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Discovery of nitroheterocycles active against African trypanosomes. In vitro screening and preliminary SAR studies

Vicente J. Arán [a], Marcel Kaiser [b,c], Christophe Dardonville [a,*]

[a] Instituto de Química Médica, CSIC, Juan de la Cierva 3, E–28006 Madrid, Spain
[b] Swiss Tropical and Public Health Institute, Socinstrasse, 57, CH-4002 Basel, Switzerland
[c] University of Basel, Basel, Switzerland

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ABSTRACT

A selection of 76 nitroheterocycles and related compounds from our in-house compound library was screened in vitro against the parasite *Trypanosoma brucei rhodesiense*, causative agent of human African trypanosomiasis (HAT). The unspecific cytotoxicity of the compounds was also evaluated against rat myoblast L6-cells to measure the selectivity of the compounds towards the parasite. This screening revealed some preliminary structure–activity relationships (SAR) among the series, and six hit compounds showing interesting activity ($IC_{50} \leqslant 10$ μM) and fair selectivity (SI >17). The 7-nitroquinoxalin-2-one and 5-nitroindazole scaffold derivatives **58** and **35**, respectively, are particularly interesting because of their established oral bioavailability in mice. These hits represent interesting starting points for a medicinal project aimed at identifying the SAR behind this class of compounds.

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease (NTD) endemic in sub-Saharan Africa. The causative agents are the protozoan parasites *Trypanosoma brucei rhodesiense* and *T. b. gambiense* which provoke acute and chronic forms of the disease, respectively. The parasites are transmitted by the bite of infected tsetse flies. In absence of treatment, the parasites present in the blood and lymph during early stage HAT invade the central nervous system (CNS) provoking the fatal meningoencephalitic (late) stage of the disease.[1,2]

The NTD status of sleeping sickness is highlighted by the fact that 3 out of the 4 drugs approved for its treatment (i.e., pentamidine, melarsoprol, suramin) were discovered more that 60 years ago. The other drug, eflornithine, was approved in 1990 for the treatment of late-stage *T. b. gambiense* infection. Other authors have reviewed the shortcomings of those drugs especially regarding toxicity, lack of oral bioavailability or lack of efficiency.[3] Apart from the recent inclusion of the nifurtimox–eflornithine combination therapy (NECT) in the WHO list of essential medicines, no new drugs have been registered for HAT in the last 20 years (http://www.who.int/selection_medicines/committees/expert/17/application/nifurtimox/en/index.html).

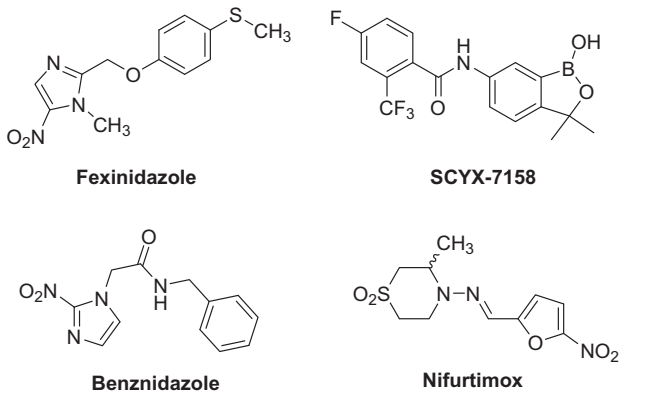

Figure 1. Drugs currently in clinical development for the oral treatment of both stages of sleeping sickness (top) and drug used clinically against *T. cruzi* infections (bottom).

Abbreviations: CNS, central nervous system; DNDi, Drugs for Neglected Diseases initiative; HAT, human African trypanosomiasis; IC_{50}, 50% inhibitory concentration; MEM, minimum essential medium; NECT, nifurtimox–eflornithine combination therapy; NTD, neglected tropical disease; SAR, structure–activity relationships; SI, selectivity index; WHO, World Health Organization.

* Corresponding author. Tel.: +34 912587490; fax: +34 915644853.
E-mail address: dardonville@iqm.csic.es (C. Dardonville).

