Published in final edited form as:

Bioorg Med Chem. 2011 January 1; 19(1): 458–470. doi:10.1016/j.bmc.2010.11.008.

Benzothieno[3,2-*b*]quinolinium and 3-(Phenylthio)quinolinium Compounds: Synthesis and Evaluation against Opportunistic Fungal Pathogens

Comfort A. Boateng^{a,#}, Suresh V. K. Eyunni^{a,#}, Xue Y. Zhu^a, Jagan R. Etukala^a, Barbara A. Bricker^a, M. K. Ashfaq^a, Melissa R. Jacob^b, Shabana I. Khan^b, Larry A. Walker^b, and Seth Y. Ablordeppey^{a,*}

^aCollege of Pharmacy and Pharmaceutical Sciences, Florida A & M University, Tallahassee, FL 32307, USA

^bThe National Center for the Development of Natural Products, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA

Abstract

Substitution around 5-methyl benzothieno[3,2-*b*]quinolinium (**2**) ring system was explored in order to identify positions of substitution that could improve its antifungal profile. The 3-methoxy (**10b**) was active against *C. albicans*, *C. neoformans* and *A. fumigatus* and the 4-chloro (**10f**) analog showed moderate increases in anti-cryptococcal and anti-aspergillus activities. The effectiveness of **10b** and **10f** were validated in murine models of candidiasis and cryptococcosis respectively. The efficacy of **10f** in reducing brain cryptococcal infection and its observation in the brain of mice injected with this quaternary compound confirm the capacity of these compounds to cross the blood-brain barrier of mice. Overall, several of the chloro and methoxy substituted compounds showed significant improvements in activity against *A. fumigatus*, the fungal pathogen prevalent in patients receiving organ transplant. Opening the benzothiophene ring of **2** to form 1-(5-cyclohexylpentyl)-3-(phenylthio)quinolinium compound (**3**) resulted in the identification of several novel compounds with over 50-fold increases in potency (cf **2**) while retaining low cytotoxicities. Thus, compound **3** constitutes a new scaffold for development of drugs against opportunistic infections.

Introduction

Infections caused by opportunistic pathogens continue to be of public health concern.¹⁻² *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* are three of the most common fungal pathogens responsible for opportunistic infections in humans. Patients with AIDS, lymphoma, those on long-term corticosteroids, organ transplant patients and even diabetes patients are at risk for opportunistic infections.³⁻⁶ Antifungal medications are used to treat opportunistic infections and intravenous therapy with amphotericin B has been and is one of the most common treatment options.⁶⁻⁸ Amphotericin B is not available orally and hence is often combined with an oral medication, such as flucytosine or one of the

*Corresponding Author..

#Designated first authors

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

azoles.⁹⁻¹⁶ The unavailability of oral forms of amphotericin B, its severe nephrotoxic side effects along with other side effects, resistance development, drug interactions associated with the azoles in particular and the paucity of biological targets support the need for newer agents.¹⁷⁻²¹

Towards this end, we embarked on structural modifications of the natural product cryptolepine²²⁻²⁹ (Chart 1) to obtain agents with improved antifungal efficacy and low toxicity. We recently reported that analogs of sulfur isosteres of cryptolepine, benzothieno[3,2-*b*]quinolinium salts (**2**), have the potential to become useful agents with broad antifungal properties and low cytotoxicity.³⁰ It was also found that while the unsubstituted 5-methyl benzothieno[3,2-*b*]quinolinium salt showed antifungal properties, the few 2-substituted analogs evaluated were disappointingly less potent. However, substitution at other positions to enhance the antifungal profile of the benzothienoquinolinium moiety has not been explored. In addition, it was of interest to explore flexible ring-opened analogs of the benzothienoquinolines (Chart 1) in order to confirm previous observations that the tetracyclic ring might not be required for its activity. Although the quaternary form of the tetracyclic structure appears to be the active form,³¹ we were concerned that the presence of a permanent positively charged atom in the N-alkylated benzothienoquinolinium salts might limit bioavailability and prevent effectiveness *in vivo*. This would be of primary concern especially when the compounds are designed to target infections of the CNS.

Hence, in this paper, we sought to synthesize and evaluate substitution at all synthetically accessible positions on the benzo[*b*]thiophene and quinoline moieties, to explore whether ring-opened analogs might offer opportunity for increased potency and selectivity and to validate the efficacy of the benzothieno[3,2-*b*]quinolinium salts in murine models of candidiasis and brain cryptococcosis, two common opportunistic infections associated particularly with AIDS.³²⁻³⁵

Results and Discussion

Synthesis

Substituted 5-methylbenzothieno[3,2-*b*]quinolinium salts were synthesized using modifications of previously published procedures,^{30,36} as outlined in Schemes 1 and 2. Briefly, suitably substituted anthranilic acids from commercial sources were acylated with (phenylthio)acetyl chloride (**5**) to obtain 1-, 3- and 4-substituted 6-(2-phenylsulfanylacetyl amino)benzoic acids, **6a-f**. To obtain the corresponding 7-, 8- and 9-substituted compounds (**14a-f**), 2-(2-bromoacetamido)benzoic acid (**13**), (prepared by condensing 2-aminobenzoic acid with bromoacetyl bromide) was reacted with suitably substituted thiophenols. In a double intramolecular cyclization reaction with polyphosphoric acid (PPA), the acylated intermediates (**6a-f** and **14a-f**) were converted to substituted-11-quinolones, which were subsequently treated with POCl₃ to form 11-chlorinated benzothieno[3,2-*b*]quinolines. Hydrogenation on Pd/C to remove the 11-substituted chlorine selectively was achieved as previously described.³⁰⁻³⁷ Attempts to prepare 5-methyl iodides of 4-substituted benzothieno[3,2-*b*]quinolines were unsuccessful. This may be due to the bulkiness of the groups at the 4-position, their proximity to the N-5 atom of the rigid tetracyclic ring and deactivation of the N atom. However, 5-methyl triflates of the quinolines **9a-c** were successfully synthesized *albeit* in poor to moderate yields depending on the reaction times and the amount of the stronger methylating MeOTf added. Addition of excess of MeOTf to **9c** and **9e** at room temperature yielded compounds **10c** and **10e** in moderate yields. Similarly, 5-methyl triflate derivatives of 1- and 3-substituted benzothienoquinolines were prepared in good yields. The 1-methoxy analog **11a** was synthesized to evaluate the effect of the counter ion (I⁻ and OTf⁻) on biological activity. Finally, N-5 methyl iodides of benzothienoquinolines, **18a-f**, were synthesized in good yields using methyl iodide at

elevated temperatures. Attempts to substitute the 5-methyl group with the more hydrophobic ω -phenyl- or ω -cyclohexyl- pentyl groups were unsuccessful as very low yields and difficult solubility problems prevented successful delivery of the products.

Synthesis of 1-substituted-3-(phenylthio)quinolinium iodides (**3**) required 3-iodoquinoline (**20**) as the starting material (Scheme 3). A Finkelstein reaction was applied to 3-bromoquinoline to exchange the bromide for the desired iodide.³⁸ Using a copper-catalyzed carbon-sulfur bond formation approach, benzenethiols were reacted with 3-iodoquinoline to obtain 3-(phenylthio)quinoline (**21**) in good yield.³⁹ Subsequent alkylation of **21** with appropriate alkyl halides provided the desired target compounds as shown in Scheme 3.

Biological Evaluation

Previous studies in our labs have revealed that substitution on the ring of cryptolepine (**1**) enhanced its potency and broadened its anti-opportunistic activity profile. Further optimization led to the identification of the sulfur isostere (**2**) of cryptolepine as a potential anti-opportunistic agent.³⁰ Thus, it was of interest to explore substitution on the 5-methyl benzothienoquinolinium compound in order to optimize its anti-opportunistic profile. The observation that introduction of a halogen on the core structure of cryptolepine enhanced potency motivated the initial decision to use a chlorine atom to probe all synthetically accessible positions on **2**. Thus, compounds **10d-f** and **18d-f** were synthesized and evaluated against *C. neoformans*, *C. albicans* and *A. fumigatus*. Substitution at position 6 was unsuccessful as ring closure consistently occurred on the side away from the 3-chloro position of the benzenethiol to form the 9-substituted product. Methoxy-substituted analogs (**10a-c**, **i** and **18a-c**) were also synthesized to provide an avenue for comparing the presence of an electron donating substituent (with the electron withdrawing chlorine) on the benzothienoquinoline structure and its effect on anti-opportunistic activities.

In-Vitro Evaluation

The results of the *in vitro* antifungal screening of the Cl and OMe-substituted benzothienoquinolines are presented in Table 1. When both IC₅₀ and MIC values are between 1 and 10 $\mu\text{g/mL}$, the compounds are considered active, moderately active between 10 – 15 $\mu\text{g/mL}$ and weak above 15 up to 20 $\mu\text{g/mL}$. Most of the compounds show only weak activities against *C. neoformans* (Cn) and *C. albicans* (Ca) except for compound **10f** (IC₅₀ = 4.0 and MIC = 10 $\mu\text{g/mL}$ against Cn) and **10b** (IC₅₀ = 1.5, MIC = 10 $\mu\text{g/mL}$ against Ca). Several of the same compounds however showed moderate to very potent activities against *A. fumigatus*, the opportunistic fungus that causes infections in organ transplant patients.⁴⁰ Clearly, the OMe-substituted benzothienoquinolinium salts show a higher level of potency against *A. fumigatus* than the Cl-substituted analogs. This observation together with the loss of activity observed for the 4-phenolic substitution (**10g**) may suggest that substituents with negative sigma and positive pi values may be associated with enhancing potency and need to be further explored. The potential of these compounds to induce toxicity in Vero cells was also investigated. None of the compounds showed cytotoxicity in Vero cells up to 4.7 $\mu\text{g/mL}$ and in fact most of them showed no cytotoxicity at 10 $\mu\text{g/mL}$. These results are very encouraging and warrant further investigation of the potential of these compounds as new anti-opportunistic infection agents. Compounds **10f** and **10b** were selected as representatives of the benzothienoquinolines for further evaluation of their *in vivo* efficacy against *C. neoformans* and *C. albicans*.

In an earlier study, it was observed that opening the benzothienoquinoline ring to form 3-phenylthioquinolinium salt which demonstrated activity against opportunistic pathogens. Compound **22a**, the unsubstituted N-methyl-3-phenylthioquinolinium salt, was synthesized and evaluated against four opportunistic pathogens as shown in Table 2 to probe this

observation. The results show that ring-opening at the benzo[*b*]thiophene moiety did not significantly impact activity. However, replacing the methyl group with an ω -phenylpentyl group, previously reported to enhance activity,²⁸ led to compound **22b** with a much enhanced activity against all the fungal pathogens including *C. krusei*. It has been reported that *C. krusei* is prevalent in bone marrow transplantation and accounts for 2-3% of all *Candida* bloodstream infections.⁴¹ Replacement of the ω -phenylpentyl group (**22b**) with the more hydrophobic ω -cyclohexylpentyl group in line with previous observations that the later produced improvement in potency²⁸ led to compound **22c**. Comparing the two unsubstituted analogs of the former (**22b**) with the later (**22c**), showed the later to be the more potent against all pathogens tested except for *A. fumigatus* where the reverse is the case. With potencies and cytotoxicities comparable to that of amphotericin B, and in particular having fungicidal activity, the compounds with the N-cyclohexylpentyl 3-phenylthioquinolinium scaffold have the potential of serving as new anti-opportunistic infection agents warranting further investigation and development. These are currently on-going.

