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Synthesis, antimalarial activity, structure–activity relationship analysis of thieno-[3,2-*b*]benzothiazine *S*,*S*-dioxide analogs

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Abstract—An improved procedure for the synthesis of 3-amino-9-arylsubstituted-thieno[3,2-*b*]benzothiazine *S*,*S*-dioxide 2-decarboxylated is reported. Thieno-[3,2-*b*]benzothiazine *S*,*S*-dioxide derivatives were investigated for their abilities to inhibit β-hematin formation, hemoglobin hydrolysis and in vivo for their efficacy in rodent *Plasmodium berghei*. Compounds **5j–o** were the most promising as inhibitors of hemoglobin hydrolysis, however, the compounds are not as efficient as chloroquine. A structure–activity relationship (SAR) study was carried out in this series. Our results allow us to determine the minimal structural requirements to produce the biological response.

1. Introduction

Malaria is one of the most important infectious disease problems for humans, particularly in tropical regions of the world. As per WHO reports, annually there are more than 500 million new cases, resulting in at least 1–2 million deaths.[1,2] In addition, since resistance to currently used antimalarials is spreading rapidly, there is a great need for new effective drugs. Thus, there is a compelling and urgent need for new antimalarials with mechanisms of action different from those of existing ones in order to replace those that are becoming obsolete and to identify new drug targets.[3] Chloroquine has recently been shown to inhibit hemozoin formation within the parasite food vacuole.[4] This process is also thought to be the molecular target of other quinoline antimalarials.[5] Hemozoin was originally considered to be formed by the polymerization of heme,[6] but it has now been demonstrated to be a crystalline cyclic dimer of ferriprotoporphyrin IX.[7] Thus, hemozoin synthesis, a process unique to the malaria parasite, offers a logical and valuable potential target for new antimalarial drug development. New drugs that attack the same vital target of chloroquine, but that are not subject to the same resistance mechanism, would be highly desirable. Fluoroquinolones, such as ciprofloxacin, gatifloxacin, moxifloxacin, and trovafloxacin, have been reported for their antimalarial activities.[8] We have recently described the preparation and antimalarial activities of several tricyclic quinolone analogs.[9]

The quinolone and benzothiazine nuclei are often found in biologically active molecules, and a large variety of methods have been employed for their synthesis.[10] Generally, these synthetic routes were carried out in solution. These methods have their merits, but all have drawbacks such as using large amounts of volatile and poisonous solvents. Moreover, the reaction times are long and the yields are not high. In recent years, microwave dielectric heating technology combined with

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solvent-free conditions has been used in many organic reactions, leading to shorter reaction times, higher yields, cleaner reaction products and environmentally benign condition compared to the classical heating.[11]

In continuation of our studies directed toward synthesis of quinolones and benzothiazine annulated with various five and six member heterocycles, we report here the synthesis of thieno-[3,2-*b*]benzothiazine *S*,*S*-dioxide analogs and their abilities to inhibit β-hematin formation and hemoglobin hydrolysis in vitro and in vivo for their efficacy in rodent *P. berghei*. A structure–activity relationship (SAR) study using molecular electrostatic potentials (MEPs) was performed in order to determine the minimal structural requirements to produce the inhibitory effect in this series.

2. Results and discussion

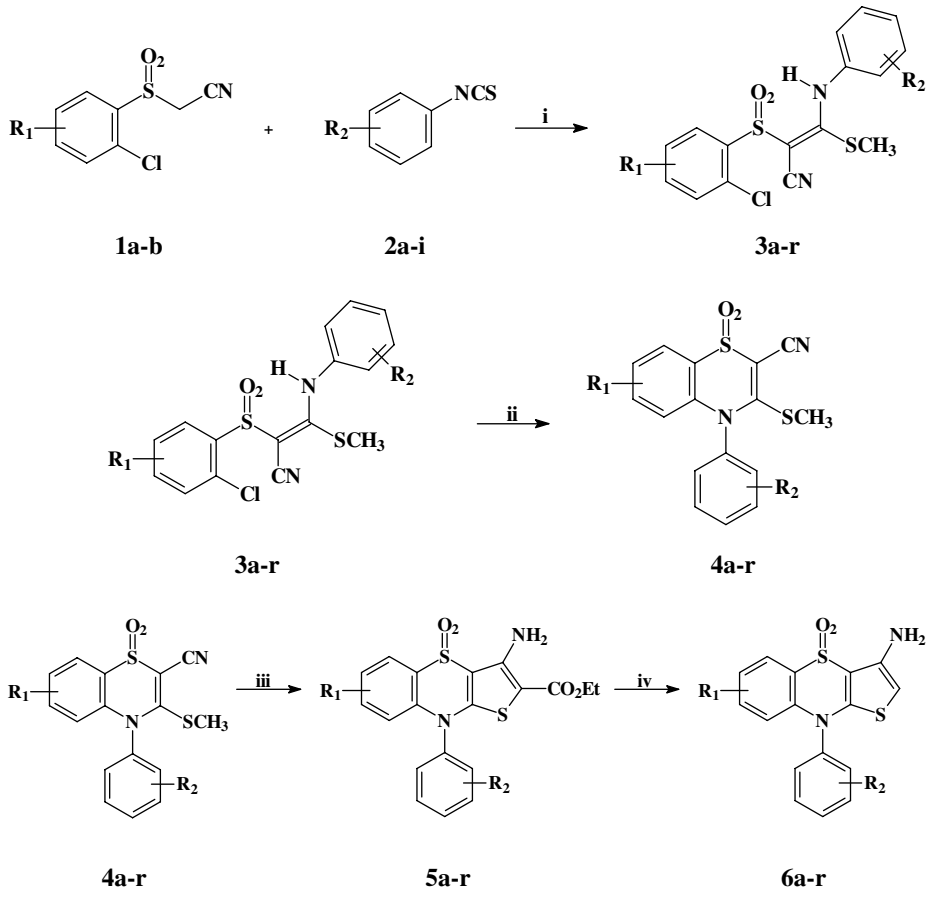
2.1. Synthesis

We first prepared the sulfone *S*,*N*-acetals **3a**–**r** by the reaction of *o*-chlorobenzenesulfonylacetonitrile **1a**–**b** with the respective phenyl isothiocyanates **2a**–**i**, methyl iodide, and potassium hydroxide in 1,4-dioxane (Scheme 1).[12] The stereochemistry around the olefinic carbon–carbon bond was established using the NMR and confirmed by X-ray diffraction studies.[13] The resulting sulfone *S*,*N*-acetals were mixed with potassium carbonate and irradiated without solvent in a domestic microwave oven to afford compounds **4a**–**r**. The target compounds were easily recovered by adding water to the final reaction mixture, removing the solid product by filtration, and recrystallization from ethanol/water 1:1 to give a pure sample. The identities of compounds **4a**–**r** were established by comparing their physical and spectroscopic properties with those in the literature and were confirmed by X-ray diffraction.[14] Products **5a**–**r** were obtained when **4a**–**r** were reacted with ethyl 2-mercaptoacetate, triethylamine in dry ethanol under an inert atmosphere of nitrogen.

The decarboxylation of the ethyl ester **5a**–**r** to obtain **6a**–**r** in a one-pot reaction was made using several conditions and concentrations of NaOH, KOH, LiOH, or HCl (Table 1). In some analog systems although the reaction is straightforward, it suffers from several drawbacks, such as long reaction times, poor atom economy (requires extra mole of base as acid scavenger), use of

Table 1. Different conditions used for decarboxylation

Base/acid	Refluxing/6 h	Room temperature/24 h
NaOH 20, 10, 5%	Decarboxylation	Starting material
KOH 20, 10, 5%	Decarboxylation	Starting material
LiOH 20, 10, 5%	Decarboxylation	Starting material
HCl 5%	Decarboxylation	Starting material



Scheme 1. Reagents and conditions: **(i)** KOH, 1,4-dioxane, MeI, rt; **(ii)** K_2CO_3, MW; **(iii)** $HSCH_2CO_2Et$, Et_3N, EtOH, Δ; **(iv)** NaOH, KOH, LiOH, or HCl, Δ.

hazardous solvents, and high temperature which results in the tar formation and reduces the yields of the desired products.[15]

All new compounds were characterized by NMR (^{1}H, ^{13}C, HETCOR and FLOCK experiments) and IR. The purity was established by TLC and microanalysis.

In the X-ray crystal structures of compounds **4d** and **5d** (Figs. 1 and 2) the bond distances are within the expected values.[16]

2.2. Biological assays

N-Phenylthieno-[3,2-*b*]benzothiazine *S*,*S*-dioxide analogs **5a**–**r** and **6a**–**r** were tested in vitro for their activity against cultured β-hematin formation and hemoglobin hydrolysis and in vivo for their efficacy in rodent *P. berghei* (Table 2). The in vitro assay was used to assess the abilities of the *N*-phenylthieno[3,2-*b*]benzothiazine derivatives to inhibit β-hematin formation. In that assay, hemin was allowed to form β-hematin under acidic conditions. Among the 36 compounds tested, none showed a measurable activity compared to chloroquine (86.6 ± 2.75%).

Consequently, compounds **5a**–**r** and **6a**–**r** were tested for inhibition of globin proteolysis, in an in vitro assay which uses rich extract of trophozoyte to digest the native hemoglobin of mice. Electrophoretic analyses indicated that compounds **5j**–**o**, were effective as inhibitors of hemoglobin degradation 78.17 ± 0.65%, 74.36 ± 0.43%, 64.98 ± 0.5%, 76.81 ± 0.69%, 55.96 ± 1.56%, and 37.01 ± 1.52%, respectively (band at MW 14.4 kDa) (Fig. 3).

Compounds **5j**–**o**, were tested in mice infected with *P. berghei* ANKA, a chloroquine-susceptible strain of murine malaria. Mice were given the compound (chloroquine or **5j**–**o**, in 20 mg kg^{-1}, ip once daily) for four consecutive days (days 0–4 post-infection). At day fourth post-infection, the parasitemia was determined; the survival days were monitored and compared with control mice receiving saline (untreated mice). Control mice died within 12 days post-infection, compound **5k** increased the survival time for 16 days, while chloroquine prolonged the survival time of the infected mice to 30 days. Compound **5k** was able to reduce and delay the progression of malaria 8.7% but did not eradicate the infection (Table 3).

It should be emphasized that compounds **5j**–**o**, which bear methyl groups on the phenyl and Cl group on position 7 of the benzothiazine, showed the highest activity. It is interesting to note that the presence of Cl group at

Table 2. Inhibition of β-hematin synthesis (IβHS) and globin proteolysis (IPG) by benzothiazine derivatives

Compound	[a]R_1, R_2	%IβHS	%IPG
5a	H	<5	0
5b	2-CH_3	<5	0
5c	4-CH_3	<5	0
5d	2,5-CH_3	<5	0
5e	3-OCH_3	<5	0
5f	4-OCH_3	<5	0
5g	3-Cl	<5	0
5h	4-Cl	<5	0
5i	3,4-Cl	<5	0
5j	H	<5	78.17 ± 0.65[b]
5k	2-CH_3	<5	74.36 ± 0.43[b]
5l	4-CH_3	<5	64.98 ± 0.50[b]
5m	2,5-CH_3	<5	76.81 ± 0.69[b]
5n	3-OCH_3	<5	55.96 ± 1.56[b]
5o	4-OCH_3	<5	37.01 ± 1.52[b]
5p	3-Cl	<5	0
5q	4-Cl	<5	0
5r	3,4-Cl	<5	<5
Leupeptin	—	—	89.06 ± 0.69
Pepstatin	—	—	92.94 ± 0.67
Chloroquine	—	86.60 ± 2.75	24.12 ± 1.16

The results are expressed by means ± standard error of the mean.
[a] R_1**5a**–**i** = **6-Cl**; **5j**–**r** = **7-Cl.**
[b] $p > 0.05$ compared to leupeptin (LEP) and pepstatin (PEP).

