European Journal of Medicinal Chemistry 44 (2009) 1303–1310

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antimalarial activity of pyrazolo and pyrimido benzothiazine dioxide derivatives

Arthur Barazarte [a], Gricela Lobo [a], Neira Gamboa [b], Juan R. Rodrigues [b], Mario V. Capparelli [c], Ángel Álvarez-Larena [d], Simón E. López [e], Jaime E. Charris [a,*]

[a] Laboratorio de Síntesis Orgánica, Universidad Central de Venezuela, Aptdo. 47206, Los Chaguaramos, 1041-A Caracas, Venezuela
[b] Unidad de Bioquímica, Facultad de Farmacia, Universidad Central de Venezuela, Aptdo. 47206, Los Chaguaramos, 1041-A Caracas, Venezuela
[c] Escuela de Química, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela
[d] Servicio de Difracción de Rayos X, Universidad Autónoma de Barcelona, Bellaterra, Spain
[e] Departamento de Química, Universidad Simón Bolívar, Caracas, Venezuela

ARTICLE INFO

Article history:
Received 10 June 2008
Received in revised form 7 August 2008
Accepted 8 August 2008
Available online 26 August 2008

Keywords:
Benzothiazines
Pyrazol
Pyrimidine
Plasmodium berghei
β-Hematin
Hemoglobin

ABSTRACT

A series of phenylsubstituted pyrazolo and pyrimido benzothiazine dioxide derivatives were synthesized and investigated for their abilities to inhibit β-hematin formation, hemoglobin hydrolysis and *in vivo* for their antimalarial efficacy in rodent *Plasmodium berghei*. Compounds 3-amino-7-chloro-9-(2′-methylpheyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide **2b** and 2,4-diamino-8-chloro-10*H*-phenyl-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide **3a** were the most promising as inhibitors of hemoglobin hydrolysis, however, their effect as inhibitors of β-hematin formation was marginal, except for compound 3-amino-7-chloro-9-(3′-chlorophenyl)-1,9dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide **2g**. The most active compound to emerge from the *in vitro* and *in vivo* murine studies was **2b**, suggesting an antimalarial activity via inhibition of hemoglobin hydrolysis, however, not as efficient as chloroquine.

1. Introduction

Malaria, a major tropical infectious disease caused primarily by the protozoan parasite *Plasmodium falciparum*, is one of the most serious health problems worldwide and is responsible for the death of over 1 million individuals every year with more than 40% of the global population at risk [1]. Since resistance to currently used antimalarials is spreading rapidly, there is a great need for new drugs. Thus, there is a compelling and urgent necessity for new antimalarials, with mechanisms of action different from the existing ones, and to identify new drug targets [2]. Chloroquine has recently been shown to inhibit hemozoin formation within the parasite food vacuole [3]. This process is also thought to be the molecular target of other quinoline antimalarials [4]. Hemozoin was originally considered to be formed by the polymerization of heme, but it has now been demonstrated to be a crystalline cyclic dimer of ferriprotoporphyrin IX [5–8]. 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 are widely used clinically. Some of these quinolones such as ciprofloxacin, gatifloxacin, moxifloxacin and trovafloxacin, display a diverse array of biological activities including antiplasmodial efficacy [9]. We have recently described the preparation and antimalarial activities of several tricyclic quinolone and benzothiazine analogs [10–12]. In continuation of our studies directed toward synthesis of quinolones and benzothiazines annelated with various five- and six-member heterocycles, we reported here the synthesis of 3-amino-6 or 7-chloro-9-(phenylsubstituted)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide **2a–t** and 2,4-diamino-7 or 8-chloro-10-(phenylsubstituted)-10*H*-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide **3a–t**, their *in vitro* abilities to inhibit β-hematin formation and hemoglobin hydrolysis and their *in vivo* efficacy against rodent *Plasmodium berghei*.

2. Chemistry

2,3-Substituted 6 or 7 chloro-*N*-phenylbenzothiazine **1a–t** were obtained following the method previously reported [10–12]. Products **2a–t** and **3a–t** were obtained when **1a–t** were reacted

* Corresponding author. Tel.: +58 212 6052722; fax: +58 212 6052707.
E-mail address: charrisj@ucv.ve (J.E. Charris).

0223-5234/$ – see front matter © 2008 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2008.08.005

with hydrazine hydrate or guanidine hydrochloride, dry pyridine or DMF under an inert atmosphere of nitrogen, respectively, (Scheme 1).

It is important to mention that in the ^{1}H NMR spectra of these compounds, protons at positions 8 and 9 from derivatives **2a–t** and **3a–t** appeared as doublets around 6.0 ppm with coupling constants ranging between 0.9–2.5 and 7.0–8.0 Hz, clearly indicating the smaller chemical shift of the proton on these positions by the effect of the phenyl group on position 9 or 10, respectively. Additional supports for these structures were obtained from ^{13}C NMR.

The molecular structure of **3p** was confirmed by X-ray crystallography (Fig. 1). The X-ray crystal structure analysis showed that all the bond distances are within expected values [13]. In the tricyclic system, the central ring displays an approximate sofa conformation, with S1 out of the plane [0.600(2) Å], and O1 and O2 in equatorial and axial positions, respectively. The N-bonded phenyl ring is approximately perpendicular to the tricyclic system [dihedral angle between mean planes: 84.83(5)°]. The molecule forms an N–H···O (sulfonyl) intramolecular hydrogen bond. In addition, in the crystal structure there are a number of intermolecular hydrogen bonds of the types N–H···O(sulfonyl), N–H···O(methoxy), N–H···Cl, N–H···S, C–H···O(sulfonyl), C–H···O(methoxy), C–H···N(amino) and C–H···N(pyrimidine, N4) which link the molecules to form a three dimensional network (see CIF file for details).

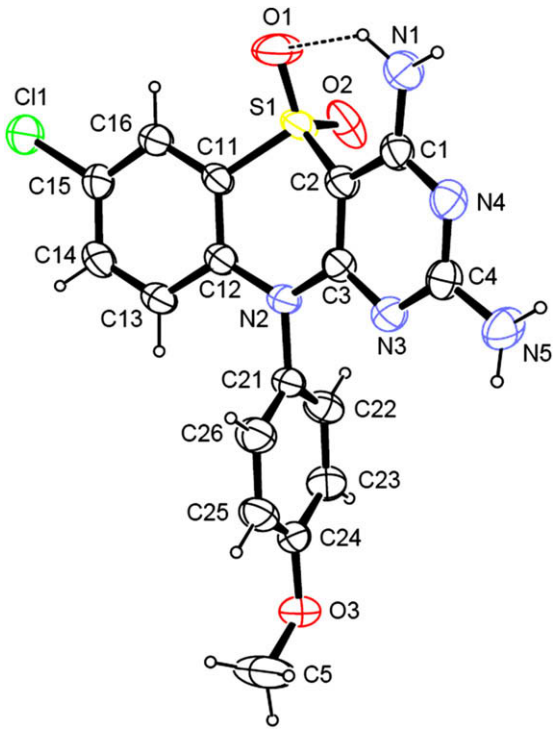
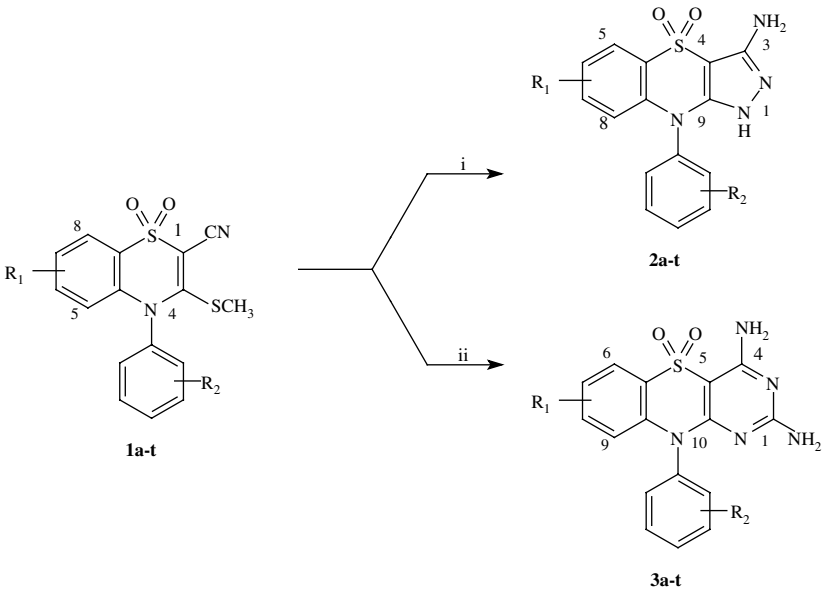


Fig. 1. Molecular structure of compound **3p** showing the atomic numbering. The displacement ellipsoids are drawn at 50% probability. A dashed line indicates an intramolecular hydrogen bond.

