Crystal structures of two N-phenylated tricyclic benzothiazines with antimalarial activity

Arthur Barazarte[a], Neira Gamboa[b], Juan Rodrigues[b], Reinaldo Atencio[c], Teresa González[c] and Jaime Charris[a]*

[a]Laboratorio de Síntesis Orgánica, [b]Laboratorio de Bioquímica, Facultad de Farmacia, Universidad Central de Venezuela, Apartado 47206, Los Chaguaramos, 1041-A Caracas, Venezuela

[c]Laboratorio de Síntesis y Caracterización de Nuevos Materiales, Instituto Venezolano de Investigaciones Científicas (IVIC) Apartado 21827, Caracas, 1020-A, Venezuela.

The crystal structures of two N-phenylated tricyclic benzothiazines were determined *3*-amino-7-chloro-9-phenyl-1,9H-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide, $C_{15}H_{11}ClN_4O_2S$ crystallises in $P2_1/c$ with a = 11.436 (4)Å, b = 10.894 (3)Å, c = 11.858 (4)Å, β = 95.297 (9)°, V = 1471.0 (8) Å^3 and Z = 4, while 2,4-diamino-8-chloro-10H-phenylpyrimido-[5,4-b]benzothiazine 5,5-dioxide, $C_{16}H_{12}ClN_5O_2S$ crystallises in $P2_1/c$ with a = 7.496 (2)Å, b = 17.728 (4)Å, c = 11.889 (2)Å, β = 91.524 (5)°, V = 1579.4 (5)Å^3 and Z = 4. Both molecules are essentially planar, including the exocyclic groups. These compounds were promising as inhibitors of hemoglobin hydrolysis, however, their effect as inhibitors of β-hematin formation was marginal. The most active compound to emerge from the *in vitro* and *in vivo* murine studies was the pyrimidobezothaizine, suggesting an antimalarial activity via inhibition of haemoglobin hydrolysis, but it was not as efficient as chloroquine.

Keywords: benzothiazine, pyrazole, pyrimidine, crystal structure, antimalarial

Malaria is believed to affect some 300 to 500 million people worldwide, and to cause one to three million deaths each year.[1] The disease is on the rise, as *Plasmodium falciparum*, responsible for the most malignant form of malaria, has developed resistance to chloroquine, the most widely used anti-malarial drug, through gene mutation.[2] Therefore, there is an urgent need for development of new antimalarial drugs.[3-5] As part of a general project aimed at finding new, more effective, antimalarial drugs, a number of quinoline, quinolone, pyrimidone, pyridopyrimidone, thiocromone, pyrazole and benzothiazine derivatives were synthesised, characterised and tested for antimalarial activity.[6-15]

3-Amino-7-chloro-9-phenyl-1,9H-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide, $C_{15}H_{11}ClN_4O_2S$ **1** and 2,4-diamino-8-chloro-10H-phenylpyrimido-[5,4-b]benzothiazine 5,5-dioxide, $C_{16}H_{12}ClN_5O_2S$ **2**, were prepared within that project and were found to have a promising *in vitro* and *in vivo* activity against a chloroquine sensible strain of *Plasmodium berghei*.

Compounds **1** and **2** were synthesised as reported elsewhere.[16,17] Crystals suitable for X-ray diffraction were obtained by the slow evaporation of solution in EtOH. In order to confirm the structure, the product was subjected to spectroscopic analysis using IR, ^{1}H and ^{13}C NMR, and elemental analysis techniques. Molecular structures of **1** and **2** are shown in the Figs. 1 and 2. Crystal data, intensity data collection parameters and final refinement are summarised in Tables 1–5. The X-ray structure determination showed that the crystals of compounds **1** and **2** contain only one organic molecule per asymmetric unit. In both compounds the bond lengths are in good agreement with the tabulated standard values[18] Tables 1, 3, and 4. The molecules are essentially planar, including the exocyclic groups (pyrazolo and pyrimidine). The r.m.s. deviations of the atoms in the tricyclic systems of 0.1207 and 0.0669Å for **1** and **2** respectively; the largest deviations from these mean planes are 0.130 (3)Å for S **1** and 0.449 (3)Å for Cl1 **2**. The aromatic rings at positions 9 and 10 of compounds **1** and **2** are roughly perpendicular to the plane defined by the atoms of the tricyclic fused-rings, with a dihedral angle between such planes of 67.67(7)º **1** and 81.94(2)° **2** respectively. Both molecules **1** and **2** form intramolecular hydrogen bonds, N-H· · · O = S = O Figs 1 and 2, and Tables 2 and 5. However, due to the geometry of the tricyclic system, similar interactions do not exist in **2** because the N· · · O distances are longer [3.498(13), 3.829(5)Å] and the N–H· · · O angle too small [113.52(2)°] in **2**. A It was found that the antimalarial activity of tricyclic benzothiazine appear to be related with some structural features, among which is the N4–H3···O2 intramolecular hydrogen