In-Vivo Evaluation

Prior to *in vivo* efficacy studies, Maximum Tolerated Dose (MTD) studies were carried out to determine the highest dose that could be delivered without any apparent adverse effects. The test compounds were dissolved in an appropriate vehicle and daily doses were administered for 3 (**10b**) or 5 (**10f**) days. Body weights and general appearances of the animals were observed daily and signs of distress were recorded. Animals were sacrificed at the termination of the scheduled experiment or earlier if signs of increased morbidity after administration of the compounds were observed. Necropsy was performed and gross lesions were recorded.

To have *in vivo* efficacy, the effect of compounds in inhibiting the growth of opportunistic pathogens must be demonstrated. To this end, cryptococcosis was induced in mice by infecting them with *C. neoformans* ATCC 52657 (5×10^5 cells) via the lateral tail vein. The mice were divided in 5 groups of 5 each and beginning 24 hours post-infection, they were treated daily with increasing doses of the test compound, amphotericin B (1.2 mg/kg) or the vehicle intraperitoneally (ip) for the next 5 days. On day 6 post-infection, all mice were sacrificed, their brains aseptically removed, and CFU of *C. neoformans* per gram brain was determined.

Compound **10f** was dissolved in DMSO:PEG:PBS (1:6:7) and injected i.p. once a day for 5 days using doses ranging from 1mg/kg to 20 mg/kg. At 10 and 20 mg/kg, mice showed decreased mobility and signs of discomfort. However, these signs were not noticeable after 2 hours post injection. The body weights of mice showed no noticeable changes up to 10 mg/kg but at 20 mg/kg, loss of weight was observed. At necropsy, no significant pathological lesions were observed at doses less than 10 mg/kg. Slight distension of the duodenum, jejunum and cecum was seen at 10 mg/kg. However, at 20 mg/kg, enlargement of the liver with fusion of the hepatic lobes was observed. The intestines were moderately distended, especially the duodenum, jejunum and cecum. The intestinal content was dark colored. No gross pathology was observed in the kidneys, spleen and other visceral organs.

Figs 1 and 2 show the effect of various doses of **10f** against *C. neoformans* infection in mice. In fig 1, the CFU per gram of brain sample is displayed for each dose while fig 2 shows the % reduction in CFU per dose of **10f**. Amphotericin B is included as the positive control while the vehicle served as a negative control. Compound **10f** showed anti-cryptococcus activity at all doses tested. Maximum activity was observed at 1 mg/kg where half of the organisms were killed in the brain tissue compared to negative control, whereas amphotericin B killed approximately 24%. Thus, at this dose (1 mg/kg), **10f** was about twice as effective as amphotericin B. At 5 mg/kg, the anti-cryptococcus activity was comparable

to that of amphotericin B. Increasing the dose to 10 mg/kg did not result in additional improvement but instead reduced activity. The mechanism by which the activities at higher concentrations of **10f** are attenuated is not known at this time but is not unique. This observation parallels the behavior reported for caspofungin⁴² (an echinocandin) and may reflect several possible events including an adverse effect on the immune system at higher doses or solubility problems leading to precipitation of the compound on injection.

Thus, the results clearly show that despite their quaternary nature, these compounds are capable of entering cells and more importantly, in crossing the blood-brain barrier (BBB) to elicit their anti-infective actions and hence providing us with the first evidence that these compounds are capable of crossing the BBB.

Murine Model of Systemic Candidiasis

Tissue Burden—Mice were infected with *Candida albicans* strain B311 ATCC 32354 (7.5×10^5 yeast cells) via the lateral tail vein and divided in 5 groups of 5 mice each. Seven, 24, and 48 hours post infection, mice were injected i.p. with various doses of the test compound, amphotericin B (1.2mg/kg) or the vehicle. At 72 hrs post-infection all mice were sacrificed, their kidneys were collected aseptically and CFU of *C. albicans* per gram of kidney was determined.

Maximum Tolerated Dose (MTD) of 10b—The compound was dissolved in DMSO:PEG:PBS (1:3:3) and injected i.p. once a day for 3 days. The compound was well tolerated by the mice and the MTD was 10 mg/kg. No significant changes in body weights were observed. At necropsy, no gross pathological lesions were observed in the visceral tissues at 1-10 mg/kg doses. However, mice injected with 20 mg/kg for 3 days showed white deposits of unknown origin in the peritoneal cavity.

Candidiasis Tissue Burden—Figs 3 and 4 show the effect of **10b** against candidiasis *in vivo*. In fig 3, the CFU per gram of kidney sample is displayed for each dose while fig 4 shows the % reduction in CFU per dose of **10b**. Amphotericin B is included as the positive control while the vehicle serves as a negative control. Amphotericin B, at a dose of 1.2 mg/kg, eliminated 99% of the infection from the kidneys compared to the vehicle while a dose of 10 mg/kg of **10b** produced 86% reduction in *C. albicans* tissue burden. At 1mg/kg and 5 mg/kg the activity of **10b** against *C. albicans* was limited to a 26% and 34% reduction, respectively.

The efficacy of these quaternary compounds especially in the brain suggests that they have the capacity to cross the blood brain barrier. To provide further proof that this is the case, mice were dosed ip with **10f** in an identical manner as in the *in vivo* efficacy studies. After 15 – 60 minutes post injection, mice were anesthetized, sacrificed, the brain perfused with phosphate buffered saline (PBS), removed and frozen until the day of extraction. An HPLC chromatogram of a perfused brain of a mouse dosed with **10f** is shown in Fig 5.

The results show the retention time and UV spectra match the **10f** standard. No peak above detection limit at the retention time of **10f** was observed in a blank brain extract treated by the same procedure. A mass spectrum of the same extract confirms **10f** with a parent peak *m/e* of 284.8 (*m/e* of a MeOH solution of **10f** was 284.4), within the resolution of the instrument (Fig 5). Another perfused brain from a rat dosed with 40 mg/kg of **10f** was macerated, extracted with 1.5 mL of 30:70 (2.3 M HClO₄:MeOH), treated with a sonic dismembrator, refrigerated, centrifuged at 10000 g for 5 minutes, and the supernatant concentrated to 300 μ L. This extract was injected into the HPLC system as described in the biological testing section, with an isocratic mobile phase of 70C:30D (where C is 90% (7.5

mM sodium heptanesulfonate + 0.1 M phosphoric acid adjusted to pH 3.00 with triethylamine) + 10% acetonitrile and **D** is 100% acetonitrile.).

Compound **10f** was identified in this extract (Fig 6A). Subsequently, the extract was spiked with a standard solution of **10f** and injected into the HPLC. The identified peak and the standard peak co-eluted providing further confirmation (Fig 6B) of the presence of **10f**.

Conclusion

The tetracyclic benzothieno[3,2-*b*]quinolinium compounds as a group have weak to good *in vitro* potency against *C. neoformans*, *C. albicans* and *A. fumigatus*. These activities were generally fungistatic below 20 µg/mL concentrations. However, selected agents (**10b** and **10f**) demonstrated significant *in vivo* efficacy against *C. albicans* and *C. neoformans* comparable to that of amphotericin B. It was further demonstrated that these compounds are able to cross not only cell membranes but also the blood brain barrier to produce their anti-infective activities. Interestingly, opening the benzo[*b*]thiophene ring and replacing the N-methyl group with N-5-phenylpentyl or 5-cyclohexylpentyl groups, to form substituted 3-(phenylthio)quinolinium compounds, produced as high as 50-fold increases in potency without increasing cytotoxicity. These analogs also demonstrated significantly higher activity against *C. krusei* than the other pathogens tested. In addition, unlike their tetracyclic counterparts, these ring-opened analogs demonstrated fungicidal activity as well.

Experimental Section

Chemistry

All the reagents were purchased from Sigma-Aldrich, Fisher Scientific or Alfa Aesar and used without further purification. Flash chromatography was performed with Davisil grade 634 silica gel. Analytical thin layer chromatography (TLC) was carried out on Merck TLC plates coated with silica gel 60 F₂₅₄ (0.25 mm layer thickness); visualization was carried out using a UV lamp (254 nm). Melting points were determined on an Electrothermal MEL-TEMP® 3.0 device and are uncorrected. Nuclear Magnetic Resonance (¹H NMR) spectra were obtained on a Varian 300 MHz Mercury NMR Spectrometer. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA, and were within 0.4% of the theory unless otherwise noted.

General Procedure for the Synthesis of Substituted 6-(2-Phenylsulfanyl-acetyl-amino)-benzoic acids 6a-f, (Method A)—A mixture of substituted anthranilic acids **4a-f** (30 mmol), NaOH (60 mmol) and phenylthioacetyl chloride (30 mmol) in H₂O (20 mL) was stirred at 0 °C for 1 h, and then at rt for 2h. The mixture was acidified with 5 % HCl (pH = 4-5) and then extracted with EtOAc (2 × 500 mL). The combined organic layer was dried over Na₂SO₄, filtered and solvent was evaporated in vacuo. The residue obtained was recrystallized using EtOAc:Hex (3:1) to give compounds **6a-f** as white solids.

1-Methoxy-6-(2-phenylsulfanyl-acetyl-amino)-benzoic acid (6a): Yield 67%. mp 97-98 °C. ¹H NMR (CDCl₃): δ 11.56 (brs, 1H, NH), 8.53-8.50 (dd, 1H, *J* = 8.7, 0.9 Hz), 7.52-7.46 (t, 1H, *J* = 8.4 Hz), 7.44-7.41 (m, 2H), 7.29-7.24 (m, 2H), 7.20-7.17 (m, 1H), 6.76 (d, 1H, *J* = 8.7 Hz), 4.07 (s, 3H), 3.81 (s, 2H).

3-Methoxy-6-(2-phenylsulfanyl-acetyl-amino)benzoic acid (6b): Yield 82%. mp 188-189 °C. ¹H NMR (CDCl₃): δ 11.92 (brs, 1H, NH), 8.41 (d, 1H, *J* = 2.4 Hz), 8.06 (d, 1H, *J* = 8.70 Hz), 7.29-7.24 (m, 2H), 7.40 (m, 2H), 20 (d, 1H, *J* = 7.5 Hz), 6.68 (dd, 1H, *J* = 9.0, 2.2 Hz), 3.88 (s, 3H), 3.82 (s, 2H).