3. Biological results and discussion

Previous reports showed that tricyclic benzothiazines exhibited antimalarial activities [10,12]. All analogs of those derivatives were tested *in vitro* for their effects as inhibitors of β-hematin formation, and inhibition of hemoglobin hydrolysis (Table 1). Only **2a**, **2g**, **3a** derivatives were tested *in vivo* for their efficacy in a murine model (Table 3). The first mentioned *in vitro* assay was used to assess the abilities of the *N*-phenylpyrazolo[4,3-*b*]benzothiazine and *N*-phenylpyrimido[5,4-*b*]benzothiazine *S*,*S*-dioxide derivatives to inhibit β-hematin formation, where hemin was allowed to form β-hematin under acidic conditions. Among the 40 compounds tested, only one **2g** showed a measurable activity (85.42 ± 6.14%) compared to chloroquine (86.6 ± 2.75%).

Compounds **2a–t** and **3a–t** were also tested for the inhibition of globin proteolysis using *in vitro* assays which used rich extracts of trophozoites to digest the native hemoglobin of mice. Electrophoretic analyses indicated that compounds **2a–j** and **3a–j** were effective as inhibitors of hemoglobin degradation; however, compounds **2b** and **3a** were the most effectives (92.32 ± 1.1 and 83.72 ± 2.13%) compared to leupeptin and pepstatin (89.06 ± 0.69 and 92.94 ± 0.67), respectively (Table 1).



Scheme 1. Synthesis of pyrazolo and pyrimido benzothiazine dioxide derivatives **2a–t**, **3a–t**. (i) N_2H_4 hydrate, pyridine, Δ. (ii) Guanidine hydrochloride, potassium carbonate, DMF, Δ. R_1: Cl; R_2: H, CH_3, OCH_3, Cl, Br.

Table 1
Inhibition of β-hematin synthesis (IβHS) and globin proteolysis (IGP) (%) by benzothiazine derivatives

No.	R_2	IβHS	IGP
2a	H	<5	76.39 ± 1.52
2b	2-CH_3	<5	92.32 ± 1.1**
2c	4-CH_3	<5	78.78 ± 0.41
2d	2,5-CH_3	<5	69.39 ± 1.17
2e	3-OCH_3	<5	47.27 ± 1.79
2f	4-OCH_3	<5	54.77 ± 1.58
2g	3-Cl	85.42 ± 6.14†	58.89 ± 2.14
2h	4-Cl	<5	55.80 ± 1.74
2i	3,4-Cl	<5	67.63 ± 1.28
2j	4-Br	<5	26.66 ± 1.31
2k	H	<5	0
2l	2-CH_3	<5	0
2m	4-CH_3	<5	0
2n	2,5-CH_3	<5	0
2o	3-OCH_3	<5	0
2p	4-OCH_3	<5	0
2q	3-Cl	<5	0
2r	4-Cl	<5	0
2s	3,4-Cl	<5	0
2t	4-Br	<5	0
3a	H	<5	83.72 ± 2.13 **
3b	2-CH3	<5	72.64 ± 2.01
3c	4-CH3	<5	50.09 ± 0.79
3d	2,5-CH3	<5	64.60 ± 0.71
3e	3-OCH3	<5	56.34 ± 1.20
3f	4-OCH3	<5	40.89 ± 1.73
3g	3-Cl	<5	0
3h	4-Cl	<5	67.19 ± 2.03
3i	3,4-Cl	<5	76 ± 1.22
3j	4-Br	<5	49.04 ± 1.41
3k	H	<5	0
3l	2-CH3	<5	0
3m	4-CH3	<5	0
3n	2,5-CH3	<5	0
3o	3-OCH3	<5	0
3p	4-OCH3	<5	0
3q	3-Cl	<5	0
3r	4-Cl	<5	0
3s	3,4-Cl	<5	0
3t	4-Br	<5	0
Leupeptin	–	–	89.06 ± 0.69
Pepstatin	–	–	92.94 ± 0.67
Chloroquine	–	86.6 ± 2.75	24.12 ± 1.16

R_1 (**2a–j**) = 7-Cl; R_1 (**2k–t**) = 6-Cl; R_1 (**3a–j**) = 8-Cl; R_1 (**3k–t**) = 7-Cl.
The results are expressed by the mean ± standard error of the mean.
†$p > 0.05$ compared to chloroquine, **$p > 0.05$ compared to leupeptin (LEP) and pepstatin (PEP).

Compounds **2b**, **2g**, and **3a** were tested in infected mice with *P. berghei* ANKA, a chloroquine-susceptible strain of murine malaria. Compounds were given to mice (chloroquine or **2b**, **2g** and **3a**, in 20 mg/kg, i.p. once daily) for four consecutive days (days 1–4 post-infection). At day fourth post-infection, the parasitemia was determined; the survival days were monitored and compared with control mice receiving a saline solution (untreated mice). Control mice died within 12 days post-infection, compound **2b** increased the survival time for 13 days, while chloroquine prolonged the survival time of the infected mice to 30 days. Compound **2b** was able to reduce and delay the progression of malaria (9.75 ± 3.01%) but did not eradicate the infection (Table 2).

It should be emphasized that compounds **2a–j** and **3a–j**, which bear methyl groups on the N-bonded phenyl ring and Cl atoms on positions 7 or 8 of the benzothiazine nucleus, showed the highest activity. It is interesting to note that the presence of Cl at positions 7 or 8 appears to be necessary, but not sufficient by itself to produce the biological response. The lack of inhibitory effect of compounds **2k–t** and **3k–t** illustrates this situation. Compounds having a Cl on positions 6 or 7 of the benzothiazine nucleus, and the same substitution pattern on the phenyl group markedly decrease the activity. Thus, the poor inhibition of β-hematin formation appears to have close relation with this substitution pattern.

Table 2
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	21.8 ± 2.31	11.66 ± 1.66
2b	9.75 ± 3.01*	13 ± 1.26
2g	10.33 ± 2.43*	12.5 ± 2.13**
3a	12.7 ± 2.11	10.8 ± 1.59
Chloroquine	1.3 ± 0.3	30

The results are expressed by the mean ± standard error of the mean.
*$p < 0.05$ and **$p < 0.01$ compared to untreated mice (saline). $n = 6$.

The presence of a methyl group as substituent in the aromatic ring appeared to be favourable for the antimalarial activity, since most of the compounds displaying this group showed measurable levels of inhibition of hemoglobin degradation, regardless of the nature of the substitutions in the aromatic ring.

Previously we observed that compounds with different substituents on phenyl ring showed different activities [12]. Thus, compounds without substituents or with methyl on phenyl ring

Table 3
Crystal data, intensity data collection parameters and final refinement results for compound **3p**

CCDC deposit No.	CCDC 685720
Crystal data	
Formula	$C_{17}H_{14}ClN_5O_3S_2$
MW	435.91
Colour	Colourless
Morphology	Prism
Specimen size (mm)	0.50 × 0.12 × 0.09
T (K)	296(2)
a (Å)	8.2872(5)
b (Å)	7.6900(5)
c (Å)	28.0940(19)
α (°)	90.000
β (°)	92.396(1)
γ (°)	90.000
V (Å^3)	1788.8(2)
Crystal system	Monoclinic
Space group (No.)	$P2_1/n$ (No. 14)
Z	4
D_c (g cm^{-3})	1.500
F(000)	832
μ (Mo Kα) (mm^{-1})	0.360
θ range (°) for cell	2.5–26.0
No. of reflections for cell	3080
Data Collection	
θ range (°)	1.5–29.0
h range	−9, 11
k range	−9, 10
l range	−38, 30
Mean Δ*I* for checks (%)	0.4
No. of reflections measured	11963
No. of reflections unique	4378
No. of reflections $I > 2\sigma(I)$	3330
Abs. correction	Multi-scan
Trans. coeff. (T_{min}, T_{max})	0.759–0.968
R_{int}	0.0229
Refinement (*last cycle*)	
Weighting scheme (*a*, *b*)	0.0614, 0.3861
No. of parameters refined	261
*R*1 [$I > 2\sigma(I)$]	0.0463
*R*1 (all data)	0.0636
*wR*2 [$I > 2\sigma(I)$]	0.1139
*wR*2 (all data)	0.1230
S (g.o.f.) (all data)	1.045
Δ/σ max	<0.0005
Δ/σ mean	<0.0005
$\Delta\rho_r$ (min., max.) (e Å^{-3})	−0.22, 0.41

having a large-size positive zone near to the phenyl group, were the most active molecules in these series. Compounds with methoxy groups have a reduced zone of positive charge due to the presence of the oxygen atom. These compounds display only mediocre activity. In contrast, compounds having electron withdrawing substituents such as Cl or Br were devoid of any inhibitory activity. These results clearly indicate that an increase of the lipophilic property, with appropriate groups on the phenyl substituent, produces good inhibitory activity for hemoglobin degradation.

The fact that the activity is markedly affected by altering the substituents on 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 (van der Waals) forces. Our results indicate that a characteristic electronic distribution on the phenyl ring might be important to produce the adequate interaction. Whereas methyl substituents produce major inhibitions, only mediocre activity was obtained with methoxy derivatives [12].

