Bioorganic & Medicinal Chemistry 22 (2014) 1626–1633

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

2-Acylamino-5-nitro-1,3-thiazoles: Preparation and in vitro bioevaluation against four neglected protozoan parasites ☆

Carlos Nava-Zuazo [a], Fabiola Chávez-Silva [a], Rosa Moo-Puc [b], Manuel Jesús Chan-Bacab [c], Benjamín Otto Ortega-Morales [c], Hermenegilda Moreno-Díaz [d], Daniel Díaz-Coutiño [d], Emanuel Hernández-Núñez [e], Gabriel Navarrete-Vázquez [a,*]

[a] Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos 62209, Mexico
[b] Unidad de Investigación Médica Yucatán, Unidad Médica de Alta Especialidad del Centro Médico Nacional Ignacio García Téllez, IMSS Mérida, Yucatán 97000, Mexico
[c] Departamento de Microbiología Ambiental y Biotecnología, Universidad Autónoma de Campeche, Campeche 24030, Mexico
[d] Instituto de Química Aplicada, Universidad del Papaloapan, Tuxtepec, Oaxaca 68301, Mexico
[e] Facultad de Ingeniería, Universidad Autónoma de Yucatán, Mérida, Yucatán 97000, Mexico

ARTICLE INFO

Article history:
Received 8 November 2013
Revised 10 January 2014
Accepted 20 January 2014
Available online 31 January 2014

Keywords:
Neglected diseases
Nitazoxanide
5-Nitrothiazole
Giardia
LiPE

ABSTRACT

The 2-acylamino-5-nitro-1,3-thiazole derivatives (**1**–**14**) were prepared using a one step reaction. All compounds were tested in vitro against four neglected protozoan parasites (*Giardia intestinalis*, *Trichomonas vaginalis*, *Leishmania amazonensis* and *Trypanosoma cruzi*). Acetamide (**9**), valeroylamide (**10**), benzamide (**12**), methylcarbamate (**13**) and ethyloxamate (**14**) derivatives were the most active compounds against *G. intestinalis* and *T. vaginalis*, showing nanomolar inhibition. Compound **13** (IC_{50} = 10 nM), was 536-times more active than metronidazole, and 121-fold more effective than nitazoxanide against *G. intestinalis*. Compound **14** was 29-times more active than metronidazole and 6.5-fold more potent than nitazoxanide against *T. vaginalis*. Ureic derivatives **2**, **3** and **5** showed moderate activity against *L. amazonensis*. None of them were active against *T. cruzi*. Ligand efficiency indexes analysis revealed higher intrinsic quality of the most active 2-acylamino derivatives than nitazoxanide and metronidazole. In silico toxicity profile was also computed for the most active compounds. A very low in vitro mammalian cytotoxicity was obtained for **13** and **14**, showing selectivity indexes (SI) of 246,300 and 141,500, respectively. Nitazoxanide showed an excellent leishmanicidal and trypanocidal effect, repurposing this drug as potential new antikinetoplastid parasite compound

1. Introduction

The neglected protozoan diseases are a group of tropical infections which are especially endemic in low-income populations, affecting hundreds of million people and animals.[1] However, these infections are also present in the United States and more developed countries. According to the Centers for Disease Control and Prevention (CDC),[2] the major neglected protozoan infections identified at this time for further action include the agents that cause Chagas disease, leishmaniasis, trichomoniasis and giardiasis.[2–4] These infections are considered neglected because relatively little attention has been devoted to their surveillance, prevention, and/or treatment.[2]

Giardia intestinalis (syn. *Giardia duodenalis*, *Giardia lamblia*) is a neglected intestinal protozoan parasite infecting humans and various other mammalian hosts.[3]

It is one of the most commonly diagnosed protozoal causes of diarrhea worldwide. Clinical resistance has been reported for current chemotherapeutics.[5,6]

Trichomonas vaginalis is the causative agent of trichomoniasis, a common sexually-transmitted disease in humans.[7] The *Leishmania* species causes a variety of diseases from self-healing cutaneous lesions to life-threatening visceral infections. Clinical manifestations depend on the infecting parasites species. There is an estimated annual 1.5–2.0 million new cases of leishmaniasis, from which approximately 500,000 belong to the visceral form, which is potentially fatal.[8] American trypanosomiasis or Chagas' disease, caused by its etiological agent *Trypanosoma cruzi*, is still one of the major causes of morbidity and mortality due to cardiovascular diseases in Latin America.[8]

☆ Taken in part from the Ph.D. thesis of C. Nava-Zuazo.

* Corresponding author. Tel./fax: +52 777 329 7089.
E-mail address: gabriel_navarrete@uaem.mx (G. Navarrete-Vázquez).

http://dx.doi.org/10.1016/j.bmc.2014.01.029
0968-0896/

Chemotherapy against parasitic neglected diseases is limited by the existence of few drugs in the market, most of which are of low efficacy, showing toxic side effects, and frequently lead to the appearance of resistant strains.[5] This reflects the need to continue searching for better antiprotozoal drugs.

On the other hand, nitazoxanide is a broad-spectrum antiparasitic compound that belongs to a nitroheterocyclic class named thiazolides.[9] Detailed in vitro and in vivo studies have currently been conducted on the efficacy of nitazoxanide and other thiazolide drugs against intracellular parasites,[8] extracellular anaerobic bacteria,[10] and virus such as hepatitis C[11] and AH_1N_1.[12]

As a part of our search for basic information about the structural requirements for new antiprotozoal molecules, we have synthesized fourteen 2-acylamino-5-nitrothiazole derivatives (Table 1). The in vitro antiparasitic activities of these compounds on intestinal unicellular parasite *Giardia intestinalis*, the urogenital tract parasite *Trichomonas vaginalis*, and the kinetoplastid parasites such as *Trypanosoma cruzi* and *Leishmania amazonensis* are reported in this work.

2. Results and discussion

2.1. Drug design of derivates 1–14

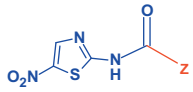
Compounds **1**–**14** were designed on the basis of the structure of the antiprotozoal drug nitazoxanide, maintaining the 5-nitrothiazole core, in addition to the acylamino substituent at position 2. Due to a number of aryl derivatives has been synthesized and tested as antibacterial agents,[10] we decided to explore the contribution to the antiprotozoal spectrum of diverse acyl analogues such as: aliphatic and aromatic ureas, homologous aliphatic amides, as well as carbamate and oxamate derivatives. Each one of the substituents mentioned before has been reported with antiprotozoal properties, but joined with different heterocycles: ureas and quinolines,[13] amides and imidazole,[14] and carbamate and benzimidazole.[15]

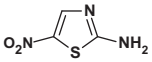
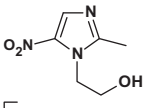
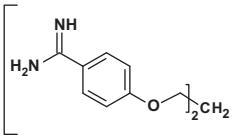
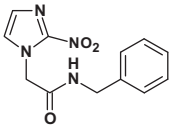
To the best of our knowledge, this is the first study reporting these kinds of groups connected with 5-nitrothiazole nucleus with demonstrated broad antiprotozoal spectrum. Recently, Soria-Arteche et al. reported hybridization between parasitophoric 5-nitrothiazole and *N*-methylbenzimidazole derivatives with high antiprotozoal activity.[16] However, these derivatives bear two heterocycles coupled by an amide bond, totally different from compounds presented in the current work.