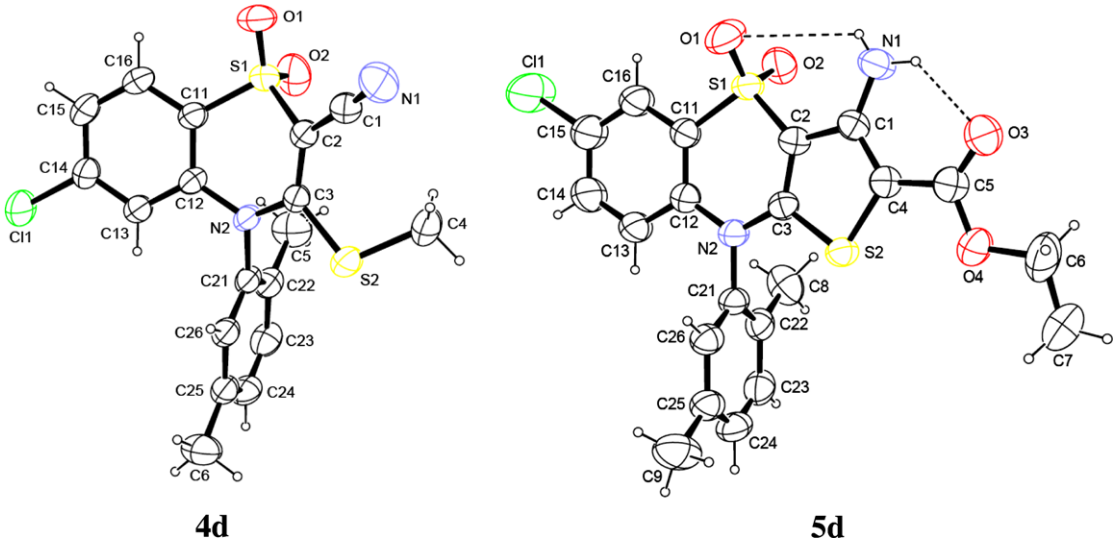


Figure 1,2. Molecular structure of compounds **4d** and **5d** showing the atomic numbering. The displacement parameters are drawn at 50% probability. Dashed lines indicate intramolecular hydrogen bonds.

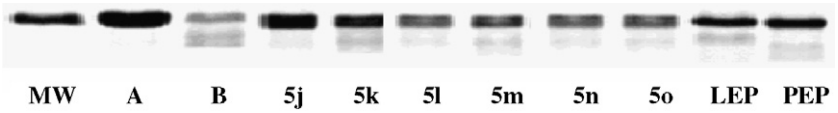


Figure 3. Standard molecular weight (MW) is expressed in kilodaltons (14.4 kDa). A, under graded globin (control hemoglobin without trophozoites); B, control hemoglobin with trophozoites of *P. berghei*; **5j–o**, benzothiazine derivatives; LEP, hemoglobin with trophozoites and leupeptin; PEP, hemoglobin with trophozoites and pepstatin.

Table 3. Effect of benzothiazine derivatives (20 mg/kg) on parasitemia at fourth day post-infection (%P) and survival days (SD) of *P. berghei* infected-mice

Treatment	%P	SD
Saline solution	21.80 ± 2.31	11.66 ± 1.66
5j	9.75 ± 3.01*	13 ± 1.26
5k	8.70 ± 2.43*	15.5 ± 2.13**
5l	15.72 ± 4.11	10.80 ± 1.59
5m	9.40 ± 3.88*	11.42 ± 2.65
5n	11.60 ± 2.69	12.16 ± 1.78
5o	11.21 ± 3.21	11.6 ± 2.34
Chloroquine	1.31 ± 0.30	30

The results are expressed by means ± standard error of the mean. *$p < 0.05$ and **$p < 0.01$ compared to untreated mice (saline). $n = 5$.

position 7 appears to be necessary, but is not by itself sufficient to produce the biological response. The lack of inhibitory effect of compounds **5p–r** illustrates this situation. Compounds that have a Cl substituent on position 6 of the benzothiazine nucleus, the same ester on position 2 or where the ester group has been removed, and the same substitution pattern on the phenyl group markedly decreases the activity. Thus, the poor inhibition of β-hematin formation appears to have close relation with this substitution pattern.

To further understand the above experimental results, we performed a conformational and electronic study on these compounds using theoretical calculations. The electronic study of these compounds was carried out using molecular electrostatic potential surfaces (MEPs).[17,18] The purpose was to obtain more precise information as to how closely these compounds resemble each other in terms of the electronic distribution and hydrophobic properties. Once the low-energy conformations for the different compounds were obtained (see Section 6 in experimental section) and in an attempt to find potentially reactive sites for the ligands, we evaluated the electronic aspects of the molecules using MEPs. The electrostatic potentials have long been applied as a guide to molecular reactive behavior;[19,20] for instance, the most negative values of MEP were interpreted as identifying and ranking sites for electrophilic attack, while its overall pattern served as the basis for qualitative analyses of biologic recognition interactions.

Figure 4 shows the MEPs obtained for compounds **5a**, **5j–5q**, and **6k**. These results account for the general characteristics of the electronic behavior of compounds reported here. The general pattern is similar for all **5** derivatives. Comparing the MEPs of **5a** with the rest, it is clear that the different positions of Cl (6 or 7) produce a different electronic distribution at the benzothiazine ring. Only compounds possessing the Cl group at position 7 displayed inhibitory activity.

The MEPs of all the active compounds exhibit two clear minima values (deep red zones) in the vicinity of the SO_2 and the COOEt groups. The region of the SO_2 is symmetrical with respect to the sulfur atom displaying two deep and extensive negative zones with $V_{(r)}$ values of about -0.079 el/au^3. The other minimum is located in the zone near to the COOEt, (red zone with $V_{(r)}$ values of about -0.078 el/au^3). A much smaller minimum, which is almost a neutral zone ($V_{(r)} \approx -0.047$ el/au^3) was found near the Cl substituent at position 7 on the benzothiazine ring. There is only one significant positive region located near to the phenyl ring (blue areas with $V_{(r)} \approx +0.035$ el/au^3). The different electronic distribution displayed for compounds of series **5** in comparison to that of series **6** might be appreciated comparing Figure 4 (compounds **5k**, **6k**). It should be noted that compound type **6** displayed a clear second positive zone near to the NH_2 group (blue zone with $V_{(r)}$ values of about +0.030 el/au^3). Thus, the lack of COOEt group in series **6** introduces a profound change in the electronic distribution which can explain the lack of biological activity obtained for these compounds.

Compounds displaying different substituents in the phenyl group showed different activities. Thus, compounds without substituents (**5j**) or with CH_3 groups (**5k–5m**) having a large-size positive zone near to the phenyl group were the most active molecules in this series. Compounds possessing OCH_3 group (**5n** and **5o**) possess a reduced positive zone by the presence of the oxygen atom giving negative zones. These compounds display only a mediocre activity. In contrast, compounds possessing electron-withdrawing substituents (compounds **5p–r**) were devoid of any inhibitory activity. These results clearly indicate that the increase of lipophilic property with appropriate groups on the phenyl substituent allows for a good activity for inhibition of hemoglobin degradation.

The fact that the activity is markedly affected by altering the substituents at the phenyl ring suggests that this aromatic ring makes a specific contribution to the binding via an aromatic ring orientation. In fact, there are various ways in which these moieties may be involved, on which we can only speculate. Thus, we may assume that a flat portion of the receptor could allow binding with this aromatic ring through dispersion forces (van der Waals). Our results indicate that a characteristic electronic distribution on the phenyl ring might be important to produce the adequate

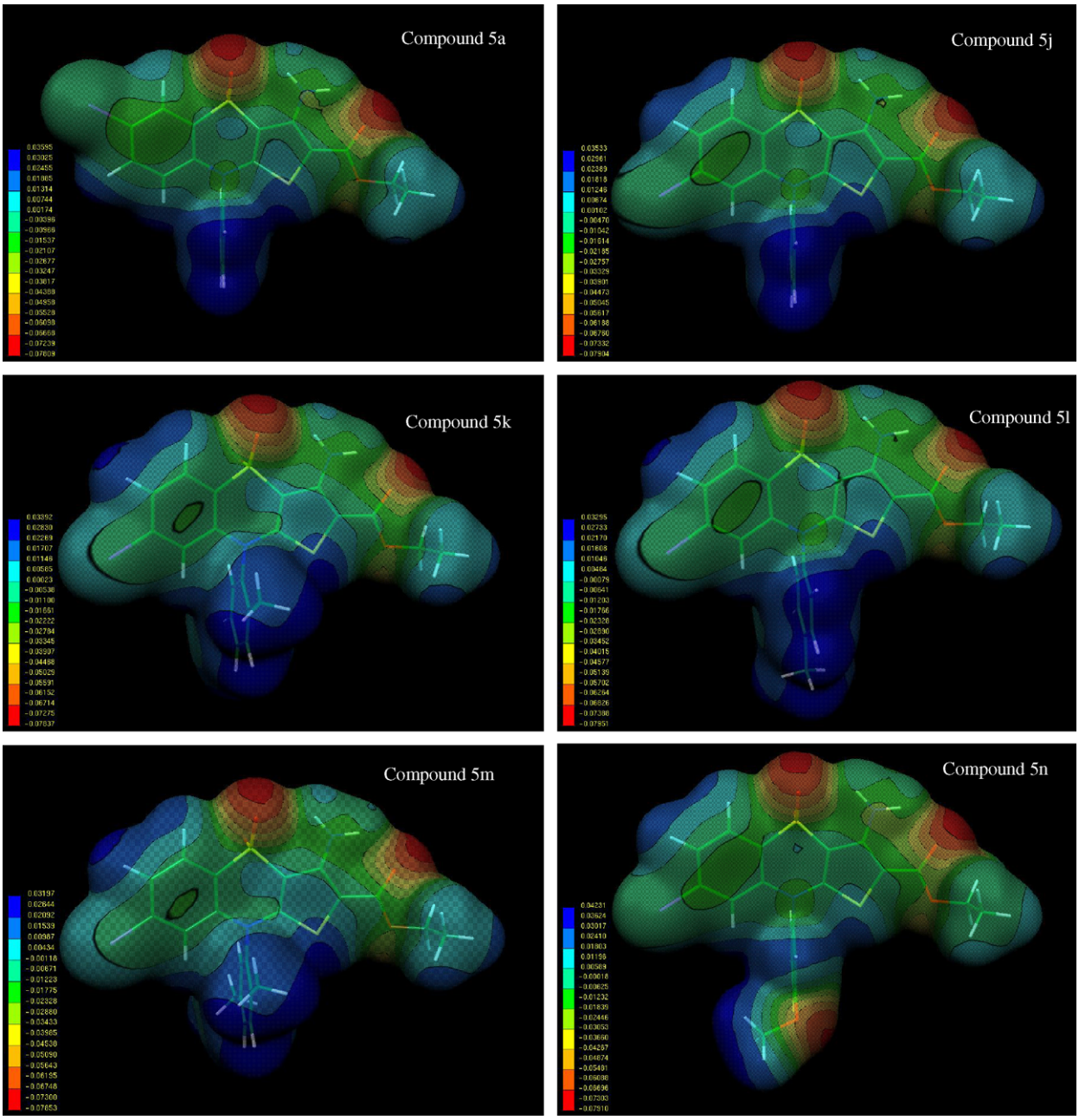


Figure 4. Electrostatic potential-encoded electrón density surfaces of the core structures of compounds **5a**, **5j**–**5q**, and **6k**. The surfaces were generated with Gaussian 03 after DFT (B3LYP) minimizations with 6-31G (d) basis set. The coloring represents electrostatic potential with red indicating the strongest attraction, a positive point charge and blue indicating the strongest repulsion. The electrostatic potential is the energy of interaction of the positive point charge with the nuclei and electron of a molecule. It provides a representative measure of overall molecular charge distribution.

interaction. Whereas methyl substituents give major inhibitions, only mediocre activity was determined for methoxy derivatives. The marginal efficacy results in the Peter test may be a result of poor bioavailability of these compounds.