Compounds **2b**, **2g** and **3a** were tested in mice infected with *P. berghei* ANKA, a chloroquine-susceptible strain of murine malaria. Mice were given the compound (chloroquine or **2b**, **2g** and **3a** in 20 mg/kg, ip once daily) for 4 consecutive days (days 1–4 post-infection). The parasitemia at fourth day post-infection and their survival times were monitored and compared with control mice receiving a saline solution (untreated mice). Control mice died at day 11.66 ± 1.66 post-infection, compound **2b** only slightly increased the survival time 13 ± 1.26 days, while **2g** and **3a** increased that time for 12.5 ± 2.13 and 10.8 ± 1.5 days, respectively (Table 2). Particular attention was paid to compound **2b** which was able to reduce and delay the progression of malaria but did not eradicate the infection 9.75 ± 3.01 days (Table 2). Although compounds **2b**, **2g** and **3a** 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, cysteine or metalloproteases due to the presence of a globin band. The 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 may be partially responsible for the poor *in vivo* activity observed.

4. Experimental

4.1. Chemistry

Melting points were determined on a Thomas micro-hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Jeol Eclipse 270 (270 MHz/67.9 MHz) spectrometer using DMSO-d_6, and are reported in parts per million downfield from the residual DMSO. Elemental analyses were performed on a Perkin Elmer 2400 CHN analyser, results were within ±0.4% of the predicted values for all compounds. Chemical reagents were obtained from Aldrich Chemical Co., USA. All solvents were distilled and dried in the usual manner. 2,3-Substituted 6 or 7 chloro-*N*-phenylbenzothiazines **3a**–**k** and **4a**–**k** were obtained following the method previously reported [12,14].

4.1.1. General procedure for the synthesis of 3-amino-6 or 7-chloro-9-(phenylsubstituted)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2a**–**t**

A mixture of the appropriate benzothiazine (1.3 mmol), hydrazine hydrate (1.3 mmol), in dry pyridine (10 mL) was refluxed for 5 h. The solvent was evaporated to dryness under reduced pressure, water was added (10 mL) and the solid thus obtained was collected by filtration. Further purification was accomplished by recrystallization from ethanol–water (4/1).

*4.1.1.1. 3-Amino-7-chloro-9-phenyl-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2a**.* Yield 65%; mp 316–318 °C; IR (KBr) cm^{-1}: 3317, 2935 (NH), 1497, 1115 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.35(s, 1H, H_8), 6.39 (br s, 2H, NH_2), 7.23 (dd, 1H, H_6, J: 8.5, 2.1 Hz), 7.43 (d, 2H, $H_{2',6'}$, J: 7.4 Hz), 7.62 (d, 2H, $H_{3',5'}$, J: 7.4 Hz), 7.65 (m, 1H, $H_{4'}$), 7.94 (d, 1H, H_5, J: 8.5 Hz), 11.64 (br s, 1H, NH); ^{13}C NMR: 87.8, 114.9, 121.1, 125.1, 125.9, 129.8, 130.2, 131.1, 137.7, 137.7, 140.8, 146.9, 147.8. Anal. $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.16. Found: C, 52.03; H, 2.97; N, 16.34%.

*4.1.1.2. 3-Amino-7-chloro-9-(2′-methylphenyl)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2b**.* Yield 53; mp 280 °C dec.; IR (KBr) cm^{-1}: 3450, 3360 (NH), 1440, 1110 (SO_2). ^{1}H NMR DMSO-d_6: δ 1.95 (s, 3H, CH_3), 6.24 (d, 1H, H_8, J: 1.5 Hz), 6.41 (br s, 2H, NH_2), 7.21 (dd, 1H, H_6, J: 8.4, 1.5 Hz), 7.48 (m, 4H, Ar), 7.96 (d, 1H, H_5, J: 8.4 Hz), 11.60 (br s, 1H, NH); ^{13}C NMR: 22.1, 92.4, 118.8, 125.8, 129.8, 130.9, 133.5, 134.9, 135.2, 137.1, 141.2, 142.0, 142.5, 144.7, 151.6. Anal. $C_{16}H_{13}ClN_4O_2S$: C, 53.26; H, 3.63; N, 15.53. Found: C, 53.37; H, 3.87; N, 15.71%.

*4.1.1.3. 3-Amino-7-chloro-9-(4′-methylphenyl)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2c**.* Yield 62%; mp 274–276 °C; IR (KBr) cm^{-1}: 3460, 3350 (NH), 1445, 1128 (SO_2). ^{1}H NMR DMSO-d_6: δ 2.42 (s, 3H, CH_3), 6.38 (br s, 3H, H_8, NH_2), 7.19 (d, 1H, H_6, J: 8.5 Hz), 7.28 (d, 2H, $H_{2',6'}$, J: 7.6 Hz), 7.42 (d, 2H, $H_{3',5'}$, J: 7.6 Hz), 7.93 (d, 1H, H_5, J: 8.5 Hz), 11.60 (br s, 1H, NH); ^{13}C NMR: 21.4, 87.7, 115.0, 120.9, 125.1, 125.4, 129.9, 131.6, 135.2, 137.5, 139.3, 141.1, 146.9, 150.2. Anal. $C_{16}H_{13}ClN_4O_2S$: C, 53.26; H, 3.63; N, 15.53. Found: C, 53.40; H, 3.45; N, 15.61%.

*4.1.1.4. 3-Amino-7-chloro-9-(2′,5′-dimethylphenyl)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2d**.* Yield 50%; mp > 320 °C; IR (KBr) cm^{-1}: 3435, 3180 (NH), 1459, 1130 (SO_2). ^{1}H NMR DMSO-d_6: δ 1.90 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 6.26 (d, 1H, H_8, J: 1.5 Hz), 6.39 (br s, 2H, NH_2), 7.14 (s, 1H, $H_{6'}$), 7.22 (dd, 1H, H_6, J: 8.7, 1.5 Hz), 7.28 (d, 1H, $H_{4'}$, J: 7.8 Hz), 7.38 (d, 1H, $H_{3'}$, J: 7.8 Hz), 7.95 (d, 1H, H_5, J: 8.7 Hz), 11.58 (s, 1H, NH); ^{13}C NMR: 16.8, 20.9, 87.6, 114.2, 121.3, 124.7, 126.0, 130.4, 130.9, 132.2, 133.8, 135.9, 138.0, 138.4, 139.9, 146.9, 147.9. Anal. $C_{17}H_{15}ClN_4O_2S$: C, 54.47; H, 4.03; N, 14.95. Found: C, 54.52; H, 4.19; N, 15.01%.

*4.1.1.5. 3-Amino-7-chloro-9-(3′-methoxyphenyl)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2e**.* Yield 48%; mp 312–314 °C; IR (KBr) cm^{-1}: 3312, 3128 (NH), 1499, 1115 (SO_2). ^{1}H NMR DMSO-d_6: δ 3.78 (s, 3H, OCH_3), 6.41 (br s, 3H, H_8, NH_2), 7.13 (m, 4H, $H_{6,2',4',6'}$), 7.53 (t, 1H, $H_{5'}$, J: 8.1 Hz), 7.95 (d, 1H, H_5, J: 8.5 Hz), 11.61 (br s, 1H, NH); ^{13}C NMR: 56.1, 87.7, 114.9, 115.6, 115.7, 121.1, 122.0, 125.1, 125.9, 131.8, 137.5, 138.9, 140.7, 147.0, 147.9. Anal. $C_{16}H_{13}ClN_4O_3S$: C, 51.00; H, 3.48; N, 14.87. Found: C, 50.89; H, 3.63; N, 15.19%.

*4.1.1.6. 3-Amino-7-chloro-9-(4′-methoxyphenyl)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2f**.* Yield 67%; mp > 320 °C; IR (KBr) cm^{-1}: 3344, 3184 (NH), 1469, 1130 (SO_2). ^{1}H NMR DMSO-d_6: δ 3.84 (s, 3H, OCH_3), 6.38 (br s, 2H, NH_2), 6.41 (d, 1H, H_8, J: 1.8 Hz), 7.15 (m, 3H, $H_{6,\ 2',6'}$), 7.33 (d, 2H, $H_{3',5'}$, J: 7.9 Hz), 7.93 (d, 1H, H_5, J: 8.2 Hz), 11.60 (br s, 1H, NH); ^{13}C NMR: 56.0, 87.5, 115.0, 116.2, 120.6, 125.1, 125.9, 130.1, 131.3, 137.4, 141.2, 146.9, 147.9, 160.0. $C_{16}H_{13}ClN_4O_3S$: C, 51.00; H, 3.48; N, 14.87. Found: C, 51.13; H, 3.48; N, 14.83%.

