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Synthesis and antiparasitic and antifungal evaluation of 2′-arylsubstituted-1*H*, 1′*H*-[2,5′]bisbenzimidazolyl-5-carboxamidines

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ABSTRACT

A series of 2′-arylsubstituted-1*H*,1′*H*-[2,5′]-bisbenzimidazolyl-5-carboxamidines were prepared in a six-step process starting from 4-amino-3-nitrobenzonitrile. The antiparasitic activity against *Trypanosoma brucei rhodesiense* (*T.b.r.*), *Plasmodium falciparum* (*P.f.*), *Leishmania donovani* (*L.d.*) and *Trypanosoma cruzi* (*T.c.*) and antifungal activity against *Candida albicans* and *Candida krusei* were evaluated in vitro. Several compounds showed promising in vitro activity against *T.b.r.*, *P.f.* and *C. albicans* and had superior activity against *P.f.* as compared to chloroquine.

1. Introduction

At least 1 billion people – one sixth of the world's population suffer from one or several neglected tropical diseases (NTDs) according to the WHO reports. Among them malaria, African (sleeping sickness) and American (Chagas disease) trypanosomiasis and leishmaniasis are widespread in tropical and subtropical areas of the world. Furthermore, these parasitic infections are frequently associated with immunocompromised patients and are thus seen also in developed countries [1].

Malaria, the most prevalent and most pernicious protozoan parasitic disease of humans is estimated to kill between one and two million people, mainly children, each year [2,3]. It is widely distributed throughout the tropic and transmitted by certain species of anopheline mosquitoes.

Four species of *Plasmodium* commonly infect humans, but particularly two of them, *Plasmodium falciparum* and *Plasmodium vivax* account for the majority of instances of morbidity and mortality [2,3]. Sleeping sickness is caused by the parasites *Trypanosoma brucei rhodesiense* (*T.b.r.*) and *T. brucei gambiense* (*T.b.g.*). More than 50 million women, men and children in over 25 countries of sub-Saharan Africa are at risk and approximately 40,000 die each year [4]. The disease is fatal if untreated.

In South America, a different trypanosome, *Trypanosoma cruzi*, causes Chagas disease [5,6]. This parasite is transmitted by blood-sucking reduviid bugs. *T. cruzi* infection is an important cause of mortality and morbidity, no vaccines or safe and effective chemotherapeutic agents are avaliable. Leishmaniasis is an another parasitic disease spread by the bite of infected sand flies [7,8]. There are several different forms of leishmaniasis. The most common forms are cutaneous leishmaniasis, which causes skin sores, and visceral leishmaniasis, which affects internal organs of the body such as spleen, liver, bone marrow. Leishmaniasis is found in 88 countries, most of them in tropical and subtropical areas [7,8].

New safe and effective drugs against these parasitic infections are desperately needed. Many of them currently in use for the treatment have major problems such as significant toxicity, variable efficacy, lack of oral bioavailability, and the need for parenteral administration [9,10]. Furthermore, drug resistance is becoming an increasing problem [9–12]. The ideal drug should target a biochemical pathway in the parasite which is not present in the human host.

Aromatic diamidines have shown promise as effective agents for the treatment of parasitic infections [9,10]. Among them pentamidine is the only compound from this class which has been significantly used in humans for the treatment of early stage human African trypanosomiasis (HAT), antimony-resistant leishmaniasis and AIDS-related *Pneumocystis jiroveci* pneumonia. Another bis-amidine

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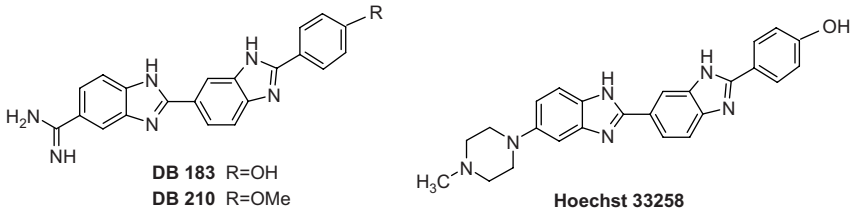
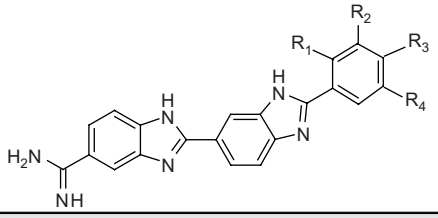
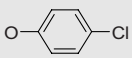
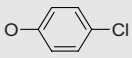
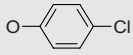
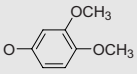


Fig. 1. DB183, DB210 and Hoechst 33258.

