - 1.61 (m, 9H), 1.39 - 1.15 (m, 10H). Anal. Calcd for $C_{24}H_{32}F_3IN_2$. $0.05H_2O$: C, 54.14; H, 6.06, N, 5.26. Found: C, 54.05; H, 6.05; N, 5.25.

3-((5-Cyclohexylpentyl)(m-tolyl)amino)-1-methylpyridinium iodide (26m)

Yellow solid (55 % yield), mp: 130–131°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 1H), 8.22 (d, 1H, *J* = 5.7 Hz), 7.58-7.53 (m, 1H), 7.43 - 7.27 (m, 3H), 7.03 (d, 2H *J* = 7.8 Hz), 4.55 (s,3H), 3.85 (t, 2H, *J* = 7.8 Hz), 2.41 (s, 3H), 1.72- 1.57 (m, 10H), 1.43 - 1.14 (m, 7H), 0.87 - 0.80 (t, 2H). Anal. Calcd for $C_{24}H_{35}IN_2$: C, 60.25; H, 7.37; N, 5.86. Found: C, 60.14; H, 7.37; N: 5.86.

3-((5-Cyclohexylpentyl)(3-methoxyphenyl)amino)-1-methylpyridinium iodide (26n)

Yellow solid (45 % yield), mp: 158–159°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.10 (s, 1H), 7.58 (s, 1H), 7.46 (s, 1H), 7.30 - 7.23 (m, 2H), 7.15 - 7.08 (m, 2H), 4.56 (s, 3H), 3.82 - 3.79 (m, 3H), 1,62 - 1.60 (m, 15H), 1.34 - 1.16 (m, 6H). Anal. Calcd for $C_{24}H_{35}IN_2O.0.1H_2O$: C, 58.30; H, 7.13; N, 5.67. Found: C, 58.09; H, 7.11; N, 5.64.

3-((3-Cyanophenyl)(5-cyclohexylpentyl)amino)-1-methylpyridinium iodide (26o)

Yellow solid (46 % yield), mp: 112–113°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, 2H, *J* = 4.8 Hz), 7.77 – 7.70 (m, 4H), 7.46 (d, 2H, 8.1 Hz), 4.60 (s, 3H), 3.91 (t, 2H, *J* = 7.5 Hz), 1.65 (d, 7H, *J* = 8.7 Hz), 1.42 – 1.15 (m, 10H), 0.87 - 0.80 (m, 2H). Anal. Calcd for $C_{24}H_{32}IN_3$: C, 58.90; H, 6.59; N, 8.59. Found: C, 59.03; H, 6.54; N, 8.56.

3-((2-Chlorophenyl)(5-cyclohexylpentyl)amino)-1-methylpyridinium iodide (26p)

Yellow solid (45 % yield), mp: 125–126°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 2H), 7.64 - 7.60 (m, 2H), 7.49 - 7.43 (m, 3H), 7.27 (s, 1H), 4.60 (s, 3H), 3.87 (t, 2H, *J* = 7.8 Hz), 1.67 - 1.61 (m, 12H), 1.39 - 1.15(m, 10H). Anal. Calcd for $C_{23}H_{32}ClIN_2$: C, 55.37; H, 6.47; N, 5.62. Found: C, 55.29; H, 6.48; N, 5.58.

3-((5-Cyclohexylpentyl)(2-(trifluoromethyl)phenyl)amino)-1-methylpyridinium iodide (26q)

Yellow solid (40 % yield), mp: 137–138°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, 1H, *J* = 5.7), 8.06 (s, 1H), 7.90 - 7.81 (m, 2H), 7.67 - 7.61 (m, 2H), 7.53 (d, 1H, *J* = 7.8 Hz), 7.17 (d, 1H, J = 8.4 Hz), 4.56 (s, 3H), 1.78 - 1.60 (m, 12H), 1.32 - 1.16 (m, 4H), 0.85(t, 1H). Anal. Calcd for $C_{24}H_{32}F_3IN_2$: C, 54.14; H, 6.06; N, 5.26. Found: C, 53.96; H, 6.00; N: 5.26.