3. Conclusions

Methods for synthesis of the benzothiazine nucleus using a microwave proceed with a significant decrease in reaction time and comparable high chemical yield, and their thiene analogs carboxylated and their decarboxylation in a one-pot reaction have been developed.

We also report a new group of thieno-[3,2-*b*]benzothiazine *S*,*S*-dioxide derivatives acting as antimalarial agents. Among them, compound **5j** and some of its congeners exhibited remarkable inhibitory activity. Although compounds **5j**–**o** were not tested as specific protease inhibitors in vitro, the mechanism of action of these compounds on hemoglobin degradation could be related to the inhibition of some aspartic, cysteinic, or metalloproteases due to the presence of a globin

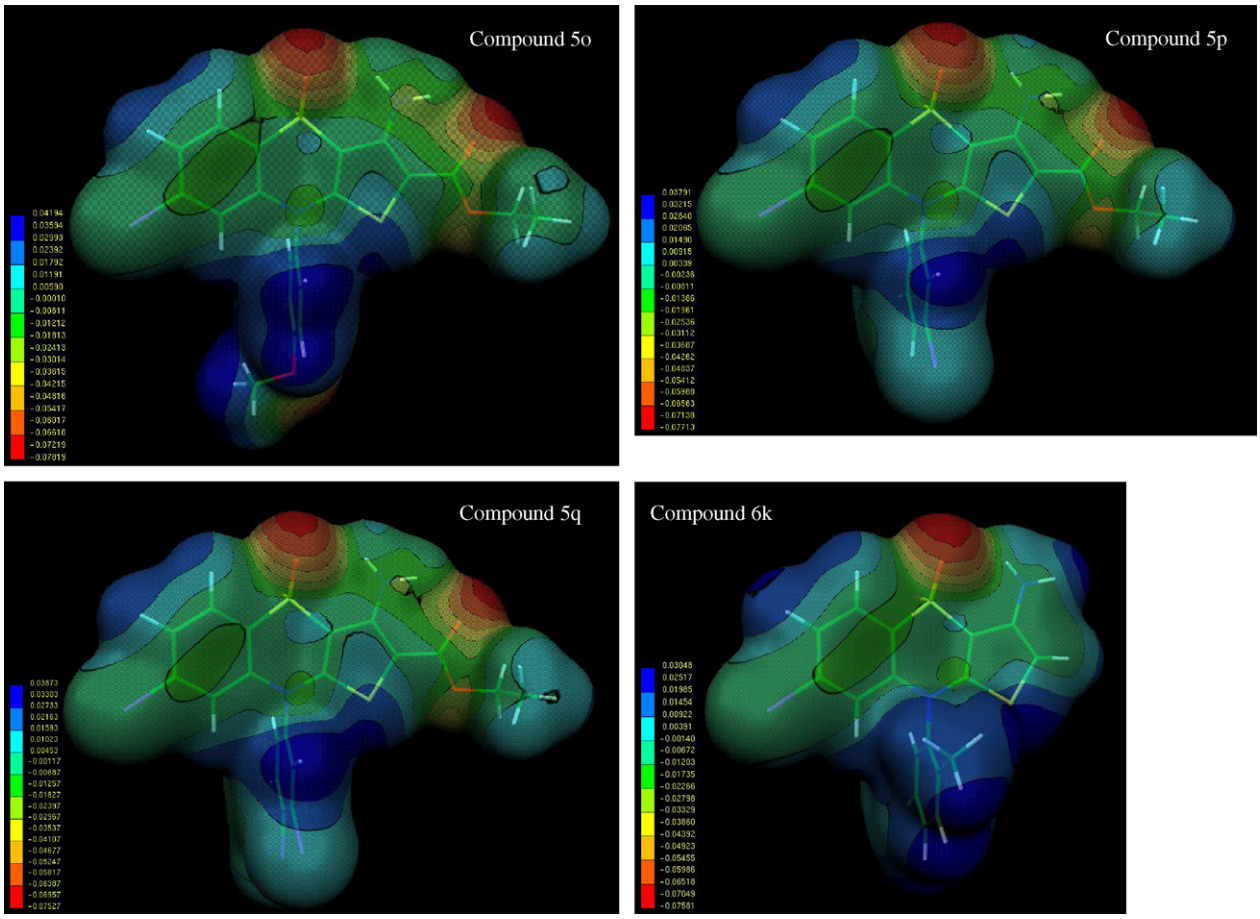


Fig. 4 (*continued*)

band. Some new benzothiazine analogs showed only marginal antimalarial activity in the Peter test against *P. berghei* by intraperitoneal administration. The poor solubility in organic solvents and water or an in vivo enzymatic hydrolysis and decarboxylation to the corresponding inactive **6j–o**, may be partially responsible for the poor in vivo activity observed.

4. Experimental

Melting points were determined with a Fischer–Johns micro hot-stage apparatus and are uncorrected. IR spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H NMR spectra were recorded using a Jeol Eclipse 270 MHz spectrometer using $CDCl_3$, and are reported in ppm downfield from the $CHCl_3$ residual. Elemental analyses were performed on a Perkin-Elmer 2400CHN analyzer, results were within ±0.4% of predicted values for all compounds. Compounds **4a**, **e**, **f**, **h**, **j**, **n**, **o**, **q** were previously described except the ^{1}H NMR spectra of **4b–d**, **g**, **i**, **k**, **l**, **p**, **r**.[14] A white-Westinghouse microwave oven equipped with a turntable and operating at 2450 MHz was used at its full power, 750 W, for all the experiments. An alumina batch (Aluminum oxide 60 G neutral, type E, Merck: 50 g; batch 4.0 cm diameter) was used as a sink inside the MW oven to irradiate the reaction mixture.

4.1. General procedure for preparation of 6 or 7-chloro-3-methylthio-4-phenyl-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4a–r)

Compounds were prepared following methods developed by us.[14]

4.1.1. 6-Chloro-3-methylthio-4-(2-methylphenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4b). Yield 76%, mp 198–200 °C; IR (KBr cm^{-1}): 2192 CN, 1453 SO_2; ^{1}H NMR δ $CDCl_3$: 1.96 (s, 3H, CH_3), 2.70 (s, 3H, SCH_3), 6.63 (d, 1H, H_5, J = 1.9 Hz), 7.26–7.29 (m, 1H, $H_{6'}$), 7.57–7.61 (m, 3H, $H_{2',3',5'}$), 7.67 (d, 1H, $H_{4'}$, J = 7.67 Hz), 7.71 (dd, 1H, H_7, J = 8.4, 1.9 Hz), 8.11 (d, 1H, H_8, J = 8.4 Hz). Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.17; H, 3.48; N, 7.43. Found: C, 53.95; H, 3.40; N, 7.23.

4.1.2. 6-Chloro-3-methylthio-4-(4-methylphenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4c). Yield 83%, mp 226–228 °C; IR (KBr cm^{-1}): 2208 CN, 1453 SO_2; ^{1}H NMR δ $CDCl_3$: 2.44 (s, 3H, CH_3), 2.68 (s, 3H, SCH_3), 6.66 (d, 1H, H_5, J = 1.8 Hz), 7.42 (d, 2H, $H_{2',6'}$, J = 8.3 Hz), 7.49 (d, 2H, $H_{3',5'}$, J = 8.3 Hz), 7.68 (dd, 1H, H_7, J = 8.5, 1.8 Hz), 8.09 (d, 1H, H_8, J = 8.5 Hz). Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.17; H, 3.48; N, 7.43. Found: C, 54.23; H, 3.27; N, 7.37.

4.1.3. 6-Chloro-3-methylthio-4-(2,5-dimethylphenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4d). Yield 92%, mp 210–212 °C; IR (KBr cm^{-1}): 2192 CN, 1456 SO_2; ^1H NMR δ $CDCl_3$: 1.92 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.72 (s, 3H, SCH_3), 6.66 (d, 1H, H_5, $J = 1.7$ Hz), 7.46–7.49 (m, 3H, $H_{3',4',6'}$), 7.71 (dd, 1H, H_7, $J = 8.5$, 1.7 Hz), 8.11 (d, 1H, H_8, $J = 8.5$ Hz). Anal. Calcd for $C_{18}H_{15}Cl_2N_2O_2S_2$: C, 55.31; H, 3.86; N, 7.16. Found: C, 54.99; H, 3.76; N, 7.23.

4.1.4. 6-Chloro-3-methylthio-4-(3-chlorophenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4g). Yield 77%, mp 200–202 °C; IR (KBr cm^{-1}): 2192 CN, 1449 SO_2; ^1H NMR δ $CDCl_3$: 2.69 (s, 3H, SCH_3), 6.70 (d, 1H, H_5, $J = 1.7$ Hz), 7.79–7.84 (m, 5H, $H_{2',4',5',6',7}$), 8.11 (d, 1H, H_8, $J = 8.4$ Hz). Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S_2$: C, 48.37; H, 2.54; N, 7.05. Found: C, 48.35; H, 2.71; N, 6.93.

4.1.5. 6-Chloro-4-methylthio-4-(3,4-dichlorophenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4i). Yield 76%, mp 224–226 °C; IR (KBr cm^{-1}): 2192 CN, 1459 SO_2; ^1H NMR δ $CDCl_3$: 2.69 (s, 3H, SCH_3), 6.89 (d, 1H, H_5, $J = 1.7$ Hz), 7.40 (dd, 1H, $H_{6'}$, $J = 8.5$, 2.5 Hz), 7.70 (dd, 1H, H_7, $J = 8.7$, 1.7 Hz), 7.96 (d, 1H, $H_{5'}$, $J = 8.5$ Hz), 8.04 (d, 1H, $H_{2'}$, $J = 2.5$ Hz), 8.10 (d, 1H, H_8, $J = 8.7$ Hz). Anal. Calcd for $C_{16}H_9Cl_3N_2O_2S_2$: C, 44.51; H, 2.10; N, 6.49. Found: C, 44.57; H, 2.07; N, 6.63.