derivative, Furamidine, also exhibits very potent activities against some parasites and its methamidoxime prodrug, Pafuramidine, is currently undergoing Phase III clinical trials against HAT [13]. This class of compounds binds to the minor-groove of DNA at AT-rich sites. Binding to DNA does not directly kill the parasite, but causes inhibition of DNA dependant enzymes or direct inhibition of transcription [14]. Recently, aromatic diguanidine types of molecules [15–19] as bioisosters of amidines were reported to exhibit significant antiprotozoal activity as well. In addition, it has already been reported that bisbenzimidazole-amidine systems, such as in compounds DB183, DB210 [20,21] (Fig. 1) with potent antifungal activity against *Candida albicans* and *Crytococcus neoformans*, provide a very favorable and flexible DNA recognition module. Benzimidazoles, for example, form the core of Hoechst 33258 (H258) [20,21] one of the most

Table 1
Formulas and in vitro antiparasitic and antifungal activities of **9–24**.



					Antiparasitic activity		Antifungal activity		Cytotoxicity
					IC_{50} (μg/ml)		MIC (μg/ml)		IC_{50} (μg/ml)
No	R_1	R_2	R_3	R_4	*T.b.r.*[a]	*P.f.*[a]	*C.a.*	*C.k.*	L-6[a]
9	F				0.08	0.007	12.5	12.5	26.8
10			F		0.04	0.010	25	12.5	2.48
11		F	F		0.33	0.015	25	25	29.8
12			$C(CH_3)_3$		1.06	0.031	1.56	12.5	13
13	OH	$C(CH_3)_3$			ND	0.211	3.12	6.25	13.4
14	Cl			CF_3	ND	0.064	NT	NT	31.8
15		CF_3		CF_3	ND	0.630	50	25	76.8
16		OCH_3		OCH_3	0.59	0.017	50	50	25.5
17		OCH_3	OCH_3		0.06	0.008	25	25	>90
18			OCH_2Ph		0.81	0.017	12.5	25	17.4
19		OCH_3	OCH_2Ph		0.57	0.023	25	50	19.8
20		OCH_2Ph	OCH_2Ph		1.06	0.061	25	50	>90
21					ND	0.193	50	50	>90
22				F	ND	0.634	50	50	>90
23					0.708	0.028	25	25	46
24					0.036	0.015	3.12	6.25	29.4
25					0.48	0.016	0.78	12.5	8.72
26					0.389	0.016	6.25	25	34.7
DB183			OH		0.017	0.013	0.78[b]	NT	4.2
DB210			OCH_3		0.009	0.015	>100[b]	NT	10.0
Mel.					0.003				
Chl.						0.086			
Flu.							0.78	25	
Pod.									0.007

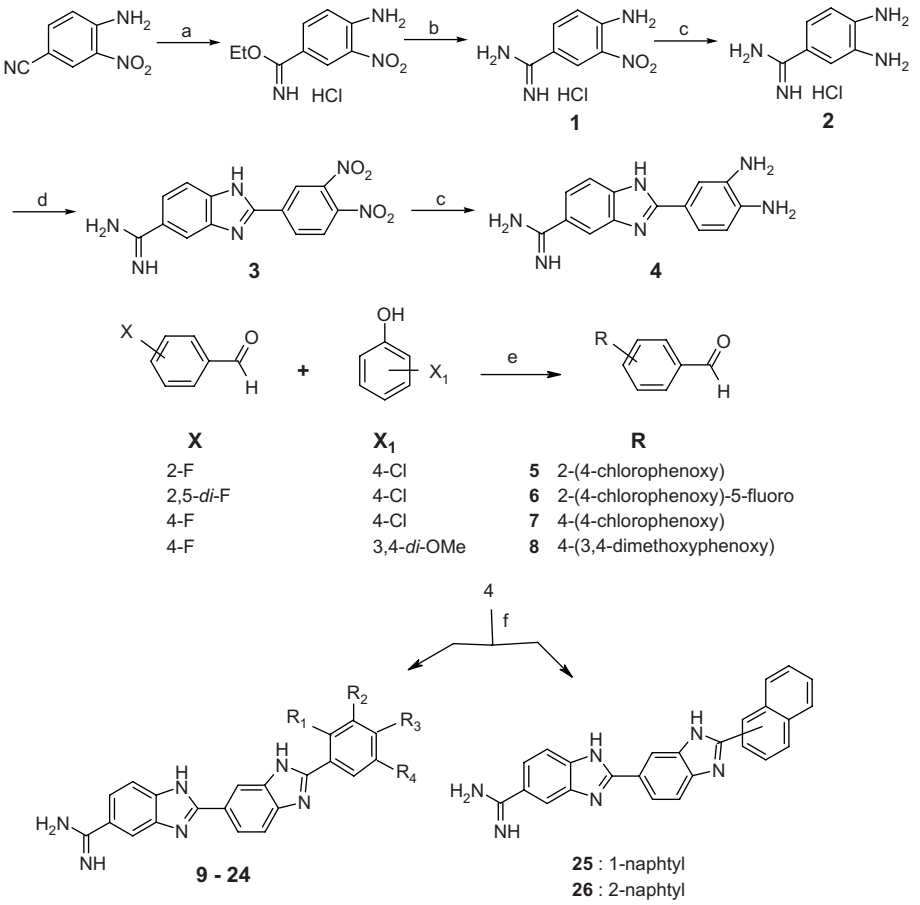
[a] Activities represent the mean of at least two independent experiments; IC_{50} values used to calculate the average for a given compound were within a factor of two. *T.b.r.*: *Trypanosoma brucei rhodesiense* STIB 900, *P.f.*: *Plasmodium falciparum* K1, *C.a.*: *Candida albicans* (ATCC 10231), *C.k.*: *Candida krusei* (ATTC 6258), Mel: Melarsoprol, Chl: Chloroquine, Flu: Fluconazole, Pod: Podophyllotoxin.

