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In Vitro Activities of Position 2 Substitution-Bearing 6-Nitro- and 6-Amino-Benzothiazoles and Their Corresponding Anthranilic Acid Derivatives against *Leishmania infantum* and *Trichomonas vaginalis*

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6-Nitro- and 6-amino-benzothiazoles bearing different chains in position 2 and their corresponding anthranilic acid derivatives were investigated for their in vitro antiparasitic properties against parasites of the species *Leishmania infantum* and *Trichomonas vaginalis* compared to their toxicity towards human monocytes. Biological investigations established that the antiprotozoal properties depended greatly on the chemical structure of the position 2 substitution-bearing group. Compound C1, 2-[(2-chloro-benzothiazol-6-yl) amino] benzoic acid, demonstrated an interesting antiproliferative activity towards parasites of the species *T. vaginalis*, while compound C11, 2-({2-[(2-hydroxyethyl) amino]-benzothiazol-6-yl} amino) benzoic acid, exhibited a promising activity against parasites of the species *L. infantum* in their intracellular amastigote form. Additional experiments established that compound C11, which was poorly toxic against the promastigote and the extracellular amastigote forms of the parasite, could improve host-protective mechanisms against *Leishmania* by preventing parasite internalization by macrophages and stimulating NO production, by means of a mechanism synergistically enhanced by the presence of gamma interferon.

During the past decade, the expansion of developmental projects such as irrigation systems and deforestation has led to a dramatic incidence of infectious diseases in most developing countries (9). Among these infections, malaria remains the most prevalent parasitic disease worldwide. Nevertheless, leishmaniases are now prevalent in 88 countries on five continents and present a new and extremely serious epidemiological profile with the emerging incidence of *Leishmania*-human immunodeficiency virus coinfections in industrialized countries of southern Europe (9, 10; also see World Health Organization, Leishmaniasis [http://www.who.int/emc/diseases/leish/]). Leishmaniases are vector-borne parasitic diseases caused by the presence of protozoa of the genus *Leishmania*, which are obligate intracellular parasites that replicate into the parasitophorous vacuoles of macrophages and produce various life-threatening clinical syndromes according to their localization in mammalian tissues, notably exemplified by visceral, cutaneous, and mucosal leishmaniasis (2). Since the 1940s, treatment with pentavalent antimonial agents has been the generally accepted therapy for all forms of leishmaniasis. However, during the past decade, antileishmanial therapy has become a bewildering subject, largely because of the complexity of the disease (10). Moreover, the continuous appearance of Glucantime-resistant *Leishmania* strains responsible for therapeutic failures in immunocompetent patients has focused on the urgent need for developing new and efficient antiparasitic molecules.

Benzothiazoles comprise a novel class of therapeutic compounds shown to exert a wide range of biological activities. Initially employed as pigments in leather tanning, most of the compounds that exhibited both high levels of fluorescence properties and a capacity to bind with cellular structures were extensively used as fluorochromes. Since the 1990s, various pharmacological investigations of newly synthesized benzothiazole derivatives demonstrated interesting pharmacological activities and led to the development of new medications for treating human diseases. Among the most efficient compounds, riluzole, sulfathiazole, mercapto-2-benzothiazole, and 2-(phenylsulfonyl)-benzothiazole revealed neuroprotective (1, 12), anticonvulsive (14), antiallergenic, and antimicrobial (7, 11, 19) activities, respectively, while other derivatives such as 2-(4-aminophenyl)-benzothiazoles exhibited potent antitumoral activity (3, 5), probably due to their capacity to bind with tumor-specific proteins. In contrast to other anticancer drugs, such as acridines, that have been extensively studied for their antileishmanial (8) and trypanocidal activities (8), benzothiazoles have been poorly investigated. Nevertheless, their potent capacity to interfere with cellular structures suggested that they are candidates for activity against protozoa. On this basis, we synthesized position 2 substitution-bearing 6-nitro- and 6-amino-benzothiazoles and their corresponding anthranilic acids and assessed the in vitro antiproliferative activity of each derivative against parasites of the genus *Leishmania* compared to its activity against another protozoan parasite, such as *Trichomonas vaginalis*, and its toxicity against human monocytes.