4.1.6. 7-Chloro-3-methylthio-4-(2-methylphenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4k). Yield 81%, mp 196–198 °C; IR (KBr cm^{-1}): 2192 CN, 1485 SO_2; ^1H NMR δ $CDCl_3$: 2.05 (s, 3H, CH_3), 2.77 (s, 3H, SCH_3), 6.65 (d, 1H, H_5, $J = 9.2$ Hz), 7.23–7.27 (m, 1H, $H_{6'}$), 7.37 (dd, 1H, H_6, $J = 9.2$, 2.5 Hz), 7.54–7.58 (m, 3H, $H_{3',4',5'}$), 8.04 (d, 1H, H_8, $J = 2.5$ Hz). Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.17; H, 3.48; N, 7.43. Found: C, 54.33; H, 3.55; N, 7.62.

4.1.7. 7-Chloro-3-methylthio-4-(4-methylphenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4l). Yield 87%, mp 223–225 °C; IR (KBr cm^{-1}): 2192 CN, 1481 SO_2; ^1H NMR δ $CDCl_3$: 2.48 (s, 3H, CH_3), 2.68 (s, 3H, SCH_3), 6.69 (d, 1H, H_5, $J = 8.9$ Hz), 7.16 (d, 2H, $H_{2',6'}$, $J = 7.7$ Hz), 7.53–7.59 (m, 3H, $H_{3',5',6}$), 8.00 (d, 1H, H_8, $J = 2.3$ Hz). Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.17; H, 3.48; N, 7.43. Found: C, 54.20; H, 3.51; N, 7.17.

4.1.8. 7-Chloro-3-methylthio-4-(2,5-dimethylphenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4m). Yield 77%, mp 210 °C d; IR (KBr cm^{-1}): 2192 CN, 1491 SO_2; ^1H NMR δ $CDCl_3$: 2.00 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.77 (s, 3H, SCH_3), 6.65 (d, 1H, H_5, $J = 9.1$ Hz), 7.29–7.35 (m, 3H, $H_{3',4',6'}$), 7.40 (dd, 1H, H_6, $J = 9.1$, 2.5 Hz), 8.03 (d, 1H, H_8, $J = 2.5$ Hz). Anal. Calcd for $C_{18}H_{15}ClN_2O_2S_2$: C, 55.31; H, 3.86; N, 7.16. Found: C, 55.22; H, 3.91; N, 7.05.

4.1.9. 7-Chloro-3-methylthio-4-(3-chlorophenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4p). Yield 71%, mp 230–232 °C; IR (KBr cm^{-1}): 2192 CN, 1483 SO_2; ^1H NMR δ $CDCl_3$: 2.69 (s, 3H, SCH_3), 6.70 (d, 1H, H_5, $J = 8.4$ Hz), 7.80–7.85 (m, 5H, $H_{2',4',5',6',6}$), 8.11 (d, 1H, H_8, $J = 2.2$ Hz). Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S_2$: C, 48.37; H, 2.54; N, 7.05. Found: C, 48.31; H, 2.78; N, 6.94.

4.1.10. 7-Chloro-4-methylthio-4-(3,4-dichlorophenyl)-1,4-benzothiazine-2-carbonitrile 1,1-dioxide (4r). Yield 83%, mp 234 °C d; IR (KBr cm^{-1}): 2192 CN, 1483 SO_2; ^1H NMR δ $CDCl_3$: 2.07 (s, 3H, SCH_3), 7.03 (d, 1H, H_5, $J = 8.7$ Hz), 6.43 (dd, 1H, $H_{6'}$, $J = 8.6$, 2.2 Hz), 6.50 (d, 1H, $H_{2'}$, $J = 2.2$ Hz), 7.24 (d, 1H, $H_{5'}$, $J = 8.6$ Hz), 7.53 (d, 1H, H_6, $J = 8.7$ Hz), 7.99 (d, 1H, H_8, $J = 2.2$ Hz). Anal. Calcd for $C_{16}H_9Cl_3N_2O_2S_2$: C, 44.51; H, 2.10; N, 6.49. Found: C, 44.63; H, 2.25; N, 6.57.

4.2. General procedure for preparation of ethyl 3-amino-9-phenylsubstituted-thieno-[3,2-*b*]-benzothiazine 4,4-dioxide 2-carboxylate (5a–r)

A mixture of appropriate benzothiazine 1,1-dioxide **4a**–**r** (1 mmol), ethyl mercaptoacetate (1 mmol), triethylamine (3 mmol) in dry ethanol (5 mL) was refluxed for 5 h under an inert atmosphere. The solvent was evaporated to dryness under reduced pressure, water was added (8 mL) and the solid obtained was collected by filtration. Further purification was accomplished by recrystallization from ethanol/water (4:1).

4.2.1. Ethyl 3-amino-6-chloro-9-phenylthieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5a). Yield 83%, mp 242–244 °C; IR (KBr cm^{-1}): 3480 NH_2, 1662 CO, 1495 SO_2; ^1H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, $J = 7.2$ Hz), 4.21 (q, 2H, CH_2, $J = 7.2$ Hz), 6.23 (br s, 2H, NH_2), 6.52 (d, 1H, H_8, $J = 9.2$ Hz), 7.32 (dd, 1H, H_7, $J = 9.2$, 2.3 Hz), 7.39 (m, 2H, $H_{2',6'}$), 7.66–7.71 (m, 3H, $H_{3',4',5'}$), 8.07 (d, 1H, H_5, $J = 2.3$ Hz); ^{13}C NMR 14.6, 60.4, 105.9, 118.2, 122.9, 126.3, 128.9, 129.3, 131.3, 131.6, 132.9, 137.5, 138.3, 149.2, 153.4, 163.8. Anal. Calcd for $C_{19}H_{15}ClN_2O_4S_2$: C, 52.47; H, 3.47; N, 6.44. Found: C, 52.71; H, 3.40; N, 6.23.

4.2.2. Ethyl 3-amino-6-chloro-9-(2′-methylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5b). Yield 47%, mp 241–243 °C; IR (KBr cm^{-1}): 3480 NH_2, 1668 CO, 1490 SO_2; ^1H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, $J = 7.0$ Hz), 2.06 (s, 3H, CH_3), 4.20 (q, 2H, CH_2, $J = 7.0$ Hz), 6.25 (br s, 2H, NH_2), 6.45 (d, 1H, H_8, $J = 9.2$ Hz), 7.28 (d, 1H, $H_{6'}$, $J = 7.7$ Hz), 7.34 (dd, 1H, H_7, $J = 9.2$, 2.2 Hz), 7.55–7.59 (m, 3H, $H_{3',4',5'}$), 8.09 (d, 1H, H_5, $J = 2.2$ Hz); ^{13}C NMR 14.6, 17.1, 60.4, 105.9, 117.6, 123.1, 126.4, 129.0, 129.1, 129.5, 131.5, 133.1, 133.1, 136.5, 136.8, 137.2, 149.5, 152.8, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_4S_2$: C, 53.51; H, 3.82; N, 6.24. Found: C, 53.51; H, 3.79; N, 6.33.

4.2.3. Ethyl 3-amino-6-chloro-9-(4′-methylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5c). Yield 67%, mp 240–242 °C; IR (KBr cm^{-1}): 3456 NH_2, 1661 CO, 1459 SO_2; ^1H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, $J = 7.0$ Hz), 2.50 (s, 3H, CH_3), 4.19 (q,

2H, CH_2, J = 7.0 Hz), 6.22 (br s, 2H, NH_2), 6.53 (d, 1H, H_8, J = 8.9 Hz), 7.23 (d, 2H, $H_{2',6'}$, J = 7.9 Hz), 7.29 (dd, 1H, H_7, J = 8.9, 1.5 Hz), 7.45 (d, 1H, $H_{3',5'}$, J = 7.9 Hz), 8.05 (d, 1H, H_5, J = 1.5 Hz); ^{13}C NMR 14.6, 21.6, 60.3, 105.7, 118.3, 122.9, 126.3, 128.5, 129.6, 132.2, 132.8, 135.7, 137.1, 141.7, 149.5, 153.7, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_4S_2$: C, 53.51; H, 3.82; N, 6.24. Found: C, 53.49; H, 3.67; N, 6.43.

4.2.4. Ethyl 3-amino-6-chloro-9-(2′,5′-dimethylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5d). Yield 43%, mp 244–246 °C; IR (KBr cm^{-1}): 3482 NH_2, 1665 CO, 1490 SO_2; ^{1}H NMR δ $CDCl_3$: 1.25 (t, 3H, CH_3, J = 7.2 Hz), 1.99 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.21 (q, 2H, CH_2, J = 7.2 Hz), 6.24 (br s, 2H, NH_2), 6.46 (d, 1H, H_8, J = 9.2 Hz), 7.07 (s, 1H, $H_{6'}$), 7.33 (dd, 1H, H_7, J = 9.2, 2.5 Hz), 7.36–7.40 (m, 2H, $H_{3',4'}$), 8.09 (d, 1H, H_5, J = 2.5 Hz); ^{13}C NMR 14.6, 16.9, 20.9, 60.4, 105.7, 117.3, 123.1, 126.3, 129.2, 129.3, 132.3, 132.8, 133.1, 133.7, 136.6, 136.6, 139.3, 149.4, 152.8, 163.8. Anal. Calcd for $C_{21}H_{19}ClN_2O_4S_2$: C, 54.48; H, 4.14; N, 6.05. Found: C, 54.66; H, 4.30; N, 6.17.

4.2.5. Ethyl 3-amino-6-chloro-9-(3′-methoxylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5e). Yield 47%, mp 227–229 °C; IR (KBr cm^{-1}): 3471 NH_2, 1672 CO, 1443 SO_2; ^{1}H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, J = 6.8 Hz), 3.79 (s, 3H, OCH_3), 4.26 (q, 2H, CH_2, J = 6.8 Hz), 6.19 (br s, 2H, NH_2), 6.59 (d, 1H, H_8, J = 9.2 Hz), 6.71 (d, 1H, $H_{2'}$ J = 2.4 Hz), 6.84 (d, 1H, $H_{4'}$, J = 7.8 Hz), 7.10 (dd, 1H, $H_{6'}$, J = 7.8, 2.3 Hz), 7.33 (dd, 1H, H_7, J = 9.2, 1.7 Hz), 7.60 (t, 1H, $H_{5'}$, J = 7.6 Hz), 8.05 (d, 1H, H_5, J = 1.7 Hz); ^{13}C NMR ^{13}C NMR 14.6, 55.8, 60.4, 105.8, 116.4, 118.3, 123.1, 126.4, 129.1, 130.2, 130.8, 132.8, 136.9, 149.7, 154.2, 161.3, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_5S_2$: C, 51.67; H, 3.68; N, 6.02. Found: C, 51.73; H, 3.59; N, 6.03.

