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Benzothieno[3,2-*b*]quinolinium and 3-(phenylthio)quinolinium compounds: Synthesis and evaluation against opportunistic fungal pathogens

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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.

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1. 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 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 cryptolepine[22–29] (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,

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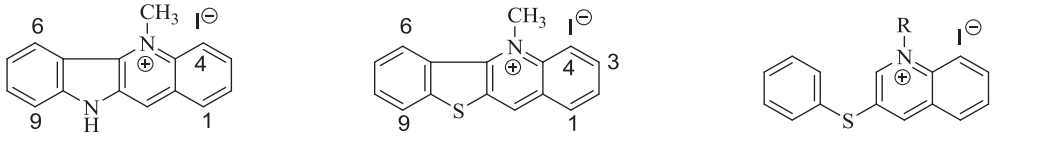


Chart 1.

two common opportunistic infections associated particularly with AIDS.[32–35]

2. Results and discussion

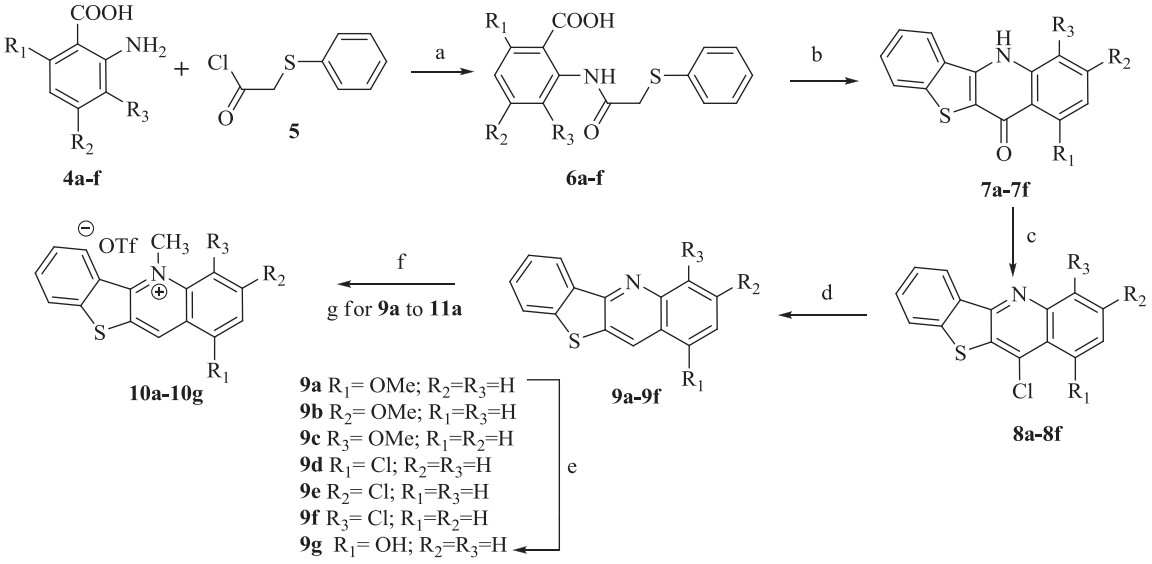
2.1. Synthesis

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-phenylsulfanylacetylamino)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 $POCl_3$ 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.

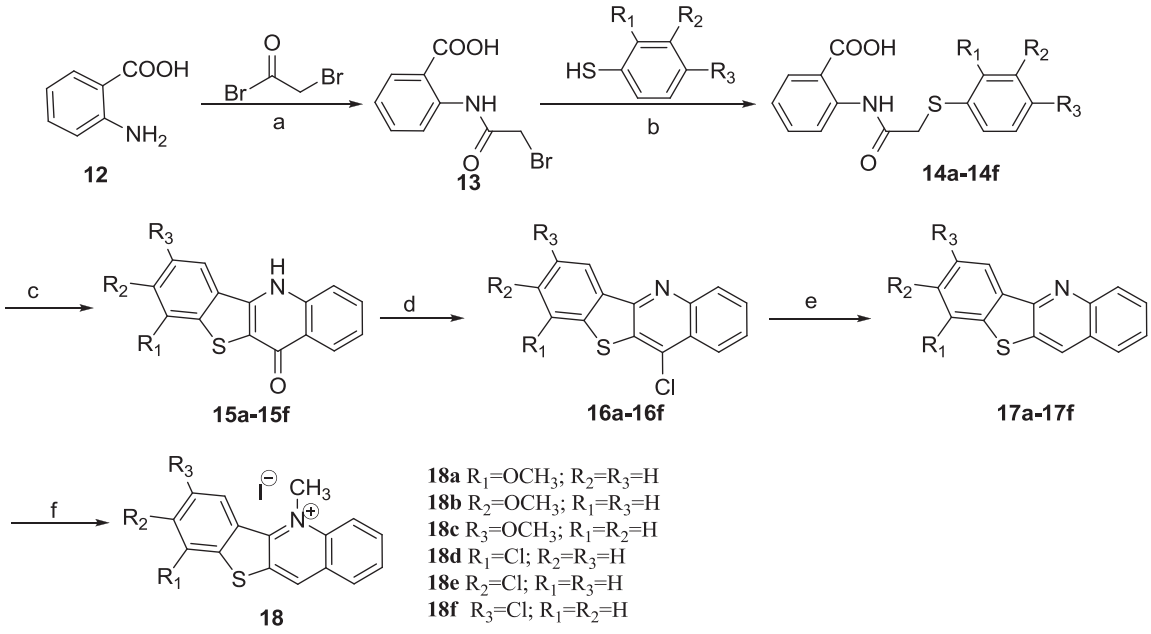
Synthesis of 1-substituted-3-(phenylthio)quinolinium iodides (**3**) required 3-iodoquinoline (**20**) as the starting material (Scheme 3). A Finkelstein reaction was applied to 3-bromoquinoline to exchange the bromide for the desired iodide.[38] Using a copper-catalyzed carbon–sulfur bond formation approach, benzenethiols was 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.

