Synthesis and biological evaluation of benzothiazole-6-carbohydrazide derivatives as antiparasitic agents

Síntesis y evaluación biológica de derivados de benzotiazol-6-carbohidrazidas como agentes antiparasitarios

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ABSTRACT

A series of N´-substituted-2-(5-nitrofuran or 5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide derivatives were synthesized and investigated for their abilities to inhibit β-hematin formation, hemoglobin proteolysis and for their antimalarial efficacy in rodent *Plasmodium berghei*. Moreover, we investigated the effect on the viability of *Trypanosoma cruzi* and *Leishmania braziliensis in vitro.* We performed a scan to evaluate the cytotoxic effects against two non-tumourogenic cell lines. We found that compounds **5a**, **6a,** and **6g** were the most promising as inhibitors of β-hematin formation, especial attention should be paid to **6a** which also inhibited hemoglobin proteolysis moderately, decreased parasitaemia and prolonged survivals in infected-mice significantly compared to vehicle controls. Finally, we demonstrated that the compound **7f** have a profound effect on the viability of *T. cruzi* (Tulahuen, CL Brener), shown an IC_{50} values of 7.7 μM and 0.2 μM respectively, without affecting the viability of the host cells. All compounds showed a marginal activity against *L. braziliensis*. Thus, compounds **6a** and **7f** showed anti-parasitic efficacy and good safety index.

Key words: Benzothiazole, malaria, *P. berghei*, Chagas, *T. cruzi*, leishmaniasis, *L. braziliensis*.

RESUMEN

Una serie de derivados de N´-sustituidas-2-(5-nitrofurano o 5-nitrotiofeno-2-il)-3H-benzo(d)tiazol-6-carbohidrazidas fueron sintetizados e investigada su capacidad para inhibir la formación de la β-hematina, proteólisis de la hemoglobina y por su eficacia antimalárica en ratones infectados con *P. berghei*. Además, nosotros investigamos el efecto sobre la viabilidad *in vitro* de *T. cruzi* y *L. braziliensis*. Se evaluó el efecto citotóxico sobre dos líneas de células tumorales. Encontramos que los compuestos **5a**, **6a,** y **6g** fueron los más prometedores como inhibidores de la formación de la β-hematina, un especial interés se prestó sobre el compuesto **6a** que inhibió moderadamente la proteólisis de la hemoglobina, disminuyó la parasitemia y prolongo el tiempo de vida de los ratones infectados de manera significativa. Por último, se pudo observar que el compuesto **7f** presento un buen efecto sobre la viabilidad de *T. cruzi* cepas (Tulahuen, CL Brener), con un valor de CI_{50} de 7,7 μM y 0,2 μM respectivamente, sin afectar la viabilidad de las células hospederas. Todos los compuestos mostraron una actividad débil contra *L. braziliensis*. Los compuestos **6a** y **7f** mostraron eficacia antiparasitaria y un buen índice de seguridad.

Palabras clave: Benzotiazol, malaria, *P. berghei*, Chagas, *T. cruzi*, leishmaniasis, *L. braziliensis*.

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1. Introduction

Neglected tropical diseases (NTDs) are the most common infections of the world´s poorest people and the leading causes of chronic disability and poverty in low and middle-income countries. NTDs especially affect children and young women of reproductive age, and consequently deprive them of their health and economic potential. The Millennium Declaration, adopted by world leaders at the United Nation in September 2000, establishes an ambitious set of eight millennium development goals to eliminate extreme poverty, hunger, and disease by 2015. The sixth goal, is related with the combat of HIV-AIDS, and the NTDs, specifically addresses the health and economic impact of infectious diseases (Hotez and Pecoul, 2010). The protozoa genera are responsible for some of the principal sicknesses, such as *Trypanosoma cruzi* (Chagas disease), *Trypanosoma b. gambiense* and *Trypanosome b. rhodesiense* (sleeping sickness), *Plasmodium* spp. (malaria), and *Leishmania* spp. (leishmaniasis). The main health organizations are concerned about those diseases due to the significant number of cases. For instance, malaria is a vector-borne infection of red blood cells caused by *Plasmodium* parasite, which is transmitted by an infective female Anopheles mosquito. There are five *Plasmodium* species that infect humans, comprising *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi. P. falciparum* accounts for 80% of the cases and 90% of the deaths. This is an infective disease transmitted to man also by blood transfusion or infected needles and syringes. According to WHO (2014), malaria kills, at least, 1 million people per year and around 500 million people by year are contaminated in an acute form in developing countries. Chagas disease, a systematic chronic parasitic infection caused by the protozoan parasite *Trypanosoma cruzi*, is endemic in South and Central America, México and southern USA, being considered one of the most serious parasite diseases in tropical regions (Hotez and Pecoul, 2010). There are between 7 to 8 million people infected and as further 28 million, mainly in Latin America, are at risk. Some public health measures such eradication of the triatomine species vectors have reduced the number of new cases. Nevertheless, the transmission of the disease by alternative modes, via blood products, organ transplant, congenital transmission, and through ingestion of food contaminated with faeces from infected bugs, constitutes a serious public health problem, mainly in urban areas of developing countries. Some cases of Chagas disease have been recently detected in countries of other continents, in particular Spain, Australia and Japan, besides Canada and non endemic regions of USA (WHO, 2014). Leishmaniasis is recognized by WHO (2014) as other of the major tropical disease that affect humans, being released in over 88 countries on 4 continents, 350 million people at risk, 12 million cases reported with a rate of approximately 1.3 million new cases annually and 20000 to 30000 deaths reported each year. Leishmaniasis is caused by 20 different pathogenic species for human and other mammals, which belong to the genus *Leishmania*, a protozoa transmitted by the bite of an infected female Phlebotomine sandfly. The cutaneous and mucocutaneous forms of the disease often result in self cure, although consequences like permanent scars and deformations in the patients can occur, which can lead to social isolation and other problems (WHO, 2014).

Despite the importance of these diseases, few therapeutic agents are available, and many of them cause side effects. For this reason, many studies are focused in the development of alternative drugs, more efficient and economic, as well as the identification and characterization of new drug targets (Serrano-Martín, 2010). Moreover, the widespread development of resistance by some parasites strains such as *P. falciparum* that can be resistant to chloroquine and to other antimalarial drugs, Human African Trypanosomiasis (HAT) to arsenical compounds, american trypanosomiasis to beznidazole and nifurtimox, and leishmaniasis to antimonial compounds, contribute to the terrible health conditions found in developing countries. New drugs that attack the same vital target but that are not subject to the same resistance mechanism would be highly desirable. The benzothiazole scaffold is one of the privileged structures in medicinal chemistry (Welsch et al., 2010). Indeed, various examples featuring this particular scaffold have been prepared, many exhibiting remarkable biological activities (Young et al., 1988; Jimonet et al., 1999; Ryu et al., 2003; Serdons et al., 2009; Khan et al., 2011).