4.2.6. Ethyl 3-amino-6-chloro-9-(4′-methoxylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5f). Yield 63%, mp 218–220 °C; IR (KBr cm^{-1}): 3456 NH_2, 1674 CO, 1459 SO_2; ^{1}H NMR δ $CDCl_3$: 1.25 (t, 3H, CH_3, J = 6.2 Hz), 3.85 (s, 3H, OCH_3), 4.21 (q, 2H, CH_2, J = 6.2 Hz), 6.21 (br s, 2H, NH_2), 6.57 (d, 1H, H_8, J = 9.1 Hz), 7.13 (d, 2H, $H_{2',6'}$, J = 8.9 Hz), 7.28 (d, 2H, $H_{3',5'}$, J = 8.9 Hz), 7.32 (dd, 1H, H_7, J = 9.1, 1.4 Hz), 8.07 (d, 1H, H_5, J = 1.4 Hz); ^{13}C NMR 14.6, 55.8, 60.3, 105.7, 116.6, 118.3, 122.9, 126.3, 129.2, 130.1, 130.8, 132.8, 137.8, 149.6, 154.1, 161.3, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_5S_2$: C, 51.67; H, 3.68; N, 6.02. Found: C, 51.88; H, 4.01; N, 5.87.

4.2.7. Ethyl 3-amino-6-chloro-9-(3′-chlorophenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5g). Yield 43%, mp 200 °C dec; IR (KBr cm^{-1}): 3488 NH_2, 1676 CO, 1459 SO_2; ^{1}H NMR δ $CDCl_3$: 1.26 (t, 3H, CH_3, J = 7.1 Hz), 4.19 (q, 2H, CH_2, J = 7.1 Hz), 6.24 (br s, 2H, NH_2), 6.53 (d, 1H, H_8, J = 9.2 Hz), 7.31–7.35 (m, 1H, $H_{6'}$), 7.36 (dd, 1H, H_7, J = 9.2, 2.4 Hz), 7.41 (s, 1H, $H_{2'}$), 7.63–7.67 (m, 2H, $H_{4',5'}$), 8.08 (d, 1H, H_5, J = 2.4 Hz); ^{13}C NMR 14.6, 60.5, 106.3, 118.0, 123.1, 126.5, 127.3, 129.4, 129.4, 129.6, 131.7, 132.6, 133.0, 137.1, 137.1, 149.1, 152.7, 163.7. Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_4S_2$: C, 48.62; H, 3.01; N, 5.97. Found: C, 48.39; H, 3.25; N, 6.29.

4.2.8. Ethyl 3-amino-6-chloro-9-(4′-chlorophenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5h). Yield 61%, mp 278–280 °C; IR (KBr cm^{-1}): 3460 NH_2, 1666 CO, 1490 SO_2; ^{1}H NMR δ $CDCl_3$: 1.26 (t, 3H, CH_3, J = 7.1 Hz), 4.22 (q, 2H, CH_2, J = 7.1 Hz), 6.22 (br s, 2H, NH_2), 6.52 (d, 1H, H_8, J = 9.1 Hz), 7.34 (d, 2H, $H_{2',6'}$, J = 8.9 Hz), 7.36 (dd, 1H, H_7, J = 9.1, 2.2 Hz), 7.65 (d, 2H, $H_{3',5'}$, J = 8.9 Hz), 8.08 (d, 1H, H_5, J = 2.2 Hz); ^{13}C NMR 14.5, 60.5, 106.2, 117.9, 123.2, 126.6, 129.6, 130.5, 131.9, 132.9, 136.7, 137.3, 137.6, 149.2, 152.8, 163.7. Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_4S_2$: C, 48.62; H, 3.01; N, 5.97. Found: C, 48.63; H, 3.12; N, 5.93.

4.2.9. Ethyl 3-amino-6-chloro-9-(3′,4′-dichlorophenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5i). Yield 55%, mp 180–182 °C; IR (KBr cm^{-1}): 3475 NH_2, 1662 CO, 1490 SO_2; ^{1}H NMR δ $CDCl_3$: 1.27 (t, 3H, CH_3, J = 6.9 Hz), 4.20 (q, 2H, CH_2, J = 6.9 Hz), 6.19 (br s, 2H, NH_2), 6.54 (d, 1H, H_8, J = 9.2 Hz), 7.27 (dd, 1H, $H_{6'}$, J = 8.4, 2.5 Hz), 7.37 (dd, 1H, H_7, J = 9.2, 2.4 Hz), 7.54 (d, 1H, $H_{2'}$, J = 2.5 Hz), 7.77 (d, 1H, $H_{5'}$, J = 8.4 Hz), 8.08 (d, 1H, H_5, J = 2.4 Hz); ^{13}C NMR 14.5, 60.6, 106.8, 117.8, 123.3, 126.7, 128.5, 129.9, 131.2, 131.5, 133.1, 137.4, 136.9, 137.2, 149.2, 153.2, 163.7. Anal. Calcd for $C_{19}H_{13}Cl_3N_2O_4S_2$: C, 45.30; H, 2.60; N, 5.56. Found: C, 45.37; H, 2.73; N, 5.81.

4.2.10. Ethyl 3-amino-7-chloro-9-phenylthieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5j). Yield 71%, mp 220–222 °C; IR (KBr cm^{-1}): 3480 NH_2, 1662 CO, 1495 SO_2; ^{1}H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, J = 7.3 Hz), 4.21 (q, 2H, CH_2, J = 7.3 Hz), 6.22 (br s, 2H, NH_2), 6.53 (d, 1H, H_8, J = 1.7 Hz), 7.27 (dd, 1H, H_6, J = 8.7, 1.7 Hz), 7.38–7.42 (m, 2H, $H_{2'}$), 7.69–7.64 (m, 4H, Ar), 8.05 (d, 1H, H_5, J = 8.7 Hz); ^{13}C NMR 14.6, 60.4, 90.7, 106.4, 116.4, 123.9, 124.1, 124.9, 128.9, 131.7, 131.9, 138.1, 138.1, 139.9, 149.3, 153.4, 163.8. Anal. Calcd for $C_{19}H_{15}ClN_2O_4S_2$: C, 52.47; H, 3.47; N, 6.44. Found: C, 52.86; H, 3.57; N, 6.68.

4.2.11. Ethyl 3-amino-7-chloro-9-(2′-methylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5k). Yield 67%, mp 180–182 °C; IR (KBr cm^{-1}): 3480 NH_2, 1668 CO, 1490 SO_2; ^{1}H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, J = 6.8 Hz), 2.07 (s, 3H, CH_3), 4.21 (q, 2H, CH_2, J = 6.8 Hz), 6.24 (br s, 2H, NH_2), 6.46 (d, 1H, H_8, J = 1.5 Hz), 7.28 (d, 1H, H_6, J = 8.4 Hz), 7.51–7.55 (m, 4H, Ar), 8.06 (d, 1H, H_5, J = 8.4 Hz); ^{13}C NMR 14.6, 17.1, 60.4, 90.8, 106.3, 115.7, 124.0, 124.1, 125.1, 129.1, 129.1, 131.6, 133.2, 136.6, 137.2, 139.5, 149.4, 152.8, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_4S_2$: C, 53.51; H, 3.82; N, 6.24. Found: C, 53.27; H, 3.81; N, 6.18.

4.2.12. Ethyl 3-amino-7-chloro-9-(4′-methylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5l). Yield 78%, mp 192–194 °C; IR (KBr cm^{-1}): 3456 NH_2, 1661 CO, 1459 SO_2; ^1H NMR δ $CDCl_3$: 1.25 (t, 3H, CH_3, J = 7.1 Hz), 2.51 (s, 3H, CH_3), 4.20 (q, 2H, CH_2, J = 7.1 Hz), 6.22 (br s, 2H, NH_2), 6.53 (d, 1H, H_8, J = 1.7 Hz), 7.27–7.31 (m, 3H, $H_{6,2',6'}$), 7.47 (d, 2H, $H_{3',5'}$, J = 8.2 Hz), 8.05 (d, 1H, H_5, J = 8.4 Hz); ^{13}C NMR 14.6, 21.6, 60.3, 91.2, 106.2, 116.4, 123.9, 124.9, 128.5, 132.3, 135.5, 139.1, 139.9, 141.8, 149.3, 153.7, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_4S_2$: C, 53.51; H, 3.82; N, 6.24. Found: C, 53.62; H, 3.60; N, 6.43.

4.2.13. Ethyl 3-amino-7-chloro-9-(2′,5′-dimethylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5m). Yield 62%, mp 218–220 °C; IR (KBr cm^{-1}): 3482 NH_2, 1665 CO, 1490 SO_2; ^1H NMR δ $CDCl_3$: 1.22 (t, 3H, CH_3, J = 6.8 Hz), 2.02 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.22 (q, 2H, CH_2, J = 6.8 Hz), 6.24 (br s, 2H, NH_2), 6.46 (d, 1H, H_8, J = 1.9 Hz), 7.08 (s, 1H, $H_{6'}$), 7.27 (dd, 1H, H_6, J = 8.7, 1.9 Hz), 7.35–7.39 (m, 2H, $H_{2',3'}$), 8.06 (d, 1H, H_5, J = 8.7 Hz); ^{13}C NMR 14.6, 16.7, 21.0, 60.0, 89.9, 107.0, 115.8, 123.9, 124.1, 125.1, 129.2, 132.9, 136.5, 139.0, 139.4, 149.2, 152.9, 163.6. Anal. Calcd for $C_{21}H_{19}ClN_2O_4S_2$: C, 54.48; H, 4.14; N, 6.05. Found: C, 54.27; H, 3.95; N, 6.33.

4.2.14. Ethyl 3-amino-7-chloro-9-(3′-methoxylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5n). Yield 62%, mp 210–212 °C; IR (KBr cm^{-1}): 3488 NH_2, 1664 CO, 1440 SO_2; ^1H NMR δ $CDCl_3$: 1.26 (t, 3H, CH_3, J = 6.8 Hz), 3.86 (s, 3H, OCH_3), 4.20 (q, 2H, CH_2, J = 6.8 Hz), 6.23 (br s, 2H, NH_2), 6.59 (d, 1H, H_8, J = 1.7 Hz), 6.87 (d, 1H, $H_{2'}$ J = 2.4 Hz), 6.94 (d, 1H, $H_{4'}$, J = 7.8 Hz), 7.19 (dd, 1H, $H_{6'}$, J = 7.8, 2.3 Hz), 7.28 (d, 1H, H_6, J = 8.5 Hz), 7.58 (t, 1H, $H_{5'}$, J = 7.6 Hz), 8.05 (d, 1H, H_5, J = 8.5 Hz); ^{13}C NMR 14.6, 55.8, 60.4, 90.1, 106.3, 113.9, 116.5, 117.3, 120.5, 123.9, 124.1, 124.8, 132.3, 138.9, 139.1, 139.8, 149.1, 152.2, 161.9, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_5S_2$: C, 51.67; H, 3.68; N, 6.02. Found: C, 51.64; H, 3.83; N, 6.15.

4.2.15. Ethyl 3-amino-7-chloro-9-(4′-methoxylphenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5o). Yield 71%, mp 250–252 °C; IR (KBr cm^{-1}): 3456 NH_2, 1674 CO, 1459 SO_2; ^1H NMR δ $CDCl_3$: 1.25 (t, 3H, CH_3, J = 6.8 Hz), 3.85 (s, 3H, OCH_3), 4.22 (q, 2H, CH_2, J = 6.8 Hz), 6.21 (br s, 2H, NH_2), 6.58 (d, 1H, H_8, J = 1.8 Hz), 7.14 (d, 2H, $H_{2',6'}$, J = 8.9 Hz), 7.27–7.33 (m, 3H, $H_{6,3',5'}$), 8.04 (d, 1H, H_5, J = 8.9 Hz); ^{13}C NMR 14.6, 55.8, 60.4, 90.9, 106.2, 116.4, 116.7, 123.9, 124.5, 124.8, 130.1, 139.1, 139.5, 140.2, 149.4, 154.1, 161.3, 163.8. Anal. Calcd for $C_{20}H_{17}ClN_2O_5S_2$: C, 51.67; H, 3.68; N, 6.02. Found: C, 51.61; H, 3.69; N, 5.98.

