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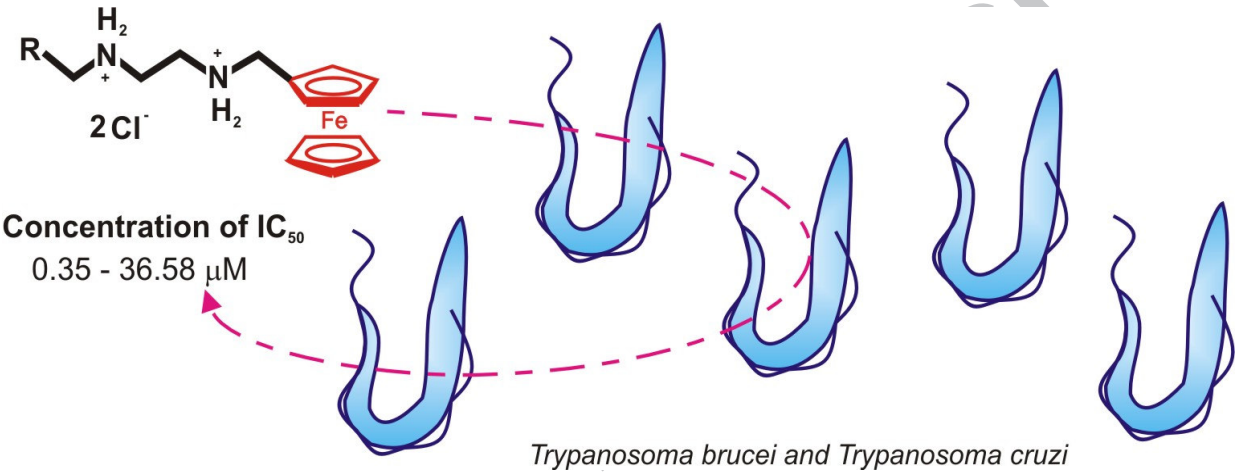

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Synthesis and tripanocidal activity of ferrocenyl and benzyl diamines against *Trypanosoma brucei* and *Trypanosoma cruzi*

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ABSTRACT

Trypanosoma brucei and *Trypanosoma cruzi* are the etiologic agents of sleeping sickness and Chagas disease, respectively, two of the 17 preventable tropical infectious diseases (NTD) which have been neglected by governments and organizations working in the health sector, as well as pharmaceutical industries. High toxicity and resistance are problems of the conventional drugs employed against trypanosomiasis, hence the need for the development of new drugs with trypanocidal activity. In this work we have evaluated the trypanocidal activity of a series of *N1*,*N2*-dibenzylethane-1,2-diamine hydrochlorides (benzyl diamines) and *N1*-benzyl,*N2*-methyferrocenylethane-1,2-diamine hydrochlorides (ferrocenyl diamines) against *T. brucei* and *T. cruzi* parasite strains. We show that incorporation of the ferrocenyl group into the benzyl diamines increases the trypanocidal activity. The molecules exhibit potential trypanocidal activity *in vitro* against all parasite strains. Cytotoxicity assay was also carried out to evaluate the toxicity in HepG2 cells.

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The *Trypanosomatidae* family, flagellated parasitic protozoa, is responsible for important infectious diseases in humans: sleeping sickness, Chagas diseases and leishmaniasis. Cost, toxicity and resistance problems of conventional drugs point to an urgent need to identify and develop new therapeutic alternatives. Chagas disease is a zoonosis that spans the Great Lakes in North America to the southeast of Patagonia[1,2] and occurs widely in Latin America[1]. The infection can be readily transmitted by blood transfusion and migration from endemic areas. The causative agent is *T. cruzi*[3]. According to the World Health Organization, WHO, an estimated 10 million people are infected worldwide, mostly in Latin America and more than 25 million people are at risk of the disease. It is estimated that in 2008 Chagas disease killed over 10.000 people[4]. The only prescription drugs in medical practice to treat Chagas disease are nifurtimox and benznidazole[5,6]. These medicines are curative only in acute or early chronic infections, and in congenital cases[7]. Benznidazole is more effective than nifurtimox in suppressing parasitemia during the chronic phase[8], but neither is effective in curing the disease at this stage[9]. Both drugs have significant side effects, probably as a consequence of reducing oxidative damage in tissues or hosts[10].