*4.1.1.7. 3-Amino-7-chloro-9-(3′-chlorophenyl)-1,9-dihydro-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **2g**.* Yield 64%; mp 306–308 °C; IR

(KBr) cm^{-1}: 3390, 3370 (NH), 1489, 1150 (SO_2). ^1H NMR DMSO-d_6: δ 6.42 (d, 1H, H_8, *J*: 1.5 Hz), 6.43 (br s, 2H, NH_2), 7.23 (dd, 1H, H_6, *J*: 8.8, 1.5 Hz), 7.65 (m, 4H, Ar), 7.95 (d, 1H, H_5, *J*: 8.9 Hz), 11.63 (br s, 1H, NH); ^{13}C NMR: 87.6, 114.9, 121.4, 125.4, 126.0, 129.2, 130.7, 132.7, 134.9, 137.5, 140.4, 146.8, 147.9. $C_{15}H_{10}Cl_2N_4O_2S$: C, 47.26; H, 2.64; N, 14.70. Found: C, 47.17; H, 2.80; N, 14.77%.

*4.1.1.8. 3-Amino-7-chloro-9-(4′-chlorophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2h***. Yield 60%; mp 248–250 °C; IR (KBr) cm^{-1}: 3444, 3317 (NH), 1499, 1125 (SO_2). ^1H NMR DMSO-d_6: δ 6.42 (m, 3H, H_8, NH_2), 7.19 (dd, 1H, H_6, *J*: 8.4, 2.0 Hz), 7.49 (d, 2H, $H_{2',6'}$, *J*: 8.6 Hz), 7.69 (d, 2H, $H_{3',5'}$, *J*: 8.6 Hz), 7.93 (d, 1H, H_5, *J*: 8.4 Hz), 11.65 (br s, 1H, NH); ^{13}C NMR: 87.9, 114.9, 121.5, 125.1, 126.1, 131.3, 132.3, 134.4, 136.4, 137.8, 140.9, 146.8, 147.8. $C_{15}H_{10}Cl_2N_4O_2S$: C, 47.26; H, 2.64; N, 14.70. Found: C, 46.95; H, 2.53; N, 14.83%.

*4.1.1.9. 3-Amino-7-chloro-9-(3′,4′-dichlorophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2i***. Yield 60%; mp > 320 °C; IR (KBr) cm^{-1}: 3395, 3399 (NH), 1455, 1124 (SO_2). ^1H NMR DMSO-d_6: δ 6.43 (br s, 2H, NH_2), 6.55 (s, 1H, H_8), 7.23 (dd, 1H, H_6, *J*: 8.4, 2.0 Hz), 7.50 (d, 1H, $H_{6'}$, *J*: 8.4 Hz), 7.89 (m, 2H, $H_{2',5'}$), 7.95 (d, 1H, H_5, *J*: 8.4 Hz), 11.65 (br s, 1H, NH); ^{13}C NMR: 87.6, 115.1, 121.6, 125.5, 126.0, 130.9, 132.8, 133.9, 133.1, 137.6, 137.6, 140.4, 147.0, 147.9. $C_{15}H_9Cl_3N_4O_2S$: C, 43.34; H, 2.18; N, 13.48. Found: C, 43.51; H, 2.33; N, 13.61%.

*4.1.1.10. 3-Amino-7-chloro-9-(4′-bromophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2j***. Yield 58%; mp > 320 °C; IR (KBr) cm^{-1}: 3367, 2922 (NH), 1499, 1122 (SO_2). ^1H NMR DMSO-d_6: δ 6.48 (m, 3H, H_8, NH_2), 7.22 (dd, 1H, H_6, *J*: 8.7, 1.9 Hz), 7.49 (d, 2H, $H_{2',6'}$, *J*: 8.4 Hz), 7.81 (d, 2H, $H_{3',5'}$, *J*: 8.4 Hz), 7.94 (d, 1H, H_5, *J*: 8.7 Hz), 11.63 (br s, 1H, NH); ^{13}C NMR: 87.7, 114.9, 121.5, 123.0, 125.1, 125.9, 132.6, 134.2, 136.9, 137.7, 140.4, 146.9, 147.9. $C_{15}H_{10}BrClN_4O_2S$: C, 42.32; H, 2.37; N, 13.16. Found: C, 42.35; H, 2.41; N, 13.23%.

*4.1.1.11. 3-Amino-6-chloro-9-phenyl-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2k***. Yield 60%; mp > 320 °C; IR (KBr) cm^{-1}: 3317, 2935 (NH), 1497, 1115 (SO_2). ^1H NMR DMSO-d_6: δ 6.42 (br s, 2H, NH_2), 6.49 (d, 1H, H_8, *J*: 9.1 Hz), 7.41 (d, 2H, $H_{3',6'}$, *J*: 7.4 Hz), 7.65 (m, 4H, $H_{7,2',4',6'}$), 7.85 (d, 1H, H_5, *J*: 2.1 Hz), 11.61 (br s, 1H, NH); ^{13}C NMR: 87.6, 118.2, 122.7, 124.9, 127.3, 129.6, 130.3, 131.0, 133.0, 135.3, 138.5, 145.9, 147.1. Anal. $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.16. Found: C, 51.73; H, 3.19; N, 16.25%.

*4.1.1.12. 3-Amino-6-chloro-9-(2′-methylphenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2l***. Yield 56%; mp 294 °C dec; IR (KBr) cm^{-1}: 3450, 3360 (NH), 1440, 1110 (SO_2). ^1H NMR DMSO-d_6: δ 1.94 (s, 3H, CH_3), 6.37 (d, 1H, H_8, *J*: 9.4 Hz), 6.40 (br s, 2H, NH_2), 7.31 (d, 1H, $H_{6'}$, *J*: 6.9 Hz), 7.47 (m, 4H, $H_{7,3',4',5'}$), 7.86 (d, 1H, H_5, *J*: 1.9 Hz), 11.59 (br s, 1H, NH); ^{13}C NMR: 16.8, 87.3, 117.4, 123,0, 125.0, 128.6, 130.8, 132.4, 133.4, 136.6, 136.9, 137.5, 137.6, 138.0, 142.6, 147.0. Anal. $C_{16}H_{13}ClN_4O_2S$: C, 53.26; H, 3.63; N, 15.53. Found: C, 52.96; H, 3.62; N, 15.49%.

*4.1.1.13. 3-Amino-6-chloro-9-(4′-methylphenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2m***. Yield 67%; mp 300 °C dec.; IR (KBr) cm^{-1}: 3460, 3350 (NH), 1445, 1128 (SO_2). ^1H NMR DMSO-d_6: δ 2.40 (s, 3H, CH_3), 6.35 (br s, 2H, NH_2), 6.51 (d, 1H, H_8, *J*: 9.4 Hz), 7.27 (d, 2H, $H_{2',6'}$, *J*: 7.9 Hz), 7.42 (d, 2H, $H_{3',5'}$, *J*: 7.9 Hz), 7.47 (dd, 1H, H_7, *J*: 9.4, 1.7 Hz), 7.84 (d, 1H, H_5, *J*: 1.7 Hz), 11.62 (br s, 1H, NH); ^{13}C NMR: 21.4, 88.0, 118.2, 122.6, 124.7, 127.2, 130.0, 131.5, 133.0, 135.4, 138.6, 139.1, 145.4, 149.0. Anal. $C_{16}H_{13}ClN_4O_2S$: C, 53.26; H, 3.63; N, 15.53. Found: C, 53.18; H, 3.65; N, 15.68%.

*4.1.1.14. 3-Amino-6-chloro-9-(2′,5′-dimethylphenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2n***. Yield 50%; mp 302 °C dec.; IR (KBr) cm^{-1}: 3345, 3180 (NH), 1459, 1130 (SO_2). ^1H NMR DMSO-d_6: δ 1.88 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.32 (br s, 2H, NH_2), 6.39 (d, 1H, H_8, *J*: 9.1 Hz), 7.12 (s, 1H, $H_{6'}$), 7.26 (d, 1H, $H_{4'}$, *J*: 7.8 Hz), 7.36 (d, 1H, $H_{3'}$, *J*: 7.8 Hz), 7.48 (dd, 1H, H_7, *J*: 9.1, 2.1 Hz), 7.85 (d, 1, H_5, *J*: 2.1 Hz), 11.73 (s, 1H, NH); ^{13}C NMR: 17.0, 21.0, 87.5, 117.5, 122.8, 125.1, 127.1, 130.0, 130.3, 130.6, 132.2, 132.9, 133.3, 134.1, 140.0, 147.1, 147.5. Anal. $C_{17}H_{15}ClN_4O_2S$: C, 54.47; H, 4.03; N, 14.95. Found: C, 54.31; H, 3.91; N, 15.17%.

*4.1.1.15. 3-Amino-6-chloro-9-(3′-methoxyphenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2o***. Yield 42%; mp 294–296 °C; IR (KBr) cm^{-1}: 3343, 3175 (NH), 1461, 1113 (SO_2). ^1H NMR DMSO-d_6: δ 3.74 (s, 3H, OCH_3), 6.47 (br s, 2H, NH_2), 6.41 (d, 1H, H_8, *J*: 9.0 Hz), 7.18 (m, 3H, $H_{2',4',6'}$), 7.36 (t, 1H, $H_{5'}$, *J*: 8.1 Hz), 7.48 (dd, 1H, H_7, *J*: 9.0, 1.8 Hz), 7.91 (d, 1H, H_5, *J*: 1.8 Hz), 11.78 (br s, 1H, NH); ^{13}C NMR: 56.9, 87.7, 118.9, 124.6, 125.7, 128.0, 130.9, 131.3, 131.8, 132.2, 133.1, 133.8, 138.9, 140.7, 147.0, 160.4. Anal. $C_{16}H_{13}ClN_4O_3S$: C, 51.00; H, 3.48; N, 14.87. Found: C, 51.12; H, 3.45; N, 14.91%.