4-Methoxy-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6c): Yield 85%. mp 167-168 °C. ^1H NMR (CD_3OD): δ 7.47-7.42 (m, 3H), 7.34-7.27 (m, 3H), 7.24-7.18 (m, 2H), 3.81 (s, 3H), 3.79 (s, 2H).

1-Chloro-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6d): Yield 82%. mp 153-154 °C. ^1H NMR (CDCl_3): δ 10.25 (brs, 1H, NH), 8.22 (d, 1H, $J = 8.4$), 7.42-7.36 (dd, 1H, $J = 8.4, 8.1$ Hz), 7.35-7.16 (m, 6H), 3.80 (s, 2H).

3-Chloro-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6e): Yield 82%. mp 161-163 °C. ^1H NMR (CDCl_3): δ 8.54 (d, 1H, $J = 2.1$), 8.02 (d, 1H, $J = 8.4$), 7.39-7.36 (m, 2H), 7.29-7.25 (m, 3H), 7.21-7.10 (m, 2H), 3.82 (s, 2H).

4-Chloro-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6f): Yield 86%. mp 178-179 °C. ^1H NMR (CDCl_3): δ 9.39 (s, 1H, NH), 7.87 (dd, 1H, $J = 7.8, 1.5$), 7.61 (dd, 1H, $J = 8.1, 1.5$ Hz), 7.41 (dd, 2H, $J = 7.8, 1.5$ Hz), 7.32-7.20 (m, 4H), 3.85 (s, 2H).

General Procedure for the Synthesis of Substituted Benzothieno[3,2-*b*]quinolin-11(5*H*)-ones, 7a-f, (Method B)—Compound **6** (20 mmol) was mixed with polyphosphoric acid (PPA, 125 g) and heated at 130-135 °C for 3h. After cooling to rt, the mixture was poured into ice/water (200 mL), neutralized with 50 % NaOH solution to give a highly insoluble dark brown solid which was washed with H_2O , EtOAc and Et_2O , dried over P_2O_5 and used as such for the next step without further purification.

1-Methoxybenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7a): Yield 95% (crude). ^1H NMR ($\text{DMSO}-d_6$): δ 12.53 (brs, 1H, NH), 8.50-8.47 (dd, 1H, $J = 7.2, 1.8$), 8.07-8.04 (dd, 1H, $J = 7.2, 1.5$), 7.61-7.52 (m, 3H), 7.33 (d, 1H, $J = 8.4$), 6.81 (d, 1H, $J = 8.10$), 3.83 (s, 3H).

3-Methoxybenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7b): Yield 95% (crude). ^1H NMR ($\text{DMSO}-d_6$): δ 8.49 (d, 1H, $J = 7.20$), 8.13 (d, 1H, $J = 9.0$ Hz), 8.08 (d, 1H, $J = 7.5$ Hz), 7.53 (m, 2H), 7.17 (d, 1H, $J = 1.80$ Hz), 6.98 (dd, 1H, $J = 8.7, 1.8$), 3.89 (s, 3H).

4-Methoxybenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7c): Yield 84% (crude). ^1H NMR ($\text{DMSO}-d_6$): δ 11.9 (brs, 1H, NH), 9.0 (d, 1H, $J = 8.10$), 8.1 (d, 1H, $J = 7.7$ Hz), 7.81 (d, 1H, $J = 7.14$ Hz), 7.65-7.53 (m, 2H), 7.38-7.30 (m, 2H), 4.08 (s, 3H).

1-Chlorobenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7d): Yield 92% (crude). ^1H NMR ($\text{DMSO}-d_6$): δ 8.51 (dd, 1H, $J = 7.8, 0.9$ Hz), 8.06 (dd, 1H, $J = 7.2, 1.2$ Hz), 7.77 (dd, 1H, $J = 8.4, 1.2$ Hz), 7.63-7.52 (m, 3H), 7.31 (dd, 1H, $J = 7.5, 0.9$ Hz).

3-Chlorobenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7e): Yield 92% (crude). ^1H NMR ($\text{DMSO}-d_6$): δ 8.50 (d, 1H, $J = 7.5$ Hz), 8.21 (d, 1H, $J = 8.7$ Hz), 7.86 (s, 1H), 8.12-8.04 (m, 1H), 7.68-7.58 (m, 2H), 7.86 (s, 1H), 7.38 (dd, 1H, $J = 8.7, 2.1$ Hz).

4-Chlorobenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7f): Yield 94% (crude). ^1H NMR ($\text{DMSO}-d_6$): δ 11.48 (s, 1H, NH), 8.91 (d, 1H, $J = 7.8$ Hz), 8.25 (d, 1H, $J = 8.10$ Hz), 8.11 (d, 1H, $J = 7.8$ Hz), 7.94 (d, 1H, $J = 7.2$ Hz), 7.67-7.55 (m, 2H), 7.42 (dd, 1H, $J = 8.10, 7.80$ Hz).

General Procedure for the Synthesis of Substituted 11-Chlorobenzothieno[3,2-*b*]quinolines 8a-f, (Method C)—A suspension of compound **7** (28 mmol) in POCl_3 (20 mL) was refluxed with stirring at 110 °C for 3 h. After evaporating the POCl_3 , the reaction mixture was poured into ice/water (50 mL) and then

neutralized with 10 % NaOH. The aqueous solution was extracted with CHCl_3 (3×200 mL) and the combined organic layer was washed with brine, dried over sodium sulfate, filtered and solvent evaporated. The residue obtained was column chromatographed over silica gel with Hex:EtOAc (3:2) as eluent to give compounds **8a-8f**.

1-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (8a): Yield 92%. mp 192-193 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 8.54 (d, 1H, $J = 7.2$ Hz), 7.86 (d, 1H, $J = 8.4$ Hz), 7.80 (d, 1H, $J = 8.4$ Hz), 7.62-7.48 (m, 3H), 6.92 (d, 1H, $J = 7.8$ Hz), 3.96 (s, 3H).

3-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (8b): Yield 78%. mp 209-210 °C. ^1H NMR (CDCl_3): δ 8.58 (d, 1H, $J = 8.10$ Hz), 8.18 (d, 1H, $J = 9.3$ Hz), 7.87 (d, 1H, $J = 7.5$ Hz), 7.64-7.53 (m, 3H), 7.34 (dd, 1H, $J = 9.3, 2.1$ Hz), 4.016 (s, 3H).

4-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (8c): Yield 94%. mp 201-202 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 8.74 (d, 1H, $J = 7.5$ Hz), 7.8-7.83 (m, 2H), 7.65-7.53 (m, 3H), 7.16 (d, 1H, $J = 7.8$ Hz), 4.19 (s, 3H).

1,11-Dichlorobenzothieno[3,2-*b*]quinoline (8d): Yield 64%. mp 200-201 °C. ^1H NMR (CDCl_3): δ 8.58 (d, 1H, $J = 7.8$ Hz), 8.26 (dd, 1H, $J = 8.7, 1.5$ Hz), 7.88 (d, 1H, $J = 7.8$ Hz), 7.72 (m, 1H), 7.66-7.55 (m, 3H).

3,11-Dichlorobenzothieno[3,2-*b*]quinoline (8e): Yield 71%. ^1H NMR (CDCl_3): δ 8.58 (d, 1H, $J = 8.1$ Hz), 8.30 (d, 1H, $J = 2.1$ Hz), 8.23 (d, 1H, $J = 9.0$ Hz), 7.86 (d, 1H, $J = 7.8$ Hz), 7.69-7.56 (m, 3H).

4,11-Dichlorobenzothieno[3,2-*b*]quinoline (8f): Yield 72%. mp 182 °C. ^1H NMR (CDCl_3): δ 8.73 (dd, 1H, $J = 7.8, 1.8$ Hz), 8.26 (dd, 1H, $J = 8.7, 1.2$ Hz), 7.94 (dd, 1H, $J = 7.5, 1.2$ Hz), 7.88 (d, 1H, $J = 8.4$ Hz), 7.69-7.64, (ddd, 1H, $J = 8.10, 7.20, 1.5$ Hz), 7.62-7.55 (m, 2H).

General Procedure for the Synthesis of Methoxy- substituted Benzothieno[3,2-*b*]quinolines 9a-c, (Method D)—Methoxy- substituted compounds, **8a-8c** (10 mmol) were each dissolved in a mixture of AcOH and NaOAc (3 g) and Pd/C (10 %, 1.5 g) was added. The resulting suspension was hydrogenated (60-65 psi) at rt for 2-4 h. The mixture was filtered to remove the Pd/C and the filtrate was concentrated to a small volume, basified with saturated aqueous NaHCO_3 , and extracted with EtOAc (2×100 mL). The combined organic layers was dried (Na_2SO_4), and filtered. The filtrate was concentrated at reduced pressure to afford the corresponding substituted benzothieno[3,2-*b*]quinolines **9a-c** as pale yellow to white solids. These intermediates were used as such for the next step without further purification.

1-Methoxybenzothieno[3,2-*b*]quinolines (9a): Yield 68%. mp 190-191 °C. ^1H NMR (CDCl_3): δ 8.94 (s, 1H), 8.59 (d, 1H, $J = 7.5$ Hz), 7.81 (dd, 2H, $J = 8.10, 9.0$ Hz), 7.59-7.44 (m, 3H), 6.80 (d, 1H, $J = 7.5$), 3.96 (s, 3H).

3-Methoxybenzothieno[3,2-*b*]quinolines (9b)⁴³: Yield 88%. mp 148-149 °C. ^1H NMR (CDCl_3): δ 8.65 (dd, 1H, $J = 7.0, 1.5$), 8.51 (s, 1H), 7.87 (dd, 1H, $J = 8.10, 1.5$ Hz), 7.80 (d, 1H, $J = 9.0$ Hz), 7.63-7.51 (m, 3H), 7.27-7.24 (m, 1H), 4.02 (s, 3H).

4-Methoxybenzothieno[3,2-*b*]quinolines (9c): Yield 78%. mp 164-165 °C. ^1H NMR (CDCl_3): δ 8.78 (dd, 1H, $J = 7.5, 1.5$ Hz), 8.58 (s, 1H), 7.86 (dd, 1H, $J = 8.4, 1.2$ Hz), 7.64-7.55 (m, 2H), 7.55-7.50 (m, 2H), 7.12-7.09 (m, 1H), 4.17 (s, 3H).

General Procedure for the Synthesis of Chloro- substituted Benzothieno[3,2-*b*]quinolines 9d-f, (Method E)—Chloro- substituted compounds **8d-f** (10 mmol) were dissolved in EtOAc. Pd/C (10 %, 1.5 g) was added to the solution. The resulting suspension was hydrogenated (60-65 psi) at rt for 6-8 hrs. The mixture was filtered, the filtrate was concentrated to dryness, and the residue was purified by column chromatography on silica gel (eluted with EtOAc-Hexane 10-15%) to afford the corresponding substituted benzothieno[3, 2-*b*]quinolines **9d-f** as pale yellow to white solids.