2.2. Biological evaluation

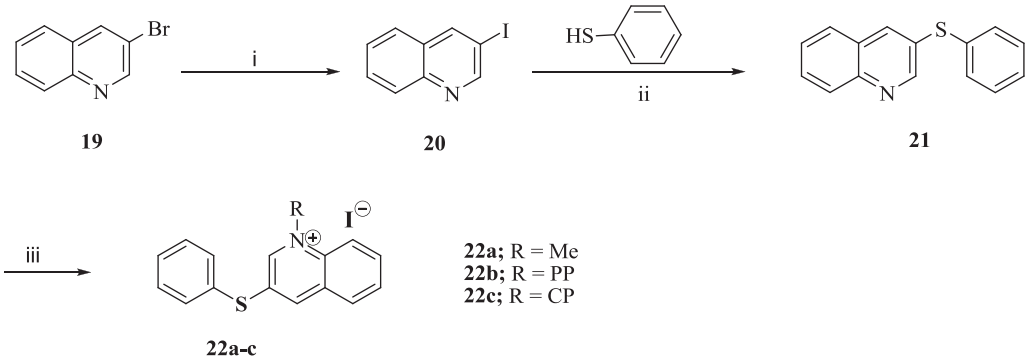
Previous studies in our labs have revealed that substitution on the ring of cryptolepine (**1**) enhanced its potency and broadened its anti-opportunistic activity profile. Further optimization led to the identification of the sulfur isostere (**2**) of cryptolepine as a potential anti-opportunistic 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



Scheme 1. Synthetic procedure for quinoline-substituted benzothienoquinolinium compounds. Reagents and conditions: (a) NaOH, H_2O, rt; (b) PPA, 130 °C, 3 h; (c) $POCl_3$, 110 °C, 3 h; (d) 10% Pd/C, EtOAc/AcOH, NaOAc, H_2 gas; (e) Py. HCl, 175 °C, 2 h; (f) MeOTf, toluene, rt, 24 h; (g) CH_3I, sulfone, 110 °C. Reflux for 12 h.



Scheme 2. Synthetic procedure for benzothiophene-substituted benzothienoquinolines. Reagents and conditions: (a) DMF/1,4-dioxane (1:1), rt, 24 h; (b) acetone, K_2CO_3, KI, reflux, 24 h; (c) PPA, 130 °C, 3 h; (d) $POCl_3$, 110 °C, 3 h; (e) 10% Pd/C, EtOAc/AcOH, NaOAc, H_2 gas; (f) CH_3I, sulfone, 110 °C. Reflux for 24 h.



Scheme 3. Synthesis of substituted 3-(phenylthio)quinolinium salts. Reagents and conditions: (i) CuI, $CH_3NHCH_2CH_2NHCH_3$, NaI, dioxane, reflux under N_2, 110 °C, 48 h; (ii) benzenethiol, CuI, $HOCH_2CH_2OH$, K_2CO_3, *i*PrOH, reflux under N_2, 110 °C, 48 h; (iii) $C_6H_5(CH_2)_4CH_2$-I (PP-I) or $C_6H_{11}(CH_2)_4CH_2$-I (CP-I) or CH_3I, sulfone, 110 °C, 12–16 h.

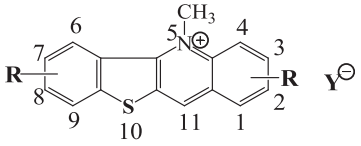
to use a chlorine atom to probe all synthetically accessible positions on **2**. Thus, compounds **10d–f** and **18d–f** were synthesized and evaluated against *C. neoformans*, *C. albicans*, and *A. fumigatus*. Substitution at position 6 was unsuccessful as ring closure consistently occurred on the 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.