3-((5-Cyclohexylpentyl)(o-tolyl)amino)-1-methylpyridinium iodide (26r)

Yellow solid (60 % yield), mp: 160–161°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, 2H, *J* = 5.7 Hz), 7.59 - 7.38 (m, 1H), 7.42 - 7.38 (m, 3H), 7.13 - 7.07 (m, 2H), 4.59 (s, 3H), 2.15 (s, 3H), 1.67 -160 (m, 10H), 1.59 - 1.32 (m, 4H), 1.39 - 1.16 (m, 10H), 0.86 (d, 1H, *J* = 9.6 Hz). Anal. Calcd for $C_{24}H_{35}IN_2$: C, 60.25; H, 7.37; N, 5.86. Found: C, 60.29; H, 7.46; N, 5.82.

3-((5-Cyclohexylpentyl)(2-methoxyphenyl)amino)-1-methylpyridinium iodide (26s)

Yellow solid (40 % yield), mp: 147–148°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, 1H, *J* = 5.7Hz), 8.10 (s, 1H), 7.59 - 7.54 (m, 1H), 7.44 (t, 1H, *J* = 8.4 Hz), 7.22 - 7.10 (m, 4H), 4.57 (s, 3H), 3.79 - 3.73 (m, 5H), 1.66 - 1.60 (m, 15H),1.35 - 0.83 (m, 4H). Anal. Calcd for $C_{24}H_{35}IN_2O.0.01H_2O$: C, 58.30; H, 7.13; N, 5.67. Found: C, 58.28; H, 7.13; N, 5.66.

3-((2-Cyanophenyl)(5-cyclohexylpentyl)amino)-1-methylpyridinium iodide (26t)

Yellow solid (48 % yield), mp: 117–118°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, 1H, *J* = 1.8 Hz), 7.91 – 7.82 (m, 2H), 7.70 (t, 2H, *J* = 6.9 Hz), 7.61 - 7.56 (m, 1H), 7.34 - 7.28 (m, 1H), 4.58 (s, 3H), 3.96 (t, 2H, *J* = 7.8 Hz), 1.76 – 1.63 (m, 8H), 1.44 – 1.13 (m, 10H), 0.86 - 0.80 (m, 2H). Anal. Calcd for $C_{24}H_{32}IN_3$: C, 58.90; H, 6.59; N, 8.59. Found: C, 58.90; H, 6.58; N, 8.46.

Antifungal Testing

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113 and *Aspergillus fumigatus* ATCC 90906. Susceptibility testing was performed using a modified version of the NCCLS methods.[19] DMSO solutions of samples were serially-diluted in saline, and transferred in duplicate to 96 well microplates. Microbial suspensions were diluted in broth to afford desired colony forming units/mL according to the 0.5 McFarland Standard [*C. albicans*: either Saboraud Dextrose broth (SDB) or RPMI 1640, *C. neoformans*: SDB, *A. fumigatus*: YM broth (for MICs) or RPMI-1640 + 5% Alamar Blue (for IC_{50} determination)]. Middlebrook 7H9 broth with OADC (Oleic Acid-Albumin-Dextrose-Catalase.) enrichment + 5% Alamar Blue. After adding microbial cultures to the samples affording a final volume of 200μL and final test concentration starting with 20μg/mL, plates were read prior to and after incubation using either fluorescence at 544ex/590em

(*A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG Lab Technologies, Germany) or optical density at 630nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont). Growth (saline only), solvent and blank (media only) controls were included on each test plate. Drug control [Amphotericin B (ICN Biomedicals, Ohio)] is included in each assay. Percent growth is calculated and plotted versus test concentration to afford the IC_{50} (sample concentration that affords 50% growth of the organism). The minimum inhibitory concentration (MIC) was determined by visually inspecting the plate, and is defined as the lowest test concentration that allows no detectable growth (for Alamar Blue assays, no color change from blue to pink).[20]