1-Chlorobenzothieno[3,2-*b*]quinolines (9d): Yield 72%. mp 173-174 °C. ¹H NMR (CDCl₃): δ 9.03 (s, 1H), 8.68 (d, 1H, *J* = 7.80 Hz), 8.24 (m, 1H), 7.89 (d, 1H, *J* = 7.2 Hz), 7.68-7.65 (m, 2H), 7.63-7.58 (m, 2H).

3-Chlorobenzothieno[3,2-*b*]quinolines (9e): Yield 42%. ¹H NMR (CDCl₃): δ 8.63 (s, 1H), 8.57 (d, 1H, *J* = 7.2 Hz), 8.29 (s, 1H), 7.84 (d, 1H, *J* = 8.1 Hz), 7.66-7.52 (m, 3H).

4-Chlorobenzothieno[3,2-*b*]quinolines (9f): Yield 52%. mp 146.0-146.5 °C. ¹H NMR (CDCl₃): δ 8.73 (dd, 1H, *J* = 7.8, 0.9 Hz), 8.27 (dd, 1H, *J* = 8.7, 1.2 Hz), 7.94 (dd, 1H, *J* = 7.2, 1.2 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 7.69-7.55 (m, 4H).

1-Hydroxybenzothieno[3,2-*b*]quinoline (9g): Compound **9a** (0.53 g, 2 mmol) was added to preheated (150 °C) pyridinium hydrochloride (10 gm) and the mixture was further heated at 175 °C for 6h. The reaction mixture was allowed to cool to rt and H₂O (100 mL) was added. The solid obtained was filtered, washed with H₂O (50 mL) and recrystallized using EtOAc:Hexane (4:1) to give a yellow solid. Yield 96%. mp 164-165 °C. ¹H NMR (DMSO-*d*₆): δ 9.86 (brs, 1H, OH), 8.97 (s, 1H), 8.75 (dd, 1H, *J* = 7.8, 1.2 Hz), 8.09 (d, 1H, *J* = 7.5 Hz), 7.71-7.59 (m, 2H), 7.50 (m, 2H), 7.18-7.13 (t, 1H, *J* = 4.2 Hz).

General Procedure for the Synthesis of Substituted 5-Methylbenzothieno[3,2-*b*]quinolin-5-ium triflates 10a-g, (Method F)—MeOTf (0.5 mL) was added to a suspension of compound **9** (100 mg) in toluene (3 mL) and stirred for 24 h at rt. The precipitate thus obtained was filtered, washed with Et₂O and recrystallized from either MeOH-Et₂O or MeOH/EtOAc to afford the desired substituted 5-methylbenzothieno[3, 2-*b*]quinolin-5-ium triflate derivatives as yellow solids in moderate to good yields.

1-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10a): Yield 72%. mp 225-227 °C. ¹H NMR (CD₃OD): δ 9.98 (s, 1H), 9.0 (d, 1H, *J* = 8.4 Hz), 8.28-8.17 (m, 3H), 7.99-7.93 (m, 2H), 7.88-7.82 (m, 1H), 7.45 (d, 1H, *J* = 7.8 Hz), 5.09 (s, 3H), 4.21 (s, 3H). Anal. *Calcd for* C₁₈H₁₄F₃NO₄S₂. 0.65H₂O: C 49.01, H 3.20, N 3.18; *Found*: C 49.03, H 3.21, N 3.06.

3-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10b): Yield 68%. mp 221-223 °C. ¹H NMR (CD₃OD): δ 9.59 (s, 1H), 9.0 (d, 1H, *J* = 8.4 Hz), 8.35 (d, 1H, *J* = 9.0 Hz), 8.29 (d, 1H, *J* = 8.4 Hz), 7.98-7.93 (m, 1H), 7.88-7.82 (m, 2H), 7.69-7.65 (dd, 1H, *J* = 9.0, 2.4 Hz), 5.08 (s, 3H), 4.22 (s, 3H). Anal. *Calcd for* C₁₈H₁₄F₃NO₄S₂. 0.25 H₂O: C 49.82, H 3.25, N 3.23; *Found*: C 49.82, H 3.27, N 3.11.

4-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10c): Yield 28%. mp 180-181 °C. ¹H NMR (CD₃OD): δ 9.62 (s, 1H), 8.95 (d, 1H, *J* = 8.4 Hz), 8.26 (d, 1H, *J* = 8.4 Hz), 7.96-7.91 (m, 3H), 7.87-7.79 (m, 2H), 5.15 (s, 3H), 4.24 (s, 3H). Anal. *Calcd for* C₁₈H₁₄F₃NO₄S₂. 0.1 H₂O: C 50.13, H 3.32, N 3.25; *Found*: C 49.85, H 3.22, N 3.10.

1-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10d): Yield 62%. mp 285-287 °C. ¹H NMR (CD₃OD): δ 10.16 (s, 1H), 9.04 (d, 1H, *J* = 8.4 Hz), 8.72 (d, 1H, *J* = 9.0 Hz), 8.35 (d, 1H, *J* = 8.4 Hz), 8.28-8.17 (m, 2H), 8.06-8.01 (m, 1H), 7.93-7.87 (m, 1H), 5.19 (s, 3H). Anal. Calcd for C₁₇H₁₁ClF₃NO₃S₂: C 47.06, H 2.56, N 3.23; Found: C 46.96, H 2.47, N 3.21.

3-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10e): Yield 65%. mp 220-221 °C. ¹H NMR (CD₃OD): δ 9.76 (s, 1H), 9.03 (d, 1H, *J* = 8.4 Hz), 8.88 (s, 1H), 8.46 (d, 1H, *J* = 8.7 Hz), 8.32 (d, 1H, *J* = 8.1 Hz), 8.07-8.00 (m, 2H), 7.92-7.86 (m, 1H), 5.13 (s, 3H). Anal. Calcd for C₁₇H₁₁ClF₃NO₃S₂·0.2H₂O: C 46.68, H 2.53, N 3.20; Found: C 46.58, H 2.52, N 3.12.

4-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10f): Yield 46%. mp 225-226 °C. ¹H NMR (CD₃OD): δ 9.77 (s, 1H), 9.01 (d, 1H, *J* = 8.4 Hz), 8.44-8.39 (m, 2H), 8.33 (d, 1H, *J* = 8.10 Hz), 8.07-7.87 (m, 3H), 5.21 (s, 3H). ¹³C NMR (DMSO-*d*₆): 152.99, 146.46, 142.31, 138.89, 138.79, 135.47, 134.81, 131.07, 130.24, 129.91, 129.33, 127.92, 127.26, 125.73, 124.54, 121.40 (q, *J* (C-F) = 322.36 Hz), 51.08. Anal. Calcd for C₁₇H₁₁ClF₃NO₃S₂: C 47.06, H 2.56, N 3.23; Found: C 46.90, H 2.59, N 3.25.

4-Hydroxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10g): Yield 68%. mp 208-209 °C. ¹H NMR (CD₃OD): δ 9.46 (s, 1H), 8.92 (d, 1H, *J* = 8.7 Hz), 8.22 (d, 1H, *J* = 8.10 Hz), 7.95-7.90 (m, 1H), 7.83-7.71 (m, 2H), 7.64-7.62 (m, 1H), 7.46 (d, 1H, *J* = 7.8 Hz), 5.27 (s, 3H). Anal. Calcd for C₁₇H₁₂F₃NO₄S₂·0.25 EtOAc: C 49.42, H 3.23, N 3.20; Found: C 49.72, H 3.29, N 3.37.

General Procedure for the Synthesis of 1-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (11a), (Method G)—CH₃I (0.5 ml) was added to a solution of compound **9a** (100 mg) in tetramethyl sulfone (0.2 mL). The mixture was heated at 110 °C for 12 hours in a sealed pressure tube. After cooling to rt, Et₂O was added to form a precipitate. The precipitate was collected, washed with Et₂O (25 mL) and recrystallized from MeOH-Et₂O as a yellow solid. Yield 82 %. mp 216-217 °C. ¹H NMR (CD₃OD): δ 10.01 (s, 1H), 9.0 (d, 1H, *J* = 8.4 Hz), 8.31-8.22 (m, 3H), 7.97 (t, 1H, *J* = 8.1, 6.9 Hz), 7.86 (t, 1H, *J* = 7.5 Hz), 7.46 (d, 1H, *J* = 7.2 Hz), 5.11 (s, 3H), 4.22 (s, 3H). Anal. Calcd for C₁₇H₁₄INOS·0.1 H₂O: C 49.70, H 3.48, N 3.41; Found: C 49.69, H 3.50, N 3.40.

General Procedure for the Synthesis of 2-(2-Bromoacetylamido)benzoic acid (13). (Method H)—A mixture of anthranilic acid (15 g, 109 mmol), DMF (35 mL), and 1,4-dioxane (35 mL) was cooled to 0 °C in a round bottom flask and bromoacetyl bromide (22 g, 109 mmol) slowly added so that the temperature did not rise above 1 °C. At the end of the addition, the temperature was maintained at 0 °C for 15 min longer and then the mixture was stirred overnight at room temperature. The contents of the flask was poured into water (400 mL), and the resulting precipitate **13** was filtered, washed with water (3 × 100 mL), and dried to yield a colorless crystalline compound **13** (20 g). No further purification was attempted. ¹H NMR: (CD₃OD): δ 8.58-8.54 (dd, 1H, *J* = 0.6, 7.8 Hz), 8.12-8.08 (dd, 1H, *J* = 1.5, 6.3 Hz), 7.60-7.54 (dtd, 1H, *J* = 1.2, 1.5 and 5.7 Hz), 7.20 (dtd, 1H, *J* = 1.2, 1.2 and 5.7 Hz), 4.10 (s, 2H).

General Procedure for the Synthesis of Substituted 2-(2-Phenylsulfanylacetylamido)benzoic acids 14a-f, (Method I)—A mixture of substituted benzenethiol (38 mmol), K₂CO₃ (2.0 g) and KI (200 mg) in acetone (100 mL) was stirred for 15 min. 2-(2-bromoacetylamido)-benzoic acid (**13**) (38 mmol) was added to the reaction mixture and refluxed for 24 h. The reaction was allowed to cool to rt and

acetone was removed on rotary evaporator. The residue obtained was dissolved in water (200 mL) and 10% HCl (100 mL) was added. The resulting white precipitate was filtered, washed with H₂O (2 × 100 mL) and dried to give the desired compounds **14a-f** in good to excellent yields. These intermediates were used as such for the next step without further purification.

2-(2-(2-Methoxyphenylsulfanyl)acetylamido)benzoic acid (14a): Yield 86%. mp 172-173 °C. ¹H NMR (CD₃OD): δ 8.53 (d, 1H, *J* = 8.1 Hz), 8.04-8.01 (m, 1H), 7.52-7.47 (m, 1H), 7.37-7.34 (m, 1H), 7.24-7.18 (m, 1H), 7.13 (t, 1H, *J* = 7.5 Hz), 6.94 (d, 1H, *J* = 8.1 Hz), 6.82 (t, 1H, *J* = 7.8 Hz), 3.83 (s, 3H), 3.75 (s, 2H).