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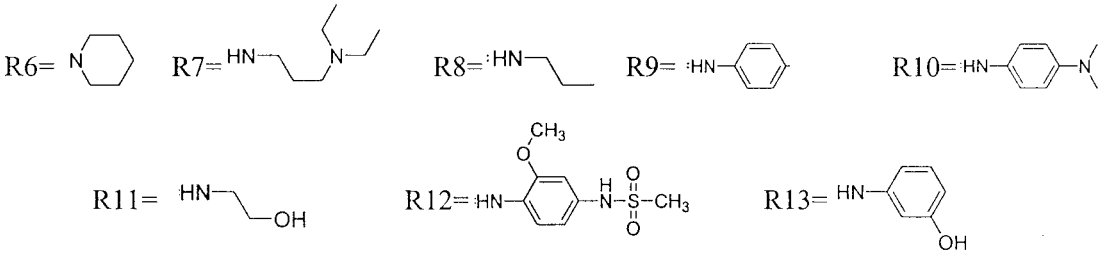
TABLE 1. Physicochemical properties and predictive values of biological activities calculated for position 2 substitution-bearing 6-nitro and 6-amino-benzothiazoles and their corresponding anthranilic acids



Compound series[a]	R	Compound designation	Lipophilicity	Solubility	Predictive value of biological activity		
					Toxicity	Antileishmanial activity	Antitrichomonal activity
A	Cl	A1	2.78	−3.65	0.387	0.469	0.387
	$NHCOCH_3$	A2	1.98	−3.83			0.426
	NH_2	A3	1.99	−2.97	0.253	0.424	0.401
	$N(CH_3)_2$	A4	2.95	−3.22	0.321	0.414	0.379
	$N(C_2H_5)_2$	A5	3.93	−3.54	0.346	0.444	0.375
	R6	A6	3.40	−3.46			0.423
	R7	A7	3.30	−4.00	0.255	0.671	0.400
	R8	A8	3.58	−3.77		0.464	0.391
	R9	A9	3.99	−4.39			0.354
	R10	A10	4.21	−4.71			0.317
	R11	A11	1.56	−3.70			0.385
	R12	A12	3.35	−4.67			0.215
	R13	A13	3.68	−4.39		0.354	0.320
B	Cl	B1	2.64	−2.73	0.377	0.438	
	$NHCOCH_3$	B2	1.11	−3.08			0.359
	NH_2	B3	0.99	−1.95		0.344	
	$N(CH_3)_2$	B4	2.09	−2.27		0.372	
	$N(C_2H_5)_2$	B5	2.99	−2.69		0.409	
	R6	B6	3.19	−2.75			0.344
	R7	B7	2.55	−3.32	0.248	0.654	0.323
	R8	B8	2.47	−2.99		0.432	0.278
	R9	B9	2.77	−3.73			
	R10	B10	3.42	−4.07	0.245		
	R11	B11	0.73	−2.93			0.266
	R12	B12	2.53	−4.22			
	R13	B13	2.56	−3.77			
C	Cl	C1	4.56	−4.75		0.398	
	$NHCOCH_3$	C2	3.45	−4.66			0.268
	NH_2	C3	3.63	−4.08		0.374	
	$N(CH_3)_2$	C4	3.91	−4.42		0.371	
	$N(C_2H_5)_2$	C5	4.56	−4.52		0.386	
	R6	C6	4.91	−4.34			0.281
	R7	C7	4.94	−4.63			
	R8	C8	4.28	−4.52		0.398	
	R9	C9	5.06	−4.99		0.346	
	R10	C10	5.32	−5.28			
	R11	C11	3.19	−4.49			0.203
	R12	C12	4.20	−4.96			
	R13	C13	4.77	−4.95			

[a] A, position 2 substitution-bearing 6-nitro-benzothiazoles; B, position 2 substitution-bearing 6-amino benzothiazoles; C, anthranilic acids.

MATERIALS AND METHODS

Strains and reagents. Position 2 substitution-bearing 6-nitro- and 6-amino-benzothiazoles, together with their corresponding anthranilic acids (Table 1), were synthesized at the Laboratoire de Valorisation de la Chimie Fine, Université d'Aix-Marseille III, site de Saint Jérome, Marseilles, France. Syntheses were performed using 2-chloro-benzothiazole (CAS 615-20-3) as starting material. The first nitro derivative, 2-chloro-6-nitro-benzothiazole (A1), was obtained using sulfuric and nitric acids according to the methodology described by Katz (11). Corresponding position 2 substitution-bearing 6-nitro-benzothiazoles (A2 to A13) were obtained by nucleophilic replacement of the chloro group by the amino group with the Meisenheimer intermediate formation. Reduction of position 2 substitution-bearing 6-nitro-benzothiazoles into position 2 substitution-bearing 6-amino-benzothiazoles (B1 to B13) was performed with Pd/C catalyst in a hydrogen atmosphere. The Ullman condensation gave the corresponding anthranilic acids (C1 to C13) in good yield by using ultrasound irradiation. Following synthesis, chemical compounds were dissolved in sterile dimethyl sulfoxide (DMSO) (analytical grade; Sigma, St. Louis, Mo.) and stored frozen at −70°C until used. For each chemical compound, purity was determined by

high-performance liquid chromatography and nuclear magnetic resonance spectroscopy. Amphotericin B (Sigma) and metronidazole were used as standard drugs for positive controls. All compounds were dissolved in sterile DMSO (analytical grade; Sigma) and stored frozen at −70°C until used. Antileishmanial activities were assessed on the referenced strain *L. infantum* (MHOM/FR/78/LEM75). Antitrichomonal activities were assessed on the referenced strain *T. vaginalis* (TVR87).