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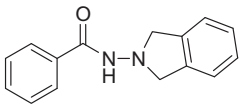
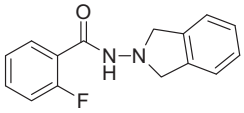
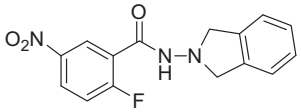
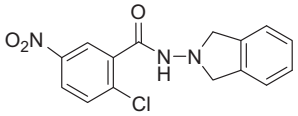
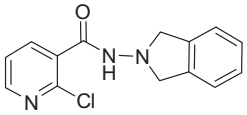
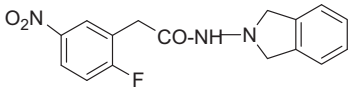
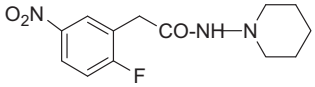
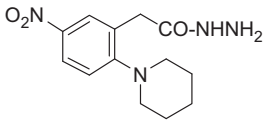
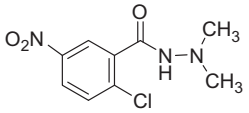
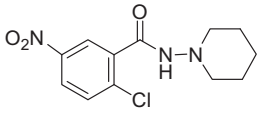
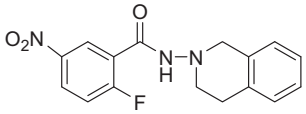
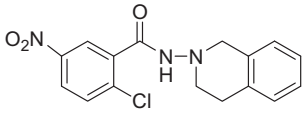
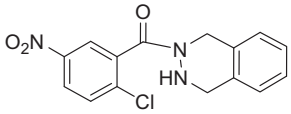
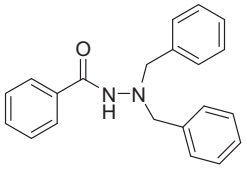
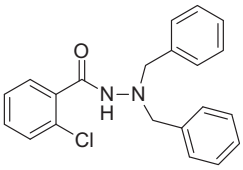
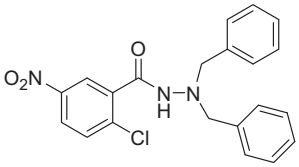
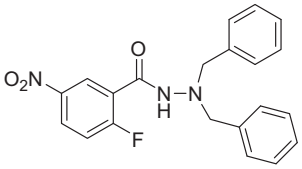
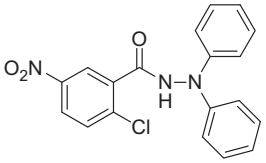
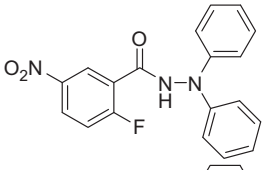
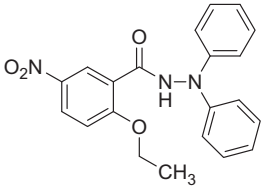
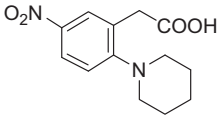
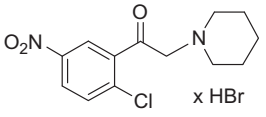
Table 1
In vitro activity of hydrazides (**1**–**20**) and related compounds (**21**–**22**) against *T. brucei rhodesiense*

Compound	Structure	*T.b.r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
1		>419	68.4	0.2	
2		166.2	241.1	1.5	
3		40.2	>331	>8.3	
4		97.3	19.8	0.2	
5		>365	>365	1.0	
6		179.8	168.1	0.9	
7		320.3	>355	1.1	12
8		154.9	>359	2.3	12,17,20
9		>410	>410	1.0	
10		211.8	>352	>1.7	
11		23.1	>317	>13.7	
12		55.5	22.0	0.4	
13		82.2	179.7	2.2	
14		>316	>316	1.0	

(*continued on next page*)