2-(2-(3-Methoxyphenylsulfanyl)acetylamido)benzoic acid (14b): Yield 78%. mp 167-169 °C. ¹H NMR (CD₃OD): δ 8.55 (d, 1H, 8.7 Hz), 8.04 (dd, 1H, *J* = 8.1, 1.8 Hz), 7.08 (m, 1H), 7.18-7.12 (m, 2H), 6.97-6.94 (m, 2H), 6.75 (dd, 1H, *J* = 9.6, 2.7 Hz), 3.82 (s, 2H), 3.69 (s, 3H).

2-(2-(4-Methoxyphenylsulfanyl)acetylamido)benzoic acid (14c): Yield 72%. mp 175-176 °C. ¹H NMR (CD₃OD): δ 8.53 (d, 1H, *J* = 8.4 Hz), 8.07 (dd, 1H, *J* = 7.8, 1.5 Hz), 7.51 (dd, 1H, *J* = 7.20, 8.4 Hz), 7.43 (dd, 2H, *J* = 9.0, 2.1 Hz), 7.14 (dd, 1H, *J* = 7.2, 7.8 Hz), 6.84 (dd, 2H, *J* = 9.0, 2.1 Hz), 3.73 (s, 3H), 3.68 (s, 2H).

2-(2-(2-Chlorophenylsulfanyl)acetylamido)benzoic acid (14d): Yield 95%. mp 171-173 °C. ¹H NMR (CD₃OD): δ 8.44-8.38 (d, 1H, *J* = 8.1 Hz), 8.04-8.00 (dd, 1H, *J* = 1.5, 6.3 Hz), 7.52-7.46 (dd, 1H, *J* = 1.5, 6.3 Hz), 7.40-7.33 (m, 1H), 7.32-7.30 (m, 1H), 7.23-7.20 (m, 1H), 7.18-7.12 (m, 1H), 7.08-7.00 (m, 1H), 3.90 (s, 2H).

2-(2-(3-Chlorophenylsulfanyl)acetylamido)benzoic acid (14e): Yield 96%. mp 167-168 °C. ¹H NMR (CD₃OD): δ 8.44-8.40 (dd, 1H, *J* = 0.9, 7.5 Hz), 8.04-7.88 (dd, 1H, *J* = 1.5, 6.0 Hz), 7.44 (t, 1H, *J* = 2.1 Hz), 7.38-7.30 (m, 2H), 7.28-7.22 (t, 1H, *J* = 7.8 Hz), 7.18-7.14 (m, 1H), 7.08-7.00 (m, 1H), 3.80 (s, 2H).

2-(2-(4-Chlorophenylsulfanyl)acetylamino)benzoic acid (14f): Yield 95%. mp 183-184 °C. ¹H NMR (CD₃OD): δ 8.56-8.50 (dd, 1H, *J* = 0.9, 7.8 Hz), 8.04-8.02 (dd, 1H, *J* = 1.5, 6.6 Hz), 7.48 (m, 1H), 7.44-7.38 (m, 2H), 7.30-7.24 (m, 2H), 7.16-7.10 (m, 1H), 3.90 (s, 2H).

Substituted benzothieno[3,2-*b*]quinoline-11(5*H*)-ones 15a-15f using General Method B—Compound **6** (20 mmol) was mixed with PPA (125 g) and heated at 130-135 °C for 3h. After cooling to rt, the mixture was poured into ice/water (200 mL), neutralized with 50 % NaOH solution to give a highly insoluble dark brown solid which was washed with H₂O, EtOAc and Et₂O, dried over P₂O₅ and used as such for the next step without further purification.

9-Methoxybenzothieno[3,2-*b*]quinoline-11(5*H*)-one (15a): Yield 52%. mp 225-227 °C. ¹H NMR (DMSO-*d*₆): δ 8.32 (d, 1H, *J* = 7.8 Hz), 8.21 (d, 1H, *J* = 8.1 Hz), 7.90-7.83 (m, 2H), 7.64 (t, 1H, *J* = 7.8 Hz), 7.48-7.43 (m, 1H), 7.31 (d, 1H, *J* = 8.1 Hz), 4.07 (s, 3H).

8-Chlorobenzothieno[3,2-*b*]quinoline-11(5*H*)-one (15e): Yield 48%. mp 217-219 °C. ¹H NMR (DMSO-*d*₆): δ 12.80 (s, 1H), 8.54-8.48 (d, 1H, *J* = 8.4 Hz), 8.28-8.26 (d, 1H, *J* = 2.1 Hz), 8.24-8.18 (d, 1H, *J* = 8.1 Hz), 7.80-7.70 (m, 2H), 7.64-7.60 (dd, 1H, *J* = 2.1, 6.9 Hz), 7.34-7.32 (m, 1H).

¹H NMR spectra of compounds **15b-d** & **f** could not be obtained because they were insoluble in DMSO/MeOH.

Substituted 11-Chlorobenzothieno[3,2-*b*]quinolines 16a-e, Using General Method C

9-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (16a): Yield 68%. mp 200-201 °C. ¹H NMR (CDCl₃): δ 8.33-8.28 (m, 2H), 8.22 (d, 1H, *J* = 8.1 Hz), 7.80 (t, 1H, *J* = 8.1 Hz), 7.70-7.65 (m, 1H), 7.53 (t, 1H, *J* = 7.8 Hz), 7.10 (d, 1H, *J* = 7.8 Hz), 4.06 (s, 3H).

8-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (16b): Yield 54%. mp 199-200 °C. ¹H NMR (DMSO-*d*₆): δ 8.25 (d, 1H, *J* = 8.7 Hz), 8.20 (d, 1H, *J* = 8.1 Hz), 7.69 (d, 1H, *J* = 8.4 Hz), 7.47-7.40 (m, 2H), 7.12 (dd, 1H, *J* = 6.9, 7.2 Hz), 7.03 (dd, 1H, *J* = 8.4, 1.1 Hz), 3.84 (s, 3H).

7-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (16c): Yield 56%. mp 207-209 °C. ¹H NMR (CDCl₃): δ 8.31 (m, 2H), 8.09 (s, 1H), 7.83 (dd, 1H, *J* = 8.4, 7.2 Hz), 7.74 (m, 1H), 7.25 (m, 2H), 4.01 (s, 3H).

9, 11-Dichlorobenzothieno[3,2-*b*]quinoline (16d): Yield 63%. mp 221-222 °C. ¹H NMR (CDCl₃): δ 8.60-8.54 (d, 1H, *J* = 7.8 Hz), 8.38-8.30 (d, 2H, *J* = 8.4 Hz), 7.88-7.82 (m, 1H), 7.76-7.70 (m, 1H), 7.68-7.62 (dd, 1H, *J* = 0.9, 6.9 Hz), 7.58-7.52 (m, 1H).

8, 11-Dichlorobenzothieno[3,2-*b*]quinoline (16e): Yield 59%. mp 252-253 °C. ¹H NMR (CDCl₃): δ 8.56-8.50 (d, 1H, *J* = 8.4 Hz), 8.36-8.26 (m, 2H), 7.88-7.80 (m, 2H), 7.74-7.68 (m, 1H), 7.58-7.52 (dd, 1H, *J* = 1.8, 6.6 Hz).

7, 11-Dichlorobenzothieno[3,2-*b*]quinoline (16f): Yield 45%. mp 69-71 °C. ¹H NMR (CDCl₃): δ 8.60 (d, 1H, *J* = 1.5 Hz), 8.38-8.26 (t, 2H, *J* = 7.8 Hz), 7.88-7.76 (m, 2H), 7.74-7.68 (m, 1H), 7.62-7.58 (dd, 1H, *J* = 2.1, 6.3 Hz).

General Procedure for the Synthesis of Methoxy- substituted Benzothieno[3,2-*b*]quinolines 17a-c, (Method D)

9-Methoxybenzothieno[3,2-*b*]quinolines (17a): Yield 62%. mp 168-169 °C. ¹H NMR (CDCl₃): δ 8.63 (s, 1H), 8.27 (t, 2H, *J* = 7.2 Hz), 7.92 (d, 1H, *J* = 8.4 Hz), 7.75 (t, 1H, *J* = 8.4 Hz), 7.60-7.50 (m, 2H), 7.09 (d, 1H, *J* = 8.1 Hz), 4.05 (s, 3H).

8-Methoxybenzothieno[3,2-*b*]quinolines (17b).⁴³ Yield 54%. mp 185-186 °C. ¹H NMR (CDCl₃): δ 8.52 (d, 1H, *J* = 8.4 Hz), 8.30 (d, 1H, *J* = 9.0 Hz), 7.81 (dd, 1H, *J* = 6.6, 8.4 Hz), 7.68 (dd, 1H, *J* = 7.8, 7.8 Hz), 7.32 (d, 1H, *J* = 2.4 Hz), 7.26 (s, 1H), 7.16 (dd, 1H, *J* = 8.7, 2.1 Hz), 3.95 (s, 3H).

7-Methoxybenzothieno[3,2-*b*]quinolines (17c): Yield 56%. mp 176-178 °C. ¹H NMR (CDCl₃): δ 8.55 (s, 1H), 8.30 (d, 1H, *J* = 8.70 Hz), 8.14 (s, 1H), 7.90 (d, 1H, *J* = 8.10 Hz), 7.78-7.68 (m, 2H), 7.59 (dd, 1H, *J* = 8.1, 6.9 Hz), 7.26 (dd, 1H, *J* = 8.7, 1.8 Hz), 4.01 (s, 3H).

General Procedure for the Synthesis of Chloro-substituted Benzothieno[3,2-*b*]quinolines 17d-f, (Method E)

9-Chlorobenzothieno[3,2-*b*]quinoline (17d): Yield 58%. mp 179-180 °C. ¹H NMR (CDCl₃): δ 8.64 (s, 1H), 8.62-8.56 (d, 1H, *J* = 7.8 Hz), 7.96-7.90 (d, 1H, *J* = 8.4 Hz), 7.82-7.76 (m, 1H), 7.64-7.58 (m, 2H), 7.56-7.50 (t, 1H, *J* = 7.5 Hz).

8-Chlorobenzothieno[3,2-*b*]quinoline (17e): Yield 52%. mp 252-253 °C. ¹H NMR (CD₃OD): δ 8.60 (s, 1H), 8.58-8.56 (d, 1H, *J* = 8.7 Hz), 8.30-8.26 (d, 1H, *J* = 8.7 Hz),

7.94-7.90 (d, 1H, $J = 8.1$ Hz), 7.82-7.74 (m, 1H), 7.64-7.58 (m, 1H), 7.56-7.52 (dd, 1H, $J = 1.8, 6.6$ Hz).

7-Chlorobenzothieno[3,2-*b*]quinoline (17f): Yield 46%. mp 190-192 °C. ^1H NMR (CD_3OD): δ 8.86 (s, 1H), 8.68 (s, 1H), 8.44-8.50 (d, 1H, $J = 8.7$ Hz), 7.98-7.92 (d, 1H, $J = 8.4$ Hz), 7.86-7.76 (m, 2H), 7.70-7.58 (m, 2H).