Predictive values of biological properties. Physicochemical values such as lipophilicity (LogP, representing the *n*-octanol–water partition coefficient) and solubility in water (LogS) were estimated by predictive mathematical methods using ALOGPS version 2.0 software according to the methodology described by Tetko et al. (18). Predictive values of antileishmanial and antitrichomonal activities, together with toxicity, were also investigated using the chemistry software server PASS (http://www.ibmh.msk.su/PASS/), according to the mathematical model and the database developed by Poroikov et al. (16) and Lagunin et al. (13), respectively.

Activity against *Trichomonas vaginalis*. Parasites were maintained in continuous culture in the *Trichomonas* medium TM 161 (Oxoid) supplemented with 8% heat-inactivated horse serum (Eurobio, Paris, France). Parasites in late log phase were incubated at an average of 10^4 cells/ml, and a range of benzothiazole concentrations were aseptically incorporated into duplicate cultures (final DMSO concentration, less than 5%). Negative controls treated by solvent (DMSO) and positive controls containing a range of metronidazole (Sigma) concentrations were added to each set of experiments. After a 48-h incubation period at 37°C, viable parasites were identified and counted microscopically on the basis of their aspect and motility and 50% inhibitory concentration (IC_{50}) values were determined.

Antileishmanial activity against promastigotes. *Leishmania infantum* promastigotes in late log phase were incubated in RPMI medium supplemented with 12% fetal calf serum at an average of 10^5 cells/ml, and a range of benzothiazole concentrations were aseptically incorporated into duplicate cultures (final DMSO concentration, less than 5%). Following a 48-h incubation period at 25°C, promastigote growth was estimated by counting parasites with a hemacytometer and IC_{50} values were determined.

Antileishmanial activity against intracellular amastigotes. Intracellular amastigote culturing was performed in human monocyte-derived macrophages according to the methodology previously described by Ogunkolade et al. (15). Maturation of monocytes into adherent macrophages was induced by treating exponentially growing monocytes (10^5 cells/ml) with 1 μM phorbol myristate acetate (Sigma). After a 48-h incubation period at 37°C (5% CO_2) in chamber slides (Fisher, Paris, France), cells were rinsed with fresh medium and suspended in RPMI medium containing stationary-phase promastigotes (cell/promastigote ratio, 1/10). After a 24-h incubation period at 37°C (5% CO_2), promastigotes were removed by four successive washes with fresh medium. Adapted dilutions of chemical compounds were added in duplicate chambers, and cultures were incubated for 96 h at 37°C (5% CO_2). Negative controls treated by solvent (DMSO) and positive controls containing a range of amphotericin B (Sigma) concentrations were added to each set of experiments. At the end of the incubation period, cells were harvested with analytical-grade methanol (Sigma) and stained with 10% Giemsa stain (Eurobio). The percentage of infected macrophages in each assay was determined microscopically at magnification of ×1,000, and IC_{50} values for the infected macrophages were determined.

Toxicity against human monocytes. In vitro toxicity of benzothiazoles was assessed for human monocytes maintained in RPMI medium (Eurobio) supplemented with 10% fetal calf serum (Eurobio) at 37°C in 5% CO_2 and replicated every 7 days. A range of benzothiazole concentrations were incorporated in late-log-phase monocytes (10^5 cells/ml), and cultures were incubated at 37°C with 5% CO_2. After a 72-h incubation period, cell growth and viability were measured by flow cytometry after staining monocytes with propidium iodide (1 μM final concentration in culture medium). IC_{50} values and 50% lethal concentration (LC_{50}) values were determined for cell growth and viability, respectively. An in vitro selective index (SI) value, corresponding to the ratio between antiparasitic and cytotoxic activities, was calculated for each parasite according to the following formula: SI = LC_{50} against human monocytes/IC_{50} against intracellular amastigotes or SI = LC_{50} against human monocytes/IC_{50} against *T. vaginalis*.