Toxicity Assay

The *in vitro* cytotoxicity was determined against mammalian kidney fibroblast (VERO) cells. The assay is performed in 96-well tissue culture-treated microplates and compounds were tested up to a highest concentration of 20 μg/ml as described earlier.[21] In brief, cells (25,000 cells/well) were seeded to the wells of the plate and incubated for 24 h. Samples were added and plates were again incubated for 48 h. The number of viable cells was determined according to neutral red assay as previously described.22, 23 IC_{50} values were determined from dose curves of growth inhibition versus concentration. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

Acknowledgments

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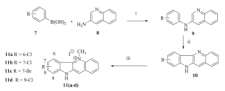
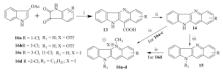


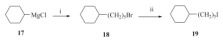
Scheme I.

Reagents and conditions: (i) CH_2Cl_2, $Cu(OAc)_2$, TEA, MS-Powder, RT, 12–24 hr; (ii) $Pd(OAc)_2$, CF_3COOH, 80 °C, 6 h; (iii) CH_3I, Toluene 110 °C, 12–24 h, sealed tube.



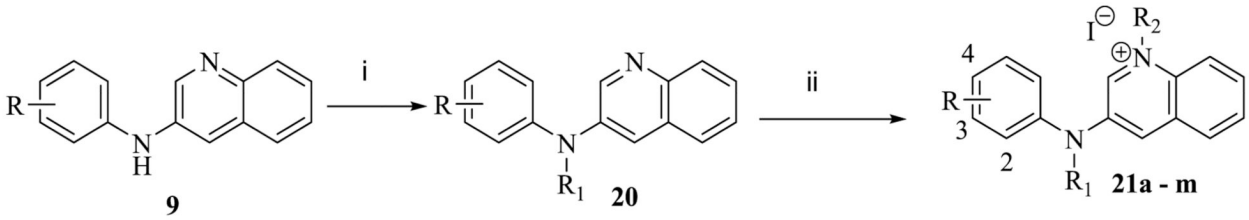
Scheme II.

(i) Aqueous KOH, 10 days. (ii) Paraffin oil, 300 °C, 3 h.; (iii) NaH, R_1-I, DME, rt, 12 h; (iv) CH_3I, Toluene, 110 °C, 12–24 h or CH_3OTf, Toluene, rt, 24 h.



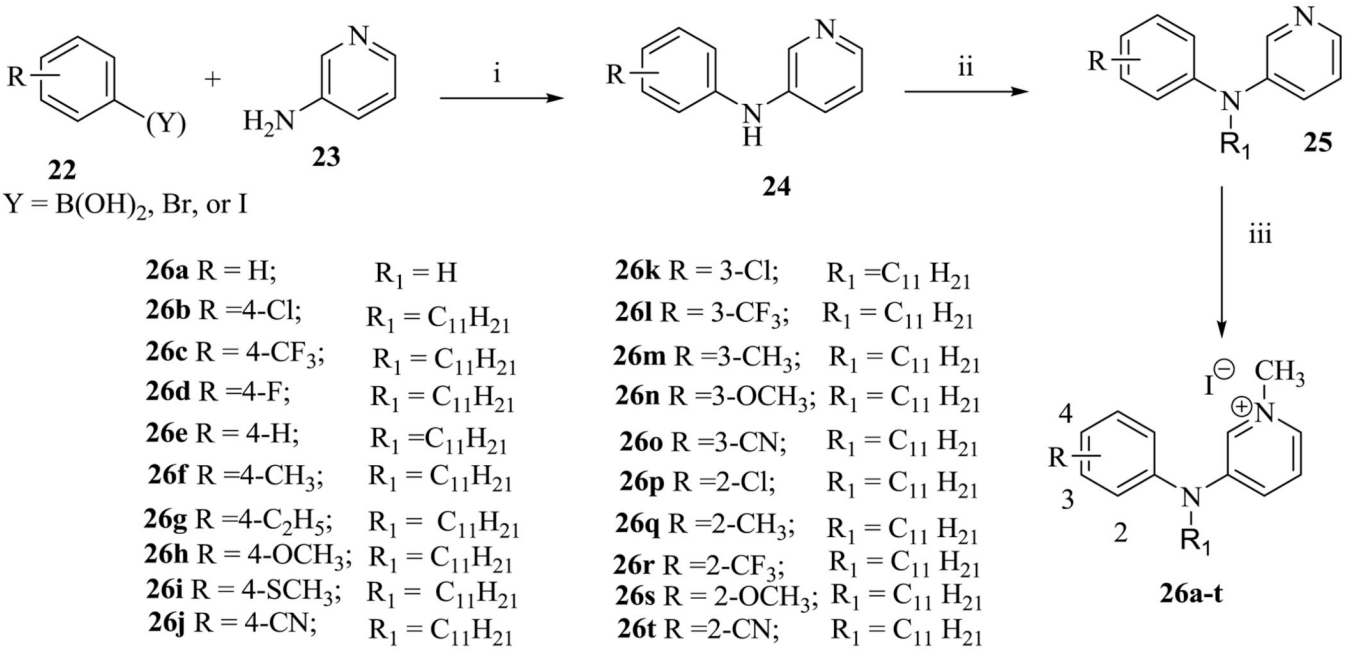
Scheme III.