*4.1.1.16. 3-Amino-6-chloro-9-(4′-methoxyphenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2p***. Yield 63%; mp 300 °C dec.; IR (KBr) cm^{-1}: 3344, 3184 (NH), 1469, 1130 (SO_2). ^1H NMR DMSO-d_6: δ 3.83 (s, 3H, OCH_3), 6.37 (br s, 2H, NH_2), 6.54 (d, 1H, H_8, *J*: 9.4 Hz), 7.13 (d, 2H, $H_{2',6'}$, *J*: 8.7 Hz), 7.31 (d, 2H, $H_{3',5'}$, *J*: 8.7 Hz), 7.47 (dd, 1H, H_7, *J*: 9.4, 2.0 Hz), 7.83 (d, 1H, H_5, *J*: 2.0 Hz), 11.59 (br s, 1H, NH); ^{13}C NMR: 56.1, 87.5, 115.0, 116.11, 18.2, 122.6, 124.6, 127.2, 131.2, 133.0, 135.1, 138.9, 146.9, 148.3, 159.9. Anal. $C_{16}H_{13}ClN_4O_3S$: C, 51.00; H, 3.48; N, 14.87. Found: C, 51.07; H, 3.41; N, 14.73%.

*4.1.1.17. 3-Amino-6-chloro-9-(3′-chlorophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2q***. Yield 54%; mp 188–190 °C; IR (KBr) cm^{-1}: 3390, 3370 (NH), 1489, 1150 (SO_2). ^1H NMR DMSO-d_6: δ 6.19 (br s, 2H, NH_2), 6.79 (d, 1H, H_8, *J*: 9.1 Hz), 7.18 (s, 1H, $H_{6'}$), 7.66 (dd, 1H, H_7, *J*: 7.1, 2.0 Hz), 7.68 (m, 3H, $H_{2',4',5'}$), 8.25 (d, 1H, H_5, *J*: 2.0 Hz), 11.10 (br s, 1H, NH); ^{13}C NMR: 87.5, 119.8, 122.0, 124.0, 127.2, 130.3, 130.8, 133.8, 134.0, 133.8, 134.5, 141.6, 143.7. $C_{15}H_{10}Cl_2N_4O_2S$: C, 47.26; H, 2.64; N, 14.70. Found: C, 47.23; H, 2.68; N, 14.77%.

*4.1.1.18. 3-Amino-6-chloro-9-(4′-chlorophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2r***. Yield 60%; mp > 320 °C; IR (KBr) cm^{-1}: 3444, 3317 (NH), 1499, 1125 (SO_2). ^1H NMR DMSO-d_6: δ 6.41 (br s, 2H, NH_2), 6.55 (d, 1H, H_8, *J*: 9.2, Hz), 7.48 (m, 3H, $H_{7,2',6'}$), 7.67 (d, 2H, $H_{3',5'}$, *J*: 8.2 Hz), 7.85 (d, 1H, H_5, *J*: 2.0 Hz), 11.64 (br s, 1H, NH); ^{13}C NMR: 87.5, 118.2, 122.7, 125.3, 127.4, 131.0, 132.4, 133.1, 134.1, 136.9, 138.3, 146.9, 147.9. $C_{15}H_{10}Cl_2N_4O_2S$: C, 47.26; H, 2.64; N, 14.70. Found: C, 47.43; H, 2.82; N, 14.73%.

*4.1.1.19. 3-Amino-6-chloro-9-(3′,4′-dichlorophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2s***. Yield 58%; mp 218–220 °C; IR (KBr) cm^{-1}: 3367, 2922 (NH), 1499, 1122 (SO_2). ^1H NMR DMSO-d_6: δ 6.28 (br s, 2H, NH_2), 7.25 (d, 1H, H_8, *J*: 9.2 Hz), 7.35 (d, 1H, $H_{6'}$, *J*: 8.9 Hz), 7.72 (m, 3H, $H_{7,2',5'}$), 8.24 (d, 1H, H_5, *J*: 2.3 Hz), 11.67 (br s, 1H, NH); ^{13}C NMR: 86.3, 117.5, 117.0, 121.1, 130.4, 131.0, 131.5, 132.5, 132.7, 134.0, 134.1, 134.3, 134.9, 135.2, 141.6, 147.6. $C_{15}H_9Cl_3N_4O_2S$: C, 43.34; H, 2.18; N, 13.48. Found: C, 43.29; H, 2.27; N, 13.60%.

*4.1.1.20. 3-Amino-6-chloro-9-(4′-bromophenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide* ***2t***. Yield 53%; mp 304–306 °C; IR (KBr) cm^{-1}: 3383, 3117 (NH), 1463, 1117 (SO_2). ^1H NMR DMSO-d_6: δ 6.35 (br s, 2H, NH_2), 7.23 (d, 1H, H_8, *J*: 9.1 Hz), 7.40 (m, 3H, $H_{7,2',6'}$), 7.93 (d, 2H, $H_{3',5'}$, *J*: 8.4 Hz), 8.19 (d, 1H, H_5, *J*: 2.0 Hz), 11.69 (br s, 1H, NH); ^{13}C NMR: 87.3, 118.5, 121.7, 129.0, 131.3, 131.9, 132.8, 135.6, 136.9, 137.9, 142.3, 145.8, 148.9. $C_{15}H_{10}BrClN_4O_2S$: C, 42.32; H, 2.37; N, 13.16. Found: C, 42.60; H, 2.35; N, 12.97%.

*4.1.2. General procedure for the synthesis of 2,4-diamino-7 or 8-chloro-10-(phenylsubstituted)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3a–t***

A mixture of the appropriate benzothiazine (1.3 mmol), guanidine hydrochloride (1.3 mmol), and potassium carbonate (1.6 mmol) in dry DMF 10 mL was refluxed for 5 h. The solvent was evaporated to dryness under reduced pressure, water-ice was added (10 mL) and the solid thus obtained was collected by filtration. Further purification was accomplished by recrystallization from ethanol–water (4/1).

*4.1.2.1. 2,4-Diamino-8-chloro-10*H*-phenyl-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3a***. Yield 59%; mp > 320 °C; IR (KBr) cm^{-1}: 3480 (NH_2), 1450, 1139 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.30 (d, 1H, H_9, *J*: 2.0 Hz), 6.64 (br s, 2H, NH_2), 7.41 (dd, 1H, H_7, *J*: 8.5, 2.1 Hz), 7.66 (m, 5H, Ar), 7.99 (d, 1H, H_6, *J*: 8.4 Hz); ^{13}C NMR: 88.3, 117.6, 123.6, 124.7, 124.9, 129.7, 130.5, 130.9, 138.4, 157.2, 160.8, 162.8. Anal. $C_{16}H_{12}ClN_5O_2S$: C, 51.41; H, 3.24; N, 18.73. Found: C, 51.37; H, 3.19; N, 18.96%.

*4.1.2.2. 2,4-Diamino-8-chloro-10*H*-(2′-methylphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3b***. Yield 63%; mp 256–258 °C; IR (KBr) cm^{-1}: 3472 (NH_2), 1450, 1139 (SO_2). ^{1}H NMR DMSO-d_6: δ 1.95 (s, 3H, CH_3), 6.26 (d, 1H, H_9, *J*: 1.9 Hz), 6.66 (br s, 2H, NH_2), 7.48–7.24 (m, 5H, $H_{7,3',4',5',6'}$), 8.01 (d, 1H, H_6, *J*: 8.2 Hz); ^{13}C NMR: 17.4, 88.3, 116.8, 123.8, 124.7, 124.9, 128.4, 129.9, 130.6, 132.2, 137.0, 137.2, 138.3, 139.5, 156.7, 160.8, 163.1. Anal. $C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06. Found: C, 52.83; H, 3.49; N, 18.20%.

*4.1.2.3. 2,4-Diamino-8-chloro-10*H*-(4′-methylphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3c***. Yield 69%; mp > 320 °C; IR (KBr) cm^{-1}: 3480 (NH_2), 1455, 1130 (SO_2). ^{1}H NMR DMSO-d_6: δ 2.42 (s, 3H, CH_3), 6.35 (d, 1H, H_9, *J*: 1.5 Hz), 6.66 (br s, 2H, NH_2), 7.24 (d, 2H, $H_{2',6'}$, *J*: 8.0 Hz), 7.37 (dd, 1H, H_7, *J*: 8.4, 1.5 Hz), 7.42 (d, 2H, $H_{3',5'}$, *J*: 8.0 Hz), 7.98 (d, 1H, H_6, *J*: 8.4 Hz); ^{13}C NMR: 21.4, 88.3, 117.7, 123.5, 124.6, 124.7, 130.2, 131.4, 135.8, 137.9, 139.1, 140.6, 157.3, 160.8, 162.8. Anal. $C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06. Found: C, 52.71; H, 3.84; N, 18.17%.