2.2. Chemistry

Ureic compounds **1**–**8** were synthesized starting from 2-amino-5-nitro-1,3-thiazole (**15**), through an addition reaction with isocyanate derivatives **15**–**23** under inert atmosphere, triethylamine and toluene as a solvent. Amide compounds **9** and **10** were obtained from the reaction of **15** with acetic anhydride or benzoyl

Table 1
In vitro antiprotozoal and cytotoxic activities of 2-acylamino-5-nitrotiazole derivatives



Compd	Z	MW	Mp (°C)	Yield	IC_{50} (μM)				CC_{50} (μM) VERO cell line
				(%)	*G. intestinalis*	*T. vaginalis*	*L. amazonensis*	*T. cruzi*	
1	Cyclohexylamine	270	200–203	86	0.359 ± 0.063	1.650 ± 0.248	>50	>50	>1000
2	Phenylamine	264	207–212	72	2.570 ± 0.152	NT	38.48 ± 1.59	>50	>1000
3	4-Chlorophenylamine	298	243 (dec)	55	0.577 ± 0.044	1.026 ± 0.128	37.18 ± 1.92	>50	>1000
4	4-Fluorophenylamine	282	211–214	94	7.826± 1.418	1.226 ± 0.019	>50	>50	>1000
5	4-Methoxyphenylamine	294	212–214	46	29.11 ± 7.959	1.300 ± 0.041	32.24 ±2.12	>50	NT
6	4-Ethoxyphenylamine	308	204–208	72	3.747 ± 0.892	0.282 ± 0.003	>50	>50	>1000
7	4-Butoxyphenylamine	336	148–153	87	1.800 ± 0.167	0.387 ± 0.101	>50	>50	58 ± 5.63
8	4-Nitrophenylamine	309	259 (dec)	80	15.34 ± 3.981	0.958 ± 0.068	>50	>50	NT
9	Methyl	187	267–270	91	0.490 ± 0.105	0.022 ± 0.007	>50	>50	>1000
10	Phenyl	249	258–261	87	0.481 ± 0.098	0.124 ± 0.065	>50	>50	>1000
11	Butyl	229	155 (dec)	67	0.398 ± 0.023	0.305 ± 0.037	>50	>50	>1000
12	Pentadecyl	383	132 (dec)	91	1.634 ± 0.894	2.584 ± 0.889	>50	>50	>1000
13	Methoxy	203	249–252	82	0.010 ± 0.001	0.081 ± 0.008	>50	>50	>1000 (2463)
14	Ethoxycarbonyl	245	252–255	90	6.410 ± 1.162	0.010 ± 0.003	>50	>50	>1000 (1415)
Nitazoxanide	2-Acetoxyphenyl	307	—	—	1.214 ± 0.012	0.068 ± 0.004	7.23 ± 1.73	18.73 ± 0.762	833 ± 1.68
15		145	—	—	29.57 ± 4.152	52.85 ± 7.452	>50	>50	441 ± 8.25
Metronidazole		171	—	—	5.360 ± 0.231	0.290 ± 0.024	>50	>50	387 ± 10.22
Pentamidine		340	—	—	4.079 ± 0.343	3.815 ± 0.368	14.32 ± 2.37	>50	47 ± 5.75
Benznidazole		260	—	—	22.58 ± 2.283	18.62 ± 2.253	>50	34.38 ± 1.584	14 ± 1.14

NT: Not tested.
dec: decomposition.

chloride, respectively. Amides **11** and **12** were achieved using a Schotten–Baumann reaction between **15** and valeroyl and palmitoyl chlorides, respectively. Carbamate compound **13** was prepared from **15** and methylchloroformate (**24**) under basic conditions, using dimethoxyethane as a solvent. The last compound (oxamate **14**) was synthesized via a coupling reaction of **15** with ethyl chlorooxoacetate (**25**), in the presence of triethylamine (Scheme 1). Title compounds were recovered with 46–94% yields and purified by recrystallization or by column chromatography. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data (NMR and mass spectra), and their purity ascertained by microanalysis. Physical constants of the title compounds are shown in Table 1.

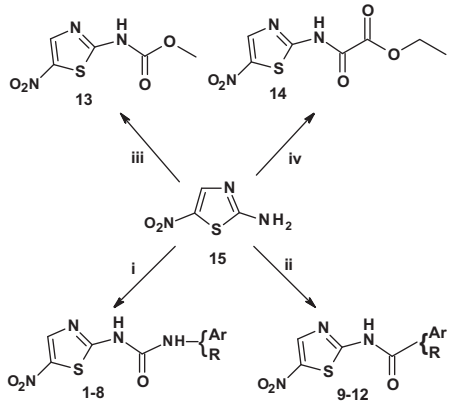
In the nuclear magnetic resonance spectra (^{1}H NMR; δ ppm), the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. All compounds showed a single signal ranging from δ 7.86 to 9.30 ppm, attributed to H-4 of the thiazole ring. For compound **1**, aliphatic signals at upfield shifts were found in displacements 1.15 to 3.45 ppm. Compounds **2** and **12** displayed characteristic signals of monosubstituted benzene. The aromatic region of the ^{1}H NMR spectrum of compounds **3**–**8** contained an A_2B_2 pattern signals ranging from δ 7.31 to 7.73 ppm (d, J_{ortho} = 8.0–9.2 Hz) and 6.86 to 8.19 ppm (d, J_{ortho} = 8.4–10 Hz) attributed to the equivalents H-2′, H-6′ and H-3′, H-5′, respectively of the benzene-4-substituted ring. Compounds **9**–**11** showed several displacements consistent with aliphatic chains. The displacement for methoxy group in **13** was found in 3.80 ppm (singlet). In the last compound (**14**), the signals for ethyl oxamate were found in δ 4.32 ppm assignable to CH_2 (quadruplet), and 1.31 attributed to CH_3 (triplet).

For the ^{13}C nuclear magnetic resonance spectra, constant signals were found for the nitrothiazole nucleus:

One signal ranging from δ_C 142.7 to 165.3 ppm, attributed to C-2, and two signals ranging from δ_C 138.9 to 151.5 and 124.0 to 142.9 ppm, assigned to C-4 and C-5, respectively. Another frequent signal was found in downfield shifts from δ_C 150.8 to 173.2 ppm endorsed to carbonyl group, founded in the fourteen compounds.

2.3. In vitro antiprotozoal assays

Compounds **1**–**14** were tested in vitro as antiprotozoal agents. Biological assays results against the four protozoa tested are summarized in Table 1. A comparison was made among new compounds and the antiprotozoal drugs of choice: nitazoxanide and metronidazole, against *G. intestinalis*, and *T. vaginalis*. In order to compare bioactivities, pentamidine (leishmanicidal drug), benznidazole (Trypanocidal drug of choice) and precursor **15** were also tested. In vitro susceptibility assays were performed using methods previously described.[8,9,13]



Scheme 1. Reagents and conditions: (i) R(Ar)—N=C=O, Et_3N, toluene; (ii) acetic anhydride or acyl chlorides, CH_2Cl_2, Et_3N; (iii) methylchloroformate, Et_3N, dimethoxyethane; (iv) ethyl chlorooxoacetate, Et_3N, CH_2Cl_2, rt.

