Bisguanidine, Bis(2-aminoimidazoline), and Polyamine Derivatives as Potent and Selective Chemotherapeutic Agents against *Trypanosoma brucei rhodesiense*. Synthesis and in Vitro Evaluation

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The in vitro screening for trypanocidal activity against *Trypanosoma brucei rhodesiense* of an in-house library of 62 compounds [i.e. alkane, diphenyl, and azaalkane bisguanidines and bis(2-aminoimidazolines)], which were chosen for their structural similarity to the trypanocidal agents synthalin (1,10-decanediguanidine) and 4,4′-diguanidinodiphenylmethane and the polyamine N^1-(3-amino-propyl)propane-1,3-diamine, respectively, is reported. The original synthetic procedure for the preparation of 21 of these compounds is also reported. Most compounds displayed low micromolar antitrypanosomal activity, with five of them presenting a nanomolar inhibitory action on the parasite: 1,9-nonanediguanidine (**1c**), 1,12-dodecanediguanidine (**1d**), 4,4′-bis[1,3-bis(*tert*-butoxycarbonyl)-2-imidazolidinylimino]diphenylamine (**28a**), 4,4′-bis(4,5-dihydro-1*H*-2-imidazolylamino)diphenylamine (**28b**), and 4,4′-diguanidinodiphenylamine (**32b**). Those molecules that showed an excellent in vitro activity as well as high selectivity for the parasite [e.g. **1c** (IC_{50} = 49 nM; SI > 5294), **28b** (IC_{50} = 69 nM; SI = 3072), **32b** (IC_{50} = 22 nM; SI = 29.5), **41b** (IC_{50} = 118 nM; SI = 881)] represent new antitrypanosomal lead compounds.

Introduction

Sleeping sickness (human African trypanosomiasis, HAT) is caused by two subspecies of African trypanosomes, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense,* responsible for the chronic and acute form of the disease, respectively.[1] Only four drugs are licensed for the treatment of HAT[2] (DFMO, suramin, pentamidine, and melarsoprol), although other drugs such as berenil (usually used against animal trypanosomiasis) or the nitrofuran nifurtimox (registered for use against Chagas' disease) have also proved useful in some limited cases. The actual situation of reemergence of sleeping sickness in sub-Saharan Africa, with an estimated 500 000 infected individuals,[3] and the drawbacks of the current HAT chemotherapy (e.g. toxicity, increasing resistance, parenteral mode of administration, price) make the search for new trypanocidal drugs urgently needed.[4]

Many diamidine, diguanidine, and polyamine compounds have been investigated for their antitrypanosomal activity as far as 65 years ago,[5−11] giving rise, for instance, to the aromatic diamidine drug pentamidine[12] which is still used nowadays for the treatment of early stage *T.b. gambiense* infections. However, this drug is unable to cross the blood−brain barrier in sufficient quantity to treat late-stage cases of HAT.[13] Other aromatic diamidines such as propamidine or berenil are used for antiprotozoal chemotherapy in cattle, and the trypanocidal activity of dicationic derivatives related to pentamidine has been reported as well (Figure 1).[9,14,15]

The polyamine metabolic pathway of trypanosomatid parasites has attracted much attention as drug target for the last 15 years.[16] This research led to the synthesis and evaluation of many polyamine analogues as chemotherapeutic agents against parasitic infections,[17−20] with trypanothione reductase being a particularly targeted enzyme (Figure 1).[21−23]

There is a clear lack of research investment in the field of tropical diseases in comparison to the number of affected population.[24,25] Thus, a reasonable approach for the discovery of new antitrypanosomal lead compounds at a lesser cost is the screening of already available molecules for antiparasitic activity. Hence, a rapid look at the structure of the polyamine, diguanidine, and diamidine compounds reported as antiprotozoal agents in the literature put into evidence the potential as possible trypanocides of a series of bisguanidine and bis(2-aminoimidazoline) compounds previously synthesized in our laboratory for other purposes.[26−28] Moreover, some of the alkanediguanidine [1,8-octanediguanidine (**1b**),[29] 1,12-dodecanediguanidine (**1d**),[6,30,31] bis(guanidinopropyl)amine (**6a**)[32]] and diphenyl compounds [4,4′-diguanidinodiphenylmethane (**31b**), 4,4′-diguanidinodiphenyl sulfone (**33b**)][8] available in our in-house library had been previously reported for their use as either trypanocide, microbicides, or fungicides. Of particular interest was the recent report on *N*,*N*′-bis(4-amidinophenyl)piperazine (Figure 1), which proved to be a very effective antitrypanosomal agent in vivo.[15]

Hence, in the search for new HAT chemotherapy, we decided to carry out an in vitro screening of a total of 62 compounds against the parasite *T. brucei rhodesiense* (Tables 1−5) taken from our in-house library and

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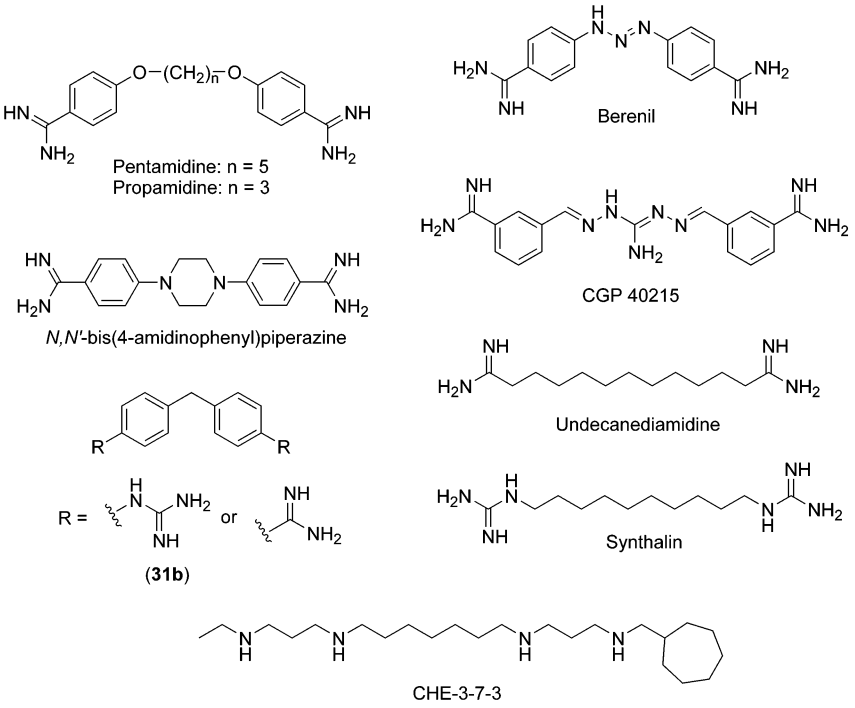
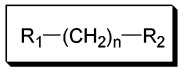


Figure 1. Examples of diamidine, guanidine, and polyamine compounds showing antiparasitic activity.