Substituted 5-Methylbenzothieno[3,2-*b*]quinolin-5-ium iodides 18a-f were synthesized using General Method E

9-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18a): Yield 68%. mp 200-201 °C. ^1H NMR (CD_3OD): δ 9.77 (s, 1H), 8.76 (d, 1H, $J = 9$), 8.60 (d, 1H, $J = 8.4$ Hz), 8.47 (d, 1H, $J = 8.4$ Hz), 8.33 (t, 1H, $J = 7.5$ Hz), 8.04 (t, 1H, $J = 7.5$ Hz), 7.83 (t, 1H, $J = 8.1$ Hz), 7.55 (d, 1H, $J = 8.4$ Hz), 5.14 (s, 3H), 4.12 (s, 3H). Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{INOS}$: C 50.13, H 3.46, N 3.44; Found: C 50.10, H 3.46, N 3.42.

8-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18b): Yield 62%. mp 228-229 °C. ^1H NMR (CD_3OD): δ 4.07 (s, 3H), 5.05 (s, 3H), 7.85 (s, 1H), 8.01-7.96 (t, 1H, $J = 7.5$ Hz), 8.30-8.24 (t, 1H, $J = 7.2$ Hz), 8.41 (d, 1H, $J = 8.10$ Hz), 8.68 (d, 1H, $J = 9.30$ Hz), 8.92 (d, 1H, $J = 9.6$ Hz), 9.56 (s, 1H). Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{INOS}$: C 50.13, H 3.46, N 3.44; Found: C 50.01, H 3.39, N 3.43.

7-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18c): Yield 78%. mp 199-200 °C. ^1H NMR (CD_3OD): δ 9.73 (s, 1H), 8.74 (d, 1H, $J = 9.3$ Hz), 8.47 (d, 1H, $J = 8.4$ Hz), 8.37-8.30 (m, 2H), 8.2 (d, 1H, $J = 9.0$ Hz), 8.06-8.01 (t, 1H, $J = 7.8$ Hz), 7.68-7.64 (dd, 1H, $J = 9.0, 1.8$ Hz), 5.18 (s, 3H), 4.06 (s, 3H). Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{INOS} \cdot 0.2 \text{H}_2\text{O}$: C 49.26, H 3.50, N 3.38; Found: C 49.27, H 3.41, N 3.33.

7-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide. (18f): Yield 56%. mp 203-204 °C. ^1H NMR (CD_3OD): δ 9.84 (s, 1H), 9.04 (d, 1H, $J = 1.5$ Hz), 8.82-8.76 (d, 1H, $J = 9.0$ Hz), 8.54-8.48 (d, 1H, $J = 8.4$ Hz), 8.40-8.32 (m, 2H), 8.12-8.06 (t, 1H, $J = 7.8$ Hz), 8.04-7.98 (dd, 1H, $J = 2.1, 6.6$ Hz), 5.20 (s, 3H). Anal. Calcd for $\text{C}_{16}\text{H}_{11}\text{ClINS}$: C 46.68, H 2.69, N 3.40. Found: C 46.39, H 2.55, N 3.30.

8-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide. (18e): Yield 61%. mp 218-219 °C. ^1H NMR: (CD_3OD): δ 9.80 (s, 1H), 9.06-9.00 (d, 1H, $J = 9.6$ Hz), 8.80-8.72 (d, 1H, $J = 9.0$ Hz), 8.52-8.48 (d, 1H, $J = 8.7$ Hz), 8.44 (d, 1H, $J = 2.1$ Hz), 8.40-8.30 (m, 1H), 8.10-8.04 (t, 1H, $J = 7.5$ Hz), 7.94-7.86 (dd, 1H, $J = 1.8, 7.2$ Hz), 5.20 (s, 3H). Anal. Calcd for $\text{C}_{16}\text{H}_{11}\text{ClINS} \cdot 0.4 \text{H}_2\text{O}$: C 45.88, H 2.84, N 3.34. Found: C 45.81, H 2.60, N, 3.38.

9-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18d): Yield 26%. mp 192-193 °C. ^1H NMR: (CD_3OD): δ 9.88 (s, 1H), 9.04-9.02 (d, 1H, $J = 8.4$ Hz), 8.80-8.78 (d, 1H, $J = 9.3$ Hz), 8.54-8.50 (d, 1H, $J = 8.1$ Hz), 8.42-8.34 (m, 1H), 8.14-8.04 (m, 2H), 7.94-7.88 (t, 1H, $J = 7.8$ Hz), 5.20 (s, 3H). Anal. Calcd for $\text{C}_{16}\text{H}_{11}\text{ClINS} \cdot 0.35 \text{CH}_3\text{OH}$: C 45.24, H 2.88, N 3.23. Found: C 44.90, H 2.56, N 3.22.

General Procedure for the Synthesis of 3-Iodoquinoline (20), (Method F)—A mixture of 3-bromoquinoline (**19**) (9.83 g, 47.3 mmol), CuI (450 mg, 2.4 mmol), NaI (14.15 g, 94.52 mmol), *N,N*-dimethylethylenediamine (0.5 mL, 415 mg, 4.7 mmol) in dioxane (47 mL) was heated, stirred, and refluxed under N_2 for 44-48 h at 110°C. The reaction was monitored by GC/MS till the conversion reached 100%. After cooling to room temperature, the mixture was diluted with 30% aqueous ammonia (20 mL), followed by extraction with EtOAc (3 $\times$ 30 mL). The combined organic layers was washed with brine (100 mL), dried

over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated by rotary evaporation under reduced pressure to yield the pure product as a pale yellow solid in almost quantitative yield. The product was used as such for the next step without further purification. ^1H NMR (CDCl_3): δ 9.03 (d, 1H, $J = 2.4$ Hz), 8.54–8.53 (m, 1H), 8.08–8.04 (m, 1H), 7.76–7.69 (m, 2H), 7.59–7.53 (m, 1H).

Synthesis of 3-(phenylthio)quinolines (21): A mixture of Cu (I) iodide (20 mg, 0.10 mmol), potassium carbonate (540 mg, 3.92 mmol), 3-iodoquinoline (500 mg, 1.96 mmol), substituted benzenethiol (0.21 mL, 1.96 mmol), ethylene glycol (0.22 mL, 3.92 mmol) and 2 mL of 2-propanol was heated at 110 °C and allowed to reflux under N_2 for 30h. After cooling to room temperature, the mixture was diluted with distilled water (20 mL) and the extracted EtOAc (3×15 mL). The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation at reduced pressure to provide crude product. The crude product was purified by column chromatography on silica gel using Hexane:EtOAc (9.8:0.2) to afford a pure 3-(phenylthio)quinoline.⁴⁴ Yield 92%. mp 78–79 °C. ^1H NMR (CDCl_3): δ 8.81 (s, 1H), 8.08–8.06 (m, 2H), 7.71–7.25 (m, 8H).

Synthesis of 1-(alkylsubstituted)-3-(phenylthio)quinolinium iodides (22a-c): 3-(Phenylthio)quinoline (100 mg, 0.39 mmol) was added to the appropriate alkyl iodide (130 mg, 0.470 mmol) in tetramethylene sulfone (0.5 mL). The mixture was heated at 110 °C for 16 h in a sealed pressure tube and then allowed to cool to rt after which EtOAc (15 mL) was added to precipitate the solid. The precipitate was collected, washed with additional EtOAc and dried to form the pure solid powder and no further purification was necessary.

1-Methyl-3-(phenylthio)quinolinium iodide (22a): Yield 81%. mp 145–146. ^1H NMR (DMSO): 9.69 (1H, s), 9.19 (1H, s), 8.48 (1H, d, $J = 7.5$ Hz), 8.37 (1H, d, $J = 8.4$ Hz), 8.25 (1H, t, $J = 8.1$ Hz), 8.03 (1H, t, $J = 8.9$ Hz), 7.50 (5H, m), 4.60 (3H, s). Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{INS}$: C 50.67, H 3.72, N 3.69; Found: 50.63, H 3.66, N 3.51.

1-(Phenylpentyl)-3-(phenylthio)quinolinium iodide (22b): Yield 75%. mp 166–167 °C. ^1H NMR (DMSO): 9.69 (1H, s), 9.17 (1H, s), 9.57 (1H, d, $J = 9.0$ Hz), 8.39 (1H, d, $J = 8.0$ Hz), 8.23 (1H, t, $J = 7.5$ Hz), 8.02 (1H, t, $J = 7.5$ Hz), 7.50 (5H, m), 7.24 (2H, t, $J = 7.0$ Hz), 7.15 (3H, m), 5.02 (2H, t, $J = 7.4$ Hz), 2.54 (2H, t, $J = 7.4$ Hz), 1.98 (2H, m), 1.60 (2H, m), 1.39 (2H, m). ^{13}C NMR (DMSO- d_6): 151.64, 148.23, 142.58, 136.99, 136.29, 132.90, 131.98, 131.39, 131.13, 130.93, 130.84, 130.56, 129.47, 128.95, 128.92, 126.37, 119.76, 58.17, 35.56, 31.03, 29.97, 25.99. Anal. Calcd for $\text{C}_{26}\text{H}_{26}\text{INS} \cdot 0.2 \text{H}_2\text{O}$: C 60.63, H 5.09, N 2.72; Found: 60.60, H 5.02, N 2.73.

1-(Cyclohexylpentyl)-3-(phenylthio)quinolinium iodide (22c): Yield: 73%. mp 174–176 °C. ^1H NMR (DMSO- d_6): δ 9.66 (d, 1H, $J = 2.1$ Hz), 9.16 (d, 1H, $J = 1.5$ Hz), 8.58 (d, 1H, $J = 9$ Hz), 8.38 (t, 1H, $J = 7.2$ Hz), 8.25–8.19 (m, 1H), 8.01 (t, 1H, $J = 7.5$ Hz), 7.56–7.41 (m, 5H), 5.01 (t, 2H, $J = 7.2$ Hz), 1.93–1.91 (m, 2H), 1.64–1.61 (m, 5H), 1.30–1.11 (m, 10H), 0.86–0.79 (m, 2H). ^{13}C NMR (DMSO- d_6): 151.60, 148.26, 137.04, 136.29, 132.88, 132.07, 131.47, 131.12, 130.95, 130.81, 130.59, 129.49, 119.71, 58.32, 37.57, 37.38, 33.52, 30.21, 26.88, 26.72, 26.52, 26.45. Anal. Calcd for $\text{C}_{26}\text{H}_{32}\text{INS}$: C, 60.34; H, 6.23; N, 2.71. Found: C, 60.48; H, 6.27; N, 2.75.

Biological testing

Antifungal testing—All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include *C. albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. neoformans* ATCC 90113 and *A. fumigatus* ATCC 90906. Susceptibility testing was

performed using a modified version of the CLSI methods 45 as described by Samoylenko et al.⁴⁶ Amphotericin B (ICN Biomedicals, Ohio) was used as a positive control in each assay.