In general, all the screened compounds showed high giardicidal bioactivity. It is interesting to note that compound **13** (methylcarbamate), was the most potent of the series (IC_{50} = 10 nM) against *G. intestinalis*. It was 536-times more potent than metronidazole. Compound **13** was also 121-fold more active than nitazoxanide.

On the other hand, ureic derivatives **1**, **3** and amides **9**–**11** were two to three-times stronger than nitazoxanide and eleven to thirteen-fold more potent than metronidazole, showing IC_{50}'s in the nanomolar range. Moreover, ureas **2**, **6**, **8**, valeroylamide **12** and ethyloxamate **14**, had potencies in the low micromolar range (<8 μM). The remaining compounds were less active than nitazoxanide and metronidazole, with IC_{50}'s >10 μM.

Compound **14** (ethyloxamate) showed nanomolar trichomonicidal potency (IC_{50} = 10 nM). It was 6.8-fold more active than nitazoxanide (IC_{50} = 68 nM), and 29-times more active than metronidazole. Acetylamide **9** was the second most active compound, three-times more potent than nitazoxanide and 13-fold more active than metronidazole. Compound **9** was previously synthesized, tested as tricomonicidal drug,[17] and formulated in pharmaceutical preparation for oral administration.[18] In the current work, we expanded the antiprotozoal spectrum of **9**, testing it against *Giardia intestinalis*, *Leishmania mexicana* and *Trypanosoma cruzi*.

Methylcarbamate **13** and benzoylamide **10** also showed nanomolar potencies against *T. vaginalis* (IC_{50} = 81 nM and 124 nM, respectively). However, they were less active than nitazoxanide, but four-times more potent than metronidazole (the trichomoniasis drug of choice). Compound **10** has already been described in the literature as antibacterial agent against *Helicobacter pylori*, *Campylobacter jejuni*, and *Clostridium difficile*.[10] The antiparasitic activity against the panel of four protozoa tested in the current research has not been reported until this work. Compound **10** structurally resembles nitazoxanide, but it lacks of 2-acetoxy substituent in the phenyl ring. This compound showed excellent giardicidal activity, being two times stronger than drug of choice.

Compounds **1**, **3**–**8** and **12** revealed trichomonicidal activities in the low micromolar range (<3 μM). Starting material 2-amino-5-nitrothiazole (**15**), exposed very low effect against *G. intestinalis* and *T. vaginalis*, although this compound was also claimed before as a trichomonicidal drug.[17] SAR analysis revealed that small substituents such as methyl, methoxy and ethoxycarbonyl derivatives, improve the antiprotozoal activity against *G. intestinalis* and *T. vaginalis*.

Antileishmanial assay was carried out using a method previously described.[8,13] Ureic derivatives **2**, **3** and **5** revealed moderate activity against *L. amazonensis* (<39 μM). However, they were almost 2-fold less active than pentamidine (second-line antileishmanial drug). The remaining compounds were inactive.

In vitro trypanocidal assay showed that compounds **1**–**15** were unable to damage this protozoon at concentration below 50 μM. It is important to emphasize that nitazoxanide had an excellent activity against both kinetoplastid protozoa. It was two-times more active than pentamidine against *L. amazonensis*, as well as benznidazole against *T. cruzi*. This is according with data previously reported by our group,[8,9] repurposing nitazoxanide as potential new antikinetoplastid parasite drug.

2.4. In vitro cytotoxicity assays

All compounds, including the most active compounds **13** and **14**, were evaluated for their cytotoxicity against mammalian VERO cell line (Table 1),[13] showing a median cytotoxic concentration (CC_{50}) of 500 and 346 μg/mL (2463 and 1415 μM, respectively).

Table 2
Selectivity indexes of **13**, **14** and nitazoxanide

Compd	Selectivity index (SI = CC_{50}/IC_{50})	
	Giardia intestinalis	*Trichomonas vaginalis*
13	246300	30407
14	220	141500
Nitazoxanide	686	12250

Nitazoxanide also showed a low cytotoxicity, meanwhile metronidazole displayed a moderated toxicity against VERO cell line, compared to **13**, **14** and nitazoxanide. It is interesting to remark that the most cytotoxic compounds were drugs of choice benznidazole, pentamidine and urea **7**, with CC_{50}s of 14, 47 and 58 μM, respectively.

The selectivity index (SI) of the compounds, defined as the ratio of cytotoxicity to biological activity (SI = CC_{50}VERO cells/IC_{50} parasites) was calculated (Table 2).

It is generally considered that biological efficacy is not due to in vitro cytotoxicity when SI $\geqslant$ 10.[9,13] Compounds **13** and **14** showed nanomolar giardicidal and trichomonicidal activities, and very low cytotoxic effects (>1000 μM), having selectivity indexes of 246,300 and 141,500, respectively. The SI calculated for these compounds versus both protozoa was $\gg$200, this fact implies that **13** and **14** are selectively toxic against the protozoa than the mammalian cells.

2.5. In silico toxicology profile

Computational prediction of toxicity has been performed in drug design and development in order to avoid the experimental study of potentially harmful substances.[19] The toxicity parameters of the most active compounds, nitazoxanide and metronidazole were calculated through the ACD/ToxSuite software, v. 2.95 (Table 3).

Cytochrome P450 isoform CYP3A4 is the major enzyme responsible for xenobiotic metabolism in human organism. Inhibition of CYP3A4 at a clinically relevant concentration (IC_{50} <10 μM) can lead to drug–drug interactions and undesirable adverse effects.[20] All compounds showed satisfactory toxicity profiles, and the predictions of inhibition for the three isoforms of CYP450 were comparable to the reference antiprotozoal drugs.

Cardiotoxicity of drug-like compounds associated with human ether-a-go-go (hERG) channel inhibition is becoming a more common cause of drug candidates' attrition.[21] All compounds showed low prediction of hERG channel blockage at clinically relevant concentrations (K_i <10 μM).

The acute toxicity of the chemical is defined as a dose that is lethal to 50% of the treated animals (LD_{50}). The acute toxicity can be viewed as a 'cumulative potential' to cause various acute effects and death of animals.[22] In these predictions, compounds **13** and **14** demonstrated similar calculated LD_{50} than nitazoxanide and metronidazole by different administration routes, showing very low toxicity profiles.