On the other hand, nitroaromatic compounds are very important group, which have been used extensively in the treatment of anaerobic infections, and are under continuum investigation. There is a direct proof that free-radical metabolites are involved in many applications including important antitumuor, antiparasitic and antibacterial agents (Kitagawa et al., 2007; Cho et al., 2008; Kim et al., 2009; Brain-Isasi et al., 2008; Sriram et al., 2009; 2010; Camacho et al., 2011). After extensive literature search, it was observed that, till date enough effort has not been made to combine these moieties as a single molecular scaffold and identify new candidates that may be value in designing new, potent, selective and less toxic antiparasitic agents.

In view of this data, we reported the synthesis of nitrofuran and nitrothiophene incorporated with bezothiazole and carbohydrazide which possessed wide variety of biological activity encouraging antimalarial activity against *P. berghei in vivo*, and *T. cruzi, L. braziliensis in vitro*. We also report the cytotoxic activity against two normal cell lines.

2. Experimental

2.1. Materials and methods

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 spectra were recorded using a Jeol Eclipse 270 (270 MHz) spectrometer using DMSO-d_6, and are reported in ppm downfield from the residual DMSO. Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer; 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. Compounds **2**, **3** and **5a-b** were synthesized following the proceeding reported previously (Charris et al., 2006).

*2.2. General procedure for the synthesis of 2-(5-nitrofuran or 5-nitrothiophe-2-yl)benzo(d)thiazole-6-carboxilic acid **5a-b***

A mixture of the 5-nitro-2-furaldehyde/5-nitro-2-thiophencarbaldehyde **4a-b** (5 mmol), 4-amino-3-mercaptobenzoic acid **3** (5 mmol), in nitrobenzene (25 mL) was refluxed for 6 h. After cooling to room temperature, hexane was added (15 mL) and the formed precipitate was filtered, washed with diethyl ether recrystallized from dioxan-water and then dried, to provide the compounds **5a-b** (Charris et al., 2006).

*2.3. General procedure for the synthesis of N´-substituted-2-(5-nitrofuran or 5-nitrothiophe-2-yl) benzo(d)thiazole-6-carbohydrazide derivatives **6, 7a-i***

A mixture of **5a-b** (0.3 mmol), thionyl chloride (1.5 mmol), and benzene 25 mL was gradually heated to boiling, whereupon the acid chloride dissolved. After refluxing for 12 h, excess of thionyl chloride and solvent were removed *in vacuo*. The acid chloride was not characterized, then to a solution of acid chloride derivatives, DMAP (1.5 mmol) in dry CH_2Cl_2 (80 mL) was slowly added a dry CH_2Cl_2 (20 mL) solution of hydrazide respective (0.4 mmol) (30 min) at 0ºC, and the mixture was stirred for 36 h at rt. The solvent was removed in vacuo and the residue was slowly added water 5% KOH at 0ºC and the mixture stirred for 30 min. The solid was washed with water, methanol and ethyl ether, recrystallized from a mixture of ethanol - water (1:1) to give **6, 7a-i**.

2.3.1. N´-Formyl-2-(5-nitrofuran-2-yl)benzo(d)thiazole-6-carbohydrazide **6a**

Yield 72%. mp > 255 °C. IR (KBr), cm^{-1}: 3439 (NH), 1649, 1607 (Ar), 1572, 1348 (NO_2). ^{1}H NMR, $DMSO_{d6}$, δ ppm: 7.72 (d, 1H, $H_{4'}$, J = 4.0 Hz); 7.90 (d, 1H, $H_{3'}$, J = 4.0 Hz); 8.14 (dd, 1H, H_5, J = 1.5, 8.8 Hz); 8.25 (d, 1H, H_4, J = 8.8 Hz); 8.80 (d, 1H, H_7, J = 1.5Hz); 10.50 (s, 1H, NH); 10.64 (s, 1H, NH); 10.90 (s, 1H, CHO). Anal. $C_{13}H_8N_4O_5S$: C 46.99, H 2.43, N 16.86; Found C 47.03, H 2.42, N 16.98.

2.3.2. N´-Benzoyl-2-(5-nitrofuran-2-yl)benzo(d) thiazole-6-carbohydrazide **6b**

Yield 63%. mp 270°C (dec). IR (KBr), cm^{-1}: 3440 (NH), 1648, 1608 (Ar), 1572, 1349 (NO_2). ^{1}H NMR, $DMSO_{d6}$, δ ppm: 7.54 (t, 2H, $H_{3'',5''}$, J = 7.7 Hz); 7.61 (t, 1H, $H_{4''}$, J = 7.3 Hz); 7.75 (d, 1H, $H_{3'}$, J = 4.4 Hz); 7.91 (d, 1H, $H_{4'}$, J = 4.4 Hz); 7.94 (d, 2H, $H_{2'',6''}$, J = 7.4 Hz); 8.12 (dd, 1H, H_5, J = 2.1, 8.6 Hz); 8.25 (d, 1H, H_4, J = 8.6 Hz); 8.81(d, 1H, H_7, J = 2.1 Hz); 10.63 (s, 1H, NH); 10.74 (s, 1H, NH). Anal. $C_{19}H_{12}N_4O_5S$: C 55.88, H 2.96, N 13.72; Found C 55.90, H 2.94, N 13.71.

2.3.3. N'-(3-Chlorobenzoyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **6c**

Yield 54%. mp >320 °C. IR (KBr), cm^{-1}: 3328 (NH), 1673, 1651 (Ar), 1516, 1350 (NO_2). ^{1}H NMR, $DMSO_{d6}$, δ ppm: 7.43 (t, 1H, $H_{5'}$, J = 7.7 Hz); 7.69 (d, 1H, Ar, J = 7.2 Hz); 7.77 (d, 1H, $H_{4'}$, J = 3.6 Hz); 7.90 (d, 1H, Ar, J = 8.1 Hz); 7.95 (d, 1H, $H_{3'}$, J = 3.6 Hz); 7.91 (s, 1H, $H_{2'}$); 8.12 (dd, 1H, H_5, J = 1.9, 8.7 Hz); 8.26 (d, 1H, H_4, J = 8.7 Hz); 8.81 (d, 1H, H_7, J = 1.9 Hz); 10.74 (s, 1H, NH); 10.78 (s, 1H, NH). Anal. $C_{19}H_{11}ClN_4O_5S$: C 51.53, H 2.50, N 12.65; Found C 51.62, H 2.53, N 12.79.