Table 1. Structure, in Vitro Trypanocidal Activity, and Cytotoxicity of Guanidine and 2-Aminoimidazoline Alkane Derivatives **1a**−**3d**



Compound	n	R_1	R_2	*T. brucei rhodesiense* IC_{50} (μM)[a]	Cytotoxicity L6-cells IC_{50} (μM)	Selectivity[b]
1a	6			8.05	>302	> 37
1b	8			0.251	>276	> 1100
1c	9			0.049	>265	> 5294
1d	12			0.047	0.63	13
2a	6			19.3	-	-
2b	8			0.453	4.2	9.4
2c	9			0.225	16	71
2d	12			0.107	2.35	22
3b	8	H		49.1	-	-
3c	9	H		19.9	-	-
3d	12	H		11.0	19.6	1.8

[a] Controls: melarsoprol, IC_{50} = 5.5 nM (SI = 3456); diminazene diaceturate, IC_{50} = 8.9 nM; CGP 40215, IC_{50} = 4.5 nM (ref 44). [b] Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells/IC_{50} *T.b. rhodesiense*].

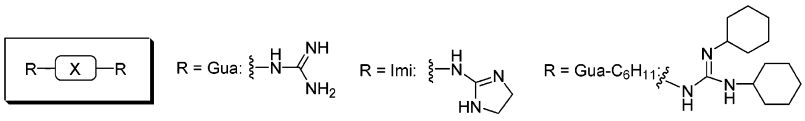
structurally related to the trypanocidal agents synthalin (1,10-decanediguanidine) and 4,4′-diguanidinodiphenylmethane (**31b**) and the polyamine N^1-(3-aminopropyl)-propane-1,3-diamine, respectively. We also describe here the original synthesis of 21 molecules that had not been previously reported.

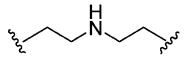
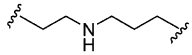
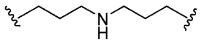
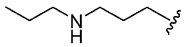
Results

Chemistry. The syntheses of the aliphatic compounds **1a**−**d**, **2a**−**d**, **3b**−**d**, **4a**,**b**, **5a**,**b**,**d**, **6a**,**b**, **7**−**9**, **11**−**13**, **21**−**24** (Tables 1−4) as well as the diphenyl derivatives **27a**−**33a**, **27b**−**33b**, and **34** (Table 5, entries 1−15) have been previously reported by us.[26,27,28]

Synthesis of the 3-Aza-1,6-hexanediamine Derivatives (Scheme 1**)**. Guanylation of 3-aza-1,6-hexanediamine with an excess of DCC in CH_3CN afforded a mixture of the disubstituted dicyclohexylguanidine derivative **5c** and the trisubstituted compound **17**. These products were separated by preparative reverse phase HPLC.

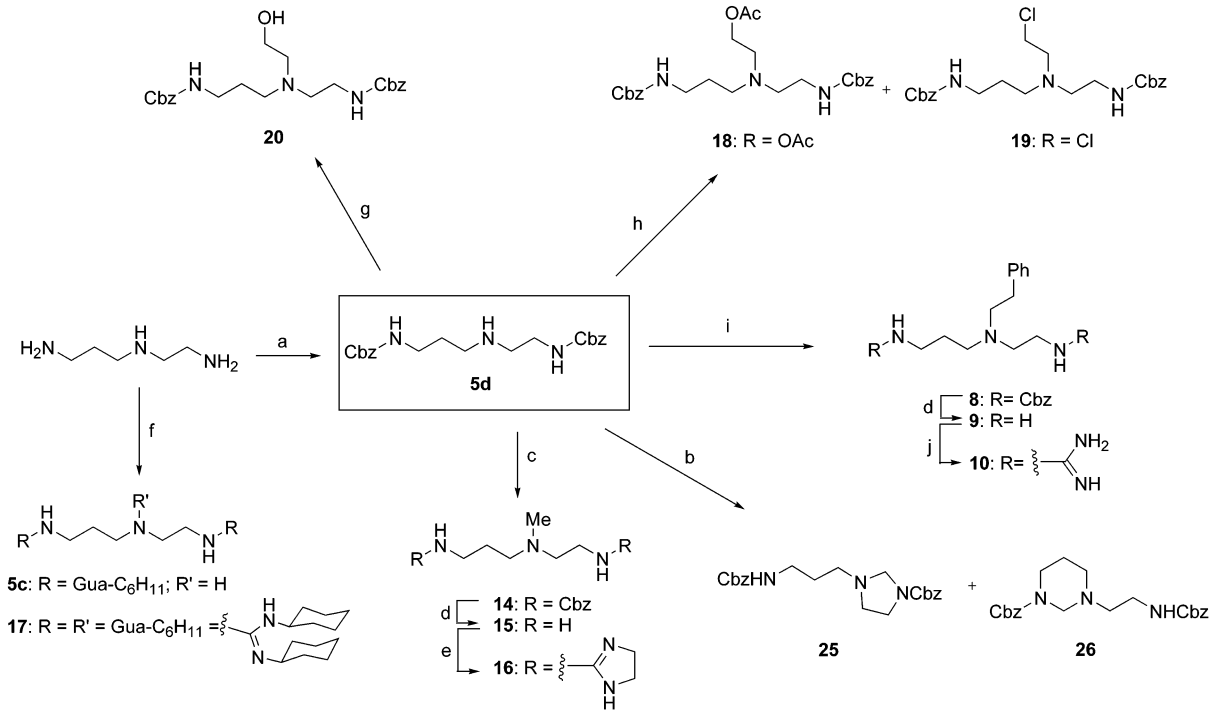
Table 2. Structure, in Vitro Trypanocidal Activity, and Cytotoxicity of Guanidine and 2-Aminoimidazoline Azaalkane Derivatives **4a**−**7**



Compd	R	X	*T. brucei rhodesiense* IC_{50} (μM)[a]	Cytotoxicity L6-cells IC_{50} (μM)
4a	Gua		16.1	-
4b	Imi		84.4	-
5a	Gua		21.4	-
5b	Imi		69.1	-
5c	Gua-C_6H_{11}		2.4	-
6a	Gua		21.2	-
6b	Imi		29.7	-
7	Imi		113.1	-

[a] Controls: melarsoprol, IC_{50} = 5.5 nM (SI = 3456); diminazene diaceturate, IC_{50} = 8.9 nM; CGP 40215, IC_{50} = 4.5 nM (ref 44). [b] Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells/IC_{50} *T.b. rhodesiense*].