[b] These data were taken from lit. [20], NT: not tested, ND: not determined since activity criteria were not fulfilled in the first screening.

Table 2
Formulas, spectroscopic data, m.p. and yields of **9**–**26**.

Compound	m.p. (°C)	Yield (%)	Formula Calculated Found	^{1}H NMR, δ ppm (DMSO-d_6, Me_4Si) (if not stated otherwise)	^{13}C NMR, δ ppm (DMSO-d_6) (if not stated otherwise)	MS (ESI+) m/z
9	>300	24	$C_{21}H_{15}FN_6 \cdot 3HCl \cdot 2.66H_2O$ C: 47.80, H: 4.45, N: 15.93 C: 48.06, H: 4.82, N: 15.40	(Base) 7.37–7.47 (m, 2H), 7.54–7.62 (m, 2H), 7.70 (d, 1H, $J_o = 8.4$), 7.76 (d, 1H, $J_o = 8.4$), 8.11 (s,1H), 8.17 (d, 1H, $J_o = 8.4$), 8.25 (t, 1H, $J = 8$), 8.52 (s, 1H)	(Base) 115.01, 115.28, 116.4, 116.55, 117.14, 117.35, 118.59, 118.7, 121.61, 121.90, 122.44, 125.1, 125.78, 131.09, 132.80, 132.88, 140.26, 141.2, 141.44, 144.32, 149.05, 156.9, 158.98, 161.47, 166.80.	371 (100)
10	>300	23	$C_{21}H_{15}FN_6 \cdot 3HCl \cdot 3H_2O$ C: 47.25, H: 4.53, N: 15.74 C: 47.57, H: 4.73, N: 15.11	(Base) 7.39 (t, 2H, $J = 8.4$), 7.52 (d, 1H, $J_o = 8.4$), 7.60 (d, 1H, $J_o = 8$), 7.66 (d, 1H, $J_o = 8.4$), 8.04 (s, 1H), 8.12 (d, 1H, $J_o = 8.4$ Hz), 8.26 (t, 2H, $J = 8$), 8.42 (s, 1H)		371 (100)
11	>300	27	$C_{21}H_{14}F_2N_6 \cdot 3HCl \cdot 3.33H_2O$ C: 45.22, H: 4.28, N: 15.07 C: 45.12, H: 4.43, N: 14.81	(CD_3OD) 7.66 (q, 1H, $J = 8.4$), 7.96 (dd, 1H, $J_o = 8.8$, $J_m = 1.6$), 8.02–8.09 (m, 3H), 8.16–8.21 (m, 1H), 8.30–8.33 (m, 2H), 8.67 (d, 1H, $J_m = 0.8$)		389 (100)
12	>300	41	$C_{25}H_{24}N_6 \cdot 3HCl \cdot 3.5H_2O$ C: 51.69, H: 5.90, N: 14.47 C: 51.52, H: 5.67, N: 14.34	(Base) 1.390 (s, 9H), 7.52–7.60 (m, 4H), 7.66 (d, 1H, $J_o = 8.8$), 8.07 (s, 1H), 8.17 (d, 3H, $J = 8.8$), 8.48 (s, 1H)	(Base) 31.67, 35.25, 113.82, 115.17, 115.81, 115.98, 120.39, 122.00, 122.12, 126.35, 126.89, 127.10, 128.46, 140.55, 142.21, 143.25, 146.51, 153.22, 153.90, 159.43, 166.28	409 (100)
13	>300	50	$C_{25}H_{24}N_6O \cdot 2HCl \cdot 2.5H_2O$ C: 55.35, H: 5.76, N: 15.49 C: 55.50, H: 5.75, N: 15.38	(CD_3OD) 1.40 (s, 9H), 6.84 (t, 1H, $J_o = 8$), 7.32 (dd, 1H, $J_o = 7.6$, $J_m = 1.2$), 7.65 (dd, 1H, $J_o = 7.6$, $J_m = 1.2$), 7.8–7.84 (m, 2H), 7.89(d, 1H, $J_o = 8.4$), 7.98 (dd, 1H, $J_o = 8$, $J_m = 2$), 8.2 (d, 1H, $J_m = 0.8$), 8.38 (d, 1H, $J_m = 1.2$)	(CD_3OD): 28.75, 34.83, 111.83, 114.44, 114.65, 115.32, 115.89, 116.42, 118.10, 123.04, 124.54, 125.61, 126.06, 130.09, 132.11, 135.58, 138.37, 153.25, 156.13, 158.05, 166.51	425 (100)