Table 4
Ligand Efficiencies analysis for compounds **1**–**14** (*G. intestinalis*)

Compd	pIC_{50} *G. intestinalis*	Log*P* <3	# Heavy atoms	MW <300	LE >0.36	LiPE 5–7	BEI>27
1	6.44	2.48	18	270	0.35	3.9	23.8
2	5.59	2.28	18	264	0.31	3.3	21.1
3	6.23	2.95	19	298	0.32	3.2	20.9
4	5.10	2.44	19	282	0.26	2.6	18.0
5	4.53	2.33	20	294	0.22	2.2	15.4
6	5.42	2.71	21	308	0.25	2.7	17.6
7	5.74	3.77	23	336	0.25	1.9	17.0
8	4.81	2.23	21	309	0.22	2.5	15.5
9	6.30	0.29	12	187	0.52	6.0	33.6
10	6.31	1.96	17	249	0.37	4.3	25.3
11	6.40	2.19	15	229	0.42	4.2	27.9
12	5.78	7.75	26	383	0.22	-2.0	15.0
13	8.00	0.78	13	203	0.61	7.2	39.4
14	5.19	0.49	16	245	0.24	4.6	21.1
NTZ	5.91	2.01	21	307	0.28	3.8	19.2
15	4.56	0.55	9	145	0.50	4.0	31.4
MNZ	5.27	-0.46	12	171	0.43	5.7	30.8

Table 5
Ligand Efficiencies analysis for compounds **1**–**14** (*T. vaginalis*)

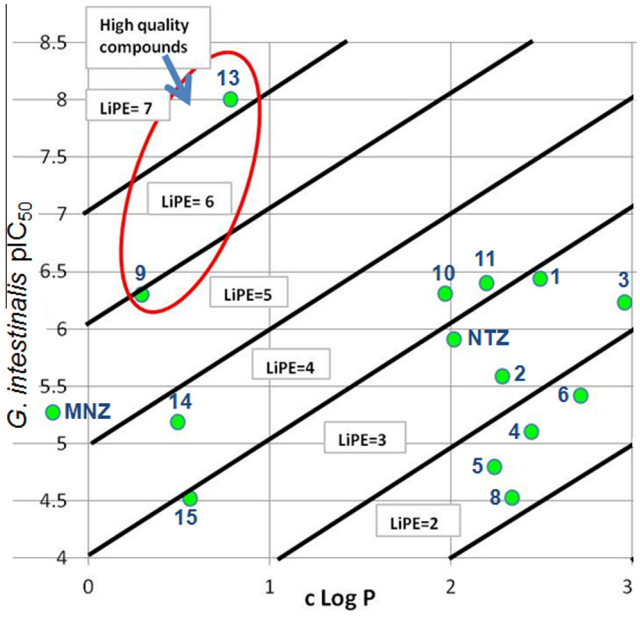
Compd	pIC_{50} *T. vaginalis*	LE >0.36	LiPE 5–7	BEI >27
1	5.7	0.32	3.3	21.4
3	5.9	0.31	3.0	20.1
4	5.9	0.31	3.4	20.9
5	5.8	0.29	3.5	20.0
6	6.5	0.31	3.8	21.2
7	6.4	0.27	2.6	19.1
8	6.0	0.28	3.7	19.4
9	7.6	0.63	7.3	40.9
10	6.9	0.40	4.9	27.7
11	6.5	0.43	4.3	28.3
12	5.5	0.21	−2.1	14.5
13	7.0	0.54	6.3	34.9
14	8.0	0.50	7.5	32.6
NTZ	7.1	0.33	5.0	23.1
15	4.2	0.47	3.7	29.4
MNZ	6.5	0.54	6.9	38.1

2.6. Ligand efficiency indexes analysis

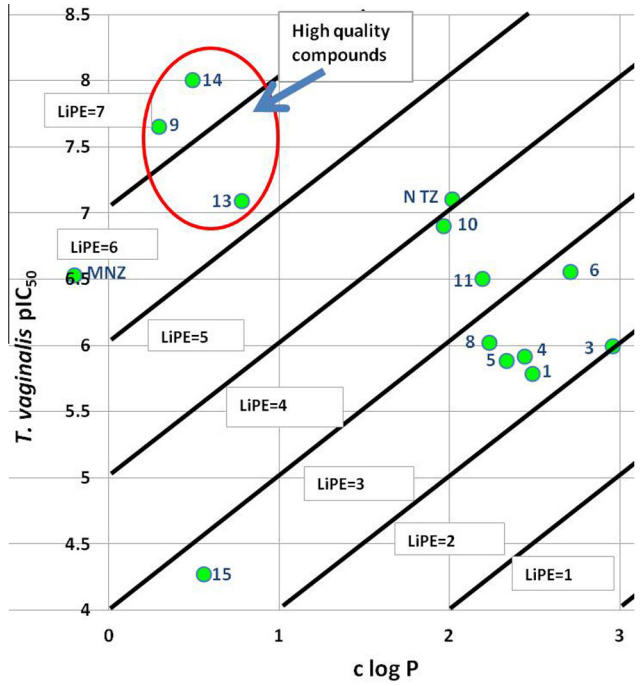
In recent years the concept of Ligand Efficiency Indexes (LEI), which combines potency (pIC_{50}), lipophilicity (log P), molecular weight (MW) and heavy atom count (HAC), have been shown to be useful tools in the lead optimization process.[23–25] To assist in the optimization of drug-like molecules, multiple efficiency indexes have been proposed:

Table 3
Toxicity profiles predicted for the most active compounds **9**, **10**, **12**–**14** and antiprotozoal drugs of choice

Compd	LD_{50} (mg/Kg)				Probability of inhibition (IC_{50} or K_i <10 μM)			
	Mouse		Rat		CYP450 isoform			
	ip	p.o.	ip	p.o.	3A4	2D6	1A2	hERG
9	280	1100	490	950	0.00	0.01	0.03	0.01
10	270	1200	550	1200	0.02	0.00	0.09	0.02
12	280	1200	670	110	0.02	0.03	0.23	0.04
13	550	1600	890	990	0.01	0.02	0.36	0.01
14	740	1200	980	3900	0.00	0.03	0.03	0.27
Nitazoxanide	500	1600	810	1600	0.22	0.05	0.02	0.01
Metronizadole	870	1660	850	1200	0.01	0.03	0.04	0.02



Scheme 2. Plot of *c*Log*P* versus *G. intestinalis* pIC_{50} for compounds **1**–**14**. Diagonal lines represent areas of equal LipE.



Scheme 3. Plot of *c*Log*P* versus *T. vaginalis* pIC_{50} for compounds **1**–**14**. Diagonal lines represent areas of equal LipE.

Ligand efficiency (LE = pIC_{50}/HAC), lipophilic efficiency (LiPE = $pIC_{50} - \log P$), binding efficiency index (BEI = pIC_{50}/MW), and ligand efficiency-dependent lipophilicity index (LELP = $\log P$/LE).[26] These parameters were calculated for compounds **1**–**14**, using biological data obtained against *G. intestinalis* (Table 4) and *T. vaginalis* (Table 5).

The calculated ligand efficiency (LE) values of **1**–**8** were all lower than 0.3 (acceptable levels of LE $\geqslant$ 0.36),[25] whereas **9**, **11**, and **13** showed LE values higher than 0.4.

In order to compare their binding efficiencies, the LE and BEI values of the reference compounds nitazoxanide (NTZ) and metronidazole (MNZ), and compounds **1**–**14** were calculated (Table 2). From BEI results, in particular from **9**, **11** and **13**, it became clear that these 2-acylamino-5-nitro-1,3-thiazoles incorporate a very efficient binding index (acceptable levels of BEI $\geqslant$ 27)[24,25] as well as a great ligand efficiency (LE >0.4), having better scores than reference compound nitazoxanide (BEI = 19, LE = 0.28).