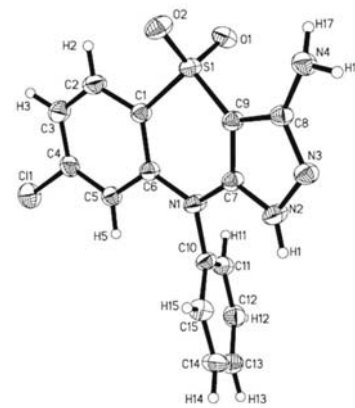

Fig. 1 Graphical representation of **1**. Rings are labelled to facilitate the discussion. Ellipsoids are given at 35% probability.

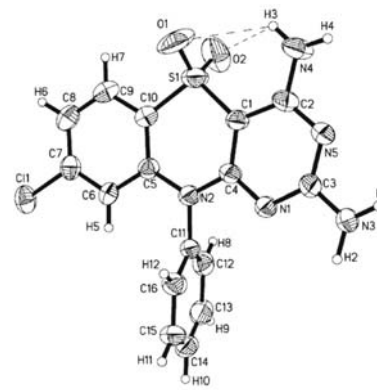

Fig. 2 Graphical representation of **2**. Rings are labelled to facilitate the discussion. Ellipsoids are given at 35% probability.

* Correspondent. E-mail:jaime.charris@ucv.ve

Table 1 Selected bond lengths (Å) and angles (°) for **1**

S(1)–O(1)	1.452(2)	C(1)–C(6)	1.396(4)	C(5)–C(6)	1.409(4)
S(1)–O(2)	1.439(2)	C(1)–C(2)	1.398(5)	C(7)–C(9)	1.402(4)
S(1)–C(1)	1.756(3)	C(2)–C(3)	1.375(5)	C(8)–C(9)	1.401(4)
Cl(1)–C(4)	1.733(3)	C(3)–C(4)	1.382(5)	C(10)–C(11)	1.378(4)
N(1)–C(7)	1.396(4)	C(4)–C(5)	1.372(5)	C(11)–C(12)	1.379(5)
N(1)–C(6)	1.401(4)	N(2)–C(7)	1.318(4)	C(12)–C(13)	1.382(5)
N(1)–C(10)	1.444(4)	N(2)–N(3)	1.380(4)	C(13)–C(14)	1.369(5)
				C(14)–C(15)	1.401(5)

O(1)–S(1)–O(2)	113.82(15)	C(7)–N(2)–N(3)	104.6(3)
O(2)–S(1)–C(9)	112.80(15)	C(8)–N(3)–N(2)	112.5(3)
O(1)–S(1)–C(9)	110.77(15)	C(6)–C(1)–C(2)	120.6(3)
O(2)–S(1)–C(1)	109.80(18)	C(6)–C(1)–S(1)	125.1(2)
O(1)–S(1)–C(1)	108.48(14)	C(2)–C(1)–S(1)	114.2(3)
C(9)–S(1)–C(1)	99.37(15)	C(3) C(2)–C(1)	120.9(3)
C(7)–N(1)–C(6)	120.0(3)	C(2)–C(3)–C(4)	118.0(3)
C(7)–N(1)–C(10)	118.1(3)	C(5)–C(4)–C(3)	122.7(3)
C(6)–N(1)–C(10)	121.8(3)	C(5)–C(4)–Cl(1)	119.3(3)
C(3)–C(4)–Cl(1)	118.0(3)	C(4)–C(5)–C(6)	119.5(3)
C(1)–C(6)–N(1)	122.8(3)	C(1)–C(6)–C(5)	118.1(3)
N(1)–C(6)–C(5)	112.0(3)	N(2)–C(7)–N(1)	122.7(3)
N(2)–C(7)–C(9)	119.1(3)	N(1)–C(7)–C(9)	125.3(3)
N(3)–C(8)–N(4)	130.6(3)	N(3)–C(8)–C(9)	105.8(3)
N(4)–C(8)–C(9)	123.6(3)	C(8)–C(9)–C(7)	105.2(3)
C(8)–C(9)–S(1)	130.1(2)	C(7)–C(9)–S(1)	124.3(2)
C(15)–C(10)–C(11)	120.6(3)	C(15)–C(10)–N(1)	120.1(3)
C(11)–C(10)–N(1)	119.2(3)	C(10)–C(11)–C(12)	119.7(3)
C(11)–C(12)–C(13)	120.3(3)	C(14)–C(13)–C(12)	119.9(3)
C(13)–C(14)–C(15)	120.1(3)	C(10)–C(15)–C(14)	119.4(3)