Toxicity of compound C11 against promastigotes and extracellular amastigotes. Promastigotes were incubated in RPMI medium supplemented with 12% fetal calf serum and incubated at 25°C. Amastigotes were obtained from human macrophages previously infected with promastigotes according to the protocol described by Ogunkolade et al. (15). They were transferred into RPMI medium supplemented with 20% fetal calf serum, titers were determined at pH 5.5, and the mixture was incubated at 37°C (5% CO_2). Under these conditions, extracellular amastigotes could be maintained for more than 1 week. Various concentrations of compound C11 were aseptically incorporated into duplicate promastigote and extracellular amastigote cultures and incubated at 25 and 37°C, respectively. Following a 48-h incubation period, parasite viability was estimated by flow cytometry after staining with 1 μM propidium iodide.

Effect of compound C11 on nitric oxide production. Maturation of human monocytes into adherent macrophages was induced by treating exponentially growing monocytes (10^5 cells/ml) with 1 μM phorbol myristate acetate (Sigma). After a 48-h incubation period at 37°C (5% CO_2) in chamber slides (Fisher), cells were rinsed with fresh medium and suspended in RPMI medium containing various concentrations of compound C11, in the presence or absence of 10 U of human recombinant gamma interferon (IFNγ)/ml. After 48 h at 37°C, NO production was measured by assessing the nitrite content of culture supernatants by the method described by Ding et al. (6). Fresh Griess reagent (100 μl) was added to equal volumes of culture supernatants, and the optical density at 540 nm was measured after 15 min of incubation at room temperature. Nitrite concentrations were determined using $NaNO_2$ diluted in Dulbecco's modified Eagle's medium as the standard.

Effect of compound C11 on phagocytic capacities of human macrophages. Assays were performed on human monocyte-derived macrophages. Maturation of monocytes into adherent macrophages was performed by treating exponentially growing monocytes (10^5 cells/ml) with 1 μM phorbol myristate acetate (Sigma). After a 48-h incubation period at 37°C (5% CO_2) in chamber slides (Fisher), cells were rinsed with fresh medium and various concentrations of compound C11 were incorporated into duplicate cultures. After a 48-h incubation period at 37°C, cells were rinsed with fresh medium and infected with RPMI medium containing stationary-phase promastigotes (cell/promastigote ratio, 1/10). After a 4-h incubation period at 37°C (5% CO_2), promastigotes were removed by four successive washes with fresh medium, fixed with methanol, and stained with 10% Giemsa stain. The percentage of macrophages containing adherent or intracellular parasites was analyzed microscopically at a magnification of ×1,000.

RESULTS

Physical properties of and predictive values for 6-nitro-benzothiazoles, 6-amino-benzothiazoles, and the corresponding anthranilic acids are summarized in Table 1. Lipophilicity was estimated by prediction of *n*-octanol–water partition coefficient LogP values (defined as the ratio of concentration in an immiscible solvent such as *n*-octanol to concentration in the aqueous phase). In practice, a LogP value of 1 signifies that the corresponding molecule could be partitioned according to the ratio 10/1 (organic solvent/aqueous phase), a LogP value of 0 demonstrates that the corresponding molecule could be partitioned according to the ratio 1/1, and a LogP value of −1 indicates that the corresponding molecule could be partitioned according to the ratio 1/10. Data showed that almost all partition coefficient values were >1 (ranging from 0.99 for compound B3 to 5.32 for compound C10), suggesting that the chemical compounds exhibited high lipophilic properties. Anthranilic acids (series C) presented the highest affinity for organic solvents, while 6-nitro- and 6-amino-benzothiazoles demonstrated lower lipophilicity. Results observed for benzothiazoles showed that replacement of 6-amino groups by 6-nitro groups led to lipophilicity decreases. As expected, data also demonstrated that all chemical compounds tested exhibited weak solubility in water. Predictive values concerning biological activities were obtained by comparing the chemical structures of the compounds with structures or substructures of more than 30,000 well-known biologically active drugs. Results of prediction are presented as estimates of the probability *Pa* that the compounds are active. For *Pa* values >0.7, the corresponding compound is very likely to reveal this activity in experiments, but in that case, the chance of the compound being the analogue of a known pharmaceutical agent is also

TABLE 2. Toxicity and antiparasitic activity of benzothiazole and anthranilic acid derivatives