*4.1.2.4. 2,4-Diamino-8-chloro-10*H*-(2′,5′-dimethylphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3d***. Yield 49%; mp 222–224 °C; IR (KBr) cm^{-1}: 3470 (NH_2), 1472, 1133 (SO_2). ^{1}H NMR DMSO-d_6: δ 1.90 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 6.28 (d, 1H, H_9, *J*: 1.8 Hz), 6.70 (br s, 2H, NH_2), 7.11 (s, 1H, $H_{6'}$), 7.27 (d, 1H, $H_{4'}$, *J*: 7.3 Hz), 7.37 (d, 1H, $H_{3'}$, *J*: 7.3 Hz), 7.41 (dd, 1H, H_7, *J*: 8.4, 1.9 Hz), 8.01 (d, 1H, H_6, *J*: 8.4 Hz); ^{13}C NMR: 17.1, 21.0, 88.3, 116.9, 123.7, 124.7, 124.9, 130.5, 130.7, 132.0, 135.5, 137.0, 137.8, 138.2, 139.5, 160.0, 160.9, 163.2. Anal. $C_{18}H_{16}ClN_5O_2S$: C, 53.80; H, 4.01; N, 17.43. Found: C, 54.05; H, 4.13; N, 17.52%.

*4.1.2.5. 2,4-Diamino-8-chloro-10*H*-(3′-methoxyphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3e***. Yield 63%; mp > 320 °C; IR (KBr) cm^{-1}: 3472 (NH_2), 1488, 1126 (SO_2). ^{1}H NMR DMSO-d_6: δ 3.80 (s, 3H, OCH_3), 6.38 (d, 1H, H_9, *J*: 1.9 Hz), 6.70 (br s, 2H, NH_2), 6.92 (d, 1H, $H_{4'}$, *J*: 8.2 Hz), 6.97 (s, 1H, $H_{2'}$), 7.12 (d, 1H, $H_{6'}$, *J*: 8.2 Hz), 7.39 (d, 1H, H_7, *J*: 8.1 Hz), 7.53 (t, 1H, $H_{5'}$, *J*: 8.2 Hz); ^{13}C NMR: 56.0, 88.3, 115.4, 116.1, 117.7, 122.4, 123.5, 124.5, 124.7, 131.5, 137.9, 139.6, 140.3, 157.1, 160.8, 161.2, 162.9. Anal. $C_{17}H_{14}ClN_5O_3S$: C, 50.56; H, 3.49; N, 17.34. Found: C, 50.61; H, 3.59; N, 17.46%.

*4.1.2.6. 2,4-Diamino-8-chloro-10*H*-(4′-methoxyphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3f***. Yield 68%; mp 262–264 °C; IR (KBr) cm^{-1}: 3490 (NH_2), 1456, 1125 (SO_2). ^{1}H NMR DMSO-d_6: δ 3.85 (s, 3H, OCH_3), 6.40 (d, 1H, H_9, *J*: 2.0 Hz), 6.65 (br s, 2H, NH_2), 7.14 (d, 2H, $H_{2',6'}$, *J*: 8.6 Hz), 7.27 (d, 2H, $H_{3',5'}$, *J*: 8.7 Hz), 7.38 (dd, 1H, H_7, *J*: 8.4, 2.0 Hz), 7.97 (d, 1H, H_6, *J*: 8.4 Hz); ^{13}C NMR: 55.9, 88.3, 115.9, 116.7, 117.7, 123.5, 124.5, 124.7, 130.9, 131.5, 137.9, 140.6, 157.3, 159.7, 160.7. Anal. $C_{17}H_{14}ClN_5O_3S$: C, 50.56; H, 3.49; N, 17.34. Found: C, 50.72; H, 3.40; N, 17.62%.

*4.1.2.7. 2,4-Diamino-8-chloro-10*H*-(3′-chlorophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3g***. Yield 43%; mp > 320 °C; IR (KBr) cm^{-1}: 3473 (NH_2), 1488, 1126 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.65 (d, 1H, H_9, *J*: 1.7 Hz), 6.75 (br s, 2H, NH_2), 7.43–7.55 (m, 3H, Ar), 7.66–7.70 (m, 1H, $H_{4'}$), 7.80 (d, 1H, H_6, *J*: 8.7 Hz); ^{13}C NMR: 88.4, 117.5, 123.7, 124.5, 125.6, 129.4, 129.9, 130.9, 132.4, 134.7, 137.9, 140.0, 157.1, 160.7, 162.8. Anal. $C_{16}H_{11}Cl_2N_5O_2S$: C, 47.07; H, 2.72; N, 17.15. Found: C, 46.93; H, 2.77; N, 17.31%.

*4.1.2.8. 2,4-Diamino-8-chloro-10H-(4′-chlorophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3h***. Yield 51%; mp 236–238 °C; IR (KBr) cm^{-1}: 3475 (NH_2), 1415, 1129 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.39 (d, 1H, H_9, *J*: 1.5 Hz), 6.70 (br s, 2H, NH_2), 7.41 (dd, 1H, H_7, *J*: 8.7, 1.5 Hz), 7.43 (d, 2H, $H_{2',6'}$, *J*: 8.4 Hz), 7.68 (d, 2H, $H_{3',5'}$, *J*: 8.4 Hz), 7.80 (d, 1H, H_6, *J*: 8.7 Hz); ^{13}C NMR: 88.4, 117.5, 123.7, 124.7, 124.9, 130.9, 132.6, 134.2, 137.3, 138.0, 140.2, 157.2, 160.8, 162.8. Anal. $C_{16}H_{11}Cl_2N_5O_2S$: C, 47.07; H, 2.72; N, 17.15. Found: C, 47.03; H, 2.75; N, 17.28%.

*4.1.2.9. 2,4-Diamino-8-chloro-10*H*-(3′,4′-dichlorophenyl)-pyrimido[5,4-*b*]benzothiazine 5,5-dioxide* ***3i***. Yield 57%; mp 242–244 °C; IR (KBr) cm^{-1}: 3460 (NH_2), 1469, 1126 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.50 (d, 1H, H_9, *J*: 2.0 Hz), 6.74 (br s, 2H, NH_2), 7.41–7.44 (m, 2H, $H_{7,6'}$), 7.85 (d, 1H, $H_{2'}$, *J*: 1.7 Hz), 7.88 (d, 1H, $H_{5'}$, *J*: 8.4 Hz), 8.00 (d, 1H, H_6, *J*: 8.7 Hz); ^{13}C NMR: 88.4, 117.5, 123.9, 124.7, 124.8, 131.2, 132.6, 132.9, 133.1, 138.1, 138.3, 139.8, 147.5, 157.0, 160.7, 162.7. Anal. $C_{16}H_{10}Cl_3N_5O_2S$: C, 43.41; H, 2.28; N, 15.82. Found: C, 43.40; H, 2.32; N, 15.91%.

*4.1.2.10. 2,4-Diamino-8-chloro-10*H*-(4′-bromophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3j***. Yield 65%; mp 238–240 °C; IR (KBr) cm^{-1}: 3472 (NH_2), 1459, 1120 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.39 (d, 1H, H_9, *J*: 1.8 Hz), 6.70 (br s, 2H, NH_2), 7.37 (d, 2H, $H_{2',6'}$, *J*: 8.4 Hz), 7.41 (d, 1H, H_7, *J*: 8.2 Hz), 7.82 (d, 2H, $H_{3',5'}$, *J*: 8.4 Hz), 7.99 (d, 1H, H_6, *J*: 8.2 Hz); ^{13}C NMR: 88.4, 117.5, 122.9, 123.7, 124.7, 124.9, 132.9, 133.8, 137.8, 138.0, 140.2, 157.1, 160.7, 162.7. Anal. $C_{16}H_{11}BrClN_5O_2S$: C, 42.45; H, 2.45; N, 15.47. Found: C, 42.63; H, 2.39; N, 15.60%.

*4.1.2.11. 2,4-Diamino-7-chloro-10*H*-phenyl-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3k***. Yield 49%; mp 268–270 °C; IR (KBr) cm^{-1}: 3480 (NH_2), 1450, 1139 (SO_2). ^{1}H NMR DMSO-d_6: δ 6.44 (d, 1H, H_9, *J*: 8.9 Hz), 6.67 (br s, 2H, NH_2), 7.36 (d, 2H, $H_{2',6'}$, *J*: 7.4 Hz), 7.36 (dd, 1H, H_8, *J*: 8.8, 1.9 Hz), 7.56–7.62 (m, 3H, Ar), 7.94 (d, 1H, H_6, *J*: 1.9 Hz); ^{13}C NMR: 87.9, 120.7, 121.4, 127.5, 129.5, 130.5, 130.7, 133.5, 138.2, 138.6, 157.1, 160.8, 162.8. Anal. $C_{16}H_{12}ClN_5O_2S$: C, 51.41; H, 3.24; N, 18.73. Found: C, 51.23; H, 3.29; N, 18.80%.