2.3.4. N'-(4-Chlorobenzoyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **6d**

Yield 89%. mp 280 °C (dec). IR (KBr), cm^{-1}: 3310 (NH), 1649, 1594 (Ar), 1540, 1349 (NO_2). ^{1}H NMR, $DMSO_{d6}$, δ ppm: 7.61 (d, 2H, $H_{3'',5''}$ J = 8.4 Hz); 7.74 (d, 1H, $H_{4'}$, J = 4.4 Hz); 7.92 (d, 1H, $H_{3'}$, J = 4.0 Hz); 7.96 (d, 2H, $H_{2',6'}$, J = 8.4 Hz); 8.12 (dd, 1H, H_5, J = 1.7, 8.0 Hz); 8.24 (d, 1H, H_4, J = 8.0 Hz); 8.81 (d, 1H, H_7, J = 1.9 Hz); 10.53 (s, 1H, NH); 10.57 (s, 1H, NH). Anal. $C_{19}H_{11}ClN_4O_5S$: C 51.53, H 2.50, N 12.65; Found C 51.53, H 2.51, N 12.67.

2.3.5. N'-(3-Methoxybenzoyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **6e**

Yield 79%. mp 260 °C (dec). IR (KBr), cm^{-1}: 3420 (NH), 1689, 1603 (Ar), 1593, 1350 (NO_2). ^{1}H NMR,

$DMSO_{d6}$, δ ppm: 3.79 (s, 3H, OCH_3); 7.13 (dd, 1H, Ar, *J* = 2.7, 7.6 Hz); 7.40-7.47 (m, 3H, Ar); 7.71 (d, 1H, $H_{4'}$, *J* = 3.9 Hz); 7.86 (d, 1H, $H_{3'}$, *J* = 3.9 Hz); 8.10 (dd, 1H, H_5, *J* = 2.1, 8.5 Hz); 8.22 (d, 1H, H_4, *J* = 8.5 Hz); 8.75 (d, 1H, H_7, *J* = 2.1 Hz); 10.57 (s, 1H, NH); 10.69 (s, 1H, NH). Anal. $C_{20}H_{14}N_4O_6S$: C 54.79, H 3.22, N 12.78; Found C 54.81, H 3.19, N 12.80.

2.3.6. N′-(3,4-Dimethoxybenzoyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **6f**

Yield 63%. mp 250 °C (dec). IR (KBr), cm^{-1}: 3360 (NH), 1648, 1598 (Ar), 1507, 1341 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 3.78 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 7.07 (d, 1H, $H_{5''}$, *J* = 8.4 Hz); 7.53 (d, 1H, $H_{2''}$, *J* = 1.8 Hz); 7.52-7.56 (m, 2H, Ar); 7.75 (d, 1H, $H_{4'}$, *J* = 3.6 Hz); 7.91 (d, 1H, $H_{3'}$, *J* = 3.6 Hz); 8.16 (dd, 1H, H_5, *J* = 2.1, 8.7 Hz); 8.25 (d, 1H, H_4, *J* = 8.4 Hz); 8.80 (d, 1H, H_4, *J* = 2.0 Hz); 10.42 (s, 1H, NH); 10.61 (s, 1H, NH). Anal. $C_{21}H_{16}N_4O_7S$: C 53.84, H 3.44, N 11.96; Found C 53.90, H 3.58, N 12.09.

2.3.7. N′-(3,4,5-Trimethoxybenzoyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **6g**

Yield 88%. mp 176 °C (dec). IR (KBr), cm^{-1}: 3536 (NH), 1669, 1651 (Ar), 1584, 1337 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 3.75 (s, 3H, OCH_3); 3.87 (s, 6H, OCH_3); 7.31 (s, 2H, $H_{2'',6''}$); 7.74 (d, 1H, $H_{4'}$, *J* = 4.0 Hz); 7.92 (d, 1H, $H_{3'}$, *J* = 8.6 Hz); 8.15 (dd, 1H, H_5, *J* = 2.0, 8.4 Hz); 8.24 (d, 1H, H_4, *J* = 8.4 Hz); 8.81 (d, 1H, H_4, *J* = 2.0 Hz); 10.47 (s, 1H, NH); 10.47 (s, 1H, NH). Anal. $C_{22}H_{18}N_4O_8S$: C 53.01, H 3.64, N 11.24; Found C 52.97, H 3.70, N 11.48.

2.3.8. N′-(3-Phenoxybenzoyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **6h**

Yield 67%. mp >260 °C. IR (KBr), cm^{-1}: 3312 (NH), 1649 (Ar), 1540, 1350 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 7.08 (d, 1H, Ar, *J* = 7.68 Hz); 7.20 (t, 1H, Ar, *J* = 7.32 Hz); 7.25 (dd, 1H, Ar, *J* = 2.2, 8.1 Hz); 7.44 (t, 2H, Ar, *J* = 7.7 Hz); 7.54-7.57 (m, 2H, Ar); 7.74-7.76 (m, 3H, Ar); 7.92 (d, 1H, $H_{3'}$, *J* = 4.0 Hz); 8.13 (dd, 1H, H_5, *J* = 1.9, 8.6 Hz); 8.25 (d, 1H, H_4, *J* = 8.6 Hz); 8.81 (d, 1H, H_7, *J* = 1.9 Hz); 10.56 (s, 1H, NH); 10.61 (s, 1H, NH). Anal. $C_{25}H_{16}N_4O_6S$: C 59.99, H 3.22, N 11.19; Found C 60.05, H 3.28, N 11.30.

2.3.9. N′-(3-Hydroxy-2-naphthyl)-2-(5-nitrofuran-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **6i**

Yield 71%. mp > 320 °C. IR (KBr), cm^{-1}: 3422 (NH), 1689 (Ar), 1536, 1344 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 7.35 (s, 1H, Ar); 7.52 (t, 1H, Ar, *J* = 6.9 Hz); 7.66 (t, 1H, Ar, *J* = 6.3 Hz); 7.74-7.76 (m, 2H, Ar); 7.90-7.92 (m, 2H, Ar); 8.12 (dd, 1H, H_5, *J* = 2.0, 8.4 Hz); 8.24 (d, 1H, H_4, *J* = 8.4 Hz); 8.58 (s, 1H, Ar); 8.81 (s, 1H, H_7); 10.73 (s, 1H, NH); 10.79 (s, 1H, NH). Anal. $C_{23}H_{14}N_4O_6S$: C 58.22, H 2.97, N 11.81; Found C 58.19, H 3.00, N 11.84.

