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Synthesis and in vitro activity of new tetrahydronaphtho[1,2-*b*]azepine derivatives against *Trypanosoma cruzi* and *Leishmania chagasi* parasites

Alirio Palma [a,*], Andrés Felipe Yépes [a], Sandra Milena Leal [b], Carlos Andrés Coronado [b], Patricia Escobar [b]

[a] *Laboratorio de Síntesis Orgánica, Escuela de Química, Universidad Industrial de Santander, A. A. 678, Bucaramanga, Santander, Colombia*
[b] *Centro de Investigación de Enfermedades Tropicales, Facultad de Salud, Escuela de Medicina, Departamento de Ciencias Básicas, Universidad Industrial de Santander, A.A. 678, Bucaramanga, Santander, Colombia*

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ABSTRACT

Series of 2-*exo*-aryl-1,4-epoxy-2,3,4,5-tetrahydronaphtho[1,2-*b*]azepines **3a**–**k** and *cis*-2-aryl-4-hydroxy-2,3,4,5-tetrahydronaphtho[1,2-*b*]azepines **4a**–**j** were synthesized and evaluated against free and intracellular live forms of *Trypanosoma cruzi* and *Leishmania chagasi* parasites using in vitro assays. Cell toxicity was also analyzed on Vero and THP-1 mammalian cell lines. The compounds **3c**, **3f**, and **4d** were the most active against both live forms of *T. cruzi* parasites with low mammalian cell toxicity. Some compounds were active on free live forms of *L. chagasi* parasites but none was active on intracellular amastigotes of *L. chagasi* infecting THP-1 macrophages.

American trypanosomiasis (Chagas' disease) and leishmaniasis have reemerged over the last few decades as important threats to human health and economic development. They constitute endemic diseases mainly distributed on the tropical and subtropical areas of the world where millions of people live on permanent risk of infection. The alternatives of treatment for the parasite-infected people are few, expensive, variable on clinical efficacy and not always available.[1] They often require long courses of parenteral administration and are associated with severe side effects and resistance.[2]

The chemotherapy for leishmaniasis has been based for over 60 years on the use of pentavalent antimonial drugs and in case of failure, amphotericine B, pentamidine isethionate, aminosidine (paromomycin), and miltefosine are commonly used.[3] The chemotherapy for Chagas' disease is more precarious, dependent on two nitroheterocyclic compounds, nifurtimox, now discontinued, and benznidazole. They are both toxic and restricted only to the acute phase of the disease.[4] There is an urgent need to develop cost-effective new drugs and discover novel molecules with a potent anti-parasitic activity and improved pharmacological characteristics.[5,6]

The tetrahydro-1-benzazepine derivatives have been extensively investigated synthetically and pharmacologically.[7–21] Compounds containing this heterocyclic unit possess a wide range of biological activities and some derivatives have been active against promastigotes and intracellular amastigotes of *L. mexicana*.[22] Other derivatives are considered as promising anti-trypanosomal agents when used as inhibitors of *Trypanosoma cruzi* dihydrofolate reductase.[23] Based on this information and, as an extension of our ongoing work toward the synthesis, structural elucidation, and functionalization of the tetrahydro-1-benzazepine ring system,[24,25] in this letter we report the anti-parasitic activity of 2-*exo*-aryl-1,4-epoxytetrahydronaphtho[1,2-*b*]azepines **3a**–**k** and *cis*-2-aryl-4-hydroxytetrahydronaphtho[1,2-*b*]azepines **4a**–**j** against *T. cruzi* and *Leishmania chagasi* parasites and Vero and THP-1 cell lines.

As illustrated in Scheme 1, the evaluated compounds were synthesized starting from the available *N*-benzyl-α-naphthylamines, which on reaction with an excess of allyl bromide in the presence of potassium carbonate in dry acetone under reflux, were converted into *N*-allyl-*N*-benzyl-substituted-α-naphthylamines **1a**–**k** in good yields (70–85%). The introduction of an allyl moiety at the *ortho*-position of the amino group was carried out via an aromatic amino-Claisen rearrangement of the *N*-allyl derivatives **1a**–**k**. Thus, rearrangement of these derivatives by heating in the presence of stoichiometric amounts of boron trifluoride diethyl

* Corresponding author. Tel./fax: +57 76 349069.
E-mail addresses: apalma@uis.edu.co, alirio.palma@gmail.com (A. Palma).