Scheme 1[a]



[a] Reagents and conditions: (a) $PhCH_2CO_2CN$ (2 equiv), CH_2Cl_2; (b) HCHO, $NaBH_3CN$, AcOH (pH <7), MeCN; (c) HCHO, $NaBH_3CN$, MeCN (pH >7); (d) H_2, Pd−C 10%, MeOH, 1 M HCl; (e) 2-methylmercapto-4,5-dihydroimidazole iodide, MeOH, reflux; (f) DCC, MeCN, rt; (g) 2-bromoethanol, MeCN, reflux; (h) chloroacetaldehyde (4 equiv), $NaBH(OAc)_3$, AcOH, MeCN, rt; (i) bromoethylbenzene, K_2CO_3, CH_3CN, reflux; (j) *S*-methylisothiouronium sulfate, CH_3CN, reflux

3-Aza-1,6-hexanediamine selectively protected on the primary amino group with benzylcyanoformate[28,33] (**5d**) was used as starting material for the preparation of the 3-substituted derivatives **8**−**10**, **14**−**16**, **18**−**20**, **25**, and **26** (Scheme 1). Alkylation of **5d** with bromoethylbenzene or 2-bromoethanol yielded **8** (83%) and **20** (69%), respectively. Hydrogenolysis of **8** with 10% Pd−C/1 M HCl/MeOH afforded the amine **9**, which was subsequently refluxed with *S*-methylisothiouronium sulfate in CH_3CN, yielding the bis-guanidine **10** (46%). When **5d** was treated with chloroacetaldehyde in excess under reductive conditions ($NaBH(OAc)_3$/AcOH/CH_3CN) at room temperature, the chloroethyl derivative **19** (35%) was obtained together with the acetate side product **18** (16%). In this reaction, the nucleophilic substitution of chloroacetaldehyde (or 2-chloroethanol derived from the reduction in situ of chloroacetaldehyde) by the secondary amine **5d**, leading to a hydroxyethyl intermediate

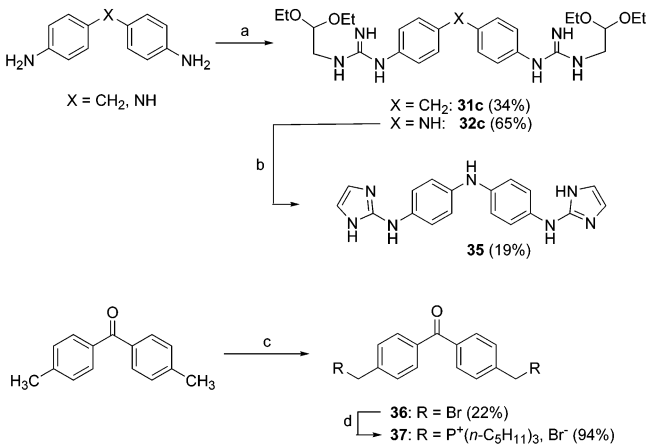
that can react with AcOH present in the reaction medium, is a potentially competitive reaction. Working at low temperature (0 °C) was unfavorable to the competitive nucleophilic substitution, and **19** could be obtained in 69% under these conditions.

An interesting feature was the methylation of **5d** with formaldehyde under reductive conditions ($NaBH_3CN$/CH_3CN).[34] Three main products were isolated depending on the pH of the reaction. Working in basic medium (i.e. pH >7, no AcOH added) yielded the methylated product **14** (51%), whereas acidic medium afforded both five- and six-member ring aminals **25** and **26**, respectively. Structural characterization of both derivatives was carried out by 1D (^{1}H, ^{13}C) and 2D NMR experiments (i.e. HSQC, HMBC). A characteristic difference between the ^{1}H NMR spectra of **25** and **26** were the aminal methylene protons, which appeared as a singlet of two protons for the six-membered ring derivative **26**, whereas two singlets (separated by 14.8 Hz) were observed for the five-membered ring counterpart **25**. This might account for the observation of two conformers of the acyl derivative **25**.[35] Study of the $^3J_{H-C}$ coupling constants between the aminal methylene protons and their neighbors allowed us to characterize both compounds.

Synthesis of the Diphenyl Derivatives (Scheme 2). Guanidines **31c** and **32c** were obtained by reaction of an ether solution of *N*-(2,2′-diethoxyethyl)carbodiimide (prepared from BrCN and aminoacetaldehyde diethyl acetal)[36] with 4,4′-diaminodiphenylamine and 4,4′-diaminodiphenymethane in the presence of CH_3SO_3H, respectively. Preparation of the 2-aminoimidazole **35** was carried out by base-catalyzed cyclization of the guanidine precursor **31c** following the methodology of Munk et al.[36] In this reaction, two cyclization products could potentially form: (1*H*-imidazol-2-yl)arylamine ("endocyclic" amino group) and 1-aryl-1*H*-imidazol-2-ylamine ("exocyclic" amino group). The ^{1}H NMR spectrum of **35** showed a unique broad singlet for the 4-H and 5-H imidazole protons at 6.76 ppm, whereas the decoupled ^{13}C NMR spectrum showed a unique signal for both 4-C and 5-C imidazole carbons, indicating the magnetic equivalence of these atoms. These data demonstrated that the expected product **35** with the endocyclic amino group was obtained (e.g. if the isomer with the exocyclic amino group were obtained, a typical AB system would be observed for the 4-H and 5-H imidazole protons).

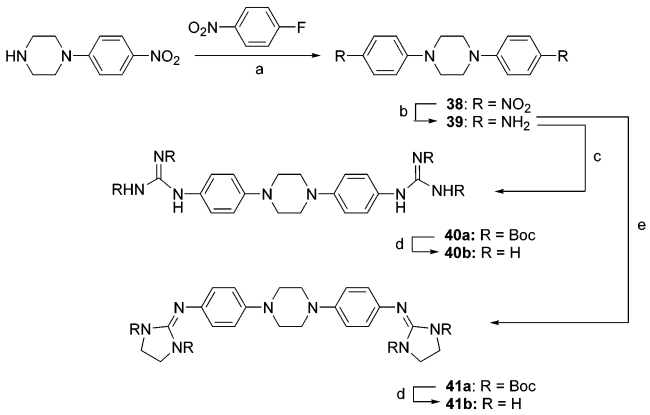
Compound **37** has been previously described[37] as a B_2 bradykinin receptor antagonist. The reported procedure was quite lengthy, so we designed a three-step synthesis starting from 4,4′-dimethylbenzophenone. Radicalar bromination of 4,4′-dimethylbenzophenone with NBS/*t*-BuOOH/CCl_4 allowed the formation of the dibromo compound **36**, which was isolated by crystallization from the reaction mixture (22% yield). The low yield obtained could be explained by the formation of a mixture of mono- and polyhalogenated derivatives (e.g. three spots were observed by TLC of the crude reaction mixture).[38] The bis-phosphonium compound **37** was obtained by nucleophilic substitution of the bromine atoms of **36** with an excess of tri-*n*-pentylphosphine in refluxing toluene. Tri-*n*-pentylphosphine was prepared by a modification of the procedure described by Davies et al.[39] The reaction of an excess of bromopentane Grignard's reagent with phosphorus trichloride working at −78 °C[40] afforded the tri-*n*-pentylphosphine, which was purified by fractional distillation.