*4.1.2.12. 2,4-Diamino-7-chloro-10*H*-(2′-methylphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3l***. Yield 57%; mp 244–246 °C; IR (KBr) cm^{-1}: 3472 (NH_2), 1450, 1130 (SO_2). ^{1}H NMR DMSO-d_6: δ 1.95 (s, 3H, CH_3), 6.38 (d, 1H, H_9, *J*: 9.2 Hz), 6.65 (br s, 2H, NH_2), 7.24–7.48 (m, 4H, Ar), 7.56 (dd, 1H, H_8, *J*: 9.2, 2.0 Hz), 7.94 (d, 1H, H_6, *J*: 1.9 Hz); ^{13}C NMR: 19.1, 87.9, 119.9, 121.5, 127.1, 127.7, 127.9, 128.3, 129.8, 130.6, 132.1, 133.8, 137.1, 137.4, 156.6, 160.8, 163.1. Anal. $C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06. Found: C, 52.67; H, 3.71; N, 18.15%.

*4.1.2.13. 2,4-Diamino-7-chloro-10*H*-(4′-methylphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3m***. Yield 59%; mp 288–290 °C; IR (KBr) cm^{-1}: 3480 (NH_2), 1455, 1130 (SO_2). ^{1}H NMR DMSO-d_6: δ 2.41 (s, 3H, CH_3), 6.47 (d, 1H, H_9, *J*: 8.9 Hz), 6.66 (br s, 2H, NH_2), 7.21 (d, 2H, $H_{2',6'}$, *J*: 7.9 Hz), 7.40 (d, 2H, $H_{3',5'}$, *J*: 7.9 Hz), 7.56 (dd, 1H, H_8, *J*: 8.9, 2.0 Hz), 7.94 (d, 1H, H_6, *J*: 1.9 Hz); ^{13}C NMR: 21.2, 87.8, 120.8, 121.4, 126.9, 128.9, 127.4, 130.3, 131.2, 133.5, 136.1, 138.3, 157.3, 160.8, 162.8. Anal.

$C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06. Found: C, 52.73; H, 3.72; N, 18.37%.

*4.1.2.14. 2,4-Diamino-7-chloro-10*H*-(2′,5′-dimethylphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3n***. Yield 47%; mp 242–244 °C; IR (KBr) cm^{-1}: 3470 (NH_2), 1472, 1133 (SO_2). ^1H NMR DMSO-d_6: δ 1.89 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.40 (d, 1H, H_9, *J*: 9.2 Hz), 6.68 (br s, 2H, NH_2), 7.08 (d, 1H, $H_{6'}$, *J*: 2.1 Hz), 7.25 (d, 1H, $H_{4'}$, *J*: 7.7 Hz), 7.36 (d, 1H, $H_{3'}$, *J*: 7.7 Hz), 7.59 (dd, 1H, H_8, *J*: 9.2, 2.0 Hz), 7.94 (d, 1H, H_6, *J*: 2.0 Hz); ^{13}C NMR: 17.2, 20.9, 87.8, 120.1, 121.5, 127.1, 127.5, 130.5, 130.6, 131.9, 132.0, 133.9, 134.0, 137.7, 156.9, 160.8, 163.1. Anal. $C_{18}H_{16}ClN_5O_2S$: C, 53.80; H, 4.01; N, 17.43. Found: C, 53.77; H, 4.23; N, 17.66%.

*4.1.2.15. 2,4-Diamino-7-chloro-10*H*-(3′-methoxyphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3o***. Yield 66%; mp 287–288 °C; IR (KBr) cm^{-1}: 3490 (NH_2), 1456, 1125 (SO_2). ^1H NMR DMSO-d_6: δ 3.87 (s, 3H, OCH_3), 6.42 (d, 1H, H_9, *J*: 9.2 Hz), 6.66 (br s, 2H, NH_2), 7.23 (m, 3H, Ar), 7.42 (t, 1H, $H_{5'}$, *J*: 8.1 Hz), 7.58 (dd, 1H, H_8, *J*: 9.1, 1.9 Hz), 7.92 (d, 1H, H_6, *J*: 1.9 Hz); ^{13}C NMR: 55.9, 88.5, 118.9, 120.6, 123.4, 128.8, 131.1, 131.3, 131.7, 133.5, 137.8, 156.7, 159.7, 161.0, 162.8. Anal. $C_{17}H_{14}ClN_5O_3S$: C, 50.56; H, 3.49; N, 17.34. Found: C, 50.62; H, 3.71; N, 17.53%.

*4.1.2.16. 2,4-Diamino-7-chloro-10*H*-(4′-methoxyphenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3p***. Yield 62%; mp 276–378 °C; IR (KBr) cm^{-1}: 3496 (NH_2), 1463, 1137 (SO_2). ^1H NMR DMSO-d_6: δ 3.67 (s, 3H, OCH_3), 6.41 (d, 1H, H_9, *J*: 9.2 Hz), 6.53 (br s, 2H, NH_2), 7.17 (d, 2H, $H_{2',6'}$, *J*: 8.7 Hz), 7.25 (d, 2H, $H_{3',5'}$, *J*: 8.7 Hz), 7.56 (dd, 1H, H_8, *J*: 9.2, 1.7 Hz), 7.91 (d, 1H, H_6, *J*: 1.7 Hz); ^{13}C NMR: 55.9, 87.9, 115.8, 120.3, 121.3, 126.9, 127.3, 131.1, 131.5, 133.5, 137.1, 157.4, 159.7, 160.8, 162.9. Anal. $C_{17}H_{14}ClN_5O_3S$: C, 50.56; H, 3.49; N, 17.34. Found: C, 50.45; H, 3.62; N, 17.41%.

*4.1.2.17. 2,4-Diamino-7-chloro-10*H*-(3′-chlorophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3q***. Yield 43%; mp 200 °C dec.; IR (KBr) cm^{-1}: 3472 (NH_2), 1488, 1126 (SO_2). ^1H NMR DMSO-d_6: δ 6.45 (d, 1H, H_9, *J*: 8.9 Hz), 6.69 (br s, 2H, NH_2), 7.35–7.43 (m, 3H, H_8, Ar), 7.60–7.66 (m, 2H, Ar), 7.90 (d, 1H, H_6, *J*: 1.7 Hz); ^{13}C NMR: 87.6, 119.8, 121.1, 127.5, 128.5, 128.6, 129.4, 132.9, 133.1, 135.6, 137.1, 137.6, 157.1, 161.0, 162.4. Anal. $C_{16}H_{11}Cl_2N_5O_2S$: C, 47.07; H, 2.72; N, 17.15. Found: C, 47.26; H, 2.73; N, 17.29%.

*4.1.2.18. 2,4-Diamino-7-chloro-10*H*-(4′-chlorophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3r***. Yield 48%; mp 262–264 °C; IR (KBr) cm^{-1}: 3475 (NH_2), 1468, 1129 (SO_2). ^1H NMR DMSO-d_6: δ 6.52 (d, 1H, H_9, *J*: 9.1 Hz), 6.68 (br s, 2H, NH_2), 7.40 (d, 2H, $H_{2',6'}$, *J*: 8.4 Hz), 7.56 (dd, 1H, H8, *J*: 9.1, 1.7 Hz), 7.67 (d, 2H, $H_{3',5'}$, *J*: 8.4 Hz), 7.94 (d, 1H, H_6, *J*: 1.7 Hz); ^{13}C NMR: 88.2, 120.7, 121.5, 127.1, 127.5, 127.6, 130.8, 132.6, 133.6, 134.0, 137.8, 157.2, 160.8, 162.8. Anal. $C_{16}H_{11}Cl_2N_5O_2S$: C, 47.07; H, 2.72; N, 17.15. Found: C, 47.22; H, 3.01; N, 17.43%.

*4.1.2.19. 2,4-Diamino-7-chloro-10*H*-(3′,4′-dichlorophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3s***. Yield 45%; mp 316 °C dec.; IR (KBr) cm^{-1}: 3472 (NH_2), 1459, 1120 (SO_2). ^1H NMR DMSO-d_6: δ 6.60 (d, 1H, H_9, *J*: 9.2 Hz), 6.72 (br s, 2H, NH_2), 7.41 (dd, 1H, $H_{6'}$, *J*: 8.5, 2.3 Hz), 7.56 (dd, 1H, H_8, *J*: 9.2, 2.0 Hz), 7.82 (d, 1H, $H_{2'}$, *J*: 2.3 Hz), 7.88 (d, 1H, $H_{5'}$, *J*: 8.5 Hz), 7.95 (d, 1H, H_6, *J*: 2.0 Hz); ^{13}C NMR: 88.2, 120.8, 121.6, 127.0, 127.7, 128.6, 131.2, 132.4, 132.5, 132.9, 133.1, 133.7, 137.5, 156.9, 160.8, 162.7. Anal. $C_{16}H_{10}Cl_3N_5O_2S$: C, 43.41; H, 2.28; N, 15.82. Found: C, 43.55; H, 2.37; N, 16.03%.