Sleeping sickness is caused by *T. b. gambiense* and *T. b. rhodesiense*, subspecies related to *T. b. brucei*, which is found in domestic animals and related to the disease called nagana[11]. WHO reports that *T. b. gambiense* accounts for 95% of reported cases of sleeping sickness[12]. The estimated number of actual cases is currently 30.000. The symptoms of sleeping sickness can be varied, but two clinical phases can be recognized during infection: an early hemolymphatic stage and a late meningoencephalitic stage. Treatment of the first phase of sleeping sickness relies on suramin and pentamidine and in the late phase, melarsoprol is active against *T. b. gambiense* and *T. b. rhodesiense*, whereas eflornithine is useful only against *T. b. gambiense*. Melarsoprol is a highly toxic arsenic-based drug, which can lead to patient death. A new combination therapy with the oral drugs nifurtimox and eflornithine is more effective when compared to nifurtimox and eflornithine monotherapies, but causes several side effects[13]. In addition, eflornithine reduces the number of leukocytes, erythrocytes and platelets[14]. These drugs are mostly antiquated, scarce, highly toxic and subject of parasite resistance[13-17]. Clearly there is an urgent need to develop new drugs to treat these diseases.

Polyamines are present in all parasitic protozoa, and in recent years, these compounds and their associated enzymes have been investigated as drug targets in search of novel parasitic therapy options[18]. A large number of *N*-alkylated polyamine analogs have been described which selectively interfere with *T. cruzi* polyamine metabolism[19]. Furthermore, in a series of recent reports, Yamanaka et al.[20] (2013) provided evidence that simpler lipophilic alkyl diamines can be leading molecules for the development of new antiparasitic drugs. They described the *in vitro* activity of several 1,2-ethane, 1,4-butane and 1,6-hexanediamine hydrochlorides against *T. cruzi*, *L. braziliensis* and *L. chagasi* and showed that in the case of *T. cruzi* the mitochondria is a target for the active compounds[21]. Differently, diaryl diamines [2-(3-(substituted-benzylamino)propylamino)-quinolin-4(1H)-ones] were shown to inhibit *T. brucei* and *T. cruzi* growth by selectively inhibiting their methionyl-tRNA synthetase[21].

Incorporation of transition metal fragments to the structure of a drug often enhances its activity[22-24]. Of special interest in this work is the ferrocenyl (Fc) group[25], which is derived from ferrocene [bis(η^5-cyclopentadienyl)iron(II)], a non-toxic and stable organometallic compound[26]. Incorporation of the Fc group into standard drugs offers new possibilities in therapeutic applications and reversal of drug resistances[27]. *E.g.* introduction of Fc into the lateral chain of chloroquine yielded ferroquine[28], with excellent *in vitro* and *in vivo* activity against malaria parasites - *Plasmodium spp.* and particularly good activity against chloroquine-resistant ones[29,30]. Furthermore substitution of a phenyl group in tamoxifen with Fc resulted in improved cytotoxic activity in breast cancer[27,31,32], and modification of ethambutol with Fc groups led to improved antimicobacterial activity (*Mycobacterium tuberculosis*)[28].

Herein we describe the synthesis of several *N1,N2*-dibenzylethane-1,2-diamine hydrochlorides (benzyl diamines) and *N1*-benzyl,*N2*-methyferrocenylethane-1,2-diamine hydrochlorides (ferrocenyl diamines) and the evaluation of their trypanocidal activity (Table 1). Compounds **2-10** (Figure 1) have been obtained as stable solids (yields ranging from 66 to 80%); their structures are fully supported by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. Compounds **2a-10a** (Figure 1), described previously, have been synthesized according to the literature[33-35]. Synthesis and full spectroscopic data are presented in the Supplementary Information.