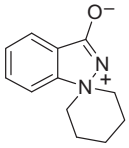
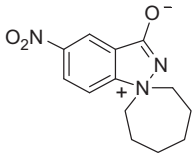
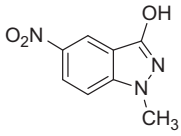
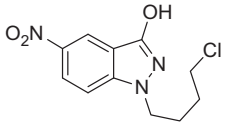
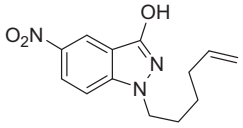
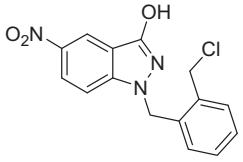
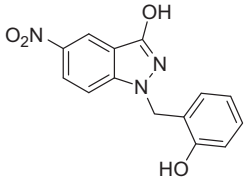
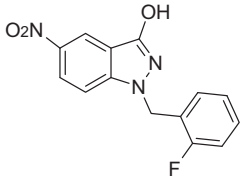
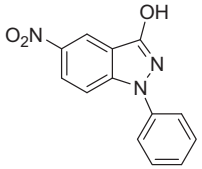
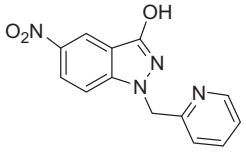
Table 1 (*continued*)

Compound	Structure	*T.b.r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
15		55.9	50.7	0.9	
16		37.6	41.7	1.1	
17		17.1	19.3	1.1	
18		7.4	13.3	1.8	
19		5.6	3.2	0.6	
20		14.2	184.7	13.0	
21		151.7	>378	2.5	12,17,20
22		126.2	104.8	0.8	20

[a] *Trypanosoma brucei rhodesiense* STIB900. Reference drug: melarsoprol, IC_{50} = 0.005 μM.
[b] Rat skeletal myoblast L6-cells. Reference drug: podophyllotoxin, IC_{50} = 0.019 μM.
[c] Bibliographic reference related to other antiparasitic activities of the compound.

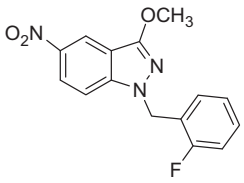
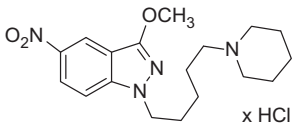
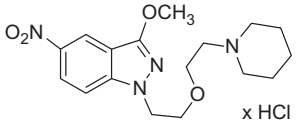
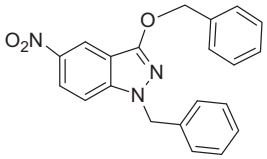
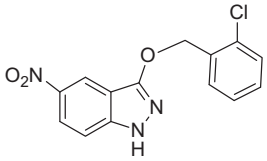
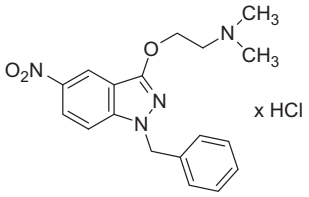
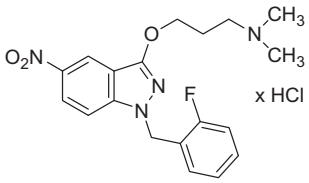
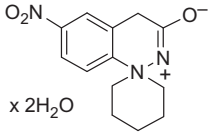
In the last years, the public-private partnership research efforts of the Drugs for Neglected Diseases initiative (DNDi) have been rewarded by the discovery of two potential drug candidates (the nitroimidazole fexinidazole and the oxaborole SCYX-7158, Fig. 1) which have entered phase I clinical development for both acute and chronic sleeping sickness.[4,5] These were discovered by screening chemical libraries of compounds against African trypanosomes. The fact that a nitroimidazole derivative, fexinidazole, is the most promising new drug candidate for the oral treatment of both stages of HAT, despite the inherent caution usually associated with nitroheterocycles as drug candidates, led us to consider our library of nitroheterocycles as a possible source of new hits against African trypanosomes. In fact, some of our nitroheterocycles and related compounds have already shown interesting in vivo and/or in vitro activity against the protozoa *Trypanosoma cruzi*, etiological agent of American trypanosomiasis (Chagas' disease),[6–13] *Trichomonas vaginalis*[13–18] and *Leishmania* spp.;[10] other compounds were effective inhibitors of biocrystallization of ferriprotoporphyrin IX (heme) to hemozoin and thus, potential antimalarial agents.[19,20] Besides, it should be kept in mind that the nitroheterocycles benznidazole and nifurtimox (Fig. 1) are currently the only drugs available for the treatment of American trypanosomiasis which emphasizes the importance of this class of chemical structures as antiprotozoal agents.