Compound	Toxicity against human monocytes		Activity against *T. vaginalis*		Activity against *L. infantum*		
	LC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	SI[a]	Promastigote IC_{50} (μM)	Intracellular amastigote IC_{50} (μM)[b]	SI[a]
A1	45.9	4.7	1.6	28.1	48.4	Toxic	
A2	325.1	42.4	>200		216.5	72.8	4.5
A3	252.5	148.7	>200		32.5	51.4	4.9
A4	248.2	129.5	>200		44.8	89.6	2.7
A5	341.5	103.5	>200		39.3	99.4	3.4
A6	310.3	4.5	189.3		37.2	103.2	3.1
A7	252.5	111.6	48.6	5.1	3.9	8.1	31.8
A8	231.4	135.4	>200		84.5	42.1	5.5
A9	184.9	3.8	186.4		36.3	36.8	5.1
A10	184.5	8.7	165.5		33.6	24.8	7.4
A11	331.5	140.4	>200		104.4	41.8	7.9
A12	257.2	98.4	83.2	3.1	17.9	50.7	5.1
A13	126.2	3.4	174.4		15.4	8.7	14.5
B1	356.6	271.7	>200		58.2	54.1	6.5
B2	246.5	146.3	>200		120.6	120.7	2.1
B3	285.5	6.3	>200		337.5	151.1	1.8
B4	128.6	5.6	>200		19.4	2.4	53.8
B5	214.1	113.3	>200		22.9	33.8	6.3
B6	42.9	4.6	214.3		10.1	Toxic	
B7	179.4	89.9	>200		26.8	114.2	1.5
B8	200.2	135.6	>200		98.4	120.7	1.6
B9	286.8	103.7	207.5		62.5	103.8	2.7
B10	186.2	183.8	133.2	1.3	73.2	Toxic	
B11	253.1	119.6	>200		143.7	125.4	2.1
B12	137.3	74.1	155.6		178.5	Toxic	
B13	124.6	38.9	>200		19.4	Toxic	
C1	81.9	80.5	16.8	4.8	32.2	48.1	1.7
C2	336.2	253.1	18.4	18.2	80.3	96.4	3.4
C3	281.6	156.4	124.7	2.2	208.5	17.5	73.2
C4	265.1	87.5	102.2	2.5	79.9	98.4	2.7
C5	141.6	18.4	2.9	48.8	32.5	10.6	13.3
C6	31.4	2.8	83.3		14.6	5.1	6.1
C7	243.3	125.6	134.2	1.8	126.5	128.1	1.8
C8	354.1	229.3	76.4	4.6	39.2	76.4	4.6
C9	265.4	91.7	41.2	6.4	29.7	27.6	9.6
C10	264.5	127.5	25.9	10.2	14.3	25.4	10.4
C11	579.5	303.9	157.6	3.6	38.5	2.5	231.8
C12	286.6	206.6	21.8	13.4	305.5	213.5	1.3
C13	395.1	265.3	134.2	2.9	236.7	295.1	1.3
Amphotericin B	20.4	12.4			0.054	0.028	1,014.2
Metronidazole	>500	>250	3.2	>150			

[a] SI, selective index corresponding to the ratio between antiparasitic and cytotoxic activities.
[b] Toxic, the compound was toxic for human adherent macrophages at concentrations that weakly inhibit intracellular amastigote growth.

high. For *Pa* values between 0.5 and 0.7, the compound is likely to reveal this activity in experiments and the compound exhibits less similarity to the known pharmaceutical agents. For *Pa* values <0.5, the compound is unlikely to reveal this activity in experiments, but if the presence of this activity is confirmed in experiments, the compound might be a new biologically active chemical entity. The results presented in Table 1 describe three biological activities: antileishmanial property, antitrichomonal capacity, and global toxicity. *Pa*-estimated toxicity values and antitrichomonal activity values were determined to be less than 0.5, which indicates that position 2 substitution-bearing 6-nitro- and 6-amino-benzothiazoles and their corresponding anthranilic acids exhibited a weak probability of showing toxicity for in vivo models and that their chemical structures exhibited low levels of similarity to those of known antitrichomonal drugs. *Pa* values estimated for antileishmanial activity were lower than 0.5 for all compounds except A7 and B7, which showed interesting predictive values (0.671 and 0.654, respectively). These results indicated that 6-nitro- and 6-amino-benzothiazoles bearing an *N*-diethylamine-propylamino group in position 2 exhibited chemical structures closely similar to known antileishmanial drugs.

Table 2 presents the results of comparisons of in vitro antiprotozoal activities to toxicity against human monocytes. Results obtained on human cells clearly demonstrated that benzothiazoles and anthranilic acids displayed weak toxicity, since most of the LC_{50} values appeared to be >100 μM. Data concerning antiproliferative properties revealed that various com-

pounds could inhibit the growth of transformed cells. This antiproliferative activity was mainly observed for 6-nitro-benzothiazoles bearing a phenylenediamino group in position 2 and for 6-amino-benzothiazoles bearing an amino or dimethylamino group in position 2. Moreover, the experiments using compounds A6, B6, and C6 demonstrated that the presence of a replacement piperidino group in position 2 was responsible for the antiproliferative activity of both molecular structures.