Reagents and conditions :(i) 1, 5-Dibromopentane, Li_2CuCl_4, THF, 0°C, 12 h, (ii) NaI, Acetone, Reflux, 12h.



21a R = H; $R_1 = H, R_2 = CH_3$
21b R = 2-Cl; $R_1 = H, R_2 = CH_3$
21c R = 3-Cl; $R_1 = H, R_2 = CH_3$
21d R = 4-Cl; $R_1 = H, R_2 = CH_3$
21e R = H; $R_1 = H, R_2 = C_{11}H_{15}$
21f R = 4-Cl; $R_1 = H, R_2 = C_{11}H_{15}$
21g R = H; $R_1 = H, R_2 = C_{11}H_{21}$
21h R =3-Cl; $R_1 = H, R_2 = C_{11}H_{21}$
21i R = 4-Cl; $R_1 = H, R_2 = C_{11}H_{21}$
21j R = 2-Cl; $R_1 = C_{11}H_{21}, R_2 = CH_3$
21k R = 3-Cl; $R_1 = C_{11}H_{21}, R_2 = CH_3$
21l R = 4-Cl; $R_1 = C_{11}H_{21}, R_2 = CH_3$
21m R =H; $R_1 = C_{11}H_{21}, R_2 = CH_3$

Scheme IV.
Reagents and conditions: (i) NaH, Cyclohexylpentyl iodide, DME, RT, 12 h; (ii) CH_3I, Toluene 110 °C, 12–24 h



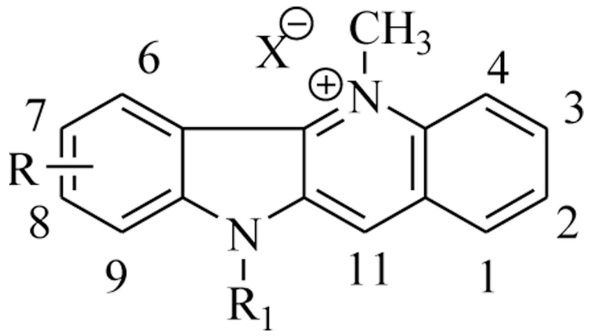
26a R = H; $R_1 = H$
26b R =4-Cl; $R_1 = C_{11}H_{21}$
26c R = 4-CF_3; $R_1 = C_{11}H_{21}$
26d R =4-F; $R_1 = C_{11}H_{21}$
26e R = 4-H; $R_1 = C_{11}H_{21}$
26f R =4-CH_3; $R_1 = C_{11}H_{21}$
26g R =4-C_2H_5; $R_1 = C_{11}H_{21}$
26h R = 4-OCH_3; $R_1 = C_{11}H_{21}$
26i R = 4-SCH_3; $R_1 = C_{11}H_{21}$
26j R = 4-CN; $R_1 = C_{11}H_{21}$
26k R = 3-Cl; $R_1 = C_{11}H_{21}$
26l R = 3-CF_3; $R_1 = C_{11}H_{21}$
26m R =3-CH_3; $R_1 = C_{11}H_{21}$
26n R =3-OCH_3; $R_1 = C_{11}H_{21}$
26o R =3-CN; $R_1 = C_{11}H_{21}$
26p R =2-Cl; $R_1 = C_{11}H_{21}$
26q R =2-CH_3; $R_1 = C_{11}H_{21}$
26r R =2-CF_3; $R_1 = C_{11}H_{21}$
26s R = 2-OCH_3; $R_1 = C_{11}H_{21}$
26t R =2-CN; $R_1 = C_{11}H_{21}$