2.3.10. N′-Formyl-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **7a**

Yield 73%. mp > 300 °C. IR (KBr) cm^{-1}: 3440 (NH), 1697, 1635 (Ar), 1516, 1340 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 8.01 (d, 1H, $H_{4'}$, *J* = 4.3 Hz); 8.11 (dd, 1H, H_5, *J* = 2.1, 8.0 Hz); 8.23 (d, 1H, H_4, *J* = 8.0 Hz); 8.25 (d, 1H, $H_{3'}$, *J* = 4.2 Hz); 8.83 (d, 1H, H_7, *J* = 2.1 Hz); 10.05 (s, 1H, NH); 10.54 (s, 1H, NH); 10.72 (s, 1H, CHO). Anal. $C_{13}H_8N_4O_4S_2$: C 44.82, H 2.31, N 16.08; Found C 44.80, H 2.33, N 16.10.

2.3.11. N′-Benzoyl-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **7b**

Yield 81%. mp 270-272 °C. IR (KBr), cm^{-1}: 3248 (NH), 1683, 1603 (Ar), 1505, 1344 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 7.54 (t, 2H, $H_{3'',5''}$, *J* = 7.7 Hz); 7.61 (t, 1H, $H_{4''}$, *J* = 7.3 Hz); 7.94 (d, 2H, $H_{2'',6''}$, *J* = 7.4 Hz); 8.03 (d, 1H, $H_{3'}$, *J* = 4.4 Hz); 8.10 (dd, 1H, H_5, *J* = 1.5, 8.8 Hz); 8.22 (d, 1H, H_4, *J* = 8.8 Hz); 8.23 (d, 1H, $H_{4'}$, *J* = 4.2 Hz); 8.76 (d, 1H, H_7, *J* = 1.5 Hz); 10.63 (s, 1H, NH); 10.74 (s, 1H, NH). Anal. $C_{19}H_{12}N_4O_4S_2$: C 53.76, H 2.85, N 13.20; Found C 53.84, H 2.86, N 13.36.

2.3.12. N′-(3-Chlorobenzoyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **7c**

Yield 79%. mp 240 °C (dec). IR (KBr), cm^{-1}: 3360 (NH), 1683, 1635 (Ar), 1539, 1323 (NO_2). ^1H NMR, $DMSO_{d6}$, *d* ppm: 7.47 (t, 1H, $H_{5''}$, *J* = 7.7 Hz); 7.70 (d, 1H, $H_{4''}$, *J* = 8.0 Hz); 7.91 (d, 1H, $H_{6''}$, *J* = 7.7 Hz); 7.97 (s, 1H, $H_{2''}$); 8.00 (d, 1H, $H_{4'}$, *J* = 4.4 Hz); 8.11 (dd, 1H, H_5, *J* = 1.5, 8.1 Hz); 8.21 (d, 1H, $H_{3'}$, *J* = 4.4 Hz); 8.24 (d, 1H, H_4, *J* = 8.1 Hz); 8.77 (d, 1H, H_7, *J* = 1.5 Hz); 10.66 (s, 1H, NH); 10.68 (s, 1H, NH). Anal. $C_{19}H_{11}ClN_4O_4S_2$: C 51.01, H 2.36, N 11.90; Found C 50.95, H 2.36, N 12.07.

2.3.13. N′-(4-Chlorobenzoyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **7d**

Yield 79%. mp 221 °C (dec). IR (KBr) cm^{-1}: 3248 (NH), 1651, 1600 (Ar), 1513, 1340 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 7.61 (d, 2H, $H_{3'',5''}$, *J* = 8.4 Hz); 7.96 (d, 2H, $H_{2'',6''}$, *J* = 8.4 Hz); 8.00 (d, 1H, $H_{4'}$, *J* = 4.8 Hz); 8.11 (dd, 1H, H_5, J = 2.0, 8.0 Hz); 8.23 (d, 1H, H_4, *J* = 8.0 Hz); 8.23 (d, 1H, $H_{3'}$, *J* = 4.6 Hz); 8.77 (d, 1H, H_7, *J* = 1.8 Hz); 10.65 (s, 1H, NH); 10.68 (s, 1H, NH). Anal. $C_{19}H_{11}ClN_4O_4S_2$: C 51.01, H 2.36, N 11.90; Found C 51.12, H 2.41, N 12.13.

2.3.14. N′-(3-Methoxybenzoyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **7e**

Yield 81%. MP 220 °C. (dec). IR (KBr) cm^{-1}: 3424 (NH), 1689, 1593 (Ar), 1578, 1344 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 3.84 (s, 3H, OCH_3); 7.18 (d, 1H, Ar,

J = 8.2 Hz); 7.45 (t, 1H, $H_{5'}$, *J* = 8.0 Hz); 7.50-7.54 (m, 2H, Ar); 8.03 (d, 1H, $H_{4'}$, *J* = 4.4 Hz); 8.12 (dd, 1H, H_5, *J* = 1.9, 8.4 Hz); 8.23 (d, 1H, $H_{3'}$, *J* = 4.4 Hz); 8.30 (d, 1H, H_4, *J* = 8.2 Hz); 8.77 (d, 1H, H_7, *J* = 2.0 Hz); 10.54 (s, 1H, NH); 10.65 (s, 1H, NH). Anal. $C_{20}H_{14}N_4O_5S_2$: C 52.86, H 3.11, N 12.33; Found C 52.89, H 3.08, N 12.30.