Data concerning antitrichomonal activity clearly indicated that 6-nitro- and 6-amino-benzothiazoles were poorly effective against parasites of the genus *Trichomonas*, while anthranilic acids displayed interesting properties. Two compounds, A1 and C5, exhibited the highest activity, with IC_{50}s of 1.6 μM and 2.9 μM, respectively. This antitrichomonal activity was associated with moderate toxicity against human monocytes, since LC_{50}s for A1 and C5 reached 45.1 μM and 141.6 μM, respectively, corresponding to SIs that averaged 28 and 48, respectively.

Antileishmanial activity was explored for both extracellular promastigote and intracellular amastigote forms. The results displayed in Table 2 show that most benzothiazoles and anthranilic acids were able to inhibit promastigote growth; however, IC_{50}s differed considerably according to the chemical nature of the replacement group in position 2. Values obtained for intracellular amastigotes demonstrated that various compounds could also inhibit parasite growth inside parasitophorous vacuoles. The results also revealed that 6-nitro-benzothiazoles and anthranilic acids appeared to exhibit more efficient activity than 6-amino-benzothiazoles. Five compounds (A7, A13, B4, C6, and C11) displayed potent antileishmanial activity, with IC_{50}s lower than 10 μM; however, only compounds B4, C3, and C11, which showed weak toxicity against human cells, exhibited interesting pharmacological selectivity, with SI values greater than 50. These three compounds were far less efficient with respect to activity levels for the promastigote form of the parasite, suggesting that their antileishmanial action could be associated with inhibition of amastigote-specific biochemical pathways or modulation of cell-mediated response.

On this basis, additional experiments, which included measurement of extracellular amastigote viability, macrophage-dependent NO production, and modulation of phagocytic properties, were conducted with compound C11 to explore possible mechanisms of action. The results for assays of toxicity against axenic amastigotes and promastigotes are presented in Fig. 1. These results show that compound C11 induced a dose-dependent decrease of parasite viability in both parasitic forms (LC_{50}s of 44.7 and 31.6 μM in promastigotes and axenic amastigotes, respectively) and indicate that C11-related antileishmanial action was not dependent on the developmental stage of the parasite. Effects of the presence of compound C11 on macrophage phagocytic properties are presented in Fig. 2. A significant dose-dependent decrease of phagocytic activity could be observed at concentrations seen to result in reduced toxicity against human cells, suggesting that compound C11 could prevent the appearance of the internalization mechanisms that occur in *Leishmania* infection. Effects of compound C11 on NO production are presented in Fig. 3. A weak drug-related NO release was observed in macrophages incubated without IFN-γ, while a twofold increase of NO content was

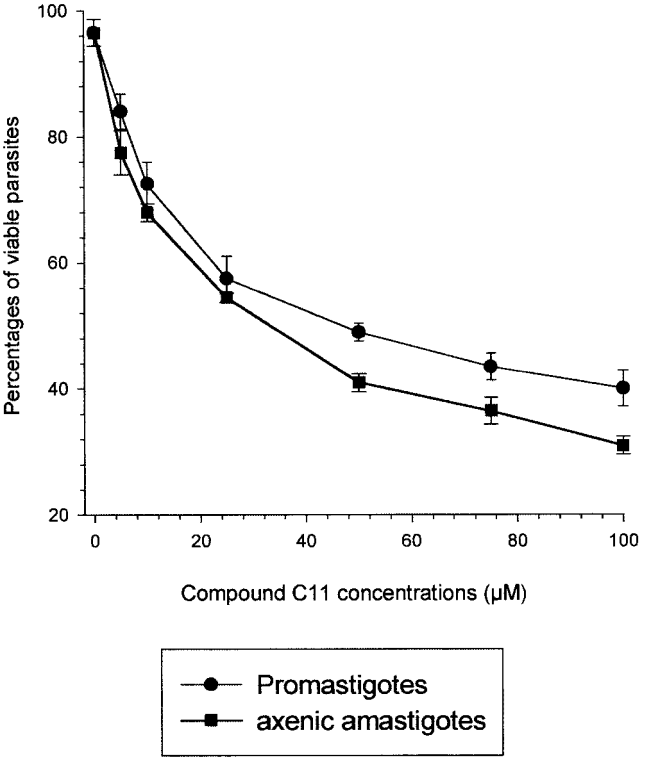


FIG. 1. Effects of compound C11 on the viability of axenic amastigotes and promastigotes.

determined in supernatants of macrophages incubated with IFN-γ. These results suggest that compound C11 interferes with host-protective mechanisms against *Leishmania* by stimulating NO production according to a mechanism synergistically enhanced by the presence of IFN-γ.