Scheme V.

Reagents and conditions: (i) (Y = $B(OH)_2$), CH_2Cl_2, $Cu(OAc)_2$, TEA, Molecular sieve, RT, 24 h, (Y = Br, I) Dioxane, $Pd(OAc)_2$, 80°C, 24 h; (ii) DME, 5-iodo-pentylcyclohexane, NaH, 0°C, RT, 12 h; (iii) CH_3I, Toluene, 110°C, 12 h.

Table 1

Antifungal activity and Cytotoxicity of Substituted Cryptolepine Analogs.



Compounds[a]	R	R_1	X	IC_{50}/MIC/MFC (μg/mL)			TC_{50} (μg/mL)
				Ca	Cn	Afu	Vero Cells
16a	1-Cl	H	OTf	0.6/>20	7.0/>20	>20	5.0
12[c]	2-Cl	H	I	0.5/5.5/>20	2.0/5.0/>20	10/>20/>20	NT
16b	3-Cl	H	OTf	3.0/>20	15/>20	>20	2.1
16c	3-Cl, 11-Cl	H	I	1.0/10/>20	6.0/10/>20	>20	10
11a	6-Cl	H	I	>20	>20	>20	>4.7
11b	7-Cl	H	I	4.5/>20	15/>20	>20	1.5
11c	7-Br	H	I	2.5/>20	2.5/20	10 (MIC)	0.93
11d	9-Cl	H	I	>20	>20	>20	3.2
16d	2-Cl	$C_{11}H_{21}$	I	0.6/1.3/>20	0.9/2.5/10	0.8/1.3/>20	1.4
Cryptolepine				20/>20	12.5[b]/20	NT	3.2[c]
Amphotericin B				0.2/0.6/1.3	1.3/0.5 /1.0	0.7 /1.3/5.0	7.6[c]

Abbreviations: Ca = *Candida albicans*, Cn = *Cryptococcus neoformans*, Afu = *Aspergillus fumigatus*. OTf = triflate; NT = Not tested.

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

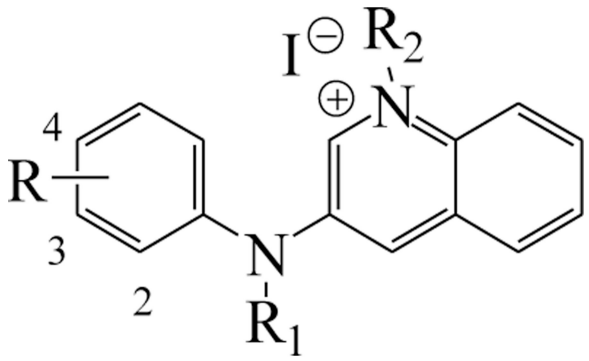
[b] MIC value; previously published [18].

[c] Data previously reported.[8]

IC_{50} = The concentration that affords 50% inhibition of growth, MIC = Minimum inhibitory concentration is the lowest test concentration that allows no detectable growth; MFC (Minimum Fungicidal Concentration) is the lowest test concentration that kills the organism; TC_{50} = The concentration that is toxic to 50% of cells.