14	>300	21	$C_{22}H_{14}ClF_3N_6 \cdot 2.5HCl \cdot 3.3H_2O$ C: 43.64, H: 3.85, N: 13.88 C: 43.69, H: 4.20, N: 13.60	(Base) 7.56 (d, 1H, $J_o = 8.4$), 7.65 (d, 1H, $J_o = 8.8$), 7.70 (d, 1H, $J_o = 8.8$), 7.79–7.85 (m, 2H), 8.06–8.08 (2H), 8.52 (d, 1H, $J_m = 1.2$), 8.48 (s, 1H)		455 (100), 457 (33)
15	>300	17	$C_{23}H_{14}F_6N_6 \cdot 3HCl \cdot 1.66H_2O$ C: 44.01, H: 3.26, N: 13.38 C: 44.10, H: 3.62, N: 12.98	(Base) 7.61 (dd, 1H, $J_o = 8.2$, $J_m = 1.8$), 7.66 (d, 1H, $J_o = 8.4$), 7.71 (d, 1H, $J_o = 8.8$), 7.96 (d, 1H, $J_o = 8.8$), 8.07–8.08 (2H), 8.41 (s, 1H), 8.83 (s, 2H)		489 (100)
16	286–290	22	$C_{23}H_{20}N_6O_2 \cdot 3HCl \cdot 2.5H_2O$ C: 48.73, H: 4.98, N: 14.83 C: 48.92, H: 4.94, N: 14.58	3.91 (s, 6H), 6.82 (s, 1H), 7.72 (s, 2H), 7.80 (d, 1H, $J_o = 8.4$), 7.92 (d, 1H, $J_o = 8.4$), 8.02 (d, 1H, $J_o = 8.4$), 8.25 (s, 1H), 8.47 (d, 1H, $J_o = 8.8$), 8.81 (s, 1H), 9.17 (s, 2H), 9.51 (s, 2H)		413 (100)
17	295–300	24	$C_{23}H_{20}N_6O_2 \cdot 3HCl \cdot 2H_2O$ C: 49.52, H: 4.88, N: 15.07 C: 49.56, H: 4.98, N: 15.05	3.76 (s, 3H), 3.81 (s, 3H), 7.16 (d, 1H, $J_o = 8.8$), 7.64 (dd, 1H, $J_o = 8.6$, $J_m = 1.2$), 7.75 (d,1H, $J_o = 8.8$), 7.86 (d, 1H, $J_o = 8.4$), 7.99 (dd, 1H, $J_o = 8.8$, $J_m = 2$), 8.08 (d, 1H, $J_m = 1.6$), 8.10 (s, 1H), 8.33 (dd, 1H, $J_o = 8.6$, $J_m = 1.2$), 8.61 (s, 1H), 9.03 (s, 2H), 9.35 (s, 2H)		413 (100)
18	270–275 bubb	20	$C_{28}H_{22}N_6O \cdot 3HCl \cdot 4H_2O \cdot 0.5C_2H_6O$ C: 52.54, H: 5.47, N: 12.68 C: 52.56, H: 5.37, N: 12.54	5.27 (s, 2H), 7.35–7.5 (m, 7H), 7.77 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz), 7.89 (d, 1H, $J_o = 8.4$), 7.99 (d, 1H, $J_o = 8.8$ Hz), 8.23 (s, 1H), 8.45–8.49 (m, 3H), 8.77 (s, 1H), 9.18 (s, 2H), 9.50 (s, 2H)		459 (100)
19	265–270	31	$C_{29}H_{24}N_6O_2 \cdot 2HCl \cdot 3.3H_2O$ C: 56.10, H: 5.29, N: 13.54 C: 56.09, H: 5.22, N: 13.27	3.82 (s, 3H), 5.09 (s, 2H), 7.20–7.34 (m, 6H), 7.62 (d, 1H, $J_o = 8.8$), 7.71 (d, 1H, $J_o = 8.8$), 7.82 (d, 1H, $J_o = 8.4$), 7.96 (d, 1H, $J_o = 8.8$), 8.08–8.09 (2H), 8.31 (d, 1H, $J_o = 8.8$), 8.57 (s, 1H), 9.07 (s, 2H), 9.37(s, 2H)	57.09, 70.79, 112.32, 113.68, 114.18, 115.19, 115.42, 116.18, 116.54, 122.95, 123.40, 124.02, 124.49, 125.46, 128.68, 128.81, 129.21, 133.55, 135.30, 136.98, 137.55, 140.66, 150.11, 151.66, 152.78, 153.45, 166.53	489 (100)
20	>300	33	$C_{35}H_{28}N_6O_2 \cdot 4HCl \cdot 3.3H_2O$ C: 54.60, H: 5.05, N: 10.92 C: 54.69, H: 5.31, N: 11.19	(Base) 5.21 (s, 2H), 5.23 (s, 2H), 7.25 (d, 1H, $J_o = 8.8$), 7.33–7.35 (m, 2H), 7.38–7.42 (m, 4H), 7.47–7.54 (m, 5H), 7.6 (d, 1H, $J_o = 8.4$), 7.65 (d, 1H, $J_o = 8$), 7.8 (dd, 1H, $J_o = 8.4$, $J_m = 1.6$), 7.97 (d, 1H, $J_m = 1.2$), 8.06 (s, 1H), 8.14 (dd, 1H, $J_o = 7.8$, $J_m = 1.4$), 8.43 (s, 1H)		565 (100)