4.2.16. Ethyl 3-amino-7-chloro-9-(3′-chlorophenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5p). Yield 48%, mp 234–236 °C; IR (KBr cm^{-1}): 3488 NH_2, 1676 CO, 1459 SO_2; ^1H NMR δ $CDCl_3$: 1.24 (t, 3H, CH_3, J = 6.8 Hz), 4.23 (q, 2H, CH_2, J = 6.8 Hz), 6.22 (br s, 2H, NH_2), 6.53 (d, 1H, H_8, J = 1.7 Hz), 7.31–7.34 (m, 2H, $H_{6,5'}$), 7.41–7.46 (m, 1H, $H_{6'}$), 7.67–7.70 (m, 2H, $H_{2',4'}$), 8.05 (d, 1H, H_5, J = 8.5 Hz); ^{13}C NMR 14.6, 60.5, 90.9, 106.8, 116.2, 124.1, 124.4, 125.1, 127.3, 129.4, 131.9, 132.7, 137.2, 138.9, 139.3, 139.5, 149.0, 152.8, 163.7. Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_4S_2$: C, 48.62; H, 3.01; N, 5.97. Found: C, 48.54; H, 3.07; N, 6.39.

4.2.17. Ethyl 3-amino-7-chloro-9-(4′-chlorophenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5q). Yield 53%, mp 218–220 °C; IR (KBr cm^{-1}): 3460 NH_2, 1666 CO, 1490 SO_2; ^1H NMR δ $CDCl_3$: 1.26 (t, 3H, CH_3, J = 6.8 Hz), 4.22 (q, 2H, CH_2, J = 6.8 Hz), 6.22 (br s, 2H, NH_2), 6.53 (d, 1H, H_8, J = 1.7 Hz), 7.29 (dd, 1H, H_6, J = 8.4, 1.7 Hz), 7.35 (d, 2H, $H_{2',6'}$, J = 8.5 Hz), 7.67 (d, 2H, $H_{3',5'}$, J = 8.5 Hz), 8.04 (d, 1H, H_5, J = 8.4 Hz); ^{13}C NMR 14.6, 60.5, 90.0, 106.7, 116.2, 124.1, 124.3, 125.1, 130.5, 132.1, 136.4, 137.7, 139.3, 139.7, 149.0, 152.9, 163.7. Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_4S_2$: C, 48.62; H, 3.01; N, 5.97. Found: C, 48.72; H, 3.33; N, 5.87.

4.2.18. Ethyl 3-amino-7-chloro-9-(3′,4′-dichlorophenyl)thieno-[3,2-*b*]benzothiazine 4,4-dioxide-2-carboxylate (5r). Yield 49%, mp 237–239 °C; IR (KBr cm^{-1}): 3472 NH_2, 1662 CO, 1495 SO_2; ^1H NMR δ $CDCl_3$: 1.27 (t, 3H, CH_3, J = 6.8 Hz), 4.21 (q, 2H, CH_2, J = 6.8 Hz), 6.24 (br s, 2H, NH_2), 6.54 (d, 1H, H_8, J = 1.7 Hz), 7.30–7.34 (m, 2H, $H_{6,6'}$), 7.55 (d, 2H, $H_{2'}$ J = 2.5 Hz), 7.79 (d, 1H, $H_{5'}$, J = 8.4 Hz), 8.05 (d, 1H, H_5, J = 8.4 Hz); ^{13}C NMR 14.6, 60.6, 90.3, 107.0, 116.0, 124.5, 125.1, 128.5, 131.2, 133.4, 135.8, 136.4, 136.9, 139.4, 139.4, 163.6. Anal. Calcd for $C_{19}H_{13}Cl_3N_2O_4S_2$: C, 45.30; H, 2.60; N, 5.56. Found: C, 45.18; H, 2.40; N, 5.68.

4.3. General procedure for preparation of 3-amino-9-phenyl-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6a–r)

Compounds **5a**–**r** (0.1 mmol) were stirred with (NaOH, KOH, LiOH, or HCl) in ethanol/water 4:1 at several conditions Table 1. The solvent was then removed under reduced pressure, dichloromethane was added (15 mL) and washed with water (2× 20 mL). Organic phase was dried over anhydrous sodium sulfate, filtered, and solvent was removed under reduced pressure to obtain starter material. Water was neutralized with hydrochloric acid (10%), dichloromethane was added, organic phase was separated, washed with water, brine, and dried with sodium sulfate, filtered, and evaporated to dryness under reduced pressure, and the solid thus obtained was collected, the crude product was recrystallized from ethanol to give a pure sample. The physical and spectra data of the compounds **6a**–**r** are as follows.

4.3.1. 3-Amino-6-chloro-9-phenylthieno-[3,2-*b*]benzothiazine 4,4-dioxide (6a). Yield 67%, mp 166–168 °C; IR (KBr cm^{-1}): 3340 NH_2, 1460 SO_2; ^1H NMR δ $CDCl_3$: 4.27 (br s, 2H, NH_2), 5.56 (s, 1H, H_2), 6.50 (d, 1H, H_8, J = 9.3 Hz), 7.29 (dd, 1H, H_7, J = 9.3, 1.9 Hz), 7.37–7.40 (m, 2H, $H_{2',6'}$), 7.66 (m, 3H, $H_{3',4',5'}$), 8.09 (d, 1H, H_5, J = 1.9 Hz). ^{13}C NMR 88.7, 107.9, 117.7,

123.2, 125.9, 129.2, 130.9, 131.5, 132.5, 137.6, 138.9, 139.3, 140.2, 150.3. Anal. Calcd for $C_{16}H_{11}ClN_2O_2S_2$: C, 52.96; H, 3.06; N, 7.72. Found: C, 53.09; H, 3.11; N, 7.83.

4.3.2. 3-Amino-6-chloro-9(2′-methylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6b). Yield 45%, mp 174–176 °C; IR (KBr cm^{-1}): 3430 NH_2, 1462 SO_2; ^1H NMR δ $CDCl_3$: 2.06 (s, 3H, CH_3), 4.02 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.41 (d, 1H, H_8, $J = 9.2$ Hz), 7.30–7.34 (m, 2H, $H_{7,6'}$), 7.50–7.55 (m, 3H, $H_{3',4',5'}$), 8.11 (d, 1H, H_5, $J = 2.2$ Hz). ^{13}C NMR 17.1, 88.5, 107.6, 117.1, 123.4, 125.8, 128.1, 128.9, 129.4, 131.1, 132.8, 132.9, 136.7, 137.3, 137.5, 140.2, 149.7. Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.18; H, 3.48; N, 7.43. Found: C, 53.99; H, 3.67; N, 7.34.

4.3.3. 3-Amino-6-chloro-9-(4′-methylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6c). Yield 59%, mp 208–210 °C; IR (KBr cm^{-1}): 3408 NH_2, 1465 SO_2; ^1H NMR δ $CDCl_3$: 2.48 (s, 3H, CH_3), 3.90 (br s, 2H, NH_2), 5.54 (s, 1H, H_2), 6.52 (d, 1H, H_8, $J = 9.2$ Hz), 7.26–7.31 (m, 3H, $H_{7,2',6'}$), 7.43 (d, 2H, $H_{3',5'}$, $J = 7.7$ Hz), 8.06 (d, 1H, H_5, $J = 2.2$ Hz). ^{13}C NMR 21.5, 88.7, 107.4, 117.8, 123.1, 125.7, 127.9, 128.9, 132.0, 132.5, 136.2, 137.7, 141.2, 140.1, 150.7. Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.18; H, 3.48; N, 7.43. Found: C, 54.31; H, 3.63; N, 7.45.

4.3.4. 3-Amino-6-chloro-9-(2′,5′-dimethylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6d). Yield 53%, mp 152–154 °C; IR (KBr cm^{-1}): 3428 NH_2, 1460 SO_2; ^1H NMR δ $CDCl_3$: 2.00 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.25 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.45 (d, 1H, H_8, $J = 8.7$ Hz), 7.07 (s, 1H, $H_{6'}$), 7.22 (dd, 1H, H_7, $J = 8.7$, 1.9 Hz), 7.32(d, 1H, $H_{4'}$, $J = 8.4$ Hz), 7.37 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 8.08 (d, 1H, H_5, $J = 1.9$ Hz). ^{13}C NMR 16.7, 21.0, 89.9, 106.9, 117.0, 123.1, 126.1, 129.1, 130.0, 131.3, 132.9, 133.1, 133.3, 136.9, 138.0, 138.5, 139.90, 151.0. Anal. Calcd for $C_{18}H_{15}ClN_2O_2S_2$: C, 53.31; H, 3.87; N, 7.16. Found: C, 53.40; H, 3.79; N, 7.45.

4.3.5. 3-Amino-6-chloro-9-(3′-methoxylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6e). Yield 61%, mp 214–216 °C; IR (KBr cm^{-1}): 3420 NH_2, 1457 SO_2; ^1H NMR δ $CDCl_3$: 3.88 (s, 3H, OCH_3), 4.11 (br s, 2H, NH_2), 5.56 (s, 1H, H_2), 6.57 (d, 1H, H_8, $J = 8.5$ Hz), 6.88 (t, 1H, $H_{2'}$ $J = 2.2$ Hz), 6.95 (d, 1H, $H_{4'}$, $J = 7.9$ Hz), 7.16 (dd, 2H, $H_{6'}$, $J = 7.9$, 2.2 Hz), 7.26 (dd, 1H, H_7, $J = 8.6$, 2.1 Hz), 7.56 (t, 1H, $H_{5'}$, $J = 7.9$ Hz), 8.07 (d, 1H, H_5, $J = 2.1$ Hz). ^{13}C NMR 55.8, 88.8, 107.4, 116.3, 117.6, 123.0, 125.3, 127.1, 129.4, 131.1, 132.5, 137.7, 140.1, 150.9, 161.2. Anal. Calcd for $C_{17}H_{13}ClN_2O_3S_2$: C, 51.97; H, 3.34; N, 7.13. Found: C, 51.97; H, 3.21; N, 6.92.

4.3.6. 3-Amino-6-chloro-9-(4′-methoxylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6f). Yield 73%, mp 224–226 °C; IR (KBr cm^{-1}): 3440 NH_2, 1465 SO_2; ^1H NMR δ $CDCl_3$: 3.90 (s, 3H, OCH_3), 4.01 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.53 (d, 1H, H_8, $J = 8.9$ Hz), 7.11 (d, 2H, $H_{2',6'}$, $J = 8.7$ Hz), 7.26–7.30 (m, 3H, $H_{7,3',5'}$), 8.06 (d, 1H, H_5, $J = 1.9$ Hz). ^{13}C NMR 55.8, 88.7, 107.4, 116.4, 117.8, 123.1, 125.7, 127.9, 130.4, 131.3, 132.5, 137.9, 140.2, 150.9, 160.9. Anal. Calcd for $C_{17}H_{13}ClN_2O_3S_2$: C, 51.97; H, 3.34; N, 7.13. Found: C, 52.13; H, 3.42; N, 6.97.