Table 2
In vitro activity of indazol-3-ol (**23**–**39**) and cinnolin-3-ol (**40**) derivatives against *T. brucei rhodesiense*

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
23		>494	>494	1.0	
24		192.1	>382	2.0	17
25		312.6	>517	1.7	
26		194.3	>370	1.9	17,20
27		191.0	222.3	1.2	17,20
28		63.9	55.4	0.9	17,20
29		146.2	216.7	1.5	15
30		168.1	210.3	1.3	17
31		109.3	157.5	1.4	
32		63.3	>370	>5.8	17,20

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
33		190.8	>331.9	1.7	
34		5.5	61.9	11.2	10,13
35		11.8	137.1	11.6	10,13
36		123.5	151.6	1.2	20
37		73.4	30.5	0.4	12,17
38		10.8	57.6	5.3	
39		5.2	32.0	6.1	
40		198.1	323.6	1.6	

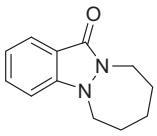
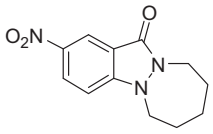
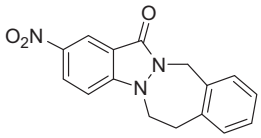
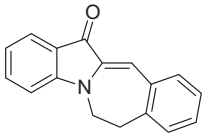
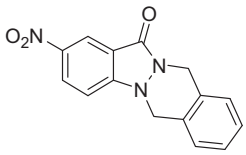
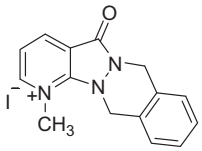
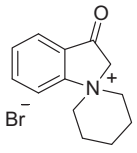
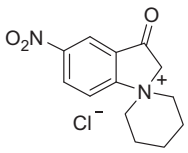
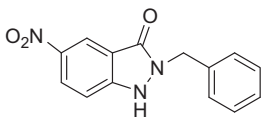
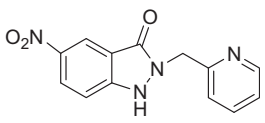
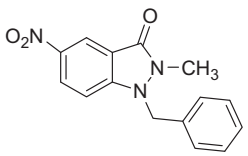
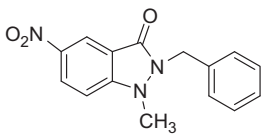
[a] *Trypanosoma brucei rhodesiense* STIB900. Reference drug: melarsoprol, IC_{50} = 0.005 μM.
[b] Rat skeletal myoblast L6-cells. Reference drug: podophyllotoxin, IC_{50} = 0.019 μM.
[c] Bibliographic reference related to other antiparasitic activities of the compound.

We report here the in vitro screening against *T. b. rhodesiense* (strain STIB900) of a selection of 76 nitro-derivatives of nitrogenated heterocycles (e.g., nitrocinnolines, nitroindoles, nitroindazoles and nitroquinoxalinones) and related compounds[13,19,21–31] using the Alamar blue growth inhibition assay.[32] Their cytotoxicity against mammalian L6-cells was also determined in vitro. This allowed the calculation of a selectivity index defined as: SI = IC_{50} (L6-cells) / IC_{50} (*T. b. r.*). This screening revealed some SAR among the series and a few hit compounds showing interesting activity and fair selectivity. These hits could be used as starting points for a medicinal chemistry optimization program.

Compounds from the hydrazide series (Table 1) had IC_{50} values against *T. brucei* in the range 5.5 to >400 μM. Seven compounds showed IC_{50} < 50 μM (**3**, **11**, **16**, **17**–**20**) but only three of those (**3**, **11**, and **20**) were selective for the parasite with SI of 8.3, 13.7, and 13, respectively. Of note is the effect of replacing the 6-halogen atom (Cl, F) by an ethoxy group in **20**; this reduced significantly the cytotoxicity (13- to 57-fold) with only a two-fold decrease in anti-trypanosomal activity with respect to **18** and **19**.