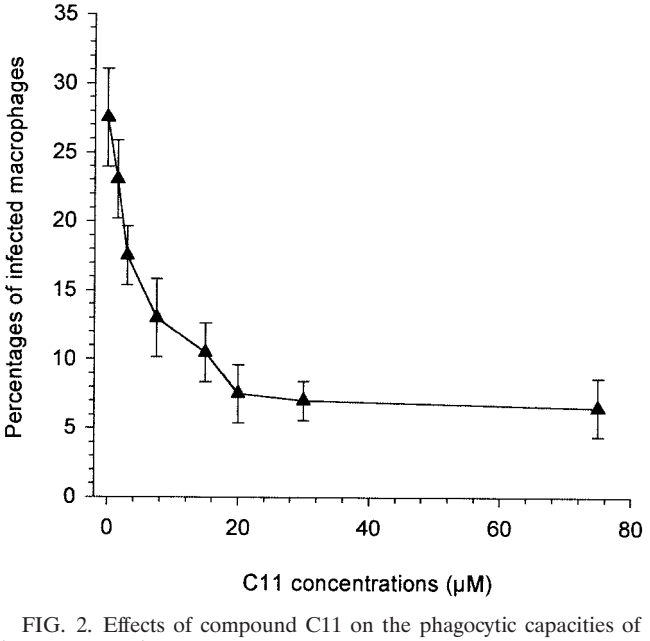


FIG. 2. Effects of compound C11 on the phagocytic capacities of human macrophages.

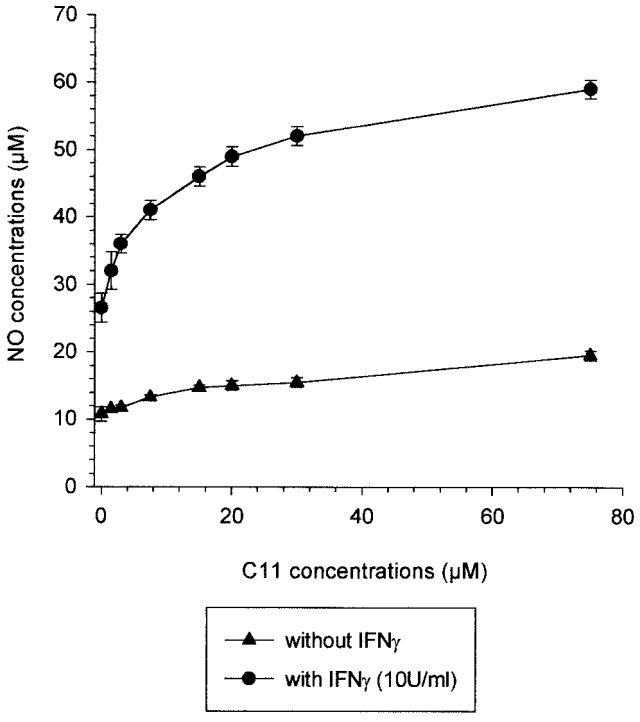


FIG. 3. Effects of compound C11 on NO production by human macrophages.

DISCUSSION

Parasitic infections due to protozoan parasites remain a major public health concern, affecting the lives of billions of people worldwide. According to the World Health Organization, 15 million people are thought to be infected by *Leishmania* parasites, with two million new cases occurring annually and 350 million people at risk of infection (World Health Organization, http://www.who.int/ctd/html/leish.html), while *T. vaginalis* infections have been associated with preterm delivery and low birth weight as well as predisposition to human immunodeficiency virus infection and cervical cancer. On the basis of these facts, experimental projects for studying parasite-specific therapeutic targets and identifying possible new and efficient drugs are of paramount importance. Results observed in the present study confirmed the hypothesis that newly synthesized position 2 substitution-bearing 6-nitro- and 6-amino-benzothiazoles and their corresponding anthranilic acids could exert antiparasitic activities against protozoa of the genus *Trichomonas* and *Leishmania*. However, they also demonstrated that biological properties differed considerably according to the chemical nature of each derivative, regardless of electrophilic properties or solubility.