Table 2 Hydrogen bonds geometry for **1**

D–H···A	D–H	H···A	D···A	D–H···A	Symmetry codes
N(2)–H(2)···O(4)	0.86	2.51	3.137(4)	131	1–*x*,1/2 + *y*,3/2–*z*
N(4)–H(16)···O(1)	0.85	2.56	3.144(4)	126	*x*,1/2–*y*,1/2 + *z*
N(4)–H(17)···N(2)	0.95	2.20	3.137(4)	171	1–*x*,–1/2 + *y*,3/2–*z*

Table 3 Crystallographic data for **1** and **2** derivatives

Empirical formula	$C_{15}H_{11}ClN_4O_2S$	$C_{16}H_{12}ClN_5O_2S$
Formula weight	346.79	371.81
Colour/Shape	Yellow/Platelet	Colourless/Block
Temperature	293 K	293
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell dimensions		
90.000 95.297 90.000	*a* = 11.437 (4) Å; α = 90.000°;	*a* = 7.496(2)Å; α = 90.000°;
	b = 10.894 (3) Å; β = 95.297(9)°;	*b* = 17.728(4)Å; β = 91.524(5)°;
	c = 11.858 (4) Å; γ = 90.000°	*c* = 11.889(2)Å; γ = 90.000°
Volume, *Z*	1471.0(8), 4	1579.4(5), 4
Density (calculated), Mg m^{-3}	1.566	1.446
Absorption coefficient, mm^{-1}	0.417	0.386
F(000)	712	704
Theta range for data collection	3.56 to 55.44°	3.42 to 55.52°
Limiting indices	$-13 \le h \le 13$, $-14 \le k \le 13$, $-15 \le l \le 15$	$-9 \le h \le 9$, $-22 \le k \le 9$, $-13 \le l \le 13$
Reflections collected	15943	18015
Independent reflections	3001 [*R*(int) = 0.0549]	3289 [*R*(int) = 0.034]
Data/restraints/parameters	8489/0/210	7655/0/226
Goodness-of-fit on F^2	1.184	1.229
Final *R* indices [$I > 2\sigma$ (I)]	R_1 = 0.0591, wR_2 = 0.189	R^1 = 0.0856, wR^2 = 0.2462
R indices (all data)	R_1 = 0.0921, wR_2 = 0.166	R^1 = 0.1611, wR^2 = 0.2136
Largest diff. Peak and hole	0.069 and –0.512eÅ^{-3}	0.064 and –0.369eÅ^{-3}

bond type previously invoked.[12,15] The activity as inhibitors of globin proteolysis could be related with this hydrogen bond, longer distances favour the inhibition of *P. berghei*, probably because weaker or no intramolecular hydrogen bonds increase the availability of the NH_2 and SO_2 groups to bind the receptor. Compounds **1** and **2** were tested for their capacity of inhibiting globin proteolysis in an *in vitro* assay, which uses rich extract of trophozoyte to digest the native haemoglobin of mice. The electrophoretic analyses (76.39 ± 1,52 and 83.72 ± 2,13) for **1** and **2** respectively (leupeptin 89.06 ± 0,69 and pepstatin 92.94 ± 0,67), indicated that compounds were moderate and good inhibitors of globin degradation. Compound **2** was tested in mice infected with *P. berghei* ANKA, a chloroquine-susceptible strain of murine malaria. Mice were given the compound (chloroquine or **2** in 20 mg/kg, ip once daily) for 4 consecutive days (days 1–4 post infection). The parasitemia at the 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 **2** only slightly increased the survival time 10.8 ± 1.5 days but was able to reduce and delay the progression of malaria but did not eradicate the infection 9.75 ± 3.01% compared to chloroquine 30 days and 1.3 ± 0.3% respectively.