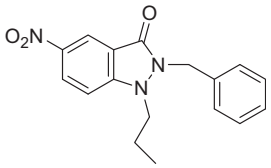
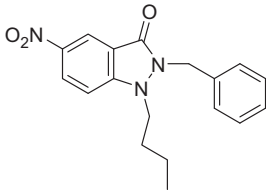
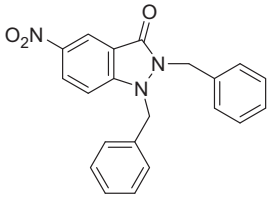
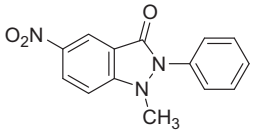
5-Nitroindazolol derivatives with free OH group (Table 2) were only weakly active against *T. b. rhodesiense* (IC_{50} >63 μM). The best results, with IC_{50} values in the range 5–12 μM and SI = 5.3–11.6,

Table 3
In vitro activity of indazolin-3-one (**41**–**43**, **45**, **49**-**56**) and indolin-3-one (**44**, **47**, **48**) derivatives and related compounds (**46**) against *T. brucei rhodesiense*

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
41		427.2	>494	1.2	12,17,20
42		208.7	>404	1.9	17,20
43		86.4	>338	>4	
44		74.4	86.5	1.2	12,17,20
45		66.8	222.2	3.3	
46		241.0	>263	1.1	
47		197.7	268.6	1.4	12,17,20
48		212.9	84.9	0.4	17
49		162.6	233.6	1.4	17
50		252.0	286.5	1.1	17
51		162.7	165.5	1.0	12
52		8.2	43.4	5.3	12

(*continued on next page*)

Table 3 (*continued*)

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
53		20.0	77.4	3.9	
54		48.9	46.7	1.0	
55		49.5	37.3	0.8	
56		138.1	66.5	0.5	

[a] *Trypanosoma brucei rhodesiense* STIB900. Reference drug: melarsoprol, IC_{50} = 0.005 μM.
[b] Rat skeletal myoblast L6-cells. Reference drug: podophyllotoxin, IC_{50} = 0.019 μM.
[c] Bibliographic reference related to other antiparasitic activities of the compound.

were obtained with compounds bearing a basic amino side chain such as 5-piperidinopentyl (**34**), 2-(2-piperidinoethoxy)ethyl (**35**), 2-(dimethylamino)ethyl (**38**), and 3-(dimethylamino)propyl.

Among indazolinone derivatives (Table 3), only four compounds (i.e., those holding a benzyl group on the *N*2 nitrogen) had IC_{50} <50 μM (**52**–**55**) although with low selectivity (SI = 0.8–5.3). Compound **52** with *N*1-methyl-*N*2-benzyl substituents was the most active and selective of this series with IC_{50} = 8.2 μM and SI = 5.3. It is worth noting that increasing the size of the *N*1-substituent decreased significantly the activity of the compound in the order: methyl > propyl > butyl ≈ benzyl (i.e., **52**–**55**). The regioisomer with *N*2-methyl-*N*1-benzyl substituents (**51**) was 20-times less active than **52**.

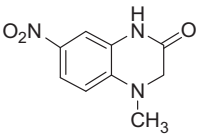
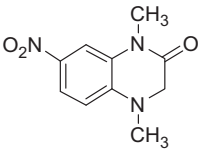
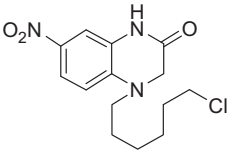
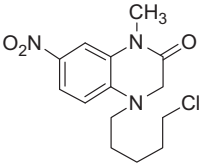
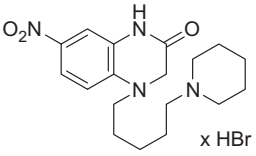
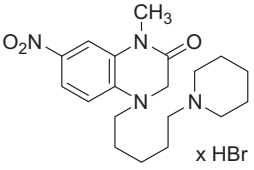
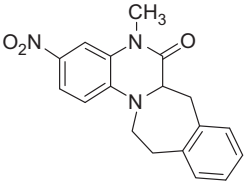
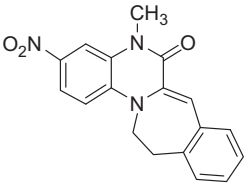
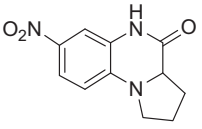
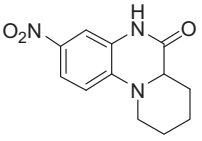
The quinoxalin-2-one scaffold gave the best antitrypanosomal compounds with 6 molecules (**57**, **58**, **59**, **60**, **62**, **65**) having IC_{50} values <15 μM and SI values in the range 3.6–39.7 (Table 4). The *N*1,*N*4-dimethyl compound **58** and the fused pyrrolidine derivative **65** were the most active compounds of this screening (IC_{50} = 3.6 and 2.7 μM, respectively). Methylation of the *N*1-nitrogen generally increased the activity by approximately 2-fold (compare **57** vs **58**, **61** vs **62**, and **69** vs **70**) except for compounds **66** and **67** where the opposite effect was observed. This substitution was generally accompagnied by an increase in cytotoxicity. Five and six methylene chloroalkyl chains at *N*4 also gave low IC_{50} values (7.5 and 6.9 μM for **59** and **60**, respectively) and fair selectivities (13.1 and 6.4, respectively). On the contrary, fused bulky rings such as piperidine (**66**–**68**), isoquinoline (**69**, **70**), or benzo[*d*]azepine (**63**, **64**) gave mostly cytotoxic compounds (i.e., SI <1).