Scheme 2[a]



[a] Reagents and conditions: (a) *N*-(2,2′-diethoxyethyl)carbodiimide (2.2 equiv); CH_3SO_3H (2 equiv); EtOH, reflux, 23 h; (b) (1) 6 M HCl, rt, 3 h; (2) 10% NaOH, rt, 1 h; (c) NBS, *t*-BuOOH, CCl_4, reflux; (d) tri-*n*-pentylphosphine (4 equiv); PhMe, reflux, 24 h.

Scheme 3[a]

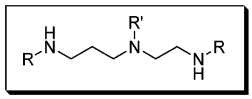


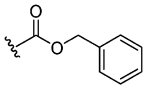
[a] Reagents and conditions: (a) DMSO, 100 °C, 60 h (73%); (b) H_2 (40 psi), 10% Pd−C, HCl, MeOH, rt (59%); (c) *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (2.2 equiv), $HgCl_2$, Et_3N, DMF, 0 °C then rt (73%); (d) TFA, CH_2Cl_2 (88%); (e) *N*,*N*′-bis(*tert*-butoxycarbonyl)imidazoline-2-thione (2.2 equiv), $HgCl_2$, Et_3N, DMF, 0 °C then rt (66%).

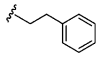
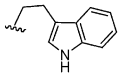
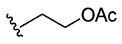
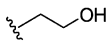
The synthetic approach for the preparation of the piperazine-based bisguanidine and bis(2-aminoimidazoline) compounds is depicted in Scheme 3. Aromatic nucleophilic substitution of 1-fluoro-4-nitrobenzene with commercially available 1-(4-nitrophenyl)piperazine in DMSO at 100 °C afforded **38**.[41] Nitro groups were reduced by catalytic hydrogenation (10% Pd−C/HCl/MeOH), affording the amine **39**.[42] Introduction of the Boc-protected guanidine and imidazoline moieties (compounds **40a** and **41a**, respectively) was carried out in good yield with *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[43] and *N*,*N*′-bis(*tert*-butoxycarbonyl)imidazoline-2-thione,[26] respectively. Removal of the Boc-protecting groups was accomplished by treatment with TFA, affording **40b** and **41b** as their trifluoroacetate salts.

Biological Results. In vitro Antitrypanosomal Activity. The results of the determination of antitrypanosomal activity against bloodstream-form trypomas-

Table 3. Structure, in Vitro Trypanocidal Activity, and Cytotoxicity of 3-Aza-1,6-hexanediamine Derivatives **8**-**20**

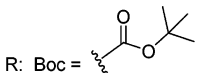


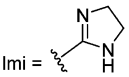
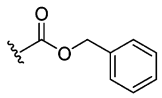
R: Gua =  Imi =  Cbz = 

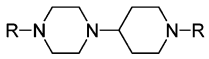
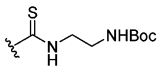
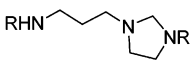
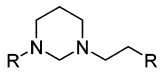
Compd	R	R'	*T. brucei rhodesiense* IC_{50} (μM)[a]	Cytotoxicity L6-cells IC_{50} (μM)	Selectivity[b]
8	Cbz		3.88	24.7	6.4
9	H		17.4	214	12
10	Gua		4.5	>199	> 43
11	Imi		27.6	-	-
12	H		13.8	-	-
13	Cbz		1.0	7.8	7.8
14	Cbz		3.1	130	41
15	H	CH_3	61.8	-	-
16	Imi		46.6	-	-
17	Gua-C_6H_{11}	Gua-C_6H_{11}	0.98	>83	> 82
18	Cbz		7.1	>191	> 26
20	Cbz		14.0	116	8.3

[a] Controls: melarsoprol, IC_{50} = 5.5 nM (SI = 3456); diminazene diaceturate, IC_{50} = 8.9 nM; CGP 40215, IC_{50} = 4.5 nM (ref 44). [b] Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells/IC_{50} *T.b. rhodesiense*].

Table 4. Structure, in Vitro Trypanocidal Activity, and Cytotoxicity of 3-Aza-1,6-hexanediamine Cyclic Derivatives **21**−**26**



Imi =  Cbz = 

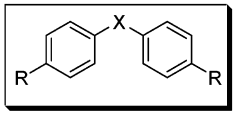
Compd	Structure	R	*T. brucei rhodesiense* IC_{50} (μM)[a]	Cytotoxicity L6-cells IC_{50} (μM)	Selectivity[b]
21		H	57.9	-	-
22		Boc	30.3	-	-
23		Imi	71.2	-	-
24			12.6	-	-
25		Cbz	3.9	106	27
26		Cbz	4.78	89	18.6

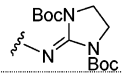
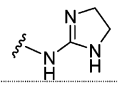
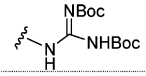
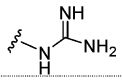
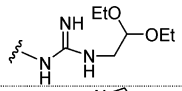
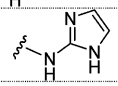
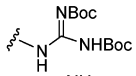
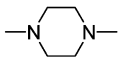
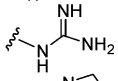
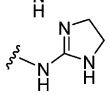
[a] Controls: melarsoprol, IC_{50} = 5.5 nM (SI = 3456); diminazene diaceturate, IC_{50} = 8.9 nM; CGP 40215, IC_{50} = 4.5 nM (ref 44). [b] Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells/IC_{50} *T.b. rhodesiense*].

tigotes of *T.b. rhodesisense* (strain STIB 900) are reported in Tables 1−5. All compounds displayed dose-dependent activities against *T.b. rhodesiense*, with IC_{50} ranging from 0.022 to 113 μM, and were selective for the parasite. Eight aliphatic (**1b**−**d**, **2b**−**d**, **13**, and **17**) and 12 diphenyl derivatives (**27b**, **28a**,**b**, **31b**,**c**, **32a**−**c**, **34**, **37**, **40b**, and **41b**) showed an IC_{50} < 1 μM. Among the latter, five compounds had an IC_{50} in the nanomolar range (**1c**, **1d**, **28a**, **28b**, and **32b**) with a selectivity index (SI) ranging from 13 (**1d**) to more than 5000 (**1c**).