Briefly, 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 Sabouraud Dextrose broth (SDB) or RPMI 1640, *C. neoformans*: SDB, *A. fumigatus*: either YM broth (for MICs) or RPMI-1640 + 5% Alamar Blue (for IC₅₀ determination). After adding microbial cultures to the samples affording a final volume of 200 μ L and final test concentration starting with 20 μ g/mL, plates were read prior to and after incubation using either fluorescence at 544ex/590em (*A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) or optical density at 630 nm 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) for fungi was included in each assay. Percent growth was calculated and plotted versus test concentration to afford the IC₅₀ (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).

Cytotoxicity assay—The *in vitro* cytotoxicity was determined against mammalian kidney fibroblast (VERO) cells. The assay was performed in 96-well tissue culture-treated microplates and compounds were tested up to a highest concentration of 23.8 μ g/mL as described earlier.⁴⁷ 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 by the neutral red assay as previously described.⁴⁷ IC₅₀ 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.

Identification of 10f in Brain Samples—Under general anesthesia, mice brains were perfused with phosphate buffered saline (PBS) until > 99.5% white. Mice were euthanized, brains were harvested and frozen, weighed, thawed at room temperature and then macerated in a silanized glass vial with a spatula before adding 0.5 M HClO₄ (2 mL) and vortex mixing. Additional 0.5 M HClO₄ was added if needed to obtain a pH of 1-1.5 on a pH paper. The mixture was then homogenized on ice with a Kinematica Polytron 3100 homogenizer at the highest setting for 2 minutes, followed by two 45 second treatments on ice with a Fisher 550 Sonic Dismembrator. Four mL MeOH (HPLC grade) was added and the mixture vortexed and sonified for 5 minutes. The supernatant was removed after centrifugation and filtered if needed into a clean silanized glass vial. The remaining brain tissue was extracted again with 2 mL of 0.5 M HClO₄ plus 4 mL MeOH at a pH of 1 – 1.5. The supernatants were combined and the pH was then adjusted to ~ 7 with conc. NH₄OH.⁴⁸⁻⁴⁹

After centrifugation if necessary, the extract was cleaned up using a 225 mg Waters Oasis WCX plus SPE cartridge as follows: The cartridge was rinsed (5 mL MeOH) and conditioned (with 5 mL of 70:30 MeOH:H₂O at pH 7). The extract (12 mL) was slowly loaded, washed with 5% NH₄OH (4 mL), then with 20% MeOH (5 mL) and another aliquot of MeOH (4 mL). The column was eluted slowly with 5% HCOOH in MeOH. Eluents were collected in 2 mL fractions (16 mL total) and analyzed by HPLC separately. Eluents were combined and then evaporated under nitrogen in a 40°C water bath. The residue was reconstituted in MeOH (100 μ L) and injected into the HPLC (Waters 2695 Alliance system with a 2996 PDA detector). The HPLC conditions were: Analytical wavelength- 294 nm,

Flowrate 1.00 mL/min, Column – Alltech Nucleosil 100, C-18, 15 cm × 4.6 cm, 5 μ with C-18 guard cartridge, Injection volume – 60 μL, Gradient elution was employed using mobile phases C, (90% 7.5 mM sodium heptanesulfonate, 0.1M H₂PO₄, adjusted to pH 3.00 with NEt₃) + 10% CH₃CN and and D (100% acetonitrile).⁵⁰

Acknowledgments

We gratefully acknowledge research support from the National Institutes of Health, National Institute of Allergy and Infectious Diseases, through AREA grant number R15 AI37976-01, Research Centers at Minority Institutions (RCMI) grant number G12 RR 03020, and Title III grant to Florida A&M University. This research was supported in part by the Pharmaceutical Research Center NIH/NCRR 1 C06-RR12512-01 Grant. We also appreciate the generous contributions of Dr. Wang Zhang, Senior Technician responsible for instrumentation for his contribution to the generation of NMR spectra of compounds in this paper and Ms. Marsha Wright for antifungal testing. Antifungal testing was supported by the NIH, NIAID, Division of AIDS, Grant No. AI 27094.

References

- (1). Pontón J, Rüchel R, Clemons KV, Coleman DC, Grillot R, Guarro J, Aldebert D, Ambroise-Thomas P, Cano J, Carrillo-Muñoz AJ, Gené J, Pinel C, Stevens DA, Sullivan DJ. *Med. Mycol* 2000;38:225.
- (2). Kaplan JE, Jones JL, Dykewicz CA. *J. Eukaryotic Micro* 2005;47:15.
- (3). Rakhmanova, A.; Gyaurgieva, OH.; Romanova, EI.; Gorkalo, GM.; Prigozhina, VK.; Vlasov, NN.; Chaika, NA. *Int. Conf. AID*; 1998; p. 308
- (4). Islam S, Das A, Islam N. *Mymensingh Med. J* 2010;19:142. [PubMed: 20046189]
- (5). Franquet T, Giménez A, Hidalgo A. *European Journal of Radiology* 2004;51:130. [PubMed: 15246518]
- (6). Ablordeppey SY, Fan P, Ablordeppey JH, Mardenborough LG. *Curr. Med. Chem* 1999;6:1151. [PubMed: 10519919]
- (7). Powderly WG. *Clin. Infect. Dis* 2008;47:131–132. [PubMed: 18505386]
- (8). Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, Sobel JD, Dismukes WE. *Clin. Infect. Dis* 2000;30:710–718. [PubMed: 10770733]
- (9). Pappas PG, Chetchotisakd P, Larsen RA, Manosuthi W, Morris MI, Anekthananon T, Sungkanuparph S, Supparatpinyo K, Nolen TL, Zimmer LO, Kendrick AS, Johnson P, Sobel JD, Filler SG. *Clin. Infect. Dis* 2009;48:1775–1783. [PubMed: 19441980]
- (10). Brouwer AE, Rajanuwong A, Chierakul W, Griffin GE, Larsen RA, White NJ, Harrison TS. *Lancet* 2004;363:1764–1767. [PubMed: 15172774]
- (11). Bennett JE, Dismukes WE, Duma RJ, Medoff G, Sande MA, Gallis H, Leonard J, Fields BT, Bradshaw M, Haywood H, McGee ZA, Cate TR, Cobbs CG, Warner JF, Alling DW. *N. Eng. J. Med* 1979;301:126–131.
- (12). van der Horst CM, Saag MS, Cloud GA, Hamill RJ, Graybill JR, Sobel JD, Johnson PC, Tuazon CU, Kerkering T, Moskovitz BL, Powderly WG, Dismukes WE. *N. Eng. J. Med* 1997;317:15–21.
- (13). Dismukes WE, Cloud G, Gallis HA, Kerkering TM, Medoff G, Craven PC, Kaplowitz LG, Fisher JF, Gregg CR, Bowles CA, Shadomy S, Stamm AM, Diasio RB, Kaufman L, Soong SJ, Blackwelder WC, National Institute of Allergy and Infectious Diseases Mycoses Studying Group. *N. Engl. J. Med* 1987;317:334. [PubMed: 3299095]
- (14). Francis P, Walsh TJ. *Clin. Infect. Dis* 1992;15:1003. [PubMed: 1457631]
- (15). Larsen RA, Bozzette SA, Jones BE, Haghighat D, Leal MA, Forthal D, Bauer M, Tilles JG, McCutchan JA, Leedom JM. *Clin. Infect. Dis* 1994;19:741. [PubMed: 7803641]
- (16). Polak A. *Prog. Drug Res* 1997;49:219–318. [PubMed: 9388389]
- (17). Ullmann AJ. *Mycoses* 2008;51:25. [PubMed: 18471158]
- (18). Girois SB, Chapuis F, Decullier E, Revol BG. *Eur. J. Clin. Microbiol. Infect. Dis* 2006;2:138. [PubMed: 16622909]

- (19). Verweij PE, Snelders E, Kema GH, Mellado E, Melchers WJ. *Lancet Infect. Dis* 2009;12:789. [PubMed: 19926038]
- (20). Johnson EM, Warnock DW, Luker J, Porter SR, Scully C. J. *Antimicrob. Chemother* 1995;35:103–114. [PubMed: 7768758]
- (21). Rex JH, Rinaldi MG, Pfaller MA. *Antimicrob. Agents Chemother* 1995;39:1. [PubMed: 7695288]
- (22). Kumar EV, Etukala JR, Ablordeppey SY. *Mini-Reviews in Med. Chem* 2008;8:538.
- (23). Ablordeppey SY, Hufford CD, Borne RF, Dwuma-Badu D. *Planta Med* 1990;5:416–417. [PubMed: 17221441]
- (24). (a) Oyekan AO, Ablordeppey SY. *Gen. Pharmacol* 1993;24:1285. [PubMed: 8270188] Oyekan AO, Ablordeppey SY. *Gen. Pharmacol* 1993;24:461. [PubMed: 7683302]
- (25). Ablordeppey SY, Fan P, Li S, Clark AM, Hufford CD. *Bioorg. Med. Chem* 2002;10:1337. [PubMed: 11886797]
- (26). Singh M, Singh MP, Ablordeppey SY. *Drug Dev. Ind. Pharm* 1996;22:377.
- (27). Cimanga K, De Bruyne T, Lasure A, Van Poel B, Pieters L, Claeys M, Vanden Berghe D, Kambu K, Tona L, Vlietinck AJ. *Planta Med* 1996;62:22. [PubMed: 8720383]
- (28). Ablordeppey SY, Fan P, Clark AM, Nimrod A. *Bioorg. Med. Chem* 1999;7:343–349. [PubMed: 10218827]
- (29). Mardenborough LG, Fan P, Ablordeppey SY, Nimrod A, Clark AM. *Med. Chem. Res* 1999;9:118.
- (30). (a) Zhu XY, Mardenborough LG, Li S, Khan A, Zhang W, Fan P, Jacob MR, Khan SI, Walker LA, Ablordeppey SY. *Bioorg. Med. Chem* 2007;15:686. [PubMed: 17134906] (b) Bierer DE, Fort DM, Mendez CD, Luo J, Imbach PA, Dubenko LG, Jolad SD, Gerber RE, Litvak J, Lu Q, Zhang P, Reed MJ, Waldeck N, Bruening RC, Noamesi BK, Hector RF, Carlson TJ, King SR. *J. Med. Chem* 1998;41:894. [PubMed: 9526563] (c) Bierer DE, Dubenko LG, Zhang P, Lu Q, Imbach PA, Garofalo AW, Phuan P-W, Fort DM, Litvak J, Gerber RE, Sloan B, Luo J, Cooper R, Reaven GM. *J. Med. Chem* 1998;41:2754. [PubMed: 9667966]
- (31). Oyekan AO, Ablordeppey SY. *Med. Chem. Res* 1996;6:602.
- (32). Busari O, A. Adeyemi A, Agboola S. *The Internet J. Infect. Dis* 2009;7:1528.
- (33). Bicanic T, Harrison TS. *Br. Med. Bull* 2005;72:99. [PubMed: 15838017]
- (34). Mwaba P, Mwansa J, Chintu C, Pobee J, Scarborough M, Portsmouth S, Zumla A. *Postgrad. Med. J* 2001;77:769. [PubMed: 11723315]
- (35). Fleming AF. *Transactions of the Royal Soc. Of Trop. Med. and Hyg* 1990;84:1.
- (36). Etukala JR, Yadav JS. *Heteroatom Chem* 2008;19:221.
- (37). Rá dl S, Konvič ka P, Vá chal P. *J. Heterocyclic Chem* 2000;37:855.
- (38). Klapars A, Buchwald SL. *J. Am. Chem Soc* 2002;124:14844. [PubMed: 12475315]
- (39). Kwong FY, Buchwald SL. *Org. Lett* 2002;4:3517. [PubMed: 12323058]
- (40). (a) Hibberd PL, Rubin RH. *Clin. Infect. Dis* 1994;19(Suppl 1):S33. [PubMed: 7948569] (b) Bodey GP, Vartivarian S. *Eur. J. Clin. Microbiol Infect. Dis* 1989;8:413. [PubMed: 2502407] (c) Symoens F, Bertout S, Piens M-A, Burnod J, Renaud F, Nolard N, Chapuis F, Grillot R, the EBGA Network. *J. Heart Lung Transplant* 2001;20:970. [PubMed: 11557192] (d) Nucci M, Anaissie E. *Clinics in Chest Medicine* 2009;30:295. [PubMed: 19375636]
- (41). (a) Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. *N. Engl. J. Med* 1991;325:1274–1277. [PubMed: 1669837] (b) Krcmery V, Barnes AJ. *J. Hosp. Infect* 2002;50:243. [PubMed: 12014897] (c) Pfaller MA, Diekema DJ. *Clin. Microbiol. Infect* 2004;10(Suppl. 1):11. [PubMed: 14748799] (d) Pfaller MA, Diekema DJ. *J. Clin. Microbiol* 2004;42:4419. [PubMed: 15472288]
- (42). Wiederhold NP, Kontoyiannis DP, Prince RA, Lewis RE. *Antimicrob. Agents Chemother* 2005;49:5146. [PubMed: 16304189]
- (43). Buu-Hoi NP, Saint-Ruf G. *Isr. J. Chem* 1963;1:369–373.
- (44). Lee JY, Lee PH. *J. Org. Chem* 2008;73:7413–7416. [PubMed: 18712924]
- (45). (a) NCCLS. *National Committee on Clinical Laboratory Standards* 1998;18(13) (b) NCCLS. *National Committee on Clinical Laboratory Standards* 2000;20:2.