2.3. 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 IC_{50} and MIC values are between 1 and 10 μg/mL, the compounds are considered active, moderately active between 10–15 μg/mL and weak above 15 up to 20 μg/mL. Most of the compounds show only weak activities against *C. neoformans* (Cn) and *C. albicans* (Ca) except for compound **10f** (IC_{50} = 4.0 and MIC = 10 μg/mL against Cn) and **10b** (IC_{50} = 1.5, MIC = 10 μg/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 μg/mL and in fact most of them showed no cytotoxicity at 10 μg/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-phenylthio-quinolinium salt which demonstrated activity against opportunistic pathogens. Compound **22a**, the unsubstituted *N*-methyl-3-phenylthioquinolinium salt,

Table 1
Physicochemical data and antifungal activities of 5-methylbenzothieno[3,2-*b*]quinolinium analogs



Compd	R	Y	Mp[b] (°C)	Empirical formula[c]	IC_{50}/MIC μg/mL			TC_{50} μg/mL
					C. neoformans	*C. albicans*	*A. fumigatus*	Vero cells
2a[a]	H	OTf	237–238	$C_{17}H_{12}F_3NO_3S_2{\cdot}0.2H_2O$	>15/>20	10/>20	6.5/>20	>23.8
2b[a]	H		215–216	$C_{16}H_{12}INS$	NT/>20/(2.5)[d]	NT/7.8/(1.0)[d]	NT/>20	>23.8
10d	1-Cl	IOTf	285–288	$C_{17}H_{11}NO_3S_2Cl$	NA	NA	3/10	NT
10h[a]	2-Cl	I	189.3	$C_{16}H_{11}ClINS{\cdot}0.2H_2O$	15/>20	2.0/>20	NT/2.5	>23.8
10e	3-Cl	OTf	220–221	$C_{17}H_{11}NO_3S_2Cl$	10/>20	NA	NA	>10
10f	4-Cl	OTf	225–226	$C_{17}H_{11}NO_3S_2Cl$	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	$C_{16}H_{11}ClINS{\cdot}0.4H_2O$	NA	2.5/>20	9.5/>20	>10
18f	9-Cl	I	192–193	$C_{16}H_{11}ClINS{\cdot}0.35CH_3OH$	15/>20	>20	6.5/10	NT
11a	1-OMe	I	216–217	$C_{17}H_{14}NOSI{\cdot}0.1H_2O$	NA	15/>20	1.5/2.5	NT
10a	1-OMe	OTf	225–227	$C_{18}H_{14}NO_4S_2F_3{\cdot}0.65H_2O$	NA	15/>20	0.8/1.3	>4.7
10i[a]	2-OMe	I	216–217	$C_{17}H_{14}INOS{\cdot}0.5H_2O$	NT/8–15	>20	20/ND	>23.8
10b	3-OMe	OTf	222–223	$C_{18}H_{14}NO_4S_2F_3{\cdot}0.25H_2O$	6.0/>20	1.5/10	0.4/0.6	>10
10c	4-OMe	OTf	180–181	$C_{18}H_{14}NO_4S_2F_3{\cdot}0.1H_2O$	15/>20	15/>20	3.0/5.0	NT
10g	1-OH	OTf	208–209	$C_{17}H_{12}NO_4S_2F_3{\cdot}0.25EtOAc$	NA	NA	NA	NT
18a	7-OMe	I	199–200	$C_{17}H_{14}NOSI{\cdot}0.2H_2O$	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

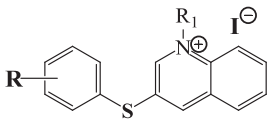
Abbreviations: NT = not tested. NA = not active at 20 μg/mL; IC_{50} = the concentration that affords 50% inhibition of growth; MIC = minimum inhibitory concentration, is the lowest test concentration that allows no detectable growth.
[a] Previously reported.[30]
[b] Melting points were uncorrected.
[c] All compounds were subjected to CHN analysis and each passed within 0.4% of the theoretical value.
[d] Data in brackets previously published.[30a]

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 potency[28] 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-phenylthio-quinolinium scaffold have the potential of serving as new anti-opportunistic infection agents warranting further investigation and development. These are currently on-going.

2.4. In vivo evaluation

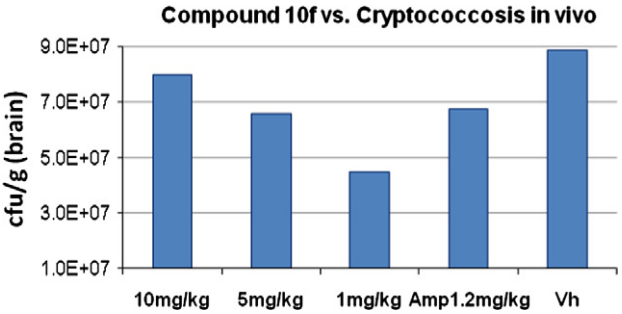
Prior to in vivo efficacy studies, maximum tolerated dose (MTD) studies were carried out to determine the highest dose that could

Table 2
Antifungal activities of *N*-alkyl 3-substituted phenylthioquinolinium analogs



Compd	R	R_1	IC_{50}/MIC/MFC (μg/mL)				TC_{50} (μg/mL)
			C. neoformans	*C. albicans*	*C. krusei*	*A. fumigatus*	Vero cells
22a	H	Me	15/20/>20	6.0/20/>20	NT	NT/>2.5/20	NT
22b	H	PP	1.5/5.0/10	5.5/10/>20	1.5/5.0/20	NT/1.3/>20	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
Cryp			12.5 (MIC)	250 (MIC)	NT	NT	3.2
2b			NT/>20/>20	NT/7.8/>20	NT	NA	>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

Abbreviations: AmB = amphotericin B; Cryp = cryptolepine; NT = not tested. NA = not active at 20 μg/mL; PP = 5-phenylpentyl-; CP = 5-cyclohexylpentyl-; 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 microorganism. TC_{50} = the concentration that is toxic to 50% of cells.