Table 4 Selected bond lengths (Å) and angles (°) for **2**

S(1)–O(2)	1.426(5)	C(6)–C(11)	1.401(6)	C(14)–C(15)	1.350(8)
S(1)–O(1)	1.436(2)	C(6)–C(7)	1.406(6)	C(15)–C(16)	1.340(8)
S(1)–C(1)	1.712(5)	C(7)–C(8)	1.382(6)	C(16)–C(17)	1.390(7)
S(1)–Cl(1)	1.729(5)	C(8)–C(9)	1.380(7)	N(2)–C(5)	1.379(6)
Cl(1)–C(8)	1.738(5)	C(9)–C(10)	1.377(7)	N(2)–C(6)	1.383(6)
C(1)–C(5)	1.399(6)	C(10)–C(11)	1.395(7)	N(2)–C(12)	1.455(6)
C(1)–C(2)	1.416(7)	C(12)–C(17)	1.374(7)	N(3)–C(4)	1.341(6)
C(2)–N(5)	1.341(6)	C(13)–C(14)	1.395(7)	N(4)–C(2)	1.354(6)
				N(5)–C(4)	1.343(6)

O(2)–S(1)–O(1)	113.7(4)	N(4)–C(2)–C(1)	121.9(5)
O(2)–S(1)–C(1)	111.3(3)	C(2)–N(5)–C(4)	115.5(4)
O(1)–S(1)–C(1)	109.8(3)	N(1)–C(4)–N(3)	116.9(4)
O(2)–S(1)–C(11)	110.3(3)	N(1)–C(4)–N(5)	127.8(4)
O(1)–S(1)–C(11)	108.5(3)	N(3)–C(4)–N(5)	115.4(4)
C(1)–S(1)–C(11)	102.7(2)	N(1)–C(5)–N(2)	115.7(4)
C(5)–N(2)–C(6)	123.3(4)	N(1)–C(5)–C(1)	120.7(4)
C(5)–N(2)–Cl(2)	118.6(4)	N(2)–C(5)–C(1)	123.5(4)
C(6)–N(2)–Cl(2)	118.1(4)	N(2)–C(6)–C(11)	123.2(4)
N(5)–C(4)–N(2)	116.5(5)	N(2)–C(6)–C(7)	119.7(4)
N(5)–C(2)–C(1)	121.5(4)	C(11)–C(6)–C(7)	117.1(4)
C(8)–C(7)–C(6)	119.9(4)	C(9)–C(8)–C(7)	122.5(5)
C(9)–C(8)–Cl(1)	119.5(4)	C(7)–C(8)–Cl(1)	118.0(4)
C(10)–C(9)–C(8)	118.5(5)	C(9)–C(10)–C(11)	120.0(5)
C(10)–C(11)–C(6)	122.0(5)	C(10)–C(11)–S(1)	115.0(4)
C(6)–C(11)–S(1)	123.0(4)	C(13)–C(12)–C(17)	120.6(5)
C(13)–C(12)–N(2)	119.8(5)	C(17)–C(12)–N(2)	119.5(5)
C(12)–C(13)–C(14)	118.6(6)	C(15)–C(14)–C(13)	120.3(6)
C(16)–C(15)–C(14)	121.4(6)	C(15)–C(16)–C(17)	119.5(6)
C(12)–C(17)–C(16)	119.6(5)		

Table 5 Hydrogen bonds geometry for **2**

D–H···A	D–H	H···A	D···A	D–H···A	Symmetry codes
N(3)–H(1)···N(5)	0.9151	2.2137	3.111(6)	166.58	–1–*x*,–*y*,1–*z*
N(3)–H(2)···O(1)	1.0000	2.5777	3.305(7)	129.50	–*x*,–*y*,1–*z*
N(4)–H(3)···O(1)	0.9666	2.5330	3.498(13)	117.18	Intra
N(4)–H(3)···O(2)	0.9666	2.5051	3.829(5)	124.20	Intra
N(4)–H(3)···O(1)	0.9666	2.2930	2.721(5)	105.83	–1/2 + *x*,1/2–*y*,*z*

Experimental

3-Amino-7-chloro-9-phenyl-1,9H-pyrazolo-[4,3-b]benzothiazine 4,4-dioxide **1** and 2,4-diamino-8-chloro-10H-phenylpyrimido-[5,4-b]benzothiazine 5,5-dioxide **2** were synthesised as reported elsewhere.[16,17] *In vitro* and *in vivo* assays of compounds **1** and **2** were tested following the procedure previously reported.[14]