2.3.15. N´-(3,4-Dimethoxybenzoyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **7f**

Yield 82%. mp 220 °C (dec). IR (KBr), cm^{-1}: 3312 (NH), 1689, 1600 (Ar), 1510, 1344 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 3.82 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 7.09 (d, 1H, $H_{5'}$, *J* = 8.4 Hz); 7.53 (d, 1H, $H_{2''}$, *J* = 1.8 Hz); 7.58 (dd, 1H, $H_{6''}$, *J* = 1.8, 8.4 Hz); 8.02 (d, 1H, $H_{4'}$, *J* = 4.4 Hz); 8.11 (dd, 1H, H_5, *J* = 2.3, 8.4 Hz); 8.22 (d, 1H, H_4, *J* = 8.4 Hz); 8.32 (d, 1H, $H_{3'}$, *J* = 4.4 Hz); 8.75 (d, 1H, H_7, *J* = 2.1 Hz); 10.48 (s, 1H, NH); 10.67 (s, 1H, NH). Anal. $C_{21}H_{16}N_4O_6S_2$: C 52.05, H 3.33, N 11.56; Found C 52.08, H 3.33, N 11.60.

2.3.16. N´-(3,4,5-trimethoxybenzoyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbohydrazide **7g**

Yield 83%. mp 250 °C (dec). IR (KBr), cm^{-1}: 3216 (NH), 1649 (Ar), 1507, 1341 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 3.76 (s, 3H, OCH_3); 3.87 (s, 6H, OCH_3); 7.30 (s, 2H, $H_{2',6'}$); 8.01 (d, 1H, $H_{4'}$, *J* = 4.4 Hz); 8.12 (dd, 1H, H_5, *J* = 2.3, 8.4 Hz); 8.21 (d, 1H, H_4, *J* = 8.4 Hz); 8.23 (d, 1H, $H_{3'}$, *J* = 4.3 Hz); 8.76 (d, 1H, H_7, *J* = 2.2 Hz); 10.47 (s, 1H, NH); 10.61 (s, 1H, NH). Anal. $C_{22}H_{18}N_4O_7S_2$: C 51.36, H 3.53, N 10.90; Found C 51.40, H 3.55, N 10.97.

2.3.17. N´-(3-Phenoxybenzoyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **7h**

Yield 70%. mp 220 °C (dec). IR (KBr), cm^{-1}: 3264 (NH), 1609 (Ar), 1510, 1349 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 7.08 (d, 2H, Ar, *J* = 7.7 Hz); 7.20 (t, 1H, Ar, *J* = 7.3 Hz); 7.25 (dd, 1H, Ar, *J* = 2.2, 8.1 Hz); 7.44 (t, 2H, Ar, *J* = 7.7 Hz); 7.54-7.57 (m, 4H, Ar); 7.74 (d, 1H, Ar, *J* = 8.1 Hz); 8.21 (d, 1H, H_4, *J* = 8.4 Hz); 8.34 (d, 1H, $H_{3'}$, *J* = 4.1 Hz); 8.75 (d, 1H, H_7, J = 2.0 Hz); 10.56 (s, 1H, NH); 10.61 (s, 1H, NH). Anal. $C_{25}H_{16}N_4O_5S_2$: C 58.13, H 3.12, N 10.85; Found C 57.99, H 3.15, N 11.01.

2.3.18. N´-(3-Hydroxy-2-naphthyl)-2-(5-nitrothiophen-2-yl)-3H-benzo(d)thiazole-6-carbo-hydrazide **7i**

Yield 66%. mp 258-260 °C. IR (KBr), cm^{-1}: 3312 (NH), 1651, 1601 (Ar), 1520, 1340 (NO_2). ^1H NMR, $DMSO_{d6}$, δ ppm: 7.35 (s, 1H, Ar); 7.52 (t, 1H, Ar, *J* = 6.9 Hz); 7.66 (t, 1H, Ar, *J* = 6.3 Hz); 7.76 (d, 1H, Ar, *J* = 8.1 Hz); 7.91 (d, 1H, Ar, *J* = 8.4 Hz); 7.98 (d, 1H, $H_{4'}$, *J* = 3.9 Hz); 8.12 (dd, 1H, H_5, *J* = 2.1, 8.7 Hz); 8.14 (d, 1H, $H_{3'}$, *J* = 3.9 Hz); 8.20 (d, 1H, H_4, *J* = 8.7 Hz); 8.58 (s, 1H, Ar); 8.79 (d, 1H, H_7, *J* = 2.1 Hz); 10.73 (s, 1H, NH); 10.79 (s, 1H, NH). Anal. $C_{23}H_{14}N_4O_5S_2$: C 56.32, H 2.88, N 11.42; Found C 56.32, H 2.90, N 11.67.

2.4. Biological assays

2.4.1. Inhibition of β-hematin formation

The heme crystallization assay was performed according to (Baelmans et al., 2000), briefly, a solution of hemin chloride in DMSO (50 µL, 5.2 µg/mL), was distributed in 96-well micro plates. Different concentrations (100-1 µM) 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 hours to allow for completion of the reaction and centrifuged (4000 RPM x 15 minutes, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed three times with DMSO (200 µL) and finally, dissolved in NaOH (200 µL, 0.2 N). The solubilized aggregates were further diluted 1:2 with NaOH (0.1N) and absorbances recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of flavoprotein (FP) crystallization with the corresponding IC_{50}.

2.4.2. 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 of the Faculty of Pharmacy, Central university of Venezuela. Mice were infected by ip injection with 1 x 10^6 *Plasmodium berghei* (ANKA strain)-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 (Dorn et al., 1995).

2.4.3. Parasite extracts

Blood of infected animals (30-50% of parasitemia), was collected by cardiac puncture with a heparinized syringe and the blood pool was centrifuged (500 g x 10 minutes, 4 °C). Plasma and buffy coat were removed and the red blood cells (RBCs) 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, 20000 g x 30 min x 4 °C) (Deharo et al., 1994). 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 saponin (0,1% in PBS x 10 min). 1 mL of cold PBS was added and the samples were centrifuged (13000 g x 5 minutes, 4 °C) to remove erythrocyte cytoplasm content (including erythrocyte hemoglobin).

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 proteolysis inhibition assay (Rosenthal, 1995).

2.4.4. 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 proteolysis assay.

2.4.5. Inhibition of hemoglobin proteolysis

The proteolytic effect of the parasite extract on the native mice hemoglobin was assayed using 96-wells 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 hours 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). DMSO, pepstatin, leupeptin and chloroquine controls were electrophoresed at the same time. Once the bands were obtained, these were quantified by densitometry. Results were expressed as inhibition of hemoglobin proteolysis.

2.4.6. *In vivo* antimalarial Activity: 4-day Suppressive Test

Balb-C mice (18-23 g) were infected i.p. with 10^6 infected red blood cells with *Plasmodium 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) and diluted with Saline-Tween 20 solution (2%). Each compound (20 mg/kg) was administered once *ip* for 4 consecutive days every 24 hours. At day four, 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 and survival days post-infection of each compound-treated group (Peters and Robinson, 1999).