The compounds were tested against *T. brucei* and *T. cruzi* strains using MTT colorimetric assay to evaluate the trypanocidal activity[36,37]. Pentamidine and benznidazole were included in the assays as positive controls for *T. brucei* and *T. cruzi*, respectively. MTT assays were carried out after 24 h (*T. brucei*) and 72 h (*T. cruzi)* incubation. The methodology is detailed in the supplementary information. A cytotoxicity assay was also carried out to evaluate the toxicity of different concentrations of the compounds in HepG2 cells. This hepatoma cell line is used as a model to simulate the hepatic function of the human organism *in vitro*, as it exhibits similar metabolic functions to the human liver[38,39].

The benzyl diamines showed higher trypanocidal effect in *T. brucei* strains than in *T. cruzi* Y (see Table 1), in accord with a previous report on *T. cruzi* strains being less responsive than *T. brucei* to this type of compounds[20]. Especially for *T. cruzi* Y the nature of the substituent strongly influenced the IC_{50} values. Compound **3a** bearing a 4-OMe group on the phenyl rings is by far the most active compound against *T. cruzi* Y (IC_{50} = 10.9 µM) and also the least toxic one of the series, with an S.I. value of about 90, higher than both pentamidine and benznidazole. It was also the most active compound of the series against both strains of *T. brucei*, with IC_{50} values comparable to that of pentamidine (8.25 and 8.63 *vs.* 6.43 µM) and lower mammalian toxicity.

Upon substitution of one of the benzyl or pyridyl groups in the structures of the benzyl diamines **2a**, **3a**, **5a**, **7a**, **8a** and **10a** for an Fc group to generate the corresponding ferrocenyl diamines **2**, **3**, **5**, **7**, **8** and **10**, respectively (Figure 1), IC_{50} values decreased considerably in *T. cruzi* (Table 1), demonstrating that the presence of the Fc group leads to increased trypanocidal activity to *T. cruzi*. In fact ferrocenyl diamines exhibited higher tripanocidal activity than the corresponding benzyl diamines on both parasites (*T. brucei* 427, *T. brucei* 29-13 and *T. cruzi* Y) which might be associated to increased lipophilicity and/or differences in electronic (redox properties) and geometric structure[29,30]. Compounds **3** (IC_{50} = 0.36 µM) and **6** (IC_{50} = 0.35 µM) were especially active against *T. brucei* 427; compound **3** (IC_{50} = 0.82 µM), against *T. brucei* 29-13 and compound **4** (IC_{50} = 2.21 µM), against *T. cruzi* Y. All ferrocenyl diamines exhibited higher mammalian toxicity compared to the corresponding benzyl diamines.

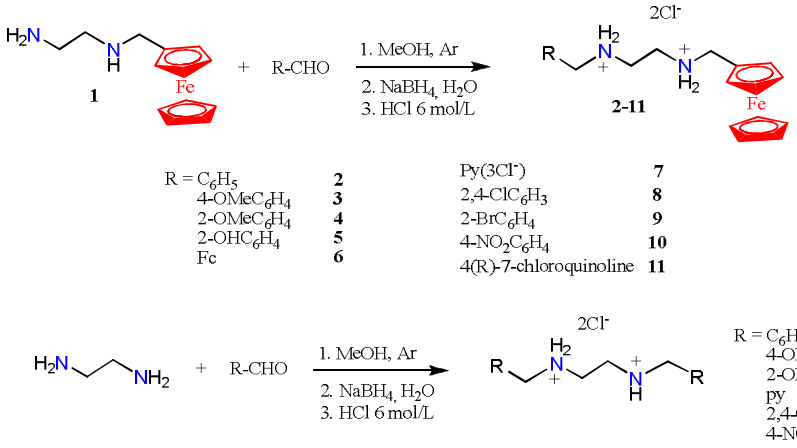

Figure 1. Synthesis of benzyl and ferrocenyl diamines.

Table 1. Antiparasitic activities and safety index of benzyl diamines and ferrocenyl diamines.