4.3.7. 3-Amino-6-chloro-9-(3′-chlorophenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6g). Yield 41%, mp 192–194 °C; IR (KBr cm^{-1}): 3360 NH_2, 1455 SO_2; ^1H NMR δ $CDCl_3$: 4.23 (br s, 2H, NH_2), 5.57 (s, 1H, H_2), 6.50 (d, 1H, H_8, $J = 9.2$ Hz), 7.31–7.34 (m, 2H, $H_{7,6'}$), 7.50 (s, 1H, $H_{2'}$), 7.60–7.64 (m, 2H, $H_{4',5'}$), 7.50 (s, 1H, $H_{2'}$), 8.08 (d, 1H, H_5, $J = 2.3$ Hz). ^{13}C NMR 88.7, 107.9, 117.5, 123.3, 125.9, 127.7, 129.7, 131.7, 132.5, 132.7, 136.9, 137.3, 139.7, 140.3, 149.6. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S_2$: C, 48.37; H, 2.54; N, 7.05. Found: C, 48.23; H, 2.67; N, 7.28.

4.3.8. 3-Amino-6-chloro-9-(4′-chlorophenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6h). Yield 48%, mp 242–244 °C; IR (KBr cm^{-1}): 3356 NH_2, 1464 SO_2; ^1H NMR δ $CDCl_3$: 4.10 (br s, 2H, NH_2), 5.56 (s, 1H, H_2), 6.49 (d, 1H, H_8, $J = 7.7$ Hz), 7.29–7.35 (m, 5H, $H_{7,2',3',5',6'}$), 8.08 (d, 1H, H_5, $J = 2.3$ Hz). ^{13}C NMR 88.8, 107.9, 117.4, 123.3, 125.2, 130.0, 131.3, 132.5, 133.2, 137.2, 139.2, 139.6, 150.2. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S_2$: C, 48.37; H, 2.54; N, 7.05. Found: C, 48.19; H, 2.37; N, 6.93.

4.3.9. 3-Amino-6-chloro-9-(3′,4′-dichlorophenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6i). Yield 37%, mp 172–174 °C; IR (KBr cm^{-1}): 3380 NH_2, 1459 SO_2; ^1H NMR δ $CDCl_3$: 4.00 (br s, 2H, NH_2), 5.58 (s, 1H, H_2), 6.51 (d, 1H, H_8, $J = 9.2$ Hz), 7.27 (dd, 1H, $H_{6'}$, $J = 8.7$, 1.9 Hz), 7.33 (dd, 1H, H_7, $J = 9.2$, 1.9 Hz), 7.53 (d, 1H, $H_{2'}$, $J = 1.9$ Hz), 7.74 (d, 1H, $H_{5'}$, $J = 8.7$ Hz), 8.08 (d, 1H, H_5, $J = 1.9$ Hz). ^{13}C NMR 88.7, 108.5, 117.3, 123.4, 126.2, 128.8, 131.5, 132.7, 133.2, 135.5, 135.8, 137.2, 137.8, 139.8, 140.4, 149.7. Anal. Calcd for $C_{16}H_9Cl_3N_2O_2S_2$: C, 44.51; H, 2.10; N, 6.49. Found: C, 44.61; H, 2.30; N, 6.73.

4.3.10. 3-Amino-7-chloro-9-phenylthieno-[3,2-*b*]benzothiazine 4,4-dioxide (6j). Yield 63%, mp 230–232; IR (KBr cm^{-1}): 3424 NH_2, 1462 SO_2; ^1H NMR δ $CDCl_3$: 4.15 (br s, 2H, NH_2), 5.56 (s, 1H, H_2), 6.51 (d, 1H, H_8, $J = 1.7$ Hz), 7.21 (dd, 1H, H_6, $J = 8.7$, 1.7 Hz), 7.37–7.40 (m, 2H, $H_{2',6'}$), 7.66–7.40 (m, 3H, $H_{3',4',5'}$), 8.07 (d, 1H, H_5, $J = 8.7$ Hz). ^{13}C NMR 88.8, 108.1, 115.7, 123.0, 123.4, 125.2, 129.2, 131.5, 138.6, 138.7, 139.9, 140.2, 150.2. Anal. Calcd for $C_{16}H_{11}ClN_2O_2S_2$: C, 52.96; H, 3.06; N, 7.72. Found: C, 52.86; H, 3.07; N, 7.68.

4.3.11. 3-Amino-7-chloro-9(2′-methylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6k). Yield 60%, mp 206–208 °C; IR (KBr cm^{-1}): 3424 NH_2, 1465 SO_2; ^1H NMR δ $CDCl_3$: 2.06 (s, 3H, CH_3), 4.02 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.43 (d, 1H, H_8, $J = 1.5$ Hz), 7.23 (dd, 1H, H_6, $J = 8.4$, 1.5 Hz), 7.28 (d, 1H, $H_{6'}$, $J = 7.2$ Hz), 7.52–7.57 (m, 3H, $H_{3',4',5'}$), 8.09 (d, 1H, H_5, $J = 8.4$ Hz). ^{13}C NMR 17.1, 88.6, 107.9, 115.1, 123.1, 123.4, 125.4, 128.9, 129.4, 131.3, 133.0, 137.4,

138.9, 139.1, 143.0, 140.2, 151.0. Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.18; H, 3.48; N, 7.43. Found: C, 54.26; H, 3.47; N, 7.38.

4.4. 3-Amino-7-chloro-9(4′-methylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6l)

Yield 71%, mp 228–230 °C; IR (KBr cm^{-1}): 3444 NH_2, 1466 SO_2; ^1H NMR δ $CDCl_3$: 2.50 (s, 3H, CH_3), 4.43 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.53 (d, 1H, H_8, J = 2.0 Hz), 7.20 (dd, 1H, H_6, J = 8.7, 2.0 Hz), 7.23 (d, 2H, $H_{2',6'}$, J = 8.4 Hz), 7.44 (d, 2H, $H_{3',5'}$, J = 8.4 Hz), 8.06 (d, 1H, H_5, J = 8.7 Hz). ^{13}C NMR 21.5, 88.8, 107.9, 115.8, 122.9, 123.4, 125.1, 128.8, 132.1, 135.9, 138.6, 140.1, 140.2, 141.4, 150.5. Anal. Calcd for $C_{17}H_{13}ClN_2O_2S_2$: C, 54.18; H, 3.48; N, 7.43. Found: C, 53.98; H, 3.67; N, 7.63.

4.5. 3-Amino-7-chloro-9(2′,5′-dimethylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6m)

Yield 47%, mp 190–192 °C; IR (KBr cm^{-1}): 3408 NH_2, 1462 SO_2; ^1H NMR δ $CDCl_3$: 2.00 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.52 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.45 (d, 1H, H_8, J = 2.0 Hz), 7.07 (s, 1H, $H_{6'}$), 7.22 (dd, 1H, H_6, J = 8.7, 2.0 Hz), 7.32 (d, 1H, $H_{4'}$, J = 8.4 Hz), 7.37 (d, 1H, $H_{5'}$, J = 8.4 Hz), 8.08 (d, 1H, H_5, J = 8.7 Hz).^{13}C NMR 16.7, 21.0, 88.6, 107.9, 115.2, 123.0, 123.4, 125.3, 129.5, 132.0, 132.7, 133.9, 136.9, 138.9, 139.1, 140.2, 149.6. Anal. Calcd for $C_{18}H_{15}ClN_2O_2S_2$: C, 53.31; H, 3.87; N, 7.16. Found: C, 53.12; H, 3.81; N, 7.18.

4.6. 3-Amino-7-chloro-9(3′-methoxylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6n)

Yield 43%, mp 158–160 °C; IR (KBr cm^{-1}): 3376 NH_2, 1462 SO_2; ^1H NMR δ $CDCl_3$: 3.85 (s, 3H, OCH_3), 4.37 (br s, 2H, NH_2), 5.56 (s, 1H, H_2), 6.57 (d, 1H, H_8, J = 1.7 Hz), 6.88 (t, 1H, $H_{2'}$ J = 2.2 Hz), 6.95 (d, 1H, $H_{4'}$, J = 7.9 Hz), 7.16 (dd, 2H, $H_{6'}$, J = 7.9, 2.2 Hz), 7.22 (dd, 1H, H_6, J = 8.7, 1.7 Hz), 7.56 (t, 1H, $H_{5'}$, J = 7.9 Hz), 8.06 (d, 1H, H_5, J = 8.7 Hz). ^{13}C NMR 55.8, 88.8, 107.0, 114.2, 115.8, 116.9, 120.8, 123.0, 123.4, 125.1, 132.1, 138.7, 139.5, 139.9, 140.1, 150.1, 161.9. Anal. Calcd for $C_{17}H_{13}ClN_2O_3S_2$: C, 51.97; H, 3.34; N, 7.13. Found: C, 52.15; H, 3.37; N, 7.23.

4.6.1. 3-Amino-7-chloro-9(4′-methoxylphenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6o).

Yield 47%, mp 226–228 °C; IR (KBr cm^{-1}): 3424 NH_2, 1465 SO_2; ^1H NMR δ $CDCl_3$: 3.91 (s, 3H, OCH_3), 4.39 (br s, 2H, NH_2), 5.55 (s, 1H, H_2), 6.54 (d, 1H, H_8, J = 1.7 Hz), 7.12 (d, 2H, $H_{2',6'}$, J = 8.9 HZ), 7.20 (dd, 1H, H_6, J = 8.7, 1.7 Hz), 7.27 (d, 2H, $H_{3',5'}$, J = 8.9 Hz), 8.05 (d, 1H, H_5, J = 8.7 Hz). ^{13}C NMR 55.7, 88.7, 108.3, 115.8, 116.5, 122.9, 123.7, 125.1, 130.4, 138.6, 138.7, 140.3, 140.4, 150.9, 161.2. Anal. Calcd for $C_{17}H_{13}ClN_2O_3S_2$: C, 51.97; H, 3.34; N, 7.13. Found: C, 51.86; H, 3.47; N, 6.90.

4.6.2. 3-Amino-7-chloro-9(3′-chlorophenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6p).

Yield 38%, mp 235–237 °C; IR (KBr cm^{-1}): 3388 NH_2, 1459 SO_2; ^1H NMR δ $CDCl_3$: 4.24 (br s, 2H, NH_2), 5.58 (s, 1H, H_2), 6.50 (d, 1H, H_8, J = 1.5 Hz), 7.23 (dd, 1H, H_6, J = 8.7, 1.5 Hz), 7.30–7.34 (m, 2H, $H_{5',6'}$), 7.42 (s, 1H, $H_{2'}$), 7.63 (d, 1H, $H_{4'}$, J = 6.0 Hz), 8.07 (d, 1H, H_5, J = 8.7 Hz). ^{13}C NMR 88.9, 108.5, 115.6, 123.4, 123.6, 125.3, 127.6, 129.7, 131.5, 132.5, 136.9, 138.9, 139.5, 139.7, 140.3, 149.6. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S_2$: C, 48.37; H, 2.54; N, 7.05. Found: C, 48.61; H, 2.43; N, 6.89.