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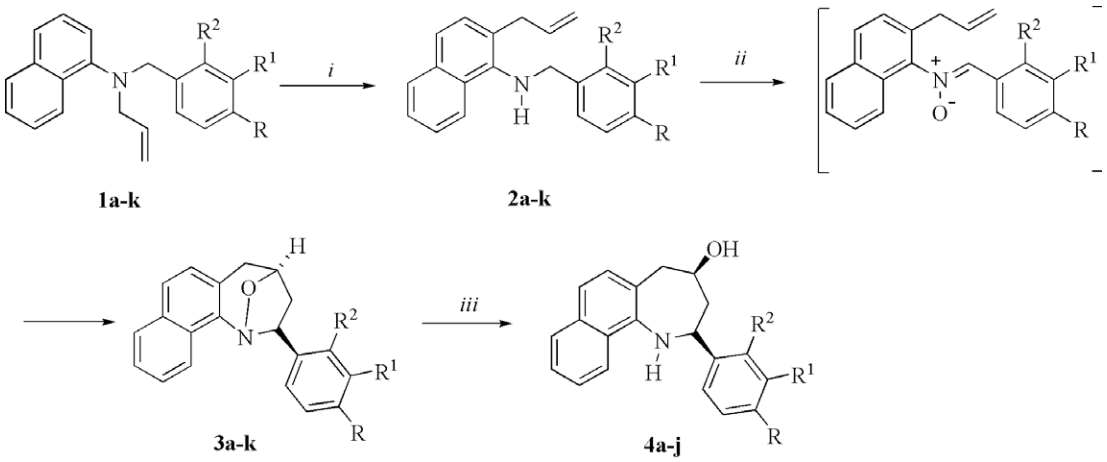

a: R = R¹ = R² = H; b: R = F, R¹ = R² = H; c: R = Cl, R¹ = R² = H; d: R = Br, R¹ = R² = H; e: R = R² = H, R¹ = Cl; f: R = R² = H, R¹ = Br; g: R = R² = H, R¹ = OCH_3; h: R = R² = H, R¹ = CH_3; i: R = R¹ = H, R² = Cl; j: R = R² = Cl, R¹ = H; k: R = R¹ = H, R² = NO_2

Scheme 1. Reagents and conditions: (i) $BF_3{\cdot}OEt_2$ (1 equiv), 115–125 °C, 1–4 h; (ii) a—30% H_2O_2 (3 equiv)/$Na_2WO_4{\cdot}2H_2O$ (5% mol), methanol, 0–25 °C, 45–50 h; b—toluene, reflux, 6–10 h; (iii) Zn/80% AcOH, 80–82 °C, 2–5 h.

ether ($BF_3{\cdot}Et_2O$), as acid catalyst, at 115–125 °C gave the rearranged products **2a–k** in excellent yields (61–88%).

Oxidation and subsequent intramolecular 1,3-dipolar cycloaddition of **2a–k** to obtain the corresponding 1,4-epoxytetrahydronaphtho[1,2-*b*]azepines **3a–k** was carried out by reacting compounds **2a–k** with an excess of hydrogen peroxide (30% H_2O_2) in the presence of catalytic amounts of sodium tungstate ($Na_2WO_4{\cdot}2H_2O$) and by heating generated *in-situ* nitrones in toluene under reflux. Cycloadducts **3a–k** were fully characterized by using ^1H and ^{13}C NMR spectroscopy at 400 MHz and 100 MHz, respectively (Bruker AM-400 apparatus), and shown to be 2-*exo*-aryl-cycloadducts as evidenced by NOESY experiments.[25] Finally, reductive cleavage of the N–O bond of **3a–j** by treating with Zn in 80% acetic acid at 80–82 °C for 2–5 h, gave, as expected, exclusively *cis*-2-aryl-4-hydroxytetrahydronaphtho[1,2-*b*]azepines **4a–j** in 64–78% yields, after column chromatography purification on silica gel. The *cis* stereochemistry of these compounds was also determined on the basis of NOESY experiments.[25]

The in vitro anti-parasitic activity of the cycloadducts **3a–k** and their reduced homologues **4a–j** were evaluated against *T. cruzi*, *L. chagasi* (free and intracellular live forms) parasites and on Vero epithelial cells and THP-1 transformed human macrophages. Nifurtimox and amphotericine B (AmB) were used as control drugs under the same assay conditions.[26,27] The obtained results are summarized in Table 1. Many of the tested compounds were active on free live forms of *T. cruzi* and *L. chagasi* parasites. Thus, fifteen compounds (**3c**,**e**,**f**,**h**,**k** and **4a–j**) were active on *T. cruzi* epimastigotes with inhibitory concentration (IC_{50}) values ranging from 0.50 ± 0.21 to 38.77 ± 1.71 μM and IC_{90} values from 11.01 ± 0.21 to 104.73 ± 17.12 μM. Likewise, nine compounds (**3c**,**f–h**,**j** and **4d**,**e**,**g**,**j**) were active on *L. chagasi* promastigotes with IC_{50} values ranging from 5.97 ± 0.64 and 35.37 ± 0.49 μM and IC_{90} values from 28.20 ± 0.08 and 67.31 ± 4.73 μM. The selectivity index (SI = CC_{50} Vero cells/IC_{50} *T. cruzi* or CC_{50} THP-1 cells/IC_{50} *L. chagasi*) from the active compounds was higher than 4. Seven compounds (**3c**,**f**,**g** and **4d**,**e**,**g**,**j**) were active on both tested parasites.