Compounds **9**–**11**, **13**–**15** showed LE values higher than 0.4, using biological data provided by *T. vaginalis* test (Table 5). LiPE and BEI scores are within the range appropriate for leadlike and druglike compounds. In the same way that in the analysis performed with *G. intestinalis*, compounds **9**, **13** and **14** emerged as promising candidates for further optimization in future projects. Conversely, compounds that show lead-like properties and efficacy must have LipE values between 5 and 7.[25] Quality drug candidates have the highest, or near highest LiPE for the series. In this case, compounds **9** and **13** showed the highest LiPE scores in *Giardia* analysis, whereas compound **14** appeared in the *Trichomonas* analysis (Schemes 2 and 3, respectively).

LiPE describes the contribution of lipophilicity (logP) to the potency. However, the LiPE index is not useful for very small and polar molecules. In order to overcome this limitation LELP index describes the price of ligand efficiency paid for increasing the potency by increasing the lipophilicity.[27] The lower limit of LE is 0.3 and the lipophilicity range is $-3 < \log P < 3$, which defines a range of optimal LELP scores as $-10 <$ LELP < 10.[28] LELP scores for compounds **9**, **13** and **14** are within the range appropriate for drug candidates (0.56, 1.28, and 2.04, respectively). From these analyses, we can conclude that nitrothiazoles **9**, **13** and **14** are lead compounds with optimal combinations of physicochemical and antiprotozoal properties.

On the other hand, the presence of nitro group in approved drugs has raised controversy. Nitroheterocyclic compounds such as metronidazole and benznidazole, generally function as prodrugs and must undergo bioreduction to mediate their antiparasitic effects.[29] A key step in this process is the reduction of the nitro group, which is attached to the imidazole, producing a nitro anion radical, a nitroso and hydroxylamine reactive intermediates, which could be cytotoxic.[30] The enzymes involved in catalytic reduction are nitroreductases,[31] and the pyruvate ferredoxin oxidoreductase (PFOR). In the case of nitazoxanide it has been reported that the mode of action is by specific inhibition of PFOR.[16] Although the nitro reduction is crucial for any biological activity, there are still no conclusive results about the incidence of the nitro position in the antiparasitic activity of thiazolides.

3. Conclusion

We report the synthesis of fourteen 2-acylamino-5-nitro-1,3-thiazole derivatives which were obtained with modest yields, and screened for the in vitro antiprotozoal effect against four neglected parasites. Several compounds of this series have shown significant inhibitory activity in the nano-molar range. Compounds **13** and **14** exhibited the most promising activities as giardicidal and trichomonicidal drugs, respectively. The findings of this study have a number of important implications for future practice, since many of the compounds displayed very low cytotoxicity and also showed activity comparable or higher than the current used antiprotozoal drugs metronidazole and nitazoxanide. Analysis of the ligand efficiency indexes of the compounds provided new insights for the design of potent and selective antiparasitic drugs. Further optimization and pharmacokinetic characterization of this series are in progress in our laboratory.

4. Experimental

4.1. Chemistry

Melting points were determined on an EZ-Melt MPA120 automated melting point apparatus from Stanford Research Systems and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated silica gel 60 F254 plates (E. Merck). ^{1}H NMR spectra were recorded on Varian Oxford (400 MHz) and ^{13}C NMR (100 MHz), as well as Varian Mercury (200 MHz) and ^{13}C NMR (50 MHz) instruments. Chemical shifts are given in ppm relative to tetramethylsilane (Me_4Si, $\delta = 0$) in DMSO-d_6; J values are given in Hz. The following abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; br s, broad signal. MS were recorded on a JEOL JMS-700 spectrometer by Fast Atom Bombarded [FAB (+)]. Starting materials were commercially available from Aldrich and used without purification.

4.1.1. General method of synthesis of ureas (1–8)

To a solution of 2-amino-5-nitro-1,3-thiazole (0.3 g, 0.0020 mol) in toluene (5 mL), was added dropwise the appropriate isocyanate (0.0031 mol, 1.5 equiv) at 25 °C. The mixture was stirred at reflux under nitrogen atmosphere for 12 h. Solvent was removed in vacuo, and the residue was suspended in water. The precipitates were filtered and dried. Crude compounds were recrystallized from a ethanol or purified by column chromatography.

4.1.1.1. *N*-Cyclohexyl-*N*-(5-nitro-1,3-thiazol-2-yl)urea (1). Yield 86%, after recrystallization from ethanol. Mp: 200–203 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.47 (1H, s, H-4), 3.45 (1H, d, J = 7.9 Hz, H-1′), 1.15–1.80 (10H, m, H-2′-6′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.5 (C-2), 152.8 (CO), 143.8 (C-4), 141.2 (C-5), 48.8 (C-1′), 32.7 (C-2′-6′), 25.5 (C-4′), 24.5 (C-3′-5′) ppm; MS (FAB$^+$) m/z 271 (M+H$^+$). Anal. Calcd for $C_{10}H_{14}N_4O_3S$: C, 44.43; H, 5.22; N, 20.73. Found: C, 44.47; H, 5.30; N, 20.79.

4.1.1.2. *N*-(5-Nitro-1,3-thiazol-2-yl)-*N*-phenylurea (2). Yield 72%, after recrystallization from ethanol. Mp: 207–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.52 (1H, s, H-4), 7.65 (2H, dd, J_o = 8.8, J_o = 2.0 Hz, H-2′-6′), 7.32 (2H, t, J_o = 8 Hz, H-3′-5′), 7.07 (1H, t, J_o = 7.6 Hz, J_o = 7.2 Hz, H-4′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ:163.9 (C-2), 152.9 (CO), 140.1 (C-4), 138.1 (C-5), 129.4 (C-3′-5′), 129.2 (C-1′), 124.0 (C-4′), 119.5 (C-2′-6′) ppm; MS (FAB$^+$) m/z 265 (M+H$^+$). Anal. Calcd for $C_{10}H_8N_4O_3S$: C, 45.45; H, 3.05; N, 21.20. Found: C, 45.50; H, 3.10; N, 21.32.

4.1.1.3. *N*-(4-Chlorophenyl)-*N*-(5-nitro-1,3-thiazol-2-yl)urea (3). Yield 55%, after recrystallization from ethanol. Mp: 243 (dec) °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (1H, s, H-4), 7.51 (2H, d, J_o = 8.8 Hz, H-2′-6′), 7.37 (2H, d, J_o = 8.8 Hz, H-3′-5′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9 (C-2), 152.7 (CO), 138.9 (C-4), 137.2 (C-5), 129.2 (C-3′-5′), 127.6 (C-1′), 125.9 (C-4′), 120.2 (C-2′-6′) ppm; MS (FAB$^+$) m/z 298 (M+H$^+$). Anal. Calcd for $C_{10}H_7ClN_4O_3S$: C, 40.21; H, 2.36; N, 18.76. Found: C, 40.01; H, 2.30; N, 18.60.

4.1.1.4. *N*-(4-Fluorophenyl)-*N*-(5-nitro-1,3-thiazol-2-yl)urea (4). Yield: 94%, after recrystallization from ethanol. Mp: 211–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.30 (1H, s, H-4), 7.48 (2H, m, J_o = 8.4 Hz, H-2′-6′), 7.17 (2H, m, J_o = 8.4 Hz, H-3′-5′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.8 (J = 130.5 Hz, C-4′), 156.8 (CO), 151.4 (C-2), 134.5 (J = 21.1 Hz, C-3′-5′), 115.6 (J = 9.1, C-2′-6′) ppm; MS (FAB$^+$) m/z 283 (M+H$^+$). Anal Calcd for $C_{10}H_7FN_4O_3S$: C, 42.55; H, 2.50; N, 19.85. Found: C, 42.65; H, 2.46; N, 19.72.