- (46). Samoylenko V, Jacob MR, Khan SI, Zhao J, Tekwani BL, Midiwo JO, Walker LA, Muhammad I. Natural Product Communications 2009;4:791. [PubMed: 19634324]
- (47). Mustafa J, Khan SI, Ma G, Walker LA, Khan IA. Lipids 2006;40:375. [PubMed: 16028720]
- (48). Madhu C, Zoltan G, Klaassen CD. J. Chromatography Bio. Appl 1995;674:193.
- (49). Corasaniti MT, Strongoli MC, Nistico G. J. Chromatogr Biomed. Appl 1990;527:189.
- (50). Chambers E, Wagrowski-Diehl DM, Lu Z, Mazzeo JR. J. Chromatogr B 2007;852:22.

Fig 1.
Effect of **10f** on *C neoformans* infection in mice.

Fig 2.
Reduction of *C. neoformans* infection by **10f** in mouse brain.

Fig 3.
Effect of **10b** on *C. albicans* infection in mice

Fig 4.
Reduction of *C. albicans* infection by **10b** in the kidney

Fig 5. HPLC chromatograms of extracts from a perfused brain of a **10f**-dosed mouse (above), a blank brain from an untreated mouse (below, left) and a mass spectrum of the extract (below, right).

Fig 6.
HPLC Chromatograms of perfused brain extracts of **10f**-dosed mouse [**6A**] and a spiked extract [**6B**].

Scheme 1.

Synthetic Procedure for Quinoline-substituted Benzothienoquinolinium Compounds

Reagents and Conditions: a) NaOH, H₂O, rt. b) PPA, 130 °C, 3 h. c) POCl₃, 110 °C, 3 h. d) 10 % Pd/C, EtOAc/AcOH, NaOAc, H₂ gas. e) Py. HCl, 175 °C, 2 h. f) MeOTf, toluene, rt, 24 h. g) CH₃I, Sulfone, 110 ° C. Reflux for 12 h.

Scheme 2.

Synthetic Procedure for benzothiophene-substituted Benzo[h]thienoquinolines

Reagents and Conditions: a) DMF: 1, 4-dioxane (1:1), r.t, 24 h. b) Acetone, K₂CO₃, KI, reflux, 24 h. c) PPA, 130 °C, 3 h. d) POCl₃, 110 °C, 3 h. e) 10 % Pd/C, EtOAc/AcOH, NaOAc, H₂ gas. f) CH₃I, Sulfone, 110 °C. Reflux for 24 h.

Scheme 3.

Synthesis of Substituted 3-(Phenylthio)quinolinium Salts Reagents and Conditions: (i) CuI, CH₃NHCH₂CH₂NHCH₃, NaI, dioxane, reflux under N₂ 110 °C, 48h; (ii) Benzenethiol, CuI, HOCH₂CH₂OH, K₂CO₃, *i*PrOH, reflux under N₂ 110 °C, 48h. (iii) C₆H₅(CH₂)₄CH₂-I (PP-I) or C₆H₁₁(CH₂)₄CH₂-I (CP-I) or CH₃-I, Sulfone, 110 °C, 12-16h.

Chart 1.

Table 1

Physicochemical Data and Antifungal Activities of 5-Methylbenzothieno[3,2-*b*]quinolinium Analogs

Compd	R	Y	MP (°C) ^b	Empirical ^c Formula	IC ₅₀ /MIC μg/mL			TC ₅₀ μg/mL
					<i>C. neoformans</i>	<i>C. albicans</i>	<i>A. fumigatus</i>	
2a ^a	H	OTf	237-238	C ₁₇ H ₁₂ F ₃ NO ₃ S ₂ ·0.2H ₂ O	>15/>20	10/>20	6.5/>20	>23.8
2b ^a	H	I	215-216	C ₁₆ H ₁₂ INS	NT/>20(2.5) ^d	NT7.8/(1.0) ^d	NT/>20	>23.8
10d	1-Cl	OTf	285-288	C ₁₇ H ₁₁ NO ₃ S ₂ Cl	NA	NA	3/10	NT
10h ^a	2-Cl	I	189.3	C ₁₆ H ₁₁ ClINS·0.2H ₂ O	15/>20	2.0/>20	NT/2.5	>23.8
10e	3-Cl	OTf	220-221	C ₁₇ H ₁₁ NO ₃ S ₂ Cl	10/>20	NA	NA	>10
10f	4-Cl	OTf	225-226	C ₁₇ H ₁₁ NO ₃ S ₂ Cl	4.0/10	NA	6.0/10.0	>10
18d	7-Cl	I	203-204	C ₁₆ H ₁₁ ClINS	NA	20/>20	NA	>10
18e	8-Cl	I	218-219	C ₁₆ H ₁₁ ClINS·0.4 H ₂ O	NA	2.5/>20	9.5/>20	>10
18f	9-Cl	I	192-193	C ₁₆ H ₁₁ ClINS·0.35 CH ₃ OH	15/>20	>20	6.5/10	NT
11a	1-OMe	I	216-217	C ₁₇ H ₁₄ NOSI·0.1 H ₂ O	NA	15/>20	1.5/2.5	NT
10a	1-OMe	OTf	225-227	C ₁₈ H ₁₄ NO ₄ S ₂ F ₃ ·0.65 H ₂ O	NA	15/>20	0.8/1.3	>4.7
10j ^a	2-OMe	I	216-217	C ₁₇ H ₁₄ INOS·0.5H ₂ O	NT/8-15	>20	20/ND	>23.8
10b	3-OMe	OTf	222-223	C ₁₈ H ₁₄ NO ₄ S ₂ F ₃ ·0.25 H ₂ O	6.0/>20	1.5/10	0.4/0.6	>10
10c	4-OMe	OTf	180-181	C ₁₈ H ₁₄ NO ₄ S ₂ F ₃ ·0.1 H ₂ O	15/>20	15/>20	3.0/5.0	NT
10g	1-OH	OTf	208-209	C ₁₇ H ₁₂ NO ₄ S ₂ F ₃ ·0.25 EtOAc	NA	NA	NA	NT
18a	7-OMe	I	199-200	C ₁₇ H ₁₄ NOSI·0.2 H ₂ O	NA	NA	8.0/20	NT
18b	8-OMe	I	228-229	C ₁₇ H ₁₄ NOSI	NA	6.0/20	1.0/2.5	NT
18c	9-OMe	I	200-201	C ₁₇ H ₁₄ NOSI	10/>20	7.0/>20	3.0/5.0	>10
Amphotericin B					1.5/2.5	0.3/0.6	1.5/2.5	6.5

Abbreviations: NT = Not Tested. NA = Not active at 20 µg/mL; IC₅₀ = The concentration that affords 50% inhibition of growth; MIC = Minimum inhibitory concentration, is the lowest test concentration that allows no detectable growth.

^aPreviously reported.³⁰

^bMelting points were uncorrected

^cAll compounds were subjected to CHN analysis and each passed within 0.4 % of the theoretical value.

^dData in brackets previously published.^{30a}

Table 2

Antifungal activities of N-Alkyl 3-Substituted Phenylthioquinolinium Analogs.

Cpd	R	R ₁	IC ₅₀ /MIC/MFC (μg/mL)					TC ₅₀ (μg/mL)
			<i>C. neoformans</i>	<i>C. albicans</i>	<i>C. krusei</i>	<i>A. fumigatus</i>	Vero cells	
22a	H	Me	15/20/>20	6.0/20/>20	NT	NT/>2.5/20	NT	NT
22b	H	PP	1.5/5.0/10	5.5/10/>20	1.5/5.0/20	NT/1.3/>20	NT	NT
22c	H	CP	0.5/1.3/2.5	2.7/5.0/10	0.7/1.3/2.5	8.6/10/>20	4.6	4.6
Cryp			12.5 (MIC)	250 (MIC)	NT	NT	3.2	3.2
2b			NT/>20/>20	NT/7.8/>20	NT	NA	>23.8	>23.8
AmB			1.5/2.5/2.5	0.3/0.6/1.3	0.5/1.3/1.3	1.5/2.5/5.0	6.5	6.5

Abbreviations: AmB = Amphotericin B; Cryp = Cryptolepine; NT = Not Tested. NA = not active at 20 μg/mL; PP = 5-Phenylpentyl-; CP = 5-Cyclohexylpentyl-; IC₅₀ = 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 microorganism. TC₅₀ = The concentration that is toxic to 50% of cells.