Drug dose	CFU/gm Brain
10 mg/kg	$7.9x10^7 \pm 1.9x10^7$
5 mg/kg	$6.58x10^7 \pm 3.2 x10^7$
1.0 mg/kg	$4.47x10^7 \pm 1.0 x10^7$
Amphotericin B 1.2 mg/kg	$6.78x10^7 \pm 2.9 x10^7$
Vehicle	$8.88x10^7 \pm 3.9 x10^7$

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

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 five groups of five each and beginning 24 h 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 ip once a day for 5 days using doses ranging from 1 mg/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 h 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.

Figures 1 and 2 show the effect of various doses of **10f** against *C. neoformans* infection in mice. In Figure 1, the CFU per gram of brain sample is displayed for each dose while Figure 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 caspofungin[42] (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.

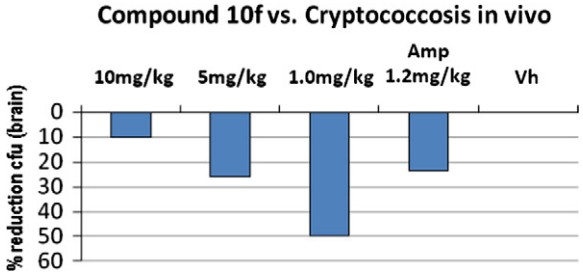
2.5. Murine model of systemic candidiasis

2.5.1. Tissue burden

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

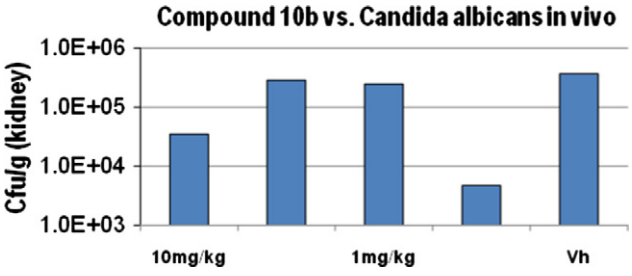
2.5.2. Maximum tolerated dose (MTD) of 10b

The compound was dissolved in DMSO/PEG/PBS (1:3:3) and injected ip 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.



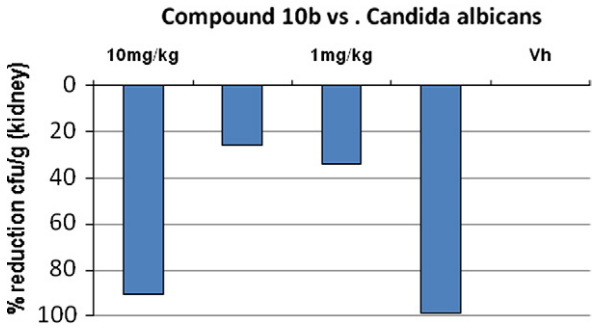
Drug dose	% Reduction CFU/gm Brain
10 mg/kg	10
5 mg/kg	26
1.0 mg/kg	50
Amphotericin B 1.2 mg/kg	24
Vehicle	0

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



Drug dose	CFU/gm Kidney
10 mg/kg	$5.39x10^4 \pm 1.7 x10^4$
5 mg/kg	$2.83x10^5 \pm 5.7x10^4$
1 mg/kg	$2.50 x10^5 \pm 1.1 x10^5$
Amphotericin B 1.2 mg/kg	$4.76x10^3 \pm 7.5x10^2$
Vehicle	$3.81x10^5 \pm 1.1 x10^5$

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



Drug dose	% Reduction CFU/gm Kidney
10 mg/kg	86
5 mg/kg	26
1 mg/kg	34
Amphotericin B 1.2 mg/kg	99
Vehicle	0

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

2.5.3. Candidiasis tissue burden

Figures 3 and 4 show the effect of **10b** against candidiasis in vivo. In Figure 3, the CFU per gram of kidney sample is displayed for each dose while Figure 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 1 mg/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 min post injection, mice were anesthesized, 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 Figure 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 $HClO_4$/MeOH), treated with a sonic dismembrator, refrigerated, centrifuged at 10,000 g for 5 min, and the supernatant concentrated to 300 μL. 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**.

3. 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.