The bisnitroindazole derivatives were poorly active against *T. brucei* (Table 5). The sole compound with interesting activity was the urea **76** which was one of the best hits of this study with IC_{50} = 7.1 μM and SI >23.6.

The discovery of fexinidazole as a potential clinical candidate for the oral treatment of both stages of sleeping sickness has led, in the last years, to a renewed interest in nitroheterocyclic compounds as possible antitrypanosomal agents. In this study, we have taken advantage of our compound library, historically rich in nitroheterocyclic compounds, and selected 76 molecules from different groups to be tested against *T. brucei* (i.e., nitrobenzohydrazide, indazolol, indazolinone, quinoxalin-2-one, bisindazolols, etc.). From the data collected in this study, the SAR for anti-*T. brucei* activity is limited even though a few significant trends could be found for the quinoxalin-2-one and indazole series. In particular, the bulk around the *N*1-position of the molecule seems to be rather restricted. Thus, a small methyl substituent is allowed (**57**, **58**) as well as fused pyrrolidine ring (**65**). Long alkylic chains are also favored (**59**, **60**) probably because they are quite flexible and can stay away from the steric restricted area around *N*1. On the contrary, bulkier fused substituents at *N*1 are detrimental to the activity. Important SAR results are depicted in Figure 2.

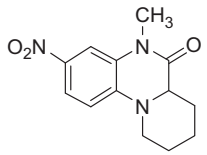
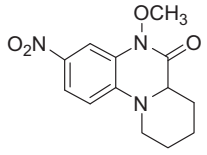
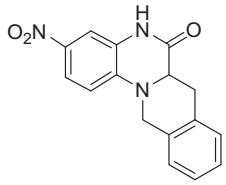
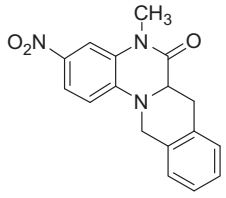
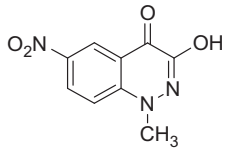
Interestingly, several of the hits identified in this screening are also lead compounds against *T. cruzi* or *T. vaginalis*. On the one hand, **58** showed in vitro activity against epimastigotes and intracellular amastigotes of *T. cruzi*, as well as in vivo suppressive activity by oral route in a murine model of acute *T. cruzi* infection.[11] In the same study, Vega et al. showed that **65** was active but more cytotoxic that **58**. Hence, despite the moderate selectivity for *T. brucei* over rat L6-cells (21.6-fold) found in our screening, **58** seems to have adequate physicochemical properties for oral bioavailability and may well be an interesting lead compound worth investigating as anti-*T. brucei* agent. On the other hand, **34** was reported to be very active against *T. vaginalis* whilst its activity against *T. cruzi* was moderate.[13] However, the 2-(2-piperidinoethoxy)ethyl analogue **35** showed similar in vitro anti-*T. cruzi* efficacy

Table 4
In vitro activity of quinoxalin-2-one (**57–70**) and cinnolin-4-one (**71**) derivatives against *T. brucei rhodesiense*