Alkane and Azaalkane Derivatives (Tables 1 and 2). The most potent compound within the alkane (Table 1) and azaalkane (Table 2) derivative series was 1,9-nonanediguanidine (**1c**), with IC_{50} = 49 nM and a remarkable selectivity for the parasite (SI > 5294). In these series, the guanidinium cation gave in general

Table 5. Structure, in Vitro Trypanocidal Activity, and Cytotoxicity of Diphenyl Derivatives **27a**−**41b**



Entry	Compd	R	X	*T. brucei rhodesiense* IC_{50} (μM)[a]	Cytotoxicity L6-cells IC_{50} (μM)	Selectivity[b]
1	**27a**		CH_2	11.6	-	-
2	**28a**		NH	0.048	9.8	202
3	**29a**		CO	7.2	-	-
4	**30a**		SO_2	2.6	56.4	21.7
5	**27b**		CH_2	0.897	63.6	71
6	**28b**		NH	0.069	212	3072
7	**29b**		CO	2.05	>214	> 104
8	**30b**		SO_2	32.4	-	-
9	**31a**		CH_2	31.7	-	-
10	**32a**		NH	0.470	3.7	7.9
11	**33a**		SO_2	24.4	-	-
12	**31b**		CH_2	0.161	2.8	17.4
13	**32b**		NH	0.022	0.65	29.5
14	**33b**		SO_2	4.3	>222	> 51
15	**34**		CO	0.206	2.7	13.1
16	**31c**		CH_2	0.316	>175	> 554
17	**32c**		NH	0.228	>175	> 767
18	**35**		NH	1.4	113	80.7
19	**36**	Br	CO	25.9	>244	> 9.4
20	**37**	$P^+(n\text{-}C_5H_{11})_3$	CO	0.414	11.8	28.5
21	**38**	NO_2		41.8	-	-
22	**39**	NH_2		1.42	>217	> 152
23	**40a**			13.8	-	-
24	**40b**			0.270	46.4	172
25	**41b**			0.118	104	881

[a] Controls: melarsoprol, IC_{50} = 5.5 nM (SI = 3456); diminazene diaceturate, IC_{50} = 8.9 nM; CGP40215, IC_{50} = 4.5 nM (ref 44). [b] Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells/IC_{50} *T.b. rhodesiense*]

more active compounds (about 2−4-fold) than its 2-aminoimidazolinium counterpart (compare **1a**−**d** vs **2a**−**d**). This was also true for the azaalkane series (Table 2, **5a**, **6a** vs **5b**, **6b**). Increasing the chain length of the methylene spacer (n = 6, 8, 9, 12) between either guanidinium or 2-aminoimidazolinium cations tended to increase the activity in the following order: 6 < 8 (ca. 30-fold) < 9 ∼ 12 (Table 1). Regarding the selectivity, the nine-methylene spacer (**1c** and **2c**) gave the best SI (5294 and 71) in both series. Noteworthy was the greater activity displayed by the dicationic derivatives **1b**−**d** and **2b**−**d** with respect to their monocationic counterparts (Table 1). This behavior was also observed for the azaalkane compound **6b**,which was 3.5-fold more active than **7** (Table 2).

Introduction of an unsubstituted nitrogen atom in the methylene chain (e.g. in the azaalkane series, Table 2) tended to reduce the activity compared to alkyl spacer. This is exemplified by the activity of compounds **1a** and **2a** (8 and 19.3 μM, respectively) and their aza-analogues **5a** and **5b** (21.4 and 69.1 μM, respectively). Another interesting result was that of the dicyclohexylguanidine **5c** (IC_{50} = 2.4 μM), which was 9-fold more active than the guanidine analogue **5a** (21.4 μM). This result might reflect better pharmacokinetic properties of the more lipophilic derivative **5c** (i.e. to cross biological membranes).

The dicyclohexylguanidine compound **17** (IC_{50} = 0.98 μM) displayed the best activity and selectivity (SI > 82) of all the 3-aza-1,6-hexanediamine derivatives (Table 3). Again, it appeared that lipophilicity was an important factor for good activity. In this series, substitution of the secondary amino group with a phenethyl, 3-(2-ethyl)indole, or methyl group afforded molecules slightly more active than the parent compound (compare the activities of **10**/**5a**, **11**/**5b**, and **16**/**5b**).[45] In addition, the amines protected with a carbobenzyloxy group (Cbz) were more active than their free amino counterparts (compare **8**/**9**, **12**/**13**, and **14**/**15**). Regarding the effect of the substituent on the secondary nitrogen in the Cbz-protected series, the following results, in order of decreasing activity, were obtained: indole (**13**, 1 μM) >

methyl (**14**, 3.1 μM) ~ phenethyl (**8**, 3.88 μM) > CH_2-CH_2OAc (**18**, 7.1 μM) > CH_2CH_2OH (**20**, 14 μM).

Worth mentioning is the result obtained for the cyclic analogues **25** and **26** (Table 4), which showed the same range of activity as the parent compound **14** (IC_{50} = 3.1 μM, Table 3) but a lower selectivity (SI = 27 and 18.6 respectively, compared to 41 for **14**). This behavior was also observed with the cyclic analogue **23** (71.2 μM), which displayed the same activity as the aliphatic parent **5b** (69.1 μM).

Diphenyl Derivatives (Table 5). In this series of bisguanidine and bis(2-aminoimidazoline) diphenyl analogues (entries 1−17), the best activities were observed for the compounds bearing a guanidinium group (from 3- to 10-fold with respect to the imidazoline analogues). However, the 2-aminoimidazoline derivatives displayed, in general, better selectivity than the guanidine counterparts (compare the activity and selectivity of **31b**/**27b**, **32b**/**28b**, **34**/**29b**, and **32a**/**28a**). In addition, replacement of the guanidine or 2-aminoimidazoline with a 2-aminoimidazole nucleus (compound **35**, entry 18) produced a loss of activity of 20- and 63-fold compared to **28b** and **32b**, respectively. Interestingly, the very lipophilic bis-phosphonium benzophenone derivative **37** showed a trypanocidal activity (IC_{50} = 0.414 μM) similar to that of the bisguanidinium diphenyl ketone **34** (0.206 mM) and a better selectivity (SI = 28.5 versus 13.1).

Notable is the effect of the N-substitution of the imidazoline and guanidine moieties (i.e. Boc, CH_2CH-$(OEt)_2$). Boc protection afforded less active compounds compared to unprotected counterparts (compare **27a**/**27b**, **29a**/**29b**, **31a**/**31b**, **32a**/**32b**, **33a**/**33b**, and **40a**/**40b**) with the exception of **28** and **30**, in which the Boc substituents produced a 1.4- and 12-fold increase in activity respectively (IC_{50} = 0.048 and 2.6 μM, respectively) compared to the free imidazolinium cation (IC_{50} = 0.069 and 32.4 μM, respectively). Moreover, the Boc substituents seemed to give somewhat less selective compounds (SI = 202 and 3072 for **28a** and **28b**, respectively; SI = 7.9 and 29.5 for **32a** and **32b**). On the contrary, the 1,1-diethoxyethane substituent produced a great increase in selectivity, superior to 26- and 32-fold for **31c** and **32c**, respectively, with only a slight loss in activity (2- and 10-fold, respectively) compared to the unsubstituted parent compounds **31b** and **32b**.

Regarding the bridge linking both phenyl rings, the same behavior was observed for the guanidinium and 2-aminoimidazolinium series, i.e., NH ≫ CH_2 > CO > SO_2 in order of decreasing activity (compare **27b**−**30b** and **31b**−**33b**, **34**). When a piperazine moiety was used as bridge between both phenyl rings (Table 5, entries 21−25), the 2-aminoimidazolinium compound **41b** showed the best activity (0.118 μM) and also a 5 times higher selectivity (SI = 881) with respect to the guanidinium analogue (SI = 172).