4.1.1.5. *N*-(4-Methoxyphenyl)-*N*-(5-nitro-1,3-thiazol-2-yl)urea (5). Yield: 46%, after recrystallization from ethanol. Mp: 212–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.52 (1H, s, H-4), 7.31 (2H, d, J_o = 9.2 Hz, H-2′-6′), 6.86 (2H, d, J_o = 10 Hz, H-3′-5′), 3.70 (3H, s, O-CH$_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.1 (C-2), 155.1 (C-4′), 150.8 (CO), 142.4 (C-4), 132.4 (C-5), 129.9 (C-1′), 120.5 (C-2′-6′), 113.5 (C-3′-5′), 54.6 (O-CH$_3$) ppm. MS (CI$^+$) m/z 295 (M+1). Anal. Calcd for $C_{11}H_{10}N_4O_4S$: C, 44.89; H, 3.43; N, 19.04. Found: C, 44.77; H, 3.56; N, 19.85.

4.1.1.6. *N*-(4-Ethoxyphenyl)-*N*-(5-nitro-1,3-thiazol-2-yl)urea (6). Yield: 72%, after recrystallization from ethanol. Mp: 204–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8. 33 (1H, s, H-4), 7.39 (2H, d, J_o = 8 Hz, H-2′-6′), 6.88 (2H, d, J_o = 8 Hz, H-3′-5′), 3.95 (2H, q, J = 7 Hz, O-CH$_2$), 1.29 (3H, t, J = 6.8 Hz, CH$_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.3 (C-2), 155.20 (C-4′), 154.1 (CO), 142.8 (C-4), 133. 3 (C-5), 131.3 (C-1′), 121.4 (C-2′-6′), 115.1 (C-3′-5′), 63.6 (O–CH$_2$), 15.1 (CH$_3$) ppm; MS (FAB$^+$) m/z 309 (M+H$^+$). Anal. Calcd for $C_{12}H_{12}N_4O_4S$: C, 46.75; H, 3.92; N, 18.17. Found: C, 46.60; H, 3.96; N, 18.23.

4.1.1.7. *N*-(4-Butoxyphenyl)-*N*-(5-nitro-1,3-thiazol-2-yl)urea (7). Yield 87%, mp: 148–153 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.54 (1H, s, H-4), 7.37 (2H, d, J_o = 9 Hz, H-2′-6′), 6.89 (2H, d, J_o = 9 Hz, H-3′-5′), 3.92 (2H, t, J = 6.4 Hz, H-1″), 1.65 (2H, q, J = 6.7 Hz, J = 7.9 Hz, H-2″), 1.41 (2H, q, J = 7.4 Hz, J = 7.6 Hz, H-3″), 0.92 (3H, t, J = 7.3 Hz, H-4″) ppm; ^{13}C NMR (50 MHz, DMSO-d_6) δ: 163.9 (C-2), 155.1 (C-4′), 151.6 (CO), 143.0 (C-4), 130.5 (C-1′), 121.0 (C-2′-6′), 114.7 (C-3′-5′), 67.3 (C-1″), 30.8 (C-2″), 18.7 (C-3″), 13.6 (C-4″) ppm; MS (FAB$^+$) m/z 337 (M+H$^+$). Anal. Calcd for $C_{14}H_{16}N_4O_4S$: C, 49.99; H, 4.79; N, 16.66. Found: C, 49.87; H, 4.79; N, 16.62.

4.1.1.8. *N*-(4-Nitrophenyl)-*N*-(5-nitro-1,3-thiazol-2-yl)urea (8). Yield: 80%, after recrystallization from ethanol. Mp: 259-desc °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.58 (1H, s, H-4), 8.19 (2H, d, J_o=8.8 Hz, H-3′-5′), 7.73 (2H, d, J_o = 8.8 Hz, H-2′-6′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.2 (C-2), 152.8 (CO), 151.5 (C-4), 144.6 (C-1′), 142.1 (C-4′), 125.0 (C-3′-5′), 117.9 (C-2′-6-′) ppm; MS (FAB$^+$) m/z 310 (M+H$^+$). Anal. Calcd for $C_{10}H_7N_5O_5S$: C, 38.84; H, 2.28; N, 22.65. Found: C, 38.80; H, 2.35; N, 22.79.

4.1.2. General method of synthesis of amides (9–12)

To a solution of 2-amino-5-nitrothiazole (0.3 g, 0.0020 mol) in dichloromethane was added 1.2 molar equiv of triethylamine (TEA). After the reaction mixture was stirred at 5 °C for 15 min, acetic anhydride (0.0100 mol, 5 equiv), or respectively acyl chlorides (0.0022 mol, 1.1 equiv) were added drop-wise. The reaction mixture was stirred at room temperature for 4–24 h. After complete conversion, the solvent was removed in vacuo and the residue was neutralized with saturated $NaHCO_3$ solution. The precipitated solids were recrystallized from a mixture of solvents.

4.1.2.1. *N*-(5-Nitro-1,3-thiazol-2-yl)acetamide (9). Yield: 91%, after recrystallization from acetonitrile/methanol 75:25. Mp: 267–270 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (1H, s, H-4), 2.21 (3H, s, CH$_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.65 (CO), 162.15 (C-2), 142.93 (C-5), 142.11 (C-4), 22.87 (CH$_3$) ppm; MS (FAB$^+$) m/z 188 (M+H). Anal. Calcd for $C_5H_5N_3O_3S$: C, 32.08; H, 2.69; N, 22.45. Found: C, 32.13; H, 2.73; N, 22.53.

4.1.2.2. *N*-(5-Nitro-1,3-thiazol-2-yl)benzamide (10). Yield 87%, after recrystallization from ethanol. Mp 258–261 °C ^{1}H NMR (200 MHz, DMSO-d_6) 7.34–7.60 (3H, m, H-3′, H-4″, H-5′), 8.09 (2H, dd, H-2′, H-6′, J_o = 8.4, J_m = 1.6 Hz), 8.33 (1H s, H-4) ppm; ^{13}C NMR (50 MHz, DMSO-d_6) 128.5 (2C, C-3′-5′), 128.7 (2C, C-2′-6′),

130.7 (C-5), 142.0 (C-1′), 142.5 (C-4), 162.4 (C-2), 166.3 (CO) ppm; MS (FAB+): *m*/*z* 250 ($M+H^+$). HRMS (FAB+) Cald for: $C_{10}H_7N_3O_3S$ [$M+H^+$] 250.0247, Found: 250.0271. Anal. Calcd for $C_{10}H_7N_3O_3S$: C, 48.19; H, 2.83; N, 16.86. Found: C, 48.20; H, 2.83; N, 16.96.