2.4.7. Antiproliferative effect on Trypanosoma cruzi and Leishmania braziliensis.

Promastigotes of *L. braziliensis* BEL 4, were cultured in LIT Medium (liver infusion tryptose medium: tryptose15 g/L, yeast extract 5 g/L, liver infusion broth 2 g/L, hemin-NaOH 0.02 g/L, glucose 4 g/L, NaCl 9 g/L, KCl 0.4 g/L and Na_2HPO_4, 7.5 g/L, pH 7.4) supplemented with inactivated 10% fetal bovine serum (GIBCO), at 29 °C, without agitation.

Epimastigotes of *T. cruzi* were cultured in LIT Medium (liver infusion tryptose medium: tryptose 15 g/L, yeast extract 5 g/L, liver infusion broth 2 g/L, hemin-NaOH 0.02 g/L, glucose 4 g/L, NaCl 9 g/L, KCl 0.4 g/L and Na_2HPO_4, 7.5 g/L, pH 7.4) supplemented with inactivated 10% fetal bovine serum (GIBCO), at 29 °C, with continuous agitation at 120 rpm. The susceptibility of parasites to different compounds was evaluated by MTT assay with fewer modifications (Muelas et al., 2000). Briefly, 500 x10^3 parasites per well were added in a 96 well plate. For each tested compound we used, at least three different concentrations to a maximum of 50 µM. The plate was incubated in the appropriated conditions for 72 h. After this time, 10 µl of MTT (10 mg/mL) solution were added and incubated in dark chamber for 4 h at 29 °C. The plate was read at 570 nm in an ELISA plate reader Synergy HT (Biotek). Those compounds with an estimated IC_{50} less than 50 µM were selected for the next experimentation phases.

2.4.8. Cell growth inhibition bioassay.

Peritoneal macrophages J774G8, were cultured in RPMI 1640 medium (GIBCO) supplemented with 5% fetal bovine serum (non-inactivated, GIBCO), at 37 °C and 5% CO_2 (Serrano-Martín et al., 2009).

Vero cells were cultured in RPMI medium (GIBCO) supplemented with 1% fetal calf serum (non-inactivated), at 37 °C and humidified atmosphere (95% air-5% CO_2) (Urbina et al., 1996).

In order to evaluate the cytotoxicity on the host cells, peritoneal macrophages J774G8 and Vero cells were exposed to the selected compounds. Briefly, 10 x10^3 cells per well were added in a 96 well plate and incubated at 37 °C in 5% CO_2 overnight in appropriated conditions. In this evaluation, there were tested four concentrations of the each selected compound (concentrations above the calculated IC_{50} for parasites). Plates were left for 76 hours under the conditions described. Then, the cell medium was removed and washed the cells with PBS, subsequently 100 µL of MTT solution (1 mg/mL) were added to each well and was incubated for 4 hours at 37 °C and 5% CO_2. The plate was read at 570 nm in an ELISA plate reader Synergy HT (Biotek).

IC_{50} of each compound is estimated in both cell lines and compared with those obtained with each parasite.

2.4.9. Data Analysis.

Data were statistically analyzed using one-way

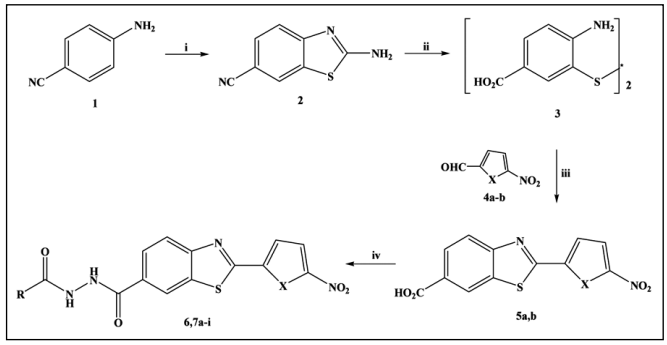
ANOVA and t-tests for specific group comparisons; assuming 95 % of confidence according GraphPad Prism 3.02 (Graph Pad Prism Software Inc, 1992-2004).

3. Results and discussion

3.1. Chemistry

Our strategy for synthesis of **6a-i** and **7a-i**, is a very simple one as illustrated in Scheme 1.

The few routes to benzothiazoles described in the literature are quite similar, typically an o-aminobenzenethiol is condensed with aldehydes, ketones, carboxylic acids or thioamides using acid, base, ethylene glycol or an organometallic catalyst in moderate to high temperatures, and long reaction times giving the corresponding benzothiazoles in moderate to good yields (Nivalkar and Mashraqui, 1996; Costa et al., 1997; Richardson et al., 1998; Lee et al., 2001; Zhong et al., 2001). In the procedure here described **5a-b** were obtained by the reaction of 4-amino-3-mercaptobenzoic acid **3** with 5-nitro-2-furaldehyde **4a** and 5-nitro-2-thiophenecarbaldehyde **4b**, to obtain the title compounds in good yields. The synthesis of the required precursor 4-amino-3-mercaptobenzoic acid **3,** was achieved starting from 4-aminobenzonitrile by its treatment with ammonium thiocyanate in the presence of bromine, giving the corresponding 2-aminobenzotiazole-6-carbonitrile **2**. The alkaline hydrolysis of **2** followed by the addition of hydrochloric acid gave in one-pot the expected 4-amino-3-mercaptobenzoic acid **3** in better yield than in a previous report (Charris et al., 2006). In this case, nitrobenzene was used as an oxidant following an analogous procedure to that reported for the synthesis of their aza counterpart's benzimidazoles (Camacho et al., 2011). Compounds **5a-b** were allowed to react with thionyl chloride to obtain the corresponding chloro derivatives, the chloro derivatives of **5a-b** were not isolated from the reaction medium, condensed with the appropriately substituted aryl acid hydrazides in DCM and DMAP to afford the target molecules. The final compounds were purified by recrystallization from ethanol, and the structure of the compounds was confirmed by IR, ^{1}H-NMR and elemental analysis. The IR spectra of target compounds **6a-i** and **7a-i** showed the broad stretching band around 3400 and 3200 cm^{-1} due to (NH), around 1650 cm^{-1} due to (CO), and around 1510-1530 and 1340-1350 cm^{-1} due (NO_2), with ^{1}H-NMR a single around 10.5 and 10.8 ppm accounted for NH vanished on D_2O exchange, doublets around 7.77 and 8.23 ppm *J*: 4-5 Hz assigned to protons $H_{3'}$ and $H_{4'}$ respectively and benzothiazole moiety protons as doublet of doublets around 8.12 and 8.15 ppm *J*: 8.0 and 2.0 Hz assigned to proton H_5, doublet around 8.20 and 8.30 ppm *J*: 8.0 assigned to proton H_4, and doublets around 8.70 and 8.80 ppm *J*: 2.0 Hz assigned to proton H_7. The synthetic route leading to the title compounds is summarized in