21	290–295 bubb	28	$C_{27}H_{19}ClN_6O\cdot 3HCl\cdot 3H_2O$ C: 50.48, H: 4.39, N: 13.08 C: 50.78, H: 4.59, N: 13.11	(Base) 7.01 (d, 1H, $J_o = 8$ Hz), 7.18–7.20 (m, 2H), 7.35(td, 1H, $J_o = 7.6$, $J_m = 1.2$),7.47–7.56 (m, 4H), 7.62 (d, 1H, $J_o = 8.8$), 7.71 (d, 1H, $J_o = 8.8$), 8.06 (d, 1H, $J_m = 1.2$), 8.15 (dd, 1H, $J_o = 8$, $J_m = 1.6$), 8.36 (dd, 1H, $J_o = 7.6$, $J_m = 2$), 8.51 (d, 1H, $J_m = 1.2$)	(Base) 115.24, 116.12, 119.34, 120.10, 121.74, 121.92, 122.09, 122.25, 124.77, 126.28, 128.69, 130.57, 131.56, 132.32, 142.43, 144.25, 145.53, 145.60, 150.29, 155.00, 155.52, 158.31, 166.42	479 (100), 481 (35)
22	288–296 bubb	42	$C_{27}H_{18}ClFN_6O\cdot 2HCl\cdot 3.3H_2O$ C: 51.53, H: 4.26, N: 13.35 C: 51.56, H: 4.18, N: 13.20	(Base) 7.10 (dd, 1H, $J_o = 8.8$, $J = 4.8$), 7.15–7.18 (m, 2H), 7.33–7.38 (m, 1H), 7.44–7.46 (m, 2H), 7.56 (dd, 1H, $J_o = 8$, $J_m = 1.6$), 7.63 (d, 1H, $J_o = 8$), 7.71 (d, 1H, $J_o = 8.8$), 8.08–8.11 (m, 2H), 8.15 (dd, 1H, $J_o = 8.6$, $J_m = 1.6$), 8.51 (s, 1H)	(Base) 114.87, 115.26, 116.12, 116.33, 117, 117.25, 118.71, 118.93, 120.87, 121.53, 121.77, 122.03, 122.10, 122.36, 123.91, 124.00, 126.48, 128.48, 130.51, 140.26, 141.29, 142.57, 145.7, 149.45, 150.93, 156.01, 157.60, 158.46, 159.9, 166.60	497 (100), 499 (33)
23	>300	38	$C_{27}H_{19}ClN_6O\cdot 3HCl\cdot 2H_2O$ C: 51.94, H: 4.20, N: 13.46 C: 51.73, H: 4.50, N: 13.14	(Base) 7.09–7.14 (m, 4H), 7.44 (d, 2H, $J_o = 8$), 7.52 (d, 1H, $J_o = 8.4$), 7.59 (d, 1H, $J_o = 8.4$), 7.64 (d, 1H, $J_o = 8$), 8.06 (s, 1H), 8.12 (d, 1H, $J_o = 8.4$), 8.23 (d, 2H, $J_o = 8.8$), 8.44 (s, 1H)	(Base) 114.03, 115.17, 116.02, 119.27, 120.81, 121.66, 121.84, 121.95, 126.12, 126.73, 128.56, 129.32, 130.73, 140.84, 142.49, 142.71, 145.90, 153.71, 155.60, 158.55, 158.83, 160.88, 166.52	479 (100), 481 (34)
24	295–300 bubb	28	$C_{29}H_{24}N_6O_3\cdot 3HCl\cdot 2H_2O$ C: 53.59, H: 4.81, N: 12.93 C: 53.47, H: 4.99, N: 12.68	3.78 (s, 3H), 3.80 (s, 3H), 6.74 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$), 6.88 (d, 1H, $J_m = 2.8$), 7.05 (d, 1H, $J_o = 8.8$), 7.23 (d, 2H, $J_o = 8.4$), 7.80 (dd, 1H, $J_o = 8.8$, $J_m = 1.6$), 7.91 (d, 1H, $J_o = 8.8$), 8.06 (d, 1H, $J_o = 8.8$), 8.25 (d, 1H, $J_m = 0.8$), 8.45–8.49 (m, 3H), 8.79 (d, 1H, $J_m = 0.8$), 9.19 (s, 2H), 9.5 (s, 2H)		505 (100)
25	>300	22	$C_{25}H_{18}N_6\cdot 3HCl\cdot 3.5H_2O$ C: 52.23, H: 4.91, N: 14.62 C: 52.38, H: 4.99, N: 14.55	(CD_3OD): 7.65–7.76 (m, 3H), 7.95 (dd, 1H, $J_o = 8.4$, $J_m = 1.2$), 8.03–8.10 (m, 3H), 8.19 (d, 2H, $J_o = 8.4$), 8.27 (d, 1H, $J_o = 8.4$), 8.33 (d, 1H, $J_m = 0.8$), 8.43 (dd, 1H, $J_o = 8.4$, $J_m = 1.2$), 8.81 (s, 1H), 9.01 (s), 9.50 (s)	(CD_3OD): 115.14, 115.21, 115.37, 116.04, 120.37, 121.26, 123.77, 125.29, 125.75, 126.16, 126.31, 127.60, 129.03, 129.20, 130.32, 130.89, 132.98, 133.04, 134.13, 134.34, 135.70, 136.34, 152.18, 152.95, 166.72	403 (100)
26	>300	31	$C_{25}H_{18}N_6\cdot 3HCl\cdot 2.5H_2O$ C: 53.92, H: 4.71, N: 15.09 C: 53.96, H: 4.86, N: 14.74	(Base) 7.51–7.61 (m,4H), 7.70 (d, 1H, $J_o = 8.4$), 7.95–8.06 (m, 4H), 8.16 (dd, 1H, $J_o = 8.8$, $J_m = 1.2$), 8.36 (dd, 1H, $J_o = 8.8$, $J_m = 1.6$), 8.48 (s, 1H), 8.77 (s, 1H)		403 (100)