4. Experimental section

4.1. 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

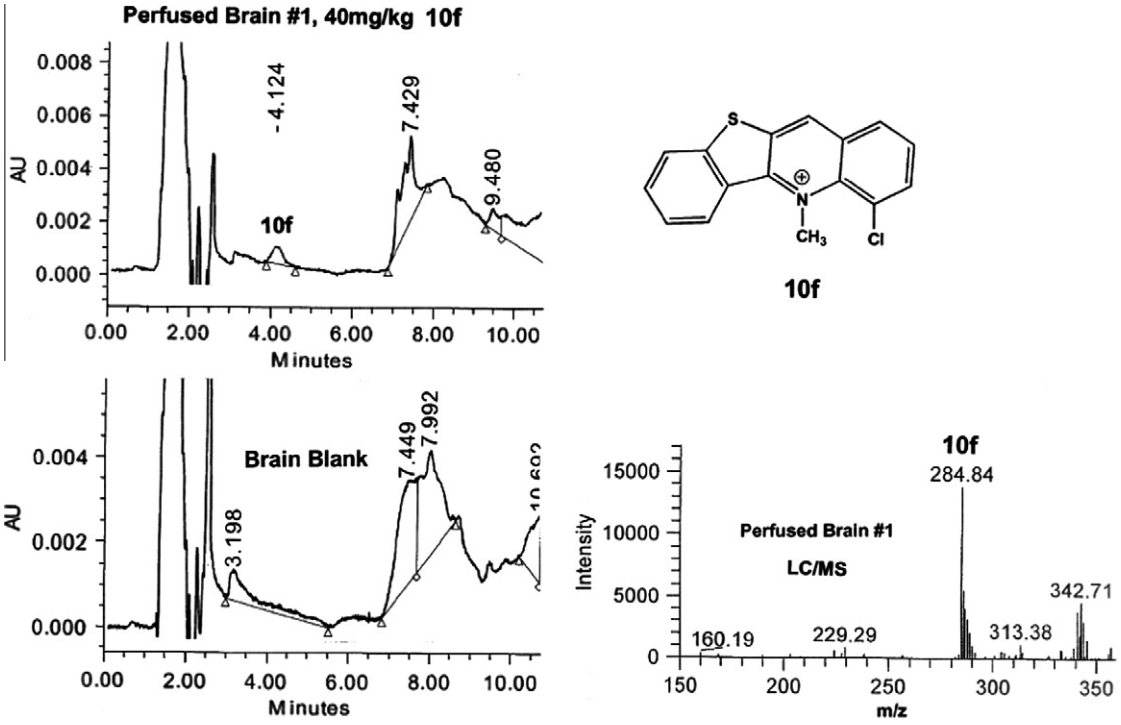


Figure 5. HPLC chromatograms of extracts from a perfused brain of a **10f**-dosed mouse (above), a blank brain from an untreated mouse (below, left) and a mass spectrum of the extract (below, right).

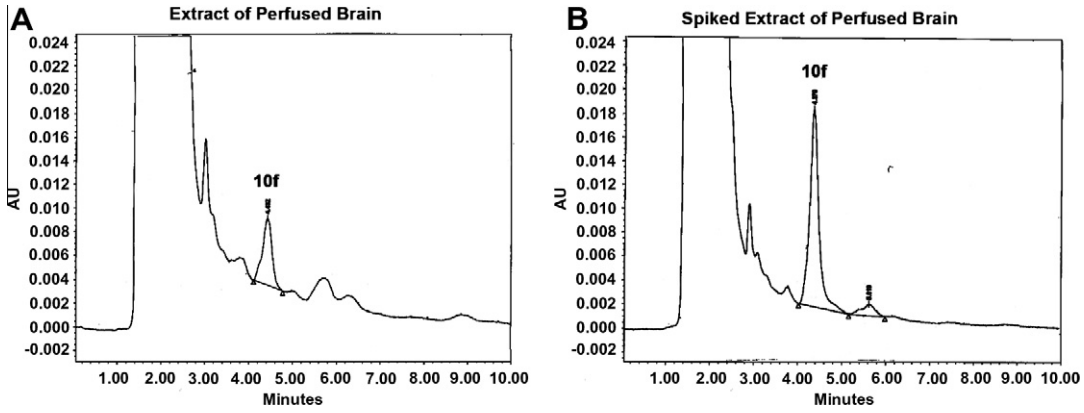


Figure 6. HPLC chromatograms of perfused brain extracts of **10f**-dosed mouse [**6A**] and a spiked extract [**6B**].

Merck TLC plates coated with Silica Gel 60 F_{254} (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 (^{1}H 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.

4.2. General procedure for the synthesis of substituted 6-(2-phenylsulfanyl-acetylamino)-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 H_2O (20 mL) was stirred at 0 °C for 1 h, and then at rt for 2 h. 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 Na_2SO_4, filtered, and solvent was evaporated in vacuo. The residue obtained was recrystallized using EtOAc/hexane (3:1) to give compounds **6a–f** as white solids.

4.2.1. 1-Methoxy-6-(2-phenylsulfanyl-acetylamino)-benzoic acid (6a)

Yield 67%. Mp 97–98 °C. ^{1}H NMR ($CDCl_3$): δ 11.56 (br s, 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).

4.2.2. 3-Methoxy-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6b)

Yield 82%. Mp 188–189 °C. ^{1}H NMR ($CDCl_3$): δ 11.92 (br s, 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).

4.2.3. 4-Methoxy-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6c)

Yield 85%. Mp 167–168 °C. ^{1}H 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).

4.2.4. 1-Chloro-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6d)

Yield 82%. Mp 153–154 °C. ^{1}H NMR ($CDCl_3$): δ 10.25 (br s, 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).

4.2.5. 3-Chloro-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6e)

Yield 82%. Mp 161–163 °C.^{1}H 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).

4.2.6. 4-Chloro-6-(2-phenylsulfanyl-acetylamino)benzoic acid (6f)

Yield 86%. Mp 178–179 °C. ^{1}H 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).

4.3. 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 3 h. 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.

4.3.1. 1-Methoxybenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7a)

Yield 95% (crude). ^{1}H NMR (DMSO-d_6): δ 12.53 (br s, 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).