Scheme 1. Synthesis of N′-substituted-2-(5-nitrofuran or 5-nitrothiophe-2-yl)benzo[d] thiazole-6-carbohydrazide derivatives 6, 7a-i.
i: NH4SCN, Br2, AcOH, rt. ii: 30% KOH/MeOH, Δ, HCl; iii: RCHO, nitrobenzene, Δ; iv: SOCl2, toluene, DMF, Δ; carbohydrazide, DMAP, DCM, 0 °C rt.

3.2. Biological

All analogs of those derivatives were tested *in vitro* for their effects as inhibitors of β-hematin formation, inhibition of hemoglobin proteolysis, and *in vivo* for their efficacy in a malaria murine model (Table I). The first mentioned *in vitro* assay was used to assess the abilities of the derivatives N′-substituted-2-(5-nitrofuran or 5-nitrothiophen-2-yl)-3H-benzo[d] thiazole-6-carbohydrazide derivatives **6a-i, 7a-i** to inhibit β-hematin synthesis. To evaluate the potential antimalarial activity of compounds, we tested the ability of these compounds to inhibit heme crystallization, considering that heme can crystallize spontaneously under acid and low oxygen condition found in the vacuole of the parasite. Results that showed more than 50% of inhibition of heme crystallization along with IC_{50}<50 μM were considered significant and compounds **5a**, **6a**, **6g** revealed strong activity inhibiting this biochemical event (Table I). The 5-nitrofuril moiety appeared to be favourable for the potential antimalarial activity, since most of the compounds possessing showed measurable levels of inhibition of β-hematin formation. Compounds with carboxylic, N′-formyl, and trimethoxyphenylhydrazide groups in position six of benzothiazole exhibited very good activity when in position two of benzothiazole is a 5-nitrofuril moiety, while phenoxy, nafthyl, mono or dimethoxy, chlorophenylhydrazide groups displayed a marginal activity against inhibition of β-hematin formation. All compounds were tested for their capacity of inhibiting hemoglobin proteolysis, in an *in vitro* assay which uses trophozoyte-rich extract to digest

Table I

***In vitro* and *in vivo* biological activities of prepared benzothiazole-6-carbohydrazide derivatives 6a-i, 7a-i**

Nº	R_1	*IbHF (50 µM)*	IC_{50} (µM)	*IHbP (%)*	*% P*	*SD*	*T. cruzi* $^{\gamma}$ IC_{50} (µM)	*T. cruzi* $^{\gamma\gamma}$ IC_{50} (µM)	*L. braziliensis* IC_{50} (µM)
5a		89.51±0.58*	9.38	0	11.44±3.05†	8.75±0.43	ND	ND	ND
5b		<5	ND	0	ND	ND	ND	ND	ND
6a	H	56.97±1.25	34.14	34.76±0.59	11.96±3.36†	10.6±0.95†	ND	≥50	≥50
6b	Ph	<5	ND	11.08±0.32	ND	ND	ND	ND	ND
6c	3-ClPh	<5	ND	0	ND	ND	ND	ND	ND
6d	4-ClPh	<5	ND	0	ND	ND	ND		ND
6e	3-MeOPh	<5	ND	0	ND	ND	ND	ND	ND
6f	3,4-MeOPh	27.65±6.92	≥50	41.42±1.2	ND	ND	ND	≥50	≥50
6g	3,4,5-MeOPh	72.91±0.25	9.16	40.01± 0.72	11.7±3.26†	9.4±0.74	ND	≥50	≥50
6h	3-PhOPh	<5	ND	3.42± 0.57	ND	ND	ND	ND	ND
6i	2-OHNaphtyl	32.43±2.08	≥50	1.97±1.53	ND	ND	ND	≥50	≥50
7a	H	<5	ND	46.15±1.16	ND	ND	18	≥50	≥50
7b	Ph	<5	ND	0	ND	ND	17.5	ND	ND
7c	3-ClPh	<5	ND	0	ND	ND	ND	ND	ND
7d	4-ClPh	<5	ND	0	ND	ND	ND	ND	ND
7e	3-MeOPh	<5	ND	0	ND	ND	17.2	ND	ND
7f	3,4-MeOPh	<5	ND	0	ND	ND	7.2^{Ω}	0.2^{Ω}	≥50
7g	3,4,5-MeOPh	<5	ND	0	ND	ND	15.5	≥50	≥50
7h	3-PhOPh	<5	ND	0	ND	ND	12.5	ND	ND
7i	2-OHNaphtyl	<5	ND	30.83±1.05	ND	ND	ND	≥50	≥50
Leu	—-	—-	—-	91.62 ± 0.69	—-	—-			
Pep	—-	—-	—-	95.45 ± 0.66	—-	—-			
CQ	—-	94.19 ± 0.36	0.92	24.12 ±1.16	1.3 ± 0. 3	>30			
Nfx	—-	—-	—-	—-	—-	—-	7.7		
V	—-	—-	—-	—-	26.2±0.97	8.2 ±0.37			

Inhibition of β-hematin formation (IbHF), hemoglobin proteolysis (IHbP) (%), effect of benzothiazole derivatives (20 mg/Kg) on parasitemia at fourth day post-infection (% P) and survival days (SD) of P. berghei infected-mice by benzothiazole derivatives. The results are expressed by the mean ± standard error of the mean. *p> 0.05 compared to chloroquine, †p< 0.05 compared to vehicle and chloroquine; γ: Tulahuen, γγ: CL Brener, Ω p< 0.05 compared to nifurtimox n=6. **X 5a, (6a-i) = O; X 5b, (7a-i) = S; Leu**=Leupeptin**; Pep**=Pepstatin**; CQ**=Chloroquine**; Nfx=** Nifurtimox**; V**=vehicle control**; 7f.** Against macrophages and VERO cell lines IC_{50}: 165 and 130 (µM) respectively.

mice-native hemoglobin. The electrophoretic analysis indicated that only compounds **6a,f,g** and **7a,i** were moderately effective inhibiting the proteolysis of hemoglobin (Table I).