X-ray analysis

Experimental crystallographic details are recorded in Table 3. Single crystals of size 0.78 × 0.58 × 0.08 mm **1** and 0.49 × 0.17 × 0.06 mm **2** were chosen for a single crystal in X-ray analysis. The X-ray data were collected on a Rigaku diffractometer provided with a CCD detector. The data were reduced and processed using CrystalClear.[19] An empirical absorption correction was applied (R. Jacobson, personal communication to the Rigaku Corporation, Tokyo, Japan 1998). The structure was solved by direct methods using SHELXTL-PLUS.[20] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in their calculated positions, except for the hydrogen atoms of the NH_2 group which were considered from the found positions. All these atoms were refined by using the Riding method. Refinements were done using SHELXTL-PLUS.[20] Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC No. CCDC 700891 and 700892). Copies of available materials can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB2 IEZ, UK (facsimile: (44) 01223 336033); e-mail: deposit@ccdc.ac.uk.

We thank the CDCH-UCV (grants PG. 06-00-6502-2006 and PI. 06-00-7078-2007), FONACIT-PCP and CYTED-RIDIMEDCHAG programmes for financial support.

Received 3 October 2008; accepted 30 October 2008
Paper 08/0208 doi:10.3184/030823409X393691
Published online: 15 January 2009

References

1 WHO *World Malaria Report* 2008 (http://rbm.who.int/wmr2008).
2 A. Sidhu, D. Verdier-Pinard and D. Fidock, *Science,* 2002, **298**, 210.
3 T. Egan, *Drug Design Rev.*-Online, 2004, **1**, 93.
4 V. Kapoor and K. Kumar, *Prog. Med. Chem*., 2005, **43**, 189
5 A. Joshi and C. Viswanathan, *Anti-Infect. Agents in Med. Chem*., 2006, **5**, 105.
6 J. Domíguez, J. Charris, M. Lobo, N. Gamboa, M. Moreno, F. Riggione, E. Sánchez, J. Olson and P. Rosenthal, *Eur. J. Med. Chem*., 2001, **36**, 555.
7 J. Charris, J. Domínguez, N. Gamboa, J. Rodrigues and J. Angel, *Eur. J. Med. Chem*., 2005, **40**, 875.
8 J. Domínguez, W. Basante, J. Charris and F. Riggione, *Farmaco*, 1996, **51**, 407.
9 J. Domínguez, J. Charris, L. Iarrusso, S. López, G. Lobo and F. Riggione, *Farmaco*, 1996, **51**, 781.
10 A. Rodríguez, L. Ruiz, E. Ferro, J. Domínguez, J. Charris, M. Girón and I. Aguilar, *J. Pharm. Sci*., 1996, **2**, 325.
11 J. Domínguez, J. Charris, M. Caparelli and F. Riggione, *Arzneim. Forsch./ Drug Res*., 2002, **52**, 482.
12 M. Caparelli, J. Charris and J. Domínguez, *J. Chem. Cryst*., 2006, **36**, 389.
13 J. Charris, J. Domínguez, N. Gamboa, J. Rodrigues and J. Angel, *Heterocyclic Commun.*, 2005, **5**, 2005.
14 J. Charris, A. Barazarte, J. Domínguez, G. Lobo, J. Camacho, R. Ferrer, N. Gamboa, J. Rodrigues and M. Capparelli, *J. Heterocyclic Chem*., 2007, **44**, 639.
15 J. Charris, A. Barazarte, R. Ferrer, J. Camacho, N. Gamboa, J. Rodrigues, R. Atencio and T. González, *J. Chem. Res*., 2007, 16.
16 A. Barazarte, J. Camacho, J. Domínguez, G. Lobo, N. Gamboa, J. Rodrigues, M. Caparelli, Á. Álvarez-Larena, S. Andujar, D. Enriz and J. Charris, *Bioorg. Med. Chem*., 2008, **16**, 3661.
17 A. Barazarte, G. Lobo, N. Gamboa, J. Rodrigues, M.V. Capparelli, Á. Álvarez-Larena, S. López and J. Charris, *Eur. J. Med. Chem*. In press 2008.
18 F. Allen and O. Kennard, *Chem. Des. Autom. News.*, 1993, **8**, 31.
19 Crystal Clear 1.3.6 (2005). Rigaku/MSC, Inc., 9009 New Trails Drive, The Woodlands, TX 77381.
20 Crystallographic software package SHELXTL-NT V5.1. PC version, Bruker Analytical X-Ray Systems. Madison, WI, USA 1998.