4.6.3. 3-Amino-7-chloro-9(4′-chlorophenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6q).

Yield 57%, mp 282–284 °C; IR (KBr cm^{-1}): 3344 NH_2, 1462 SO_2; ^1H NMR δ $CDCl_3$: 4.23 (br s, 2H, NH_2), 5.58 (s, 1H, H_2), 6.50 (d, 1H, H_8, J = 1.9 Hz), 7.23 (dd, 1H, H_6, J = 8.7, 1.9 Hz), 7.34 (d, 2H, $H_{2',6'}$, J = 8.7 Hz), 7.64 (d, 2H, $H_{3',5'}$, J = 8.7 Hz), 8.07 (d, 1H, H_5, J = 8.7 Hz). ^{13}C NMR 88.4, 107.9, 115.76, 123.3, 124.1, 125.9, 131.9, 132.0, 132.1, 137.6, 137.6, 139.8, 141.6, 149.8. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S_2$: C, 48.37; H, 2.54; N, 7.05. Found: C, 48.57; H, 2.57; N, 7.17.

4.6.4. 3-Amino-7-chloro-9(3′,4′-dichlorophenyl)-thieno-[3,2-*b*]benzothiazine 4,4-dioxide (6r).

Yield 49%, mp 230–232 °C; IR (KBr cm^{-1}): 3430 NH_2, 1464 SO_2; ^1H NMR δ $CDCl_3$: 4.24 (br s, 2H, NH_2), 5.59 (s, 1H, H_2), 6.51 (d, 1H, H_8, J = 1.9 Hz), 7.25 (dd, 1H, H_6, J = 8.7, 1.9 Hz), 7.28 (dd, 1H, $H_{6'}$, J = 8.4, 2.2 Hz), 7.54 (d, 1H, $H_{2'}$, J = 2.2 Hz), 7.76 (d, 1H, $H_{5'}$, J = 8.4 Hz), 8.06 (d, 1H, H_5, J = 8.7 Hz). ^{13}C NMR 88.9, 108.7, 115.4, 123.6, 123.7, 125.4, 128.8, 131.5, 133.3, 135.6, 135.9, 137.5, 138.9, 139.5, 140.4, 149.3. Anal. Calcd for $C_{16}H_9Cl_3N_2O_2S_2$: C, 44.51; H, 2.10; N, 6.49. Found: C, 44.37; H, 1.87; N, 6.37.

5. X-ray crystallography

Crystals suitable for X-ray diffraction for all compounds were obtained by slow evaporation of solutions in ethanol. Crystal data, intensity data collection parameters and final refinement results are summarized in Table 4.

Unit cell and intensity measurements were carried out on a Bruker Smart CCD area-detector diffractometer, using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods and refined on F^2 by full-matrix least-squares, using all reflections, anisotropic displacement parameters and weights $w = [\sigma^2(F_o^2) + (aP)^2 + bP]^{-1}$, with $P = (F_o^2 + 2F_c^2)/3$. The C- and N(imino)-bonded H atoms were placed in calculated positions, and refined using a riding atom model with fixed C–H [0.93 Å for $C(sp^2)$, 0.96 Å for $C(sp^3)$] and N–H [0.86 Å] distances, and Uiso = p Ueq(parent atom) [p = 1.2 for $C(sp^2)$ and N; 1.5 for $C(sp^3)$]. The N(amino)-bonded H atoms were located in difference Fourier syntheses and refined isotropically. The final difference Fourier syntheses were featureless.

The following computer programs were used: data collection, SMART;[21a] data reduction and cell refinement, SAINT;[21b] absorption correction, SADABS;[21c] struc-

Table 4. Crystal data, intensity data-collection parameters, and final refinement results

Compound	**4d**	**5d**
CCDC deposit No.	CCDC 650290	CCDC 650291
Crystal data		
Formula	$C_{18}H_{15}ClN_2O_2S_2$	$C_{21}H_{19}ClN_2O_4S_2$
MW	390.89	462.95
Color	Colorless	Colorless
Morphology	Prism	Prism
Specimen size (mm)	$0.24 \times 0.18 \times 0.13$	$0.30 \times 012 \times 0.11$
T (K)	296(2)	296(2)
a (Å)	9.8708(8)	11.1580(8)
b (Å)	9.9252(8)	10.1225(7)
c (Å)	11.7038	19.1247(14)
α (°)	91.235(2)	
β (°)	111.7120(10)	91.851(2)
γ (°)	116.8240(10)	
V (Å^3)	925.75(13)	2158.9(3)
Crystal system	Triclinic	Monoclinic
Space group (No.)	$P-1$ (#2)	$P2_1/c$ (#14)
Z	2	4
D_c (g cm^{-3})	1.402	1.424
$F(000)$	404	960
μ(Mo-Kα) (mm^{-1})	0.446	0.401
θ range (°) for cell	2.4–26.7	2.3–21.5
No. refls. for cell	1897	2139
Data collection		
θ range (°)	1.9–29.0	1.8–29.0
h range	−13, 13	−14, 8
k range	−13, 13	−13, 13
l range	−10, 15	−25, 25
Mean ΔI for checks (%)	<1	<1
No. refls. measured	6438	14649
No. refls. unique	4320	5328
No. refls. $I > 2\sigma(I)$	3099	3341
R_{int}	0.0147	0.0368
Refinement (last cycle)		
Weighting scheme (a,b)	0.0586, 0.1562	0.0636, 0.5070
Trans. coeff. (T_{min}, T_{max})	0.919–0.944	0.913–0.957
No. params. refined	229	282
R_1 [$I > 2\sigma(I)$]	0.0464	0.0567
R_1 (all data)	0.0664	0.0998
wR_2 [$I > 2\sigma(I)$]	0.1153	0.1286
wR_2 (all data)	0.1253	0.1482
S (g.o.f.) (all data)	1.043	1.029
Δ/σ max.	0.014	0.001
Δ/σ mean	<0.0005	<0.0005
$\Delta\rho_r$ (min, max) (e Å^{-3})	−0.29, 0.42	−0.59, 0.65

ture solution, SHELXS-97;[22] structure refinement, SHELXL-97;[23] molecular graphics, ORTEP 3.[24] The structure solutions, the refinements and the drawings were carried out with the aid of the WinGX[25] suite of programs.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication Nos. CCDC 650290 and 650291. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.

6. Computational methods

All the calculations reported here were carried out using the program Gaussian 03.[26] In the first step, a conformational search was carried out using exploratory RHF/3-21G calculations. Then more accurate B3LYP/6-31G (d) calculations were carried out to confirm the critical points obtained. Minima were characterized through harmonic frequency analysis using B3LYP/6-31G (d) calculations. The electronic study of the molecules was carried out using molecular electrostatic potentials (MEPs).[17,18] MEPs were calculated from B3LYP/6-31G(d) wave functions. MEP graphical presentations were created using the Molekel program.[27]

7. Biological assays

7.1. Inhibition of β-hematin formation

The β-hematin formation assay was performed with a solution of hemin chloride (50 μL, 4 mM), dissolved in DMSO (5.2 mg/mL), distributed in 96-well microplates.[28] Different concentrations (50–5 mM) of the compounds dissolved in DMSO were added in triplicate in test wells (50 μL). Controls contained either water (50 μL) or DMSO (50 μL). β-Hematin formation was initiated by the addition of acetate buffer (100 μL, 0.2 M, pH 4.4). Plates were incubated at 37 °C for 48 h to allow for completion of the reaction and centrifuged (4000 RPM × 15 min, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with DMSO (200 μL) and finally, dissolved in NaOH (200 μL, 0.2 N). The solubilized aggregates were further diluted 1:2 with NaOH (0.1 N) and absorbance recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of β-hematin formation.

7.2. Parasite, experimental host, and strain maintenance

Male Balb-C mice, weighing 18–22 g were maintained on a commercial pellet diet and housed under conditions approved by the Ethics Committee. *Plasmodium berghei* (ANKA strain chloroquine sensible), a rodent malaria parasite, was used for infection. Mice were infected by ip passage of 1×10^6 infected erythrocytes diluted in phosphate buffered saline solution (PBS, 10 mM, pH 7.4, 0.1 mL). Parasitemia was monitored by microscopic examination of Giemsa stained smears.[29]

7.3. Parasite extracts

Blood of infected animals with a high level of parasitemia (30–70%), was collected by cardiac puncture with a heparinized syringe and the blood pool was centrifuged (500g × 10 min, 4 °C). Plasma and buffy coat were removed and the red blood cell (RBC) pellet was washed twice with chilled PBS–Glucose (5.4%). The washed RBC pellet was centrifuged on a discontinuous percoll gradient (80–70% percoll in PBS–Glucose, 20,000g × 30 min × 4 °C).[30] The upper band (mature forms)

was removed by aspiration, collected in eppendorf tubes and washed twice with chilled PBS–Glucose and the infected erythrocytes were lysed with the non-ionic detergent saponin (0.1% in PBS × 10 min). Cold PBS (1 mL) was added and the samples were centrifuged (13,000*g* × 5 min, 4 °C) to remove erythrocyte cytoplasm content (including erythrocyte hemoglobin). The free parasites were mixed in PBS–Glucose (5.4%), and subjected to three freeze–thaw cycles (−70 °C + 37 °C). The final homogenate was used in the hemoglobin hydrolysis inhibition assay.[31]

7.4. Mice native hemoglobin

Native hemoglobin from non-infected mice was obtained by treating one volume of pellet erythrocytes with two volumes of water. The resulting solution was used as the substrate in the inhibition of the hemoglobin hydrolysis assay.

7.5. Inhibition of hemoglobin hydrolysis

The proteolytic effect of the parasite extract on the native mice hemoglobin was assayed using 96-well tissue culture plate (Greiner Bio-One). The assay mixture contained: mice native hemoglobin (10 μL), parasite extract (50 μL), GSH (10 μL, 10 μM), and acetate buffer (0.2 M, pH 5.4) to a final volume of 200 μL. The compounds, chloroquine, leupeptin, and pepstatin (2.5 mM) were incorporated into the incubation mixture dissolved in DMSO. The incubations were carried out at 37 °C for 18 h and the reactions were stopped by the addition of reduced sample buffer. The degree of digestion was evaluated electrophoretically by SDS–PAGE by visual comparison of the globin bands (14.4 kDa). A DMSO control was electrophoresed at the same time. Once the bands were obtained, the densitometer registered the band densities reported as intensity/mm^2 ± SD, so we proceeded to check the densities in order to have a percentage of inhibition of hemoglobin hydrolysis.

7.6. Four-day suppressive test

NIH mice (18–22 g) were infected ip with 1×10^7 *P. berghei* parasites. Two hours after infection, treatment began with the best compounds tested in the globin hydrolysis assay. These were dissolved in DMSO (0.1 M), diluted with Saline–Tween 20 solution (2%). Each compound (20 mg/kg) was administered once by ip for 4 days. At day four, the parasitemia was counted by examination of Giemsa stained smears. The chloroquine (25 mg/kg) was used as a positive control. The survival time after infection was recorded. The results were expressed as a percentage of parasitemia at four days post-infection and survival time after infection compared to control mice.[32]

7.7. Data analysis

Data were statistically analyzed using *t*-tests for specific group comparisons, assuming 95% confidence according GraphPad Prism 3.02.[33]

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