Discussion

Some of the compounds described in this paper were available in our in-house library. Since few of these molecules had been previously reported in the literature for their anti-trypanosomal activity (e.g. **1d**,[5] **31b**,[8] **33b**[10]), we anticipated that our compounds would display trypanocidal action. Indeed, simple aliphatic diguanidines were potent and selective trypanocides, with **3c** (SI > 5294) being more selective than the control melarsoprol (SI = 3456). The potency of **3c** is to be compared with that of synthalin (1,10-decanediguanidine)[7] or 1,11-undecanediamidine, a trypanocidal drug that proved able to cure mice and rabbits infected with a strain of *T.b. rhodesiense*.[6] The 1,9-nonanediguanidine **3c** could be considered as the bio-isostere of 1,11-undecanediamidine with the supplementary amino groups of the guanidine moieties playing the role of the two supplementary methylene units, thus keeping approximately the same chain length in both molecules.

In these series, the guanidine moiety afforded in general better trypanocidal drugs than the 2-aminoimidazoline one. Moreover, the presence of two cations was required for potent activity, which is in agreement with the results previously obtained by King et al.[6] This assumption could probably be extended to the diphenyl series, according to the previous findings reported for aromatic diamidines and diguanidines,[6,9,10] although this hypothesis was not tested here because monocationic aromatic compounds were not available in our library.

For short methylene chains (n = 5−7), introduction of a secondary nitrogen atom into the alkyl spacer afforded less active molecules, although further substitution of this nitrogen could increase slightly the activity (compounds **8**−**20**). Conformational restriction of the azaalkane molecules did not affect nor increase the trypanocidal action compared to their linear analogues (compounds **23**, **25**, and **26**). The importance of the lipophilicy of these molecules, facilitating drug uptake by the parasite by passive diffusion, was exemplified by the higher antitrypanosomal activity of **5c** with respect to **5a**.

The most interesting results probably came from the diphenyl series with a NH bridge. The bis(2-aminoimidazoline) derivative **28b** was extremely potent (IC_{50} = 69 nM) and also highly selective for the parasite (SI = 3072). The Boc-protected counterpart **28a** had the same range of activity but a lower selectivity index (SI = 202). This result in particular might be relevant because of the higher lipophilicity of the Boc-protected compound **28a**. Late-stage cases of HAT involve central nervous system (CNS) infection and hence require drugs able to cross the blood−brain barrier. However, the Boc-protecting group is probably stable under the conditions of the in vitro assay but potentially could be metabolized in vivo to afford the unprotected derivative **28b**.

Changing the 2-aminoimidazolinium cations for guanidinium ones led to the most active compound of this screening, **32b** (22 nM, SI = 29.5). The nature of the bridge linking both phenyl rings had a clear influence on the trypanocidal action of these compounds. Electron-donating groups such as NH, piperazine, or CH_2 afforded better trypanocides than electron-withdrawing groups such as C=O or SO_2. Such behavior was consistent with the findings of Donkor et al. in the pentamidine congener series, where electron-rich phenyl groups (e.g. phenoxy) afforded better trypanocides than electron-poor phenyl rings (e.g. acetylated aniline or pyridine).[15]

If we compare the different cationic species studied (i.e. guanidinium, 2-aminoimidazolinium, phosphonium), the good activity and selectivity displayed by the bis-phosphonium derivative **37** (IC_{50} = 0.414 μM, SI =

28.5) is of particular interest. These results suggest that lipophilic bis-phosphonium diphenyl derivatives might be a good alternative (with potentially better pharmacokinetic properties) to the guanidine or 2-aminoimidazoline derivatives. With respect to the guanidine cation, N-substitution with a diethoxyethane moiety afforded highly selective antitrypanosomal agents (SI > 554 and 767 for **31c** and **32c**, respectively).

The antitrypanocidal efficacy of a drug depends on its effective uptake by the parasite. It is known that diamidines such as pentamidine, which have a very slow rate of diffusion across biological membranes, can be transported into the cell by a P2-amino-purine transporter that specifically recognizes the main $H_2N-C(R_1)=NR_2$ motif.[46,47] The guanidine molecules reported here also present this recognition motif. In the case of the diphenyl derivatives, most of the Boc-protected molecules (i.e. the most lipophilic) showed a weaker activity than the charged, unprotected, guanidinium analogues. This might account for a more efficient transport of the unprotected derivatives through the P2 transporter, although affinity assays for this transporter remain to be done.

It is still too early to propose a mode of action of the compounds presented here, and further studies are needed. However, a number of dicationic molecules belonging to the diamidine family (e.g. pentamidine) are known to bind to the minor-groove of DNA, and their antiprotozoal activity is thought to be the result of that interaction (e.g. inhibition of DNA-dependent enzymes or inhibition of transcription).[48−50] In a recent article, Donkor et al. studied the trypanocidal activity of a series of conformationally restricted congeners of pentamidine.[15] Although a direct correlation between the DNA binding affinity and the trypanocidal activity was not observed, the authors concluded that compounds with strong DNA affinity generally showed good trypanocidal activity in that series. In particular, *N*,*N*′-bis(4-amidinophenyl)piperazine (Figure 1) and *N*,*N*′-bis(4-imidazolinophenyl)piperazine were the most potent trypanocides and also the strongest DNA binders in this series. According to the results of Donkor et al., we might expect good DNA binding affinity for compounds **40b** and **41b**, which are the guanidine and 2-aminoimidazoline analogues of these congeners, respectively (vide infra). However, this hypothesis will need experimental confirmation.

Several compounds presented in this paper were first and foremost studied for different activities on the CNS (i.e. α_1-adrenergic antagonism, I_2-imidazoline binding site affinity or analgesic properties). The knowledge of these interactions (i.e. possible side effects) is of importance, because useful antitrypanocidal agents are expected to penetrate the CNS to cure late-stage cases of HAT. The diphenyl compounds (Table 5, entries 5−8 and 12−15) present α_1-adrenergic antagonist activity in various tissues.[26,51] In particular, the blood pressure and heart rate responses of two compounds (**29b** and **31b**) had been tested on rats in vivo, suggesting a smaller magnitude of cardiovascular effects than the α_1-adrenergic antagonist doxazosin at the same dose.[52] On the other hand, the alkane derivatives (Table 1, **1a**−**d** and **2a**−**d**) showed a moderate to good affinity for the I_2-imidazoline binding sites and α_2-adrenoceptors in human brain membranes.[27] Finally, several azaalkane derivatives (Tables 2 and 3, **4a**,**b**, **5a**,**b**, **6a**,**b**, **7**, **10**, **11**, **23**) were tested for analgesic activity in mice.[28] These data are relevant and should be taken into consideration when choosing possible lead compounds for in vivo assays.