4.1.2.3. *N*-(5-Nitro-1,3-thiazol-2-yl)pentanamide (11). Yield 67%, after recrystallization from ethanol. Mp 155 (dec) °C. ^{1}H NMR (200 MHz, $CDCl_3$-DMSO-d_6) δ: 12.33 (1H, s, NH), 8.19 (1H, s, H-4), 2.42–2.46 (2H, m, H-2′) 1.58–1.67 (2H, m, H-3′), 1.17–1.27 (2H, m, H-4′), 0.72–0.84 (3H, m, H-5′) ppm. ^{13}C NMR (50 MHz, $CDCl_3$-DMSO-d_6) δ: 173.2 (CO), 162.4 (C-2), 142. 7 (C-5), 141.7 (C-4), 35.6 (C-1′), 27.1 (C-2′), 22.4 (C-3′), 13.9 (C-4′) ppm. MS/FAB$^+$: *m*/*z* 230 ($M+H^+$). Anal. Calcd for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.84; N, 18.33. Found: C, 41.62; H, 4.78; N, 17.95.

4.1.2.4. *N*-(5-Nitro-1,3-thiazol-2-yl)hexadecanamide (12). Yield 91%, after recrystallization from acetone/ethanol 50:50. Mp 132 (dec) °C ^{1}H NMR (200 MHz, DMSO-d_6). 12.29 (s, 1H, NH), 7.86 (1H, s, H-4), 2.06 (2H, t, *J* = 7.6 Hz, H-2′), 1.23 (2H, t, *J* = 6.4 Hz, H-3′), 0.78 (24H, bs, H-3′-15′), 0.41 (3H, t, *J* = 6.2 Hz, H-16′) ppm; ^{13}C NMR (50 MHz, $CDCl_3$-DMSO-d_6) δ: 172.6 (CO), 161.7 (C-2), 140.9 (C-4), 124.0 (C-5), 13. 7 (C-16), 22.1, 24.3, 28.5, 28.7, 29.1, 31.3, 35.0, 38.7, 39.1, 140.9, 172.6 ppm; MS/FAB$^+$: *m*/*z* 384 ($M+H^+$). Anal. Calcd for $C_{18}H_{33}N_3O_3S$: C, 59.50; H, 8.67; N, 10.96. Found: C, 59.87; H, 8.57; N, 11.12.

4.1.3. Synthesis of methyl 5-nitro-1,3-thiazol-2-ylcarbamate (13)

To a solution of 2-amino-5-nitro-1,3-thiazole (0.0027 mol) in ethyleneglycol dimethyleter, was added triethylamine (0.0034 mol, 1.25 equiv). The reaction mixture was stirred at 5 °C for 30 min. After that, methyl chloroformiate (0.0034 mol, 1.25 equiv) was added droopingly. The reaction mixture was stirred at reflux for 5 h. After complete conversion as indicated by TLC, the solvent was removed in vacuo, the residue was neutralized with saturated $NaHCO_3$ solution, and the aqueous layer was extracted with ethyl acetate (3 × 15 mL), washed with water and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the precipitated solids were recrystallized from ethanol. Yield: 82%, mp: 243–244 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.53 (1H, s, H-5), 3.80 (3H, s, OCH_3) ppm; ^{13}C NMR (50 MHz, DMSO-d_6) δ: 164.9 (C-2), 154.9 (CO) 143.8 (C-4), 142.5 (C-5), 54.3 (OCH_3) ppm; MS (FAB$^+$) *m*/*z* 204 ($M+H^+$). Anal. Calcd for $C_5H_5N_3O_4S$: C, 29.56; H, 2.48; N, 20.68. Found: C, 29.49; H, 2.53; N, 20.79.

4.1.4. Synthesis of ethyl [(5-nitro-1,3-thiazol-2-yl)amino](oxo)acetate (14)

To a solution of 2-amino-5-nitro-1,3-thiazole (0.0015 mol) in dichloromethane, was added triethylamine (1.2 equiv). The reaction mixture was stirred at 5 °C for 15 min. After that, a solution of ethyl chlorooxoacetate (0.0018 mol, 1.2 equiv) was added droopingly. The reaction mixture was stirred at room temperature for 6 h. After complete conversion as indicated by TLC, the solvent was removed in vacuo, the residue was neutralized with saturated $NaHCO_3$ solution, and the aqueous layer was extracted with ethyl acetate (3 × 15 mL), washed with water (3 × 20 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the precipitated solids were recrystallized from a mixture of acetonitrile/methanol. Yield: 90%, mp: 252–255 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.67 (1H, s, H-4), 4.32 (2H, q, O-CH_2) 1.31 (3H, t, CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.7 (C-2), 158.4 (CO-OR), 157.7 (RNH-CO), 143.3 (C-5), 142.7 (C-4), 63.4 (O–CH_2), 14.1 (CH_3) ppm; MS (FAB$^+$) *m*/*z* 246 ($M+H^+$). Anal. Calcd for $C_7H_7N_3O_5S$: C, 34.29; H, 2.88; N, 17.14. Found: C, 34.19; H, 2.83; N, 17.09.

4.2. Biological assays

4.2.1. In vitro giardicidal and trichomonicidal assay

G. intestinalis strain IMSS:0696:1 was cultured in TYI-S-33 modified medium, supplemented with 10% calf serum and bovine bile, *T. vaginalis* strain GT3 was cultured in TYI-S-33 medium, supplemented with 10% bovine serum. In vitro susceptibility assays were performed using a method previously described.[9,13] Briefly: 4×10^4 trophozoites of *G. lamblia* or *T. vaginalis* were incubated for 48 h at 37 °C with increasing concentrations of synthesized compounds, nitazoxanide and metronidazole. As the negative control, trophozoites were incubated in culture medium with DMSO used in the experiments. After the incubation, trophozoites were washed and subcultured for another 48 h in fresh medium alone. At the end of this period, trophozoites were counted and the 50% inhibitory concentration (IC_{50}) was calculated by Probit analysis. Experiments were carried out in triplicate and repeated at least twice.

4.2.2. In vitro antileishmanial and trypanocidal assay

The growth inhibition test was performed on promastigotes of *L. amazonensis* (IFLA/BR/67/PH-8; clinical strain originally isolated from a patient with diffuse cutaneous leishmaniasis) and epimastigotes of *T. cruzi* (MHOM/MX/1994/Ninoa; clinical strain originally isolated from a patient with the disease in acute phase).[8] Parasites were cultivated at 26 °C in Schneider's drosophila medium, supplemented with 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 μg/mL). Biological assays were performed in 96-well plates and all compounds were evaluated in duplicate. Compounds were solubilized in DMSO and diluted in a liquid medium. A mixture of 100 μL of compounds solution and 100 μL of culture medium containing 10,000 *Leishmania* promastigotes or 20,000 *T. cruzi* epimastigotes was added to obtain concentrations of 10, 5, 2.5, 1.25 μg/mL. Benznidazole (first-line antichagasic drug) and pentamidine (second-line antileishmanial drug) were used as positive controls. Cultures containing parasites without compound solution were also included. The plate was incubated at 26 °C for 72 h and the leishmanicidal and trypanocidal activity of compounds were determined by direct count of parasites in a Neubauer chamber.[32] The concentration required to inhibit 50% of the parasites grow (IC_{50}) was calculated by probit analysis.