Scheme 1. Reagents, a: HCl(g)/EtOH, b: NH_3(g)/EtOH, c: H_2/PdC, d: 3,4-dinitrobenzoic acid/PPA, e: K_2CO_3/DMAC, f: $Na_2S_2O_5$ adduct of compounds **5**–**8** and other aldehydes.

extensively studied minor-groove binding agents, as well as of a variety of other related DNA binding compounds.

These findings prompted us to synthesize new analogues of a series of novel 2′-arylsubstituted-1*H*,1′*H*-[2,5′]bis-benzimidazolyl-5-carboxamidine derivatives in order to develop new antiparasitic and/or antifungal agents.

2. Chemistry

Compounds **9**–**26** (Tables 1 and 2) were prepared using the methods outlined in Scheme 1. The nitrile group of 4-amino-3-nitrobenzonitrile was converted to imidate esters (the reason of the instability they were not characterized), with dry HCl gas in absolute ethanol, by Pinner reaction, and then treatment of imidate ester with NH_3 gas in ethanol gave the amidine **1**. Reduction of nitro group yielded **2**. Compound **3** was obtained by condensation of **2** with 3,4-dinitrobenzoic acide in PPA. Reduction of both nitro groups of **3** afforded **4**. Condensation of 4-chlorophenol with 2-fluorobenz-aldehyde, 4-fluorobenzaldehyde, 2,5-difluorobenzaldehyde and 3,4-dimethoxyphenol with 4-fluorobenzaldehyde yielded the corresponding substituted-phenyloxybenzaldehydes, **5**–**8** in dimethylacetamide. The final compounds **9**–**26** were prepared by condensation of **4** with $Na_2S_2O_5$ adduct of **5**–**8** and other aldehydes [22] in dimethylformamide.