6-Nitro-benzothiazoles (compounds A1 to A13) displayed interesting predictive values for antitrichomonal activities. Probably due to the presence of levels of 6-nitro known to play an important role in the toxicity of metronidazole against *Trichomonas,* these values were not confirmed by experimental data, since all IC_{50}s except those of compound A1 were higher than 10 μM. They indicated that antitrichomonal properties depended greatly on the nature of the replacement group at position 2; on this basis of this finding, association of 6-nitro and 2-chloro groups was shown to enhance toxicity against *T. vaginalis*. As expected, this antitrichomonal property was associated with increased antiproliferative activity against human cells (IC_{50} = 4.5 μM). Concerning antileishmanial activity, various 6-nitro-benzothiazoles presented interesting predictive biological values, especially compound A7 (bearing a 2-*N*-diethylamine-propylamino group). This group obtained a predictive *Pa* value higher than 0.8, indicating that its chemical structure was closely similar to those of known antileishmanial drugs. Experimental results confirmed that the presence of the 2-*N*-diethylamine-propylamino group was responsible for antileishmanial activity of 6-nitro-benzothiazoles (IC_{50} = 8.1 μM, SI = 31.8); nevertheless, the results also demonstrated that this property disappeared when the 6-nitro group was reduced into a 6-amino group (IC_{50} = 117.2 μM, SI = 1.5).

6-Amino-benzothiazoles exhibited weak predictive biological values, suggesting that their chemical structures were highly different from those of known active compounds. Experimental data confirmed these values, showing that 6-amino-benzothiazoles were poorly effective at inhibiting the growth of *T. vaginalis* and *L. infantum*. Nevertheless, compound B4, bearing a replacement *N*-dimethylamino group at position 2, exhibited an interesting antileishmanial effect (IC_{50} = 2.4 μM, SI = 53.8). Interestingly, compounds B3 and B5, bearing 2-*N*-amino and 2-*N*-diethylamino groups, respectively, appeared far less active, suggesting that conformational properties could play an important role in antiparasitic activity.

Anthranilic acids displayed low predictive biological values; nevertheless, most of these derivatives exhibited antitrichomonal and antileishmanial effects. Compound C5, bearing a 2-*N*-diethylamino group, exerted significant selective antitrichomonal activity (IC_{50} = 2.9 μM, SI = 48.8), while compound C11, bearing a 2-ethanolamino group, exhibited a potent selective antileishmanial action (IC_{50} = 2.5 μM, SI = 231). This effect was highly specific for the intracellular amastigote stage of the parasite, since weak toxicity could be observed against the promastigote and the extracellular axenic amastigote forms, suggesting that the molecule could modulate host-specific mechanisms. Additional experiments confirmed this hypothesis, since they demonstrated that compound C11 could protect human macrophages from *Leishmania* parasitism by two different mechanisms: reduction of parasite internalization by inhibition of macrophage phagocytosis and killing of intracellular amastigotes by enhanced NO production.

In mammals, *Leishmania* multiply almost exclusively as amastigotes inside cells of the mononuclear phagocytic system. After the mammal has been inoculated with infective promastigotes by the bite of a parasite-carrying sand fly through the dermis, the binding of the parasites to the macrophage cell surface occurs through numerous receptors. In physiological conditions, the main receptors appear to be complement receptor type I (CR1) and CR3 (17). Moreover, although some active participation of the parasite in host-cell entry cannot be completely excluded, it is generally accepted that phagocytosis is the basic mechanism for endocytosis of *Leishmania* (2, 17). Immediately following phagocytosis, *Leishmania* are located in compartments that are delimited by membrane originating from the macrophage plasmalemma. Survival of *Leishmania* parasites within the mammalian host has been shown to de-

pend greatly on the capacity for the parasite to adhere and internalize into resident or recruited macrophages. On this basis, chemically induced modulation of macrophage phagocytic properties could influence parasite infection, and various compounds, including oxidant molecules and neuropeptides, demonstrated in vivo antileishmanial activities along with inhibition of macrophage phagocytosis (17).

Within the parasitophorous vacuole, the promastigotes are transformed into aflagellated amastigote forms which multiply asexually in resting macrophages and, after rupture of the parasitized cells, disseminate to the other cells of the reticuloendothelial system (2). However, macrophages may prevent parasite development by using protective mechanisms for killing intracellular amastigotes. Among these mechanisms, production of NO by inducible NO synthase has been shown to represent an essential way of inducing the intracellular destruction of amastigotes (4). Based on these lines of evidence, new techniques using NO-generating compounds have been envisaged for the treatment of human infection and have given encouraging results (4).

Compound C11, which produced a 50% decrease of parasite internalization and a twofold increase of NO production in activated macrophages, can be considered a promising member of this new class of protective drugs. On this basis, experiments should be completed using new chemical syntheses in order to explore the role of each radical on toxicity and antiparasitic or protective abilities and a complete evaluation of biological effects should be performed by in vivo assays of rodents infected with *L. infantum*.

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