4.3.2. 3-Methoxybenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7b)

Yield 95% (crude). ^{1}H NMR (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).

4.3.3. 4-Methoxybenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7c)

Yield 84% (crude). ^{1}H NMR (DMSO-d_6): δ 11.9 (br s, 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).

4.3.4. 1-Chlorobenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7d)

Yield 92% (crude). ^{1}H NMR (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).

4.3.5. 3-Chlorobenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7e)

Yield 92% (crude). ^{1}H NMR (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).

4.3.6. 4-Chlorobenzothieno[3,2-*b*]quinolin-11(5*H*)-one (7f)

Yield 94% (crude). ^{1}H NMR (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).

4.4. 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–f**.

4.4.1. 1-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (8a)

Yield 92%. Mp 192–193 °C. ^{1}H NMR (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).

4.4.2. 3-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (8b)

Yield 78%. Mp 209–210 °C. ^{1}H 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).

4.4.3. 4-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (8c)

Yield 94%. Mp 201–202 °C. ^{1}H NMR (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).

4.4.4. 1,11-Dichlorobenzothieno[3,2-*b*]quinoline (8d)

Yield 64%. Mp 200–201 °C. ^{1}H 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).

4.4.5. 3,11-Dichlorobenzothieno[3,2-*b*]quinoline (8e)

Yield 71%. ^{1}H 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).

4.4.6. 4,11-Dichlorobenzothieno[3,2-*b*]quinoline (8f)

Yield 72%. Mp 182 °C. ^{1}H 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).

4.5. General procedure for the synthesis of methoxy-substituted benzothieno[3,2-*b*]quinolines 9a–c, (Method D)

Methoxy-substituted compounds, **8a–c** (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.

4.5.1. 1-Methoxybenzothieno[3,2-*b*]quinolines (9a)

Yield 68%. Mp 190–191 °C. ^{1}H 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).

4.5.2. 3-Methoxybenzothieno[3,2-*b*]quinolines (9b)[43]

Yield 88%. Mp 148–149 °C. ^{1}H 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).

4.5.3. 4-Methoxybenzothieno[3,2-*b*]quinolines (9c)

Yield 78%. Mp 164–165 °C. ^{1}H 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).

4.6. 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 h. 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.

4.6.1. 1-Chlorobenzothieno[3,2-*b*]quinolines (9d)

Yield 72%. Mp 173–174 °C. ^{1}H NMR ($CDCl_3$): 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).

4.6.2. 3-Chlorobenzothieno[3,2-*b*]quinolines (9e)

Yield 42%. ^{1}H NMR ($CDCl_3$): 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).

4.6.3. 4-Chlorobenzothieno[3,2-*b*]quinolines (9f)

Yield 52%. Mp 146.0–146.5 °C. ^{1}H NMR ($CDCl_3$): 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).

4.6.4. 1-Hydroxybenzothieno[3,2-*b*]quinoline (9g)

Compound **9a** (0.53 g, 2 mmol) was added to preheated (150 °C) pyridinium hydrochloride (10 g) and the mixture was further heated at 175 °C for 6 h. The reaction mixture was allowed to cool to rt and H_2O (100 mL) was added. The solid obtained was filtered, washed with H_2O (50 mL) and recrystallized using EtOAc/hexane (4:1) to give a yellow solid. Yield 96%. Mp 164–165 °C. ^{1}H NMR (DMSO-d_6): δ 9.86 (br s, 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).

4.7. 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 Et_2O and recrystallized from either MeOH-Et_2O 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.

4.7.1. 1-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10a)

Yield 72%. Mp 225–227 °C. ^{1}H NMR (CD_3OD): 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 $C_{18}H_{14}F_3NO_4S_2{\cdot}0.65H_2O$: C, 49.01; H, 3.20; N, 3.18. Found: C, 49.03; H, 3.21; N, 3.06.

4.7.2. 3-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10b)

Yield 68%. Mp 221–223 °C. ^{1}H NMR (CD_3OD): 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 $C_{18}H_{14}F_3NO_4S_2{\cdot}0.25H_2O$: C, 49.82; H, 3.25; N, 3.23. Found: C, 49.82; H, 3.27; N, 3.11.

4.7.3. 4-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10c)

Yield 28%. Mp 180–181 °C. ^{1}H NMR (CD_3OD): 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 $C_{18}H_{14}F_3NO_4S_2{\cdot}0.1H_2O$: C, 50.13; H, 3.32; N, 3.25. Found: C, 49.85; H, 3.22; N, 3.10.

4.7.4. 1-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10d)

Yield 62%. Mp 285–287 °C. ^{1}H NMR (CD_3OD): 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 $C_{17}H_{11}ClF_3NO_3S_2$: C, 47.06; H, 2.56; N, 3.23. Found: C, 46.96; H, 2.47; N, 3.21.

4.7.5. 3-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10e)

Yield 65%. Mp 220–221 °C. ^{1}H NMR (CD_3OD): 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 $C_{17}H_{11}ClF_3NO_3S_2{\cdot}0.2H_2O$: C, 46.68; H, 2.53; N, 3.20. Found: C, 46.58; H, 2.52; N, 3.12.