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
57		10.6	419.4	39.7	6,16
58		3.6	78.2	21.6	11,16
59		7.5	97.8	13.1	11,16,20
60		6.9	44.3	6.4	11,16,20
61		21.2	97.1	4.6	7,18
62		14.6	52.3	3.6	7,18
63		63.4	15.1	0.2	
64		191.1	>311	1.6	
65		2.7	46.7	17.4	11,16
66		38.0	113.7	3.0	11,16

(*continued on next page*)

Table 4 (*continued*)

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
67		153.5	101.8	0.7	
68		121.5	67.4	0.6	
69		123.3	21.8	0.2	11,16
70		61.1	18.7	0.3	
71		255.4	>452	1.8	17,20

[a] *Trypanosoma brucei rhodesiense* STIB900. Reference drug: melarsoprol, IC_{50} = 0.005 μM
[b] Rat skeletal myoblast L6-cells. Reference drug: podophyllotoxin, IC_{50} = 0.019 μM
[c] Bibliographic reference related to other antiparasitic activities of the compound.

Table 5
In vitro activity of bisindazole derivatives (**72**–**75**) and 1,3-disubstituted urea **76** against *T. brucei rhodesiense*

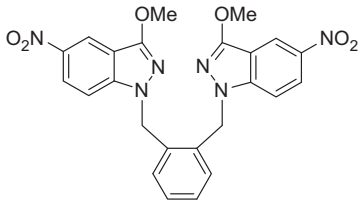
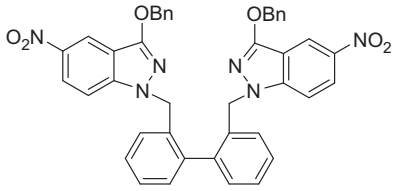
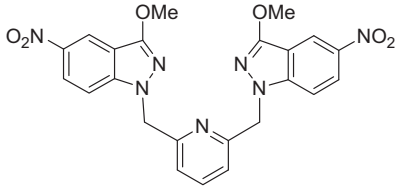
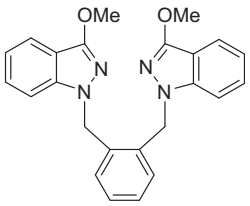
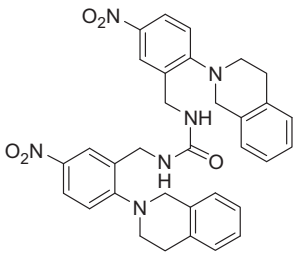
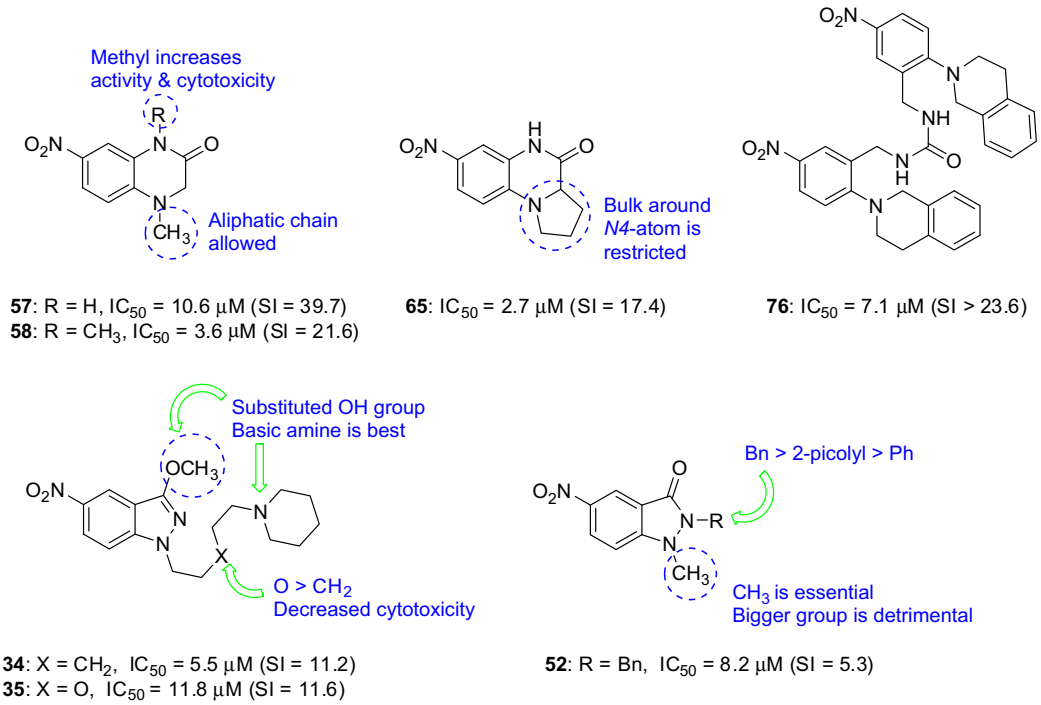
Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
72		119.2	>204	1.7	19
73		80.5	>139	1.7	19
74		57.6	151.0	2.6	19