Conclusion

We have reported here the screening for trypanocidal activity against *T.b. rhodesiense* of an in-house library of 62 compounds [i.e. alkane, diphenyl, and azaalkane bisguanidine and bis(2-aminoimidazoline)] that were chosen for their structural similarity to the trypanocidal agents synthalin (1,10-decanediguanidine), and 4,4′-diguanidinodiphenylmethane and the polyamine N^1-(3-aminopropyl)propane-1,3-diamine, respectively. The original synthetic procedure for the preparation of 21 of these compounds was also reported.

The results of the determination of in vitro antitrypanosomal activity allowed drawing some conclusions about the SAR of these series of molecules. Most compounds displayed low micromolar antitrypanosomal activity, with five of them presenting a nanomolar inhibitory action on the parasite (**1c**, **1d**, **28a**, **28b**, and **32b**). A few of these compounds, which showed an excellent in vitro activity as well as high selectivity, e.g. **1c** (IC_{50} = 49 nM; SI > 5294), **28b** (IC_{50} = 69 nM; SI = 3072), **32b** (IC_{50} = 22 nM; SI = 29.5), **41b** (IC_{50} = 118 nM; SI = 881), are promising lead compounds for antitrypanosomal chemotherapy. The results of in vivo activity of these molecules will be reported in due course.

Tropical diseases mainly affect Third-World countries, which usually lack research capacities and financial resources for investigation. The lack of available funds and research in this field put into light the importance of screening in-house libraries of molecules already available in order to save time and money in the discovery of new lead compounds for neglected diseases such as HAT.

Experimental Section

Chemistry. All reaction solvents were purchased anhydrous and used as received. Other solvents used were reagent grade. Reactions were monitored by TLC using precoated silica gel 60 F_{254} plates. Chromatography was performed either with silica gel 60 PF_{254} (particle size 40−63 μm) or with a medium-pressure chromatography system using KP-Sil 40S or 40M cartridges (particle size 32−63 μm, 60 Å). All reactions requiring anhydrous conditions or an inert atmosphere were performed under a positive pressure of N_2. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, unless otherwise noted. Chemical shifts of the ^{1}H NMR spectra were internally referenced to the residual proton resonance of the deuterated solvents: $CDCl_3$ (7.26 ppm), D_2O (δ 4.6 ppm), CD_3OD (3.49 ppm), and DMSO (δ 2.49 ppm). Chemical shifts of ^{13}C NMR and ^{31}P spectra were referenced with a capilar of DMSO-d_6 (δ 39.5 ppm) and H_3PO_4 (δ 0 ppm), respectively. IR spectra were recorded as KBr pellets or neat. Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Mass spectra were recorded on a Hewlett-Packard Series 1100 MSD spectrometer (ES, APCI) and on a VG Autospec spectrometer (FAB). Elemental analysis was performed on a Heraeus CHN-O rapid analyzer. Analytical results were within ±0.4% of the theoretical values unless otherwise noted. Analytical HPLC was run on a Beckman LC-168 HPLC with either a Waters Delta Pak 5μ-C18, 100 Å (3.9 × 150 mm) (column I) or a Varian Microsorb-MV-C18, 100Å

column (column II) using the following conditions: gradient time = 40 and 15 min for columns I and II, respectively, H_2O/CH_3CN (100:0 → 0:100) (TFA 0.1%), flow rate = 1 mL/min, λ = 214 and 254 nm. Preparative HPLC (compounds **5c** and **17**) was carried out using a Waters Deltaprep apparatus with a Waters prepak-RCM Base column and detection at 214 nm.

Compounds **1a**−**d**, **2a**−**d**, **3b**−**d, 4a**−**6a**,[53] **4b**, **5b,d, 6b**, **7**−**9**, **11**−**13**, **21**−**24**, **27a**−**33a**, **27b**−**33b**, and **34** were prepared as previously reported.[26−28,53]

***N*-{3-[(2-Guanidinoethyl)phenethylamino]propyl}guanidine (10).** A solution of **9** (0.5 mmol) and *S*-methylisothiouronium sulfate (148 mg, 0.53 mmol) in dry MeOH (7 mL) was heated for 12 h at reflux. The solvent was removed by reduce pressure, and the crude product dissolved in a mixture of H_2O/EtOH was treated with a few drops of 5% H_2SO_4. The solution was allowed to stand for 3 days in the refrigerator and the supernatant was discarded. Acetone was added and the oily residue was triturated with a spatula until the product crystallized. The solid was dried in vacuo, affording **10** as a highly hygroscopic colorless solid (104 mg, 46%): ^{1}H NMR (D_2O) δ 7.3−7.0 (m, 5H), 3.6−2.8 (m, 12H), 1.87 (m, 2H); ^{13}C NMR (D_2O) δ 155.9 (br), 135.0, 128.1, 127.7, 126.4, 53.0, 50.6, 49.9, 37.1, 35.4, 28.2, 22.7. LRMS (ES^+) *m*/*z* 307 [(M + H)], 100].

{3-[(2-Benzyloxycarbonylaminoethyl)methylamino]propyl}carbamic Acid Benzyl Ester (14). $NaBH_3CN$ (100 mg, 1.49 mmol) was added to a solution of amine **5d** (443 mg, 1.15 mmol) and 37% aqueous formaldehyde (0.4 mL, 4.6 mmol). The reaction was stirred for 4 h at room temperature, and the solvents were removed by reduce pressure. The crude residue was partitioned between $CHCl_3$ and water. The organic phase was collected and the aqueous phase was extracted three times with $CHCl_3$. Organic extracts were washed with brine, dried (Na_2SO_4), and concentrated by reduce pressure. Flash chromatography (40S cartridge) with CH_2Cl_2/MeOH (95:5) afforded the methylated amine **14** as a colorless solid (236 mg, 51%): mp 60−62 °C; IR (KBr) ν 3300, 2900, 2725, 1665, 1515, 1250, 1120, 960, 720, 685, 670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.4−7.2 (m, 10H), 5.6 (br, NH), 5.4 (br, NH), 5.07 (s, 2H), 5.05 (s, 2H), 3.3−3.1 (m, 4H), 2.5−2.3 (m, 4H), 2.17 (s, 3H), 1.62 (quint, 2H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 157.1, 137.3, 137.2, 129.0, 128.6, 128.5, 67.1, 67.0, 57.3, 55.9, 42.2, 40.1, 38.9, 27.4; LRMS (ES^+) *m*/*z* 400 [(M + H), 100]. Anal. ($C_{22}H_{29}N_3O_4$) C, H, N.