4.7.6. 4-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10f)

Yield 46%. Mp 225–226 °C. ^{1}H NMR (CD_3OD): 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). ^{13}C 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 $C_{17}H_{11}ClF_3NO_3S_2$: C, 47.06; H, 2.56; N, 3.23. Found: C, 46.90; H, 2.59; N, 3.25.

4.7.7. 4-Hydroxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium triflate (10g)

Yield 68%. Mp 208–209 °C. ^{1}H NMR (CD_3OD): 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 $C_{17}H_{12}F_3NO_4S_2{\cdot}0.25$EtOAc: C, 49.42; H, 3.23; N, 3.20. Found: C, 49.72; H, 3.29; N, 3.37.

4.8. General procedure for the synthesis of 1-methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (11a), (Method G)

CH_3I (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 h in a sealed pressure tube. After cooling to rt, Et_2O was added to form a precipitate. The precipitate was collected, washed with Et_2O (25 mL) and recrystallized from MeOH–Et_2O as a yellow solid. Yield 82%. Mp 216–217 °C. ^{1}H NMR (CD_3OD): 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 $C_{17}H_{14}INOS{\cdot}0.1H_2O$: C, 49.70; H, 3.48; N, 3.41. Found: C, 49.69; H, 3.50; N, 3.40.

4.9. 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. ^{1}H NMR: (CD_3OD): 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).

4.10. General procedure for the synthesis of substituted 2-(2-phenylsulfanylacetylamido)benzoic acids 14a–f, (Method I)

A mixture of substituted benzenethiol (38 mmol), K_2CO_3 (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 H_2O (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.

4.10.1. 2-(2-(2-Methoxyphenylsulfanyl)acetylamido)benzoic acid (14a)

Yield 86%. Mp 172–173 °C. ^{1}H NMR (CD_3OD): 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).

4.10.2. 2-(2-(3-Methoxyphenylsulfanyl)acetylamido)benzoic acid (14b)

Yield 78%. Mp 167–169 °C. ^{1}H NMR (CD_3OD): 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).

4.10.3. 2-(2-(4-Methoxyphenylsulfanyl)acetylamido)benzoic acid (14c)

Yield 72%. Mp 175–176 °C; ^{1}H NMR (CD_3OD): 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).

4.10.4. 2-(2-(2-Chlorophenylsulfanyl)acetylamido)benzoic acid (14d)

Yield 95%. Mp 171–173 °C. ^{1}H NMR (CD_3OD): 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).

4.10.5. 2-(2-(3-Chlorophenylsulfanyl)acetylamido)benzoic acid (14e)

Yield 96%. Mp 167–168 °C. ^{1}H NMR (CD_3OD): 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).

4.10.6. 2-(2-(4-Chlorophenylsulfanyl)acetylamino)benzoic acid (14f)

Yield 95%. Mp 183–184 °C. ^{1}H NMR (CD_3OD): 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).

4.11. Substituted benzothieno[3,2-*b*]quinoline-11(5*H*)-ones 15[a]–15f using General Method B

Compound **6** (20 mmol) was mixed with PPA (125 g) and heated at 130–135 °C for 3 h. 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.

4.11.1. 9-Methoxybenzothieno[3,2-*b*]quinoline-11(5*H*)-one (15a)

Yield 52%. Mp 225–227 °C. ^{1}H 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).

4.11.2. 8-Chlorobenzothieno[3,2-*b*]quinoline-11(5*H*)-one (15e)

Yield 48%. Mp 217–219 °C. ^{1}H 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).

^{1}H NMR spectra of compounds **15b–d and f** could not be obtained because they were insoluble in DMSO/MeOH.

4.12. Substituted 11-chlorobenzothieno[3,2-*b*]quinolines 16a–e, using General Method C

4.12.1. 9-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (16a)

Yield 68%. Mp 200–201 °C. ^{1}H NMR ($CDCl_3$): 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).

4.12.2. 8-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (16b)

Yield 54%. Mp 199–200 °C. ^{1}H 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).

4.12.3. 7-Methoxy-11-chlorobenzothieno[3,2-*b*]quinoline (16c)

Yield 56%. Mp 207–209 °C. ^{1}H NMR ($CDCl_3$): 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).

4.12.4. 9,11-Dichlorobenzothieno[3,2-*b*]quinoline (16d)

Yield 63%. Mp 221–222 °C. ^{1}H NMR ($CDCl_3$): 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).

4.12.5. 8, 11-Dichlorobenzothieno[3,2-*b*]quinoline (16e)

Yield 59%. Mp 252–253 °C. ^{1}H NMR ($CDCl_3$): 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).

4.12.6. 7,11-Dichlorobenzothieno[3,2-*b*]quinoline (16f)

Yield 45%. Mp 69–71 °C. ^{1}H NMR ($CDCl_3$): 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).

4.13. General procedure for the synthesis of methoxy-substituted benzothieno[3,2-*b*]quinolines 17a–c, (Method D)

4.13.1. 9-Methoxybenzothieno[3,2-*b*]quinolines (17a)

Yield 62%. Mp 168–169 °C. ^{1}H NMR ($CDCl_3$): 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).

4.13.2. 8-Methoxybenzothieno[3,2-*b*]quinolines (17b)[43]

Yield 54%. Mp 185–186 °C. ^{1}H NMR ($CDCl_3$): 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).