3. Results and discussion

All described amidinobisbenzimidazoles **9**–**26**, were tested in vitro for antiparasitic activity against *T.b. rhodesiense*, *Leishmania donovani*, *P. falciparum* and *T. cruzi*. As a first step, to clarify the antiparasitic effect of the synthesized compounds, % inhibition values were determined at two different concentrations (4.85 μg/mL and 0.81 μg/mL). Activity was defined as ≥50% inhibition at the lower concentration and/or ≥70% inhibition at the higher concentration for *T.b.r.*, *L.d.*, *P.f.* and for *T.c.*, respectively. Subsequently, the IC_{50} values were determined for the compounds which achieved these criteria. None of the compounds fulfilled the activity criteria in the first screening for *L.d.* and *T.c.*, and hence their IC_{50} values were not determined. The IC_{50} values of the selected compounds against *T.b.r.* ranged from 0.036 to 1.06 μg/mL (Table 1). Among them the best activity were obtained with **10** ($IC_{50} = 0.041$ μg/mL) and **24** ($IC_{50} = 0.036$ μg/mL) which are having 4-fluorophenyl and 4-(3,4-dimethoxyphenoxy)phenyl groups at the C-2′ position of the amidinobisbenzimidazole moiety. While compound **17** (having a 3,4-dimethoxyphenyl group at the same position) exhibited good result with an IC_{50} value of 0.06 μg/mL, the inhibitory activity is dramatically decreased in **16** ($IC_{50} = 0.594$ μg/mL) which is having a 3,5-dimethoxyphenyl group at the C-2′ position.

The inhibitory activity of these compounds against *P.f.* is very important, since most of them have superior activity to that of the reference compound chloroquine. The IC_{50} values range from 0.007 to 0.634 μg/mL against *P.f.* with the most active antimalarials being **9**, **10**, **17** with IC_{50} values of 0.007, 0.010 and 0.008 μg/mL, respectively. The antifungal activity of the compounds were tested in vitro against *C. albicans* and *Candida krusei* by the well known tube dilution method. The results are given as MIC values (Minimum Inhibitory Concentration) in Table 1. The data show that, introduction of 1-naphtyl group at C-2′ gives a good profile of antifungal activity (**25**, MIC value of 0.78 μg/mL against *C. albicans*). It is surprising that the introduction of a 2-naphtyl group at the same

position (**26**), lead to a 8-fold reduction in antifungal activity (MIC 6.25 μg/mL). However, similiar structure–activity relationships are also observed for their cytotoxicity. With the exception of **10** and **25**, the compounds do not show cytotoxicity and thus are reasonably selective for *P. falciparum*.

4. Conclusions

This work demonstrates that 2′-arylsubstituted-1*H*,1′*H*-[2,5′]bis-benzimidazolyl-5-carboxamidine derivatives show a good activity profile especially against *P f.* and *C. albicans*. Most of them showed higher activity against *P.f.* that chloroquine and compound **25** exhibited the greatest activity against *C. albicans* comparable to fluconazole. *In vivo* studies of compounds **9**, **10**, **17**, **25** for efficacy in an animal model of infection and the establishment of pharmacokinetic profiles are in progress.

5. Experimental

5.1. General methods

Uncorrected melting points were measured on an Büchi B-540 capillary melting point apparatus. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded employing a Varian Mercury 400 MHz FT spectrometer, chemical shifts (δ) are in ppm relative to TMS, and coupling constats (J) are reported in Hertz. Mass spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC, using ESI(+) method, with C-18 column. Elemental analyses were performed by Leco CHNS-932. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or ethanol of solvation; for compound **18**, proton NMR confirmed the presence of solvent. All chemical and solvents were purchased from Aldrich Chemical Co. or Fischer Scientific. Compounds **1** and **2** were synthesized as described in our previous study [23].

5.1.1. 2-(3,4-Dinitrophenyl)-1H-benzimidazole-5-carboxamidine (**3**)

3,4-Diaminobenzamidine HCl **2**, 0.575 g (30 mmol) and 3,4-dinitrobenzoic acid 0.635 g (30 mmol) in polyphosphoric acid (25 g) was heated to 160 °C for 4 h. After cooling to room temperature, the resultant reaction solution was poured into a mixture of ice and water, and the solution was basified with 40% NaOH solution. The resultant red precipitate was filtered, washed with water; yield 46%; m.p.: >300 °C; ^{1}H NMR δ ppm (DMSO-d_6 + D_2O): 7.36 (dd, 1H, H-6′, J_o = 8.4, J_m = 1.6), 7.59 (d, 1H, H-5′, J_o = 8.4), 8.05 (d, 1H, H-2′, J_m = 1.2), 8.21 (d, 1H, H-7, J_o = 8.4), 8.62 (dd, 1H, H-6, J_o = 8.4, J_m = 2), 8.76 (d, 1H, H-4, J_m = 2), ^{13}C NMR δ ppm (DMSO-d_6 + D_2O): 117.05 (C-5′), 117.83 (C-2′), 118.85 (C-6′), 118.96, 122.82 (C-4), 127.01 (C-7), 130.69 (C-6), 140.22, 143.98, 144.25, 148.40, 153.34, 160.96(C-2), 167.33 (C-amidine), MS (ESI+) m/z: 327 (M + H, 100%).