Table 5 (*continued*)

Compound	Structure	*T. b. r.*[a]	Cytotoxicity[b]	SI	Ref.[c]
		IC_{50} (μM)			
75		100.6	44.7	0.4	19
76		7.1	>168	**>23.6**	17

[a] *Trypanosoma brucei rhodesiense* STIB900. Reference drug: melarsoprol, IC_{50} = 0.005 μM.
[b] Rat skeletal myoblast L6-cells. Reference drug: podophyllotoxin, IC_{50} = 0.019 μM.
[c] Bibliographic reference related to other antiparasitic activities of the compound.



57: R = H, IC_{50} = 10.6 μM (SI = 39.7)
58: R = CH_3, IC_{50} = 3.6 μM (SI = 21.6)

65: IC_{50} = 2.7 μM (SI = 17.4)

76: IC_{50} = 7.1 μM (SI > 23.6)

34: X = CH_2, IC_{50} = 5.5 μM (SI = 11.2)
35: X = O, IC_{50} = 11.8 μM (SI = 11.6)

52: R = Bn, IC_{50} = 8.2 μM (SI = 5.3)

Figure 2. Summary of the hit compounds active against *T. b. rhodesiense* and selective over L6-cells. Important SAR features for anti-*T. brucei* activity are highlighted.

with reduced cytotoxicity compared to **34**;[10] this finding is confirmed here as **34** has 2-fold higher cytotoxicity against L6-cells than **35**. Besides, Boiani et al. have shown that **35** was active in vivo by oral administration in a murine model of acute Chagas' infection indicating adequate bioavailability for this compound.[10] These data indicate that **35** may also be a good trypanocidal lead for HAT.

At this stage, the target and mode of action of these compounds against African trypanosomes remain unknown. In *T. cruzi*, the action of this kind of molecules seems to be associated with the production of reduced species of the 5-NO_2 moiety similarly to what happens with benznidazole[8,9] or nifurtimox.[33] Thus, the antitrypanosomal action of these compounds, like fexinidazole, could possibly involve bioreductive activation by parasite type I nitroreductase leading to reactive intermediates that would provoke cellular damage.[5,34,35] Hall & Wilkinson have shown recently that the bioactivation of benznidazole by *T. brucei* type I nitroreductase generates the reactive dialdehyde glioxal which in turn leads to glyoxal-guanosine adducts. The authors suggest that the trypanocidal activity of benznidazole is the result of DNA damage by these metabolites.[36] However, the nitroheterocycles reported here cannot be metabolized to glioxal by reductive activation. This means that other mechanism of action (or metabolites) may be involved. This hypothesis will need experimental confirmation.

Altogether, the results of our in vitro screening against *T. brucei* take their importance in the light of the previous data of antiprotozoal activity reported for those compounds and related nitroimidazoles. For instance, the lead compound fexinidazole, which is currently in clinical trials for HAT, has IC_{50} values in the range 1.71–2.93 μM against *T. b. rhodesiense* STIB900 in the same assay.[5] Even though fexinidazole selectivity (SI >322) is higher than that of our compounds, some of the hits presented here could be good starting points for an optimization program directed towards the discovery of new antitrypanosomal agents for the treatment of sleeping sickness. This is the case, in particular, of the 7-nitroquinoxalin-2-one **58** and the 5-nitroindazole **35** that have demonstrated low micromolar activity and in vivo bioavailability in mice .

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