4.13.3. 7-Methoxybenzothieno[3,2-*b*]quinolines (17c)

Yield 56%. Mp 176–178 °C. ^{1}H NMR ($CDCl_3$): 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).

4.14. General procedure for the synthesis of chloro-substituted benzothieno[3,2-*b*]quinolines 17d–f, (Method E)

4.14.1. 9-Chlorobenzothieno[3,2-*b*]quinoline (17d)

Yield 58%. Mp 179–180 °C. ^{1}H NMR ($CDCl_3$): 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).

4.14.2. 8-Chlorobenzothieno[3,2-*b*]quinoline (17e)

Yield 52%. Mp 252–253 °C. ^{1}H NMR (CD_3OD): 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).

4.14.3. 7-Chlorobenzothieno[3,2-*b*]quinoline (17f)

Yield 46%. Mp 190–192 °C. ^{1}H 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).

4.15. Substituted 5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodides 18–f were synthesized using General Method E

4.15.1. 9-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18a)

Yield 68%. Mp 200–201 °C. ^{1}H 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 $C_{17}H_{14}INOS$: C, 50.13; H, 3.46; N, 3.44. Found: C, 50.10; H, 3.46; N, 3.42.

4.15.2. 8-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18b)

Yield 62%. Mp 228–229 °C. ^{1}H 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 $C_{17}H_{14}INOS$: C, 50.13; H, 3.46; N, 3.44. Found: C, 50.01; H, 3.39; N, 3.43.

4.15.3. 7-Methoxy-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18c)

Yield 78%. Mp 199–200 °C. ^{1}H 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 $C_{17}H_{14}INOS{\cdot}0.2H_2O$: C, 49.26; H, 3.50; N, 3.38. Found: C, 49.27; H, 3.41; N, 3.33.

4.15.4. 7-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18f)

Yield 56%. Mp 203–204 °C. ^{1}H 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 $C_{16}H_{11}ClINS$: C, 46.68; H, 2.69; N, 3.40. Found: C, 46.39; H, 2.55; N, 3.30.

4.15.5. 8-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18e)

Yield 61%. Mp 218–219 °C. ^{1}H 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 $C_{16}H_{11}ClINS{\cdot}0.4H_2O$: C, 45.88; H, 2.84; N, 3.34. Found: C, 45.81; H, 2.60; N, 3.38.

4.15.6. 9-Chloro-5-methylbenzothieno[3,2-*b*]quinolin-5-ium iodide (18d)

Yield 26%. Mp 192–193 °C. ^{1}H 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 $C_{16}H_{11}ClINS{\cdot}0.35CH_3OH$: C, 45.24; H, 2.88; N, 3.23. Found: C, 44.90; H, 2.56; N, 3.22.

4.16. 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 × 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. ^{1}H 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).

4.17. 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 30 h. 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. ^{1}H NMR ($CDCl_3$): δ 8.81 (s, 1H), 8.08–8.06 (m, 2H), 7.71–7.25 (m, 8H).

4.18. 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

4.18.1. 1-Methyl-3-(phenylthio)quinolinium iodide (22a)

Yield 81%. Mp 145–146. ^{1}H 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 $C_{16}H_{14}INS$: C 50.67, H 3.72, N 3.69; Found: 50.63, H 3.66, N 3.51.

4.18.2. 1-(Phenylpentyl)-3-(phenylthio)quinolinium iodide (22b)

Yield 75%. Mp 166–167 °C. ^{1}H 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 $C_{26}H_{26}INS{\cdot}0.2H_2O$: C, 60.63; H, 5.09; N, 2.72. Found: 60.60; H, 5.02; N, 2.73.

4.18.3. 1-(Cyclohexylpentyl)-3-(phenylthio)quinolinium iodide (22c)

Yield: 73%. Mp 174–176 °C. ^{1}H 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 $C_{26}H_{32}INS$: C, 60.34; H, 6.23; N, 2.71. Found: C, 60.48; H, 6.27; N, 2.75.

4.19. Biological testing

4.19.1. 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 Saboraud Dextrose broth (SDB) or RPMI 1640, *C. neoformans*: SDB, *A. fumigatus*: either YM broth (for MICs) or RPMI-1640 + 5% Alamar Blue (for IC_{50} determination). After adding microbial cultures to the samples affording a final volume of 200 μL and final test concentration starting with 20 μg/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 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 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).

4.19.2. 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 μg/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] 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.

4.19.3. 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 $HClO_4$ (2 mL) and vortex mixing. Additional 0.5 M $HClO_4$ 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 min, followed by two 45 s treatments on ice with a Fisher 550 Sonic Dismembrator. Four mL MeOH (HPLC grade) was added and the mixture vortexed and sonified for 5 min. 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 $HClO_4$ plus 4 mL MeOH at a pH of 1–1.5. The supernatants were combined and the pH was then adjusted to ~7 with concd NH_4OH.[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/H_2O at pH 7). The extract (12 mL) was slowly loaded, washed with 5% NH_4OH (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 μL) 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, 0.1 M H_2PO_4, adjusted to pH 3.00 with NEt_3) + 10% CH_3CN and D (100% acetonitrile).[50]

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