Compounds **5a, 6a** and **6g** were tested in *P. berghei*-infected mice, a chloroquine-susceptible strain of murine malaria. Compounds were given to mice (**5a, 6a, 6g** in 20 mg kg^{-1}, i.p. once daily) for four consecutive days using vehicle and chloroquine (25 mg/kg) as comparative controls. At day four post-infection, the parasitemias were determined. Survival days were monitored and registered. Vehicle control mice died within 8.2 ± 0.37 days post-infection and tested compounds slightly increased the survival rates, while chloroquine prolonged the survival time of the infected mice up to 30 days when animals were euthanized. Compounds were able to reduce and delay the progression of malaria which was observed also by the determination of parasitemias, showing that compound **6a** was in general, the most active analogue *in vivo* revealing significant increases in the survival rates and decreases in parasitemias compared to vehicle control; however, the tested derivatives did not eradicate the infection (Table I). Thus, it is important to remark that **6a** is able to inhibit β-hematin formation (>50%) and globin proteolysis moderately. The synergistic effects of this substance involving two different mechanisms of action would lead to a partial aminoacid deprivation in parasites as well as the inhibition in the heme detoxification process, results that are in concordance with the potential anti-plasmodial effect by the increase in infected-mice survival after **6a** treatment.

To evaluate the activities anti *T. cruzi* and anti *L. brazilensis* of compounds **5a-b**, **6a-i, 7a-i**, we performed *in vitro* susceptibility assays using a method previously described. Cultures of parasites were exposed to different compounds concentrations and their viability was measured by MTT assay (Table I). Compound tested against *L. braziliensis* showed a marginal value ≥ 50 µM. Meanwhile, the compound **7f** generated an important effect on the proliferation of *T. cruzi* (Tulahuen, CL Brener) showing IC_{50} values of 7.2 and 0.2 µM respectively compared with nifurtimox (Nfx) 7.7 µM (Table I).

Macrophages and VERO cells were exposed to different compounds concentrations and their viability was measured by MTT assay. The results shown that the selected compound **7f**, slightly affect the viability of host cells, compared with the effect on parasites IC_{50} value of 165 µM on macrophages and IC_{50} value of 130 µM on VERO cells. These results demonstrate that **7f** was 650 times more effective against *T. cruzi* than on their host cells. The 5-nitrothiophen moiety appeared to be favourable for the potential anti *T. cruzi* activity, since most of the compounds tested showed measurable activity against *T. cruzi* strain *in vitro*. Compounds with aryloxy or methoxyphenylhydrazide groups in position six of benzothiazole exhibited very good activity when in position two of benzothiazole is a 5-nitrothiophen moiety %IC 100 and IC_{50} between 0.2 and 17.2 µM, while compounds with carboxylic, N'-formyl, napthyl or chlorophenylhydrazide groups and 5-nitrofuril or 5-nitrothiophen moieties in position two of benzothiazole displayed a marginal activity against proliferation of *T. cruzi* strain.

3.3. Structure-activity relationship study

Having confirmed the activity of compound **5a**, we embarked on a hit-to-lead exploration program focusing on CO_2H of benzothiazole nucleus. The first step toward lead optimization was incorporation of formylhydrazido group **6a**. The inhibition of heme crystallization study data of this compound showed a less activity that **5a**; however, the activity *in vivo* against *P. berghei*-infected mice was moderate, especially for **6a**, the cytotoxic, anti *T. cruzi* and anti *L. braziliensis* activities were marginal. One significant problem with accurate measurements of kinetic constant and other activities for these compounds has been solubility. Hence we planned to introduce the aromatic group with electron withdrawing and donating groups at different position to study its influence on activity. Different analogs with H, chlorine, hydroxyl, aryloxy, and methoxy groups were synthesized. Compounds having H, chlorine, phenoxy and mono and dimethoxy groups and a 5-nitrofuranyl or 5-nitrothiophene-2-yl moiety in position 2 of benzothiazole exhibited marginal activity as antimalarial, cytotoxic, anti *T. cruzi* and anti *L. braziliensis*. However, compounds with trimethoxy substituent and a 5-nitrofuranyl moiety in position 2 of benzothiazole exhibited good activity *in vitro* as inhibitors against heme polymerization study but a marginal activity as cytotoxic, anti *T. cruzi* and anti *L. braziliensis*. On the other hand, we can to see that activity as anti *T. cruzi* was favored with donating groups as aryloxy, and methoxyphenylhydrazide and a 5-nitrothiophene-2-yl moiety in position 2 of benzothiazole, but was not favored by a chlorine group. Compound **7f** showed an excellent safety index as anti *T. cruzi*.

4. Conclusions

The present study describes the synthesis and the *in vitro* and *in vivo* antiparasitic and cytotoxic activities of tripartite hybrids from pharmacophores benzothiazole, 5-nitrofuran or 5-nitrothiophene and substituted aryl acid hydrazides. In summary, some of them showed good selectivity index between the parasite, and normal cells. Compounds **5a**, **6a**, **6g** exhibited potential effects as inhibitors of β-hematin formation. The study confirms that the antimalarial mechanism of action could be similar to that chloroquine, as most of the compounds form an association complex with β-hematin and thereby inhibit hemozoin formation. The results provide basic information to establish that a 5-nitrothiophene-2-yl moiety on position two of the bezothiazole is not very essential for an inhibitory activity of heme dimerization; however, a 5-nitrofuryl moiety is essential and opens new views for design of new antimalarial agents. Compounds **7b**, **7e-h** exhibited potential effect as anti *T. cruzi*, however, compound **7f** was exceptionally as potent as nifurtimox with a safety index of 650. The results provide basic information to establish that a 5-nitrofuryl moiety on position two of the bezothiazole is not very essential for an activity against *T. cruzi*, however, a 5-nitrothiophene-2-yl moiety is essential and opens new views for design of new anti *T. cruzi* agents. Rationally, such a combination of antiprotozoal pharmacophores and other functionalities offers many attractive features for accelerating antimalarial, and anti-trypanosomatid. Due to the overall good effects as inhibitor of β-hematin formation and *in vivo* antimalarial activity compound **6a**, and against *T. cruzi* (*Tulahuen, CL Brener*) strains *in vitro*, compound **7f**, have been selected for further development. Studies to acquire more information about their molecular mechanism, structure-activity relationships and pharmacokinetics are in progress in our laboratories.

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