*4.1.2.20. 2,4-Diamino-7-chloro-10*H*-(4′-bromophenyl)-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide* ***3t***. Yield 52%; mp 267–269 °C; IR (KBr) cm^{-1}: 3417 (NH_2), 1451, 1135 (SO_2). ^1H NMR DMSO-d_6: δ 6.67 (d, 1H, H_9, *J*: 9.1 Hz), 6.75 (br s, 2H, NH_2), 7.43 (d, 2H, $H_{2',6'}$, *J*: 8.4 Hz), 7.60 (dd, 1H, H_8, *J*: 9.0, 1.9 Hz), 7.67 (d, 2H, $H_{3',5'}$, *J*: 8.4 Hz), 8.16 (d, 1H, H_6, *J*: 2.0 Hz); ^{13}C NMR: 88.3, 120.5, 121.7, 127.8, 128.4, 130.9, 131.4, 131.9, 134.7, 136.9, 137.9, 138.5, 142.3, 155.9, 161.2, 163.0. Anal. $C_{16}H_{11}BrClN_5O_2S$: C, 42.45; H, 2.45; N, 15.47. Found: C, 42.51; H, 2.63; N, 15.75%.

4.2. X-ray crystallography

Crystals of **3p** suitable for X-ray diffraction were obtained by slow evaporation of a solution in ethanol. Crystal data, intensity data collection parameters and final refinement results are summarised in Table 3.

Unit cell and intensity measurements were carried out on a Bruker Smart CCD area-detector diffractometer, using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). The structure was 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-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) and 0.96 Å for C(sp^3)] distances, and $U_{iso} = pU_{eq}$(parent atom) [$p = 1.2$ for C(sp^2) and 1.5 for C(sp^3)]. The N-bonded H atoms were located in difference Fourier syntheses and refined isotropically.

The following computer programs were used: data collection, SMART [15]; data reduction and cell refinement, SAINT [16]; absorption correction, SADABS [17]; structure solution, SHELXS-97 [18]; structure refinement, SHELXL-97 [19]; geometrical calculations, PLATON [20]; molecular graphics, ORTEP-3 [21]. The structure solution, the refinement and the drawings were carried out with the aid of the WinGX [22] suite of programs.

Comprehensive crystallographic data (excluding structure factors) for the structural analysis of **3p** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data (CIF file) can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44-(0)1223-336033, or from http://www.ccdc.cam.ac.uk/products/csd/request/, quoting deposition No. CCDC 685720.

4.3. Biological assays

4.3.1. Inhibition of heme polymerization

The heme polymerization assay was performed according to Ref. [23], briefly, a solution of hemin chloride (50 μL, 4 mM), dissolved in DMSO (5.2 mg/mL), was distributed in 96-well micro plates. Different concentrations (100−5 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). The 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 absorbances recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of flavoprotein (FP) polymerization.

4.3.2. 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 Ethics Committee. *P. berghei* (ANKA strain), a rodent malaria parasite, was used for infection. Mice were infected by ip injection with 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 [24].

4.3.3. Parasite extracts

Blood of infected animals, at a high level of parasitemia (30–50%), was collected by cardiac puncture with an heparinized syringe and the blood pool was centrifuged (500 g × 10 min, 4 °C). Plasma and buffy coat were removed and the red blood cell (RBC) pellets were 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, 20,000 g × 30 min × 4 °C) [25]. 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 nonionic detergent saponin (0.1% in PBS × 10 min). One milliliter of cold PBS 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 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 [26].

4.3.4. 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.

4.3.5. 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 100 μL. The compounds (10 μM) were incorporated in the incubation mixture dissolved in DMSO. The incubations were carried out at 37 °C for 18 h and the reactions were stopped by addition of reduced sample buffer. The degree of digestion was evaluated electrophoretically by SDS-PAGE by visual comparison of the globin bands (14 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.

4.3.6. 4-Day suppressive test

Balb-C mice (18–23 g) were infected i.v. (using caudal vein) with 10^6 infected red blood cells with *P. berghei* ($n = 6$). Two hours after infection, treatment began with the best compounds tested in the *in vitro* assays. 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 4, the parasitemia was counted by examination of Giemsa stained smears. Chloroquine (25 mg/kg) was used as a positive control. The survival time beyond the control group (without drug treatment) was recorded. The results were expressed as percentage of parasitemia (% of parasitemia) and survival days of each compound-treated group over the control (non-treated group) [27].

5. Data analysis

Data were statistically analyzed using one-way ANOVA and *t*-tests for specific group comparisons; assuming 95% of confidence according GraphPad Prism 3.02 [28].

Acknowledgments

We thank the IIF-FF and CDCH-UCV (grants IIF: 03.2006, PG. 06-00-6502-2006), FONACIT-PCP and CYTED-RIDIMEDCHAG programmes for financial support.

References

[1] [WHO. World Malaria Report. Available at: <http://rbm.who.int/wmr>, 2005.
[2] L.H. Miller, D.I. Baruch, K. Marsh, O.K. Doumbo, Nature 415 (2002) 673.
[3] (a) T.J. Egan, K.K. Ncokazi, J. Inorg. Biochem. 99 (2005) 1532;
(b) A.A. Joshi, C.L. Viswanathan, Anti Infect. Agents Med. Chem. 5 (2006) 105.
[4] L. Tilley, P. Loria, M. Foley, Chloroquine and Other Quinoline Antimalarials, in: P.J. Rosenthal (Ed.), Antimalarial Chemotherapy, Humana Press Inc., Totowa, N.J., 2001, p. 87.
[5] D.J. Sullivan, I.Y. Gluzman, D.G. Russell, D.E. Goldberg, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 11865.
[6] A. Slater, W. Swiggard, B. Orton, W. Flitter, D. Goldberg, A. Cerami, G. Henderson, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 325.
[7] S. Pagola, P. Stephens, D. Bohle, A. Kosar, S. Madesen, Nature (Lond) 404 (2000) 307.
[8] T.J. Egan, J. Inorg. Biochem. 100 (2006) 916.
[9] G. Anquetin, M. Rouquayrol, N. Mahmoudi, M. Santillana-Hayat, R. Gonzalbes, J. Greiner, K. Farhati, F. Derouin, R. Guedj, P. Vierling, Bioorg. Med. Chem. Lett. 14 (2004) 2773.
[10] J.E. Charris, J.N. Domínguez, N. Gamboa, J. Rodrigues, J.E. Angel, Heterocycl. Commun. 11 (2005) 419.
[11] J. Charris, A. Barazarte, J. Domínguez, G. Lobo, J. Camacho, R. Ferrer, N. Gamboa, J. Rodrigues, M. Capparelli, J. Heterocycl. Chem. 44 (2007) 639.
[12] A. Barazarte, J. Camacho, J. Domínguez, G. Lobo, N. Gamboa, J. Rodrigues, M.V. Capparelli, Á. Álvarez-Larena, S. Andujar, D. Enriz, J. Charris, Bioorg. Med. Chem. 16 (2008) 3661.
[13] F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, R. Taylor, J. Chem. Soc. Perkin Trans. 2 (1987) S1–S19.
[14] J. Charris, A. Barazarte, J. Domínguez, N. Gamboa, J. Chem. Res. (2005) 27.
[15] Bruker, SMART: Version 5.630, Bruker AXS Inc., Madison, Wisconsin, USA, 2002.
[16] Bruker, Saint: Version 6.22, Bruker AXS Inc., Madison, Wisconsin, USA, 2001.
[17] Bruker, SADABS: Version 2.05, Bruker AXS Inc., Madison, Wisconsin, USA, 2003.
[18] G.M. Sheldrick, SHELXS – A Program for Crystal Structure Solution. Release 97-2, University of Göttingen, Germany, 1997.
[19] G.M. Sheldrick, SHELXL – A Program for Crystal Structure Refinement. Release 97-2, University of Göttingen, Germany, 1997.
[20] A.L. Spek, A. Platon, Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands, 1998.
[21] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565.
[22] L.J. Farrugia, J. Appl. Crystallogr. 32 (1999) 837.
[23] R. Baelmans, E. Deharo, V. Muñoz, M. Sauvain, H. Ginsburg, Exp. Parasitol. 96 (2000) 243.
[24] A. Dorn, R. Stoffel, H. Matile, A. Bubendorf, R. Ridley, Nature (Lond) 374 (1995) 269.
[25] E. Deharo, P. Gautret, H. Ginsburg, A. Chabaud, I. Landau, Parasitol. Res. 80 (1994) 159.
[26] P. Rosenthal, Exp. Parasitol. 80 (1995) 272.
[27] W. Peters, B.L. Robinson, Parasitic Infection Models, in: O. Zak, M. Sande (Eds.), Handbook of Antimalarial Models of Infection, Academic Press, London, 1999, p. 757.
[28] Graph Pad Prism Software Inc. 4.02 for Windows. May 17th 1992–2004.