5.1.2. 2-(3,4-Diaminophenyl)-1H-benzimidazole-5-carboxamidine (**4**)

A solution of 2-(3,4-dinitrophenyl)-1*H*-benzimidazole-5-carboxamidine (**3**) (400 mg, 15 mmol) in EtOH (30 ml) was subjected to hydrogenation using 40 psi of H_2 and 10% Pd-C (0.100 g) until of H_2 uptake ceased. The catalyst was filtered on a bed of Celite, washed with EtOH, and the filtrate was concentrated in vacuo; yield 73%; m.p.: 98–105 °C bubb.; ^{1}H NMR δ ppm (DMSO-d_6): 4.67 (s, 2H, NH_2), 4.99 (s, 2H, NH_2), 6.59(d, 1H, J_o = 8), 7.25 (dd, 1H, J_o = 8.4, J_m = 2), 7.41 (d, 1H, J_m = 2), 7.47 (d, 1H, J_o = 8.4), 7.54 (dd, 1H, J_o = 8.4, J_m = 1.6), 7.9 (s, 1H). MS (ESI+) m/z: 267 (M + H, 100%).

5.1.3. General procedure for synthesis of aryloxybenzaldehydes (**5**–**8**)

A mixture of substituted phenols (10 mmol), fluorobenzaldehyde (10 mmol) and anhydrous K_2CO_3 (10 mmol) in dimethylacetamide (5 ml) were heated for 4 h at 140 °C. The cooled reaction mixture was treated with water (30 mL) and extracted with $CHCl_3$ (60 mL). The organic layer was dried ($MgSO_4$), and evaporated in vacuo. Further purification methods are given in Table 3.

5.1.4. General procedure for synthesis of amidinobisbenzimidazoles (**9**–**26**)

The corresponding benzaldehydes (15 mmol) were dissolved in EtOH (50 ml) and sodium metabisulfite (1.6 g) in H_2O (10 mL) was added in portions. The reaction mixture was stirred vigorously and more EtOH was added. The mixture was kept in a refrigarator for several hours. The precipitate was filtered and dried. The mixture of these salts (1 mmol) and **4** (1 mmol) in DMF (1–2 mL) was heated at 120 °C for 4 h. The reaction mixture was cooled, poured into water and the solid was filtered. Crude product was purified by column chromatography, using the mixture of Chloroform:NH_3(g) saturated Methanol (12:3) solvent system. For the HCl salts of the synthesized compounds, the free bases were dissolved in ethanol and dry HCl gas was passed through the solution.

5.2. Biology

5.2.1. Antifungal activity

Antifungal activity against *C. albicans* and C. *krusei* was determined by the macro-broth dilution [26] assay. The synthesized compounds and reference drugs were dissolved in water or DMSO–water (40%) at a concentration of 400 μg/mL. The concentration was adjusted to 100 μg/mL by 4-fold dilution with culture medium and fungi solution at the first tube. Data was not taken for the initial solution because of the high DMSO concentration (10%).

5.2.2. Antiparasitic activity

In vitro assays with *T.b. rhodesiense* STIB 900, *P. falciparum* K1, *T. cruzi* Tulahen Lac Z C4 and *L. donovani* MHOM-ET/67/L82 were carried out as previously reported [27]. For *T. cruzi* and *L. donovani* a medium

Table 3
Formulas, spectroscopic data, m.p. and yields of aryloxybenzaldehydes **5**–**8**.

Compound	Formula	^{1}H NMR, δ ppm ($CDCl_3$)	m.p. (°C)	Yield (%)	Purification, column chromatography
5	$C_{13}H_9ClO_2$		55 Lit [24] 56.5–57		
6	$C_{13}H_8ClFO_2$	6.91 (dd, 1H, J = 9.2, J = 4), 6.97 (d, 2H, J_o = 8.8), 7.22–7.27 (m, 1H), 7.31 (d, 2H, J_o = 9.2), 7.58 (dd, 1H, J = 8.4, J = 3.6), 10.37 (d, 1H, J = 2.8)	87–88	44	*n*-Hexane:EtOAc (1:1)
7	$C_{13}H_9ClO_2$		47 Lit [25] 47–48.5		
8	$C_{15}H_{14}O_4$	3.84 (s, 3H), 3.89 (s, 3H), 6.65 (m, 2H), 6.85 (d, 1H, J_o = 8.8), 7.01 (d, 2H, J_o = 8.4), 7.8 (d, 2H, J_o = 8.4), 9.90 (s, 1H)	115	41	*n*-Hexane:EtOAc (2:1)

throughput assay was used with only two different concentrations of the synthesized compounds (4.85 and 0.81 μg/mL).

5.2.3. Cytotoxicity studies

Cytotoxicity was evaluated using cultured L-6 rat myoblast cells and an Alamar Blue assay as previously reported [27].

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