Table 2

Antifungal activity and Cytotoxicity of 3-Phenylaminoquinolinium Analogs



Compounds[a]	R	R_1	R_2	IC_{50} /MIC/MFC (μg/mL)			TC_{50} (μg/mL)
				Ca	Cn	Afu	Vero Cells
21a	H	H	CH_3	NA	NA	NA	NA
21b	2-Cl	H	CH_3	NA	NA	NA	NA
21c	3-Cl	H	CH_3	NA	NA	NA	NA
21d	4-Cl	H	CH_3	NA	NA	NA	NA
21e	4-Cl	H	$C_{11}H_{15}$	15/>20/>20	4.0/6.3/12.5	10/>20/>20	>25
21f	H	H	$C_{11}H_{21}$	3.5/10/>20	1.5/2.5/10	1.0/2.5/10	NT
21g	3-Cl	H	$C_{11}H_{21}$	2.0/6.3/13	2.0/3.1/50	3.5/6.3/>20	>10
21h	4-Cl	H	$C_{11}H_{21}$	2.0/>20	1.0/1.6/>20	NA	>10
21i	2-Cl	$C_{11}H_{21}$	CH_3	0.63/1.3/1.3	0.2/0.3/0.6	3.1/5.0/20	NT
21j	3-Cl	$C_{11}H_{21}$	CH_3	0.9/2.5/10	0.9/1.3/5.0	1.0/2.5/10	>10
21k	4-Cl	$C_{11}H_{21}$	CH_3	0.5/1.3/5.0	0.4/0.6/2.5	0.8/1.3/10	10
21*l*	H	$C_{11}H_{21}$	CH_3	1.2/2.5/2.5	0.2/0.3/0.6	4.6/10/10	9.8
Cryptolepine				20/250/NT	12.5[b]/NT/NT	NT	3.2[c]
Amphotericin B				0.2/0.6/1.3	1.3/0.5 /1.0	0.7 /1.3/5.0	7.6[c]

Abbreviations: Ca = *Candida albicans*, Cn = *Cryptococcus neoformans*, Afu = *Aspergillus fumigatus*. Otf = triflate; Crypto = Cryptolepine. Amph B = Amphotericin B; NT = Not tested. NA = not active at 20 μg/mL;

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

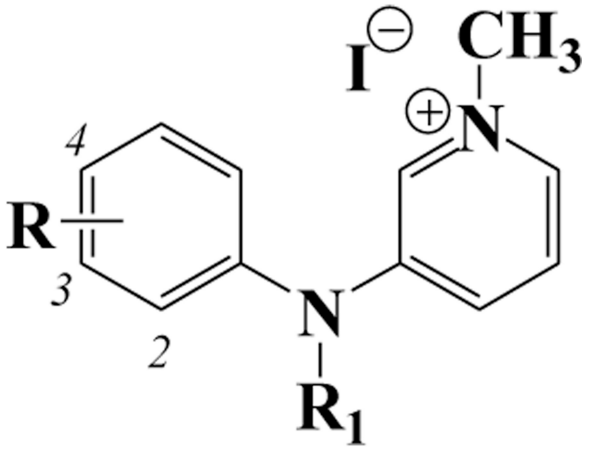
[b] MIC value; previously published [18].

[c] Data previously reported [2, 11, 19]

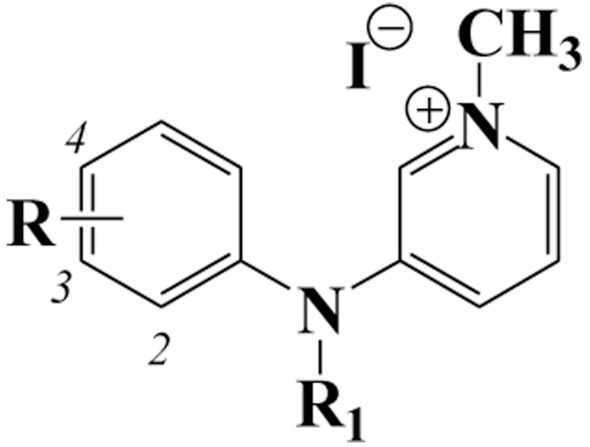
IC_{50} = The concentration that affords 50% inhibition of growth, MIC = Minimum inhibitory concentration is the lowest test concentration that allows no detectable growth; MFC (Minimum Fungicidal Concentration) is the lowest test concentration that kills the organism; TC_{50} = The concentration that is toxic to 50% of cells.