On the other side, few compounds were active on parasite intracellular live forms. Only **3c** (IC_{50} 16.22 ± 1.12 μM and IC_{90} 83.37 ± 10.37 μM; SI > 17.7), **3f** (IC_{50} 18.35 ± 0.49 μM and IC_{90} 76.29 ± 16.82 μM; SI > 13.6), and **4d** (IC_{50} 9.89 ± 0.10 μM and IC_{90} 29.70 ± 0.08 μM; SI 6.7) compounds were active on *T. cruzi* amastigotes infecting Vero cells. None of the tested molecules was active on intracellular *L. chagasi* parasites at any of the evaluated concentration (1–100 μM). Intracellular amastigotes of *L. chagasi* were susceptible only to the AmB referential drug with activities of IC_{50} 0.03 ± 0.0009 and IC_{90} 0.19 ± 0.002 μM (data not shown). About the mammalian cell toxicity, the 2-*exo*-aryl-1,4-epoxytetrahydronaphtho[1,2-*b*]azepines **3a–k** were, in general, less toxic than their reduced analogs **4a–j**.

In a search for structural parameters which might enhance the anti-parasitic activity, we introduced chlorine, bromine, fluorine, methoxy, methyl, and nitro substituents onto the phenyl ring of compounds **3** and **4**. The presence of the bromine atom on compounds **3d**,**f** and **4d**,**f** showed interesting behavior. Since **3f** compound was active on free and intracellular live forms of *T. cruzi* and on *L. chagasi* promastigotes without toxicity on Vero or THP-1 cells, compound **4d** was also active but with moderate toxicity on mammalian cells. In contrast, compounds **3d** and **4f** lost activity on intracellular forms of *T. cruzi*. Among the chlorine and fluorine containing molecules, **3c**, **4c**, **3e**, **4e**, and **4b** were highly active on epimastigotes of *T. cruzi* (even higher than the reference nifurtimox drug). The compounds **3c** and **4b** showed the best activity, being active on free and intracellular live forms of *T. cruzi* and on *L. chagasi* promastigotes without (for **3c**) or moderate (for **4b**) toxicity on mammalian cells. The obtained values also indicate that halogen, methyl, and nitro substitution at 2′-position as well as methyl or methoxy substitution at 3′-position resulted in a loss of activity. Dichloro substitution at 2′, 4′-positions presented in addition a higher cell toxicity.

In conclusion, the most active compounds to emerge from this study were 2-*exo*-(4′-chlorophenyl)-1,4-epoxytetrahydronaphtho[1,2-*b*]azepine **3c**, 2-*exo*-(3′-bromophenyl)-1,4-epoxytetrahydronaphtho[1,2-*b*]azepine **3f**, and *cis*-2-(4′-bromophenyl)-4-hydroxytetrahydronaphtho[1,2-*b*]azepine **4d**. They were active against free and intracellular forms of *T. cruzi* with SI higher than 6. Although some of the tested compounds were active on *L. chagasi* promastigotes, none of them was active on intracellular amastigotes infecting THP-1 cells. The results obtained offer new

Table 1
In vitro activity of 2-*exo*-aryl-1,4-epoxytetrahydronaphtho[1,2-*b*]azepines **3a–k** and *cis*-2-aryl-4-hydroxy-tetrahydronaphtho[1,2-*b*]azepines **4a–j** against *Trypanosoma cruzi* and *Leishmania chagasi*