	Compound	R	HepG2 cells [a]	*T. brucei* 427 [a]	S.I. [b] *T. brucei* 427	*T. brucei* 29-13 [a]	S.I. [b] *T. brucei* 29-13	*T. cruzi* Y [a]	S.I. [b] *T. cruzi* Y
Ferrocenyl diamines	**2**	C_6H_5	30.99	0.51	60.76	4.04	7.67	6.03	5.14
	3	4-$OMeC_6H_4$	18.60	0.36	51.67	0.82	22.68	4.23	4.40
	4	2-$OMeC_6H_4$	32.26	1.78	18.12	7.69	4.20	2.21	14.60
	5	2-OHC_6H_4	34.49	1.27	27.16	5.33	6.47	9.58	3.60
	6	Fc	9.20	0.35	26.29	4.81	1.91	5.59	1.65
	7	Py($3Cl^-$)	85.45	7.46	11.45	10	8.55	3.74	22.85
	8	2.4-ClC_6H_3	21.94	0.45	48.76	4.58	4.79	3.98	5.51
	9	2-BrC_6H_4	26.20	0.41	63.90	1.13	23.19	3.37	7.77
	10	4-$NO_2C_6H_4$	22.68	2.28	9.95	3.56	6.37	6.07	3.74
	11	4(R)-7-chloroquinoline	20.31	10.42	1.95	6.48	3.13	12.84	1.58
Benzyl diamines	**2a**	C_6H_5	223.74	11.08	20.19	9.99	22.40	159.61	1.40
	3a	4-$OMeC_6H_4$	990.62	8.25	120.08	8.63	114.79	10.9	90.88
	5a	2-$OMeC_6H_4$	148.21	4.61	32.15	5.16	28.72	108.9	1.36
	7a	py	86.91	13.88	6.26	14.11	6.16	258.97	0.34
	8a	2.4-ClC_6H_3	280.57	12.35	22.72	16.78	16.72	51.44	5.45
	10a	4-$NO_2C_6H_4$	590.18	36.58	16.13	19.22	30.71	122.01	4.84
	Pentamidine	---	159.71	6.43	24.84	6.43	24.84	4.58	34.87
	Benznidazole	---	2126.16	207.57	10.24	149.58	14.21	34.00	62.53

[a] IC_{50} (μM): half maximal inhibitory concentration

[b] S.I.: Safety index. OBS: The underlined S.I. values indicate promising compounds, i.e. those with low mammalian toxicity and high toxicity to parasites.

Although pentamidine and other diamidines, such as furamidine, exhibit high potency, they display poor oral bioavailability and unfavorable side effects[40]. These dicationic compounds have been studied primarily against sleeping sickness and few have been assayed as anti-*T. cruzi* candidates[41]. Nevertheless, in this work, pentamidine was active against the epimastigote forms of *T. cruzi* Y strain (IC_{50} = 4.58 μM).

In their study, de Almeida et al.[20] evaluated the *in vitro* trypanocidal activity of several lipophilic diamines against *T. cruzi* epimastigote (IC_{50} = 11.9–15.1 μM) and amastigote forms (IC_{50} = 1.6–23.6 μM), and observed mitochondrial depolarization in *T. cruzi* epimastigote forms treated with the most active compounds of the series, which suggested that this mechanism may be involved in the trypanocidal effect.

In biological systems ferrocene is oxidized by H_2O_2 in the presence of peroxidases to the ferrocenium cation, which forms charge-transfer complexes with protein donor groups[42]. For parasite survival and growth, trypanosomatids possess a defense mechanism against oxidative stress. Superoxide dismutase (SOD), old yellow enzyme (OYE) and peroxiredoxine (Prx) are involved in various mitochondrial activities in certain life cycle stages of trypanosomatids[16]. The ferrocenyl group could therefore interfere with the defense mechanisms of the parasites.

According to the IC_{50} values in HepG2 cells (Table 1) the ferrocenyl amines showed lower toxic activity on mammalian cells, as compared with their toxicity against parasites, which is very interesting for new drugs evaluation.

These ferrocenyl diamines could therefore be potential trypanocidal drug candidates. Studies on the mechanism of action of the ferrocenyl diamines against both *T. cruzi* and *T. brucei* are underway, and so are the tests with other *T. cruzi* strains, due to genetic variability, and with the *T. cruzi* amastigote form and the bloodstream forms of *T. brucei*.

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Supplementary Information

Supplementary information associated with this article can be found, in the online version, at doi:

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