Table 3

Antifungal activity and Cytotoxicity Data of 3-Phenylaminopyridinium Analogs



Compounds[a]	R	R_1	IC_{50} /MIC/MFC (μg/mL)			TC_{50} (μg/mL)
			Ca	Cn	Afu	Vero Cells
26a	H	H	NA	NA	NA	NA
26b	4-Cl	$C_{11}H_{21}$	1.5/3.1/NT	1.0/1.6/NT	1.0/1.6/NT	>10
26c	4-CF_3	$C_{11}H_{21}$	1.0/2.5/10.0	1.5/2.5/5.0	1.5/2.5/5.0	>10
26d	4-F	$C_{11}H_{21}$	9.6/20.0/NA	1.4/2.5/2.5	NA/NA/NA	>10
26e	H	$C_{11}H_{21}$	8.3/20.0/NA	1.5/2.5/2.5	19.7/NA/NA	>10
26f	4-CH_3	$C_{11}H_{21}$	1.5/2.5/10.0	1.5/2.5/5.0	1.5/5.0/5.0	>10
26g	4-C_2H_5	$C_{11}H_{21}$	1.3/2.5/5.0	0.4/0.6/5.0	2.7/5.0/ND	10
26h	4-OCH_3	$C_{11}H_{21}$	5.1/10/20	1.4/2.5/2.5	NA	>10
26i	4-SCH_3	$C_{11}H_{21}$	2.0/5.0/10	1.2/1.3/2.5	6.4/10.0/10.0	>10
26j	4-CN	$C_{11}H_{21}$	19/20/>20	2.6/5.0/5.0	NA	>10
26k	3-Cl	$C_{11}H_{21}$	3.0/5.0/10	0.7/1.3/1.3	12.4/20.0/20.0	>10
26l	3-CF_3	$C_{11}H_{21}$	3.0/5.0/20	1.5/2.5/5.0	1.5/2.5/10.0	>10



Compounds[a]	R	R_1	IC_{50} /MIC/MFC (µg/mL)			TC_{50} (µg/mL)
			Ca	Cn	Afu	Vero Cells
26m	3-CH_3	$C_{11}H_{21}$	3.0/5.0/10	1.4/2.5/2.5	8.7/10.0/20.0	>10
26n	3-OCH_3	$C_{11}H_{21}$	8.9/20/>20	3.6/5.0/5.0	NA	>10
26o	3-CN	$C_{11}H_{21}$	NA	3.5/5.0/5.0	NA	>10
26p	2-Cl	$C_{11}H_{21}$	2.8/5.0/5.0	2.0/2.5/2.5	18/>20/>20	NT
26q	2-CF_3	$C_{11}H_{21}$	2.6/5.0/5.0	2.3/5.0/5.0	NA	NT
26r	2-CH_3	$C_{11}H_{21}$	2.6/5.0/5.0	1.5/2.5/2.5	19>20>20	NT
26s	2-OCH_3	$C_{11}H_{21}$	5.0/10.0/10	3.0/5.0/5.0	NA	NT
26t	2-CN	$C_{11}H_{21}$	NA	6.6/10/20	NA	>10
Cryptolepine			20/250/NT[b]	12.5 (MIC)[b]	NT	3.2[b]
Amphotericin B			0.2/0.6/1.3	1.3/0.5 /1.0	0.7 /1.3/5.0	7.6

Abbreviations: Ca = *Candida albicans*, Cn = *Cryptococcus neoformans*, Afu = *Aspergillus fumigatus*. NT = Not tested. NA = not active at 20 µg/mL;

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

[b] Data previously reported [2, 11, 19]

IC_{50} = The concentration that affords 50% inhibition of growth, MIC = Minimum inhibitory concentration is the lowest test concentration that allows no detectable growth; MFC (Minimum Fungicidal Concentration) is the lowest test concentration that kills the organism; TC_{50} = The concentration that is toxic to 50% of cells.