Compound	R	R^1	R^2	μM							
				Trypanosoma cruzi[a]				*Leishmania chagasi*[b]		Mammalian cells	
				Epimastigote forms		Intracellular amastigotes		Promastigote forms		Vero cells[c]	THP-1 cells[d]
				IC_{50}[e]/IC_{90}	SI[g]	IC_{50}/IC_{90}	SI	IC_{50}/IC_{90}	SI	CC_{50}[f]/CC_{90}	CC_{50}/CC_{90}
3a	H	H	H	11.5	6.3	>100	<0.7	21.2	>16.5	72.9	>350
				>350		>100		175.2		>350	>350
4a	H	H	H	5.7	18.9	19.3	5.6	46.0	2.2	108.0	101.9
				35.1		>30		67.1		>268.3	>300
3b	F	H	H	90.5	>3.3	>100	>3.0	70.4	>4.3	>300	>300
				>300		>100		112.7		>300	>300
4b	F	H	H	1.1	91.4	14.8	6.8	21.0	3.9	100.5	81.9
				11.6		>30		120.1		>250	>250
3c	Cl	H	H	38.8	7.4	16.2	>17.7	35.4	>8.5	286.7	>300
				104.7		83.4		43.1		>300	>300
4c	Cl	H	H	3.2	29.7	>100	<1.0	25.9	1.4	95.0	36.2
				24.8		>100		52.9		>250	122.2
3d	Br	H	H	11.4	21.9	>100	2.5	73.4	>3.4	>250	>250
				175.5		>100		>250		>250	>250
4d	Br	H	H	10.2	6.5	9.9	6.7	18.5	4.3	66.1	79.0
				15.9		29.7		37.1		>200	>200
3e	H	Cl	H	12.6	>23.8	13.1	>22.9	32.7	>9.2	>300	>300
				80.1		>100		>300		>300	>300
4e	H	Cl	H	1.4	21.9	23.4	1.3	6.0	7.2	30.7	43.2
				54.9		>30		67.3		>200	176.8
3f	H	Br	H	17.5	>14.3	18.4	>13.6	10.4	>24.0	>250	>250
				82.3		76.3		31.3		>250	>250
4f	H	Br	H	10.7	4.6	>100	0.5	18.3	2.3	49.5	41.7
				19.4		>100		55.7		>250	>250
3g	H	OCH_3	H	82.7	>3.6	89.6	>3.3	16.4	>18.3	>300	>300
				>300		>100		49.5		>300	>300
4g	H	OCH_3	H	14.0	7.0	24.3	4.0	17.9	10.9	97.6	195.0
				48.5		>30		58.7		>200	>300
3h	H	CH_3	H	17.0	>17.6	>100	>3.0	35.2	>8.5	>300	>300
				44.1		>100		41.2		>300	>300
4h	H	CH_3	H	16.5	5.3	33.4	2.6	41.4	2.2	87.4	89.9
				42.7		>30		134.0		>250	115.6
3i	H	H	Cl	100.2	0.4	>100	<0.4	183.8	>1.6	37.2	>300
				292.6		>100		>300		>300	>300
4i	H	H	Cl	0.5	193.0	10.6	9.1	30.6	2.2	96.5	66.1
				11.01		>30		64.6		>250	>300
3j	Cl	H	Cl	52.7	1.0	>250	<0.2	22.1	>11.3	55.00	>250
				>250		>250		45.8		>250	>250
4j	Cl	H	Cl	1.0	45.4	>30	<1.5	11.0	7.2	45.4	79.4
				19.43		>30		28.2		>200	92.7
3k	H	H	NO_2	1.4	28.7	>100	<0.4	40.9	>7.3	40.2	>300
				29.6		>100		208.7		>300	>300
Nfx[h]				4.8	23.7	1.4	81.1	ND	—	113.6	ND
				17.0		11.0		ND		>300	ND
AmB[i]				ND[k]	—	ND	—	0.01	17.0	ND	0.170
								0.03			0.180

[a] *Trypanosoma cruzi* strain 320I04 was treated for 72 h at 27 °C with compounds.
[b] *Leishmania chagasi* strain MHOM/BR/74/PP75 was treated for 72 h at 27 °C with compounds.
[c] African green monkey kidney cell line (Vero, ATCC) was treated for 72 h at 37 °C with compounds.
[d] Transformed human acute monocytic leukemia cell line (THP-1, ATCC) was treated for 72 h at 37 °C with compounds.
[e] Inhibitory concentration IC_{50} or IC_{90} was the concentration required for 50% or 90% growth inhibition. All reported values are representative of one experiment from three.
[f] Cytotoxic concentration CC_{50} or CC_{90} was the concentration required for 50% or 90% mammalian cell killing.
[g] Selectivity index (SI) was obtained by dividing CC_{50} of Vero or THP-1 cells by IC_{50} of *T. cruzi* and *L. chagasi* parasites.
[h] Reference drug: nifurtimox (Nfx).
[i] Reference drug: amphotericine B (AmB).
[k] ND, not determined.

possibilities for further improvements concerning the anti-parasitic activity of other closely related derivatives of these series of compounds. A more complete structure–activity relationship is currently pursued in our laboratory.

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