4.2.3. Cytotoxicity on VERO cell line

The cytotoxicity assay was performed as reported previously,[13] where 1.5×10^4 viable cells from the VERO cell line were seeded in a 96-well plate and incubated for 24–48 h. VERO cells were grown in DMEM media supplemented with 10% (v/v) Fetal Bovine Serum with 100 UI/mL penicillin and 100 mg/mL streptomycin and maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. When cells reached >80% confluence, the medium was replaced and the cells were treated with the compounds at 0.097–100 μg/mL dissolved in DMSO at a maximum concentration of 0.05%. After 48 h of incubation, viability of the cell lines was evaluated by the sulforhodamine B (SRB) method.[33,34] Metronidazole was used as a positive control, whereas untreated cells were used as negative controls. The concentration of the extract that killed 50% of the cells (CC_{50}) was calculated by nonlinear fit (GraphPad Prism 4 software). All concentrations were evaluated in duplicate, and each experiment was performed in triplicate.

Acknowledgments

This work was supported in part by internal funds from Facultad de Farmacia, UAEM. We are grateful to Victoria Labastida and Maria Medina from the Centro de Investigaciones Químicas, UAEM

for the determination of mass spectra. C.N.-Z. acknowledges the fellowship awarded by CONACyT (266444) to carry out graduate studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.029. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

1. Pink, R.; Hudson, A.; Marie-Annick, M.; Bendig, M. *Nat. Rev. Drug Disc.* **2005**, *4*, 727.
2. Center for Disease Control. http://www.cdc.gov/parasites/npi.html (January 5, 2014).
3. Savioli, L.; Smith, H.; Thompson, A. *Trends Parasitol.* **2006**, *22*, 203.
4. Escobedo, A. A.; Almirall, P.; Robertson, L. J.; Franco, R. M.; Hanevik, K.; Mørch, K.; Cimerman, S. *Infect. Disord. Drug Targets* **2010**, *10*, 329.
5. Argüello-García, R.; Cruz-Soto, M.; Romero-Montoya, L.; Ortega-Pierres, G. *Infect. Genet. Evol.* **2009**, *9*, 1057.
6. Upcroft, P. *Drug Resist. Updat.* **1998**, *1*, 166.
7. Schwebke, J. R.; Burgess, D. *Clin. Microbiol. Rev.* **2004**, *17*, 794.
8. Chan-Bacab, M. J.; Hernández-Núñez, E.; Navarrete-Vázquez, G. *J. Antimicrob. Chemother.* **2009**, *63*, 1292.
9. Navarrete-Vázquez, G.; Chávez-Silva, F.; Argotte-Ramos, R.; Rodríguez-Gutiérrez, M. C.; Chan-Bacab, M. J.; Cedillo-Rivera, R.; Moo-Puc, R.; Hernández-Núñez, E. *Bioorg. Med. Chem. Lett.* **2011**, *15*, 3168.
10. Ballard, T. E.; Wang, X.; Olekhnovich, I.; Koerner, T.; Seymour, C.; Hoffman, P. S.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3537.
11. Rossignol, J. F.; Elfert, A.; El-Gohary, Y.; Keeffe, E. B. *Gastroenterology* **2009**, *136*, 856.
12. Rossignol, J. F.; La Frazia, S.; Chiappa, L.; Ciucci, A.; Santoro, M. G. *J. Biol. Chem.* **2009**, *284*, 29798.
13. Nava-Zuazo, C.; Estrada-Soto, S.; Guerrero-Álvarez, J.; León-Rivera, I.; Molina-Salinas, G. M.; Said-Fernández, S.; Chan-Bacab, M. J.; Cedillo-Rivera, R.; Moo-Puc, R.; Miron-Lopez, G.; Navarrete-Vázquez, G. *Bioorg. Med. Chem.* **2010**, *18*, 6398.
14. Hernández-Núñez, E.; Tlahuext, H.; Moo-Puc, R.; Torres-Gómez, H.; Reyes-Martínez, R.; Cedillo-Rivera, R.; Nava-Zuazo, C.; Navarrete-Vazquez, G. *Eur. J. Med. Chem.* **2009**, *44*, 2975.
15. Aguayo-Ortiz, R.; Méndez-Lucio, O.; Romo-Mancillas, A.; Castillo, R.; Yépez-Mulia, L.; Medina-Franco, J. L.; Hernández-Campos, A. *J. Mol. Graph. Model.* **2013**, *45*, 26.
16. Soria-Arteche, O.; Hernández-Campos, A.; Yépez-Mulia, L.; Trejo-Soto, P. J.; Hernández-Luis, F.; Gres-Molina, J.; Maldonado, L. A.; Castillo, R. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6838.
17. Cuckler, C. A. U.S. 2998480, 1961.
18. Kupferberg, B. A.; Mende C. W.; Millman, N.; Singher O. H. U.S. 2735798, 1956.
19. Hidalgo-Figueroa, S.; Ramírez-Espinosa, J. J.; Estrada-Soto, S.; Almanza-Pérez, J. C.; Román-Ramos, R.; Alarcón-Aguilar, F. J.; Hernández-Rosado, J. V.; Moreno-Díaz, H.; Díaz-Coutiño, D.; Navarrete-Vázquez, G. *Chem. Biol. Drug Des.* **2013**, *81*, 474.
20. Xu, L.; Chen, Y.; Pan, Y.; Skiles, G. L.; Shou, M. *Drug Metab. Dispos.* **2009**, *37*, 2330.
21. Taboureau, O.; Jørgensen, F. S. *Comb. Chem. High Throughput Screen.* **2011**, *14*, 375.
22. Navarrete-Vázquez, G.; Alaniz-Palacios, A.; Hidalgo-Figueroa, S.; González-Acevedo, C.; Ávila-Villarreal, G.; Estrada-Soto, S.; Webster, S. P.; Medina-Franco, J. L.; López-Vallejo, F.; Guerrero-Álvarez, J.; Tlahuext, H. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3244.
23. Abad-Zapatero, C. *Expert Opin. Drug Discov.* **2007**, *2*, 469.
24. Abad-Zapatero, C.; Metz, J. T. *Drug Discovery Today* **2005**, *10*, 464.
25. Meanwell, N. A. *Chem. Res. Toxicol.* **2011**, *24*, 1420.
26. Shultz, M. D. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5992.
27. Tarcsay, A.; Nyíri, K.; Keseru, G. M. *J. Med. Chem.* **2012**, *55*, 1252.
28. Keserü, G. M.; Makara, G. M. *Nat. Rev. Drug Disc.* **2009**, *8*, 203.
29. Wilkinson, S. R.; Bot, C.; Kelly, J. M.; Hall, B. S. *Curr. Top. Med. Chem.* **2011**, *11*, 2072.
30. Hall, B. S.; Wilkinson, S. R. *Antimicrob. Agents Chemother.* **2012**, *56*, 115.
31. Müller, J.; Schildknecht, P.; Müller, N. *J. Antimicrob. Chemother.* **2013**, *68*, 1781.
32. Encarnacion-Dimayuga, R.; Murillo-Álvarez, J. I.; Christophersen, C.; Chan-Bacab, M.; García Reiriz, M. L.; Zacchino, S. *Nat. Prod. Commun.* **2006**, *1*, 541.
33. Moo-Puc, R.; Robledo, D.; Freile-Pelegrin, Y. *Cienc. Mar.* **2009**, *35*, 345.
34. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl Cancer Inst.* **1990**, *82*, 1107.