***N*^{1}-(2-Aminoethyl)-*N*^{1}-methylpropane-1,3-diamine (15).** Catalytic hydrogenation of a suspension of **14** (230 mg, 0.57 mmol), 10% Pd−C (23 mg), and 1 M HCl (1 mL) in MeOH (30 mL) under 36 psi hydrogen pressure for 24 h at room temperature afforded the HCl salt of **15** as a colorless oil (118 mg, quantitative): ^{1}H NMR (D_2O) δ 3.0−2.8 (m, 4H), 2.58 (t, 2H, J = 6.8 Hz), 2.46 (t, 2H, J = 7.6 Hz), 2.16 (s, 3H), 1.74 (quint, 2H, J = 7.6 Hz); ^{13}C NMR (D_2O) δ 54.8 (t), 54.5 (t), 41.0 (q), 38.5 (t), 37.0 (t), 24.5 (t); LRMS ($APCI^+$) *m*/*z* 132 [(M + H), 100]. Anal. ($C_6H_{20}N_3Cl_3$·0.3H_2O) Calcd: C, 29.47; H, 8.49; N, 17.19. Found: C, 29.35; H, 8.40; N, 17.00.

***N*,*N*′-Bis[2-(4,5-dihydro-1*H*-imidazol-2-ylamino)ethyl]-*N*′-methylpropane-1,3-diamine (16).** A solution of **15** (110 mg, 0.84 mmol) and 2-methylmercapto-4,5-dihydro-1*H*-imidazole iodide (410 mg, 1.76 mmol) in EtOH (10 mL) was heated for 24 h at reflux (**CAUTION**: the noxious gas CH_3SH is evolved during the reaction and it should be trapped with a concentrated aqueous NaOH solution). The solvent was removed by reduce pressure and the crude compound was purified by formation of its picrate salt: a hot solution of picric acid (400 mg in 5 mL of H_2O) was added to the hot reaction mixture and the flask was allowed to stand in the refrigerator for 1 week. The crystals were collected by filtration and rinsed successively with water, hexane, and Et_2O. Picrate of **16**: yellow solid (302 mg, 53%); mp 81−83 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (br s, 1H), 9.25 (br s, 1H), 8.54 (s, 4H), 8.18 (t, 1H, J = 5.7 Hz), 8.13 (t, 1H, J = 4.8 Hz), 7.65 (br, 1H), 3.80 (s, 3H), 3.58 (s, 4H), 3.55 (s, 4H), 3.1 (m, 4H), 2.8−2.7 (m, 4H), 1.8 (br, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 170.5, 160.9 (s), 159.4 (s), 141.5 (s), 125.3 (d), 124.8 (s), 53.6 (br, t), 53.0 (t), 45.2 (t), 42.6 (t), 40.2 (q), 37.2 (t); LRMS (FAB^+) *m*/*z* 268 [(M + H)]. Anal. ($C_{24}H_{31}N_{13}O_{14}$) C, H, N.

3-Azahexane-1,7-(*N*,*N*′-dicyclohexyl)diguanidine (5c). A solution of 3-(2-aminoethylamino)propylamine (1 mL, 8.5 mmol) and DCC (3.7 g, 17.9 mmol) in dry CH_3CN (25 mL) was stirred for 4 days at room temperature under argon atmosphere. The solvent was removed by reduce pressure and the crude oil was dissolved in Et_2O. A current of HCl_g was bubbled into the solution for 2 min. The white precipitate was collected, rinsed with Et_2O, and dried in vacuo, affording a mixture of the di- and trisubstituted compounds **5c** and **17,** which were separated by preparative HPLC using the following eluent system: H_2O/CH_3CN (100:0 → 0:100) (TFA 0.1%). Trifluoroacetate of **5c**: white solid; mp 88−93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45 (t, 2H, J = 6.3 Hz), 3.6−3.25 (m, 10H), 3.18 (t, 2H, J = 7 Hz), 2.95 (br t, 2H), 1.82 (q, 2H, J = 7.8 Hz), 1.69 (br s, 8H), 1.60 (br d, 8H), 1.46 (br d, 4H), 1.16 (t, 16H, J = 10 Hz), 1.0 (br m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.7 (TFA), 154.0 (s), 154.0 (s), 117.6 (TFA), 52.6 (d), 52.5 (d), 47.3 (t), 46.5 (t), 39.3 (t), 38.9 (t), 33.4 (t), 33.4 (t), 26.5 (t), 25.8 (t), 25.7 (t); LRMS (ES^+) *m*/*z* 265.9 [(M + 2H), 100], 644.6 [(M + TFA)], 758.6 [(M + 2TFA)]; HPLC (column II) t_R = 9.37 min (100%).

3-Azahexane-1,3,7-(*N*,*N*′-dicyclohexyl)triguanidine (17). Trifluoroacetate of **17**: white flocculent solid; ^{1}H NMR (500 MHz, CD_3OD) δ 3.73−3.60 (m, 8H), 3.56−3.44 (m, 6H), 2.14−1.8 (m, 30H), 1.7−1.3 (m, 32H); ^{13}C NMR (125 MHz, CD_3OD) δ 160.1 (s), 154.2 (s), 56.0 (d), 52.6 (d), 52.6 (d), 49.8 (t), 47.7 (t), 39.9 (t), 39.7 (t), 34.5 (t), 33.9 (t), 33.9 (t), 28.2 (t), 26.2 (t), 26.2 (t), 26.1 (t), 26.1 (t), 26.0 (t); LRMS (ES^+) *m*/*z* 369 [(M + 2H), 100], 246.5 [(M + 3H)]; HPLC (column I) t_R = 30.55 min (99.58%).

{3-[(2-Benzyloxycarbonylaminoethyl)(2-chloroethyl)amino]propyl}carbamic Acid Benzyl Ester (19). Chloroacetaldehyde (50% in water, 1.5 mL, 11.6 mmol) was added to a solution of **5d** (1.11 g, 2.9 mmol) in CH_3CN (20 mL). After a few minutes, AcOH (0.5 mL, 8.5 mmol)) was added, followed 5 min later by $NaBH(OAc)_3$ (1.24 g, 5.8 mmol). The reaction mixture was stirred for 4 h at room temperature, and the pH was adjusted to 5−6 with AcOH during the course of the reaction. The reaction was quenched by careful addition of 5% $NaHCO_3$ and diluted with CH_2Cl_2. The organic phase was separated and the aqueous phase was extracted three times with CH_2Cl_2. Combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated by reduce pressure. Chromatography (40M cartridge) with petroleum ether/acetone (80:20) yielded the acetyl side product **18** (16%) and the expected chloro derivative **19** as an oil that solidified as a yellowish pasty residue (450 mg, 35%); IR (KBr) ν 1680, 1415, 1240, 755, 713, 675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.29 (br s, 10H), 5.67 (br, NH), 5.60 (br, NH), 5.09 (s, 2H), 5.08 (s, 2H), 3.43 (t, 2H, J = 6.1 Hz), 3.3−3.1 (m, 4H), 2.68 (t, 2H, J = 6.3 Hz), 2.6−2.4 (m, 4H), 1.56 (quint, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 157.0 (2C), 137.1, 128.8, 128.3, 66.8, 56.0, 54.0, 52.4, 42.5, 39.7, 39.3, 27.5; LRMS (ES^+) *m*/*z* 448.5 [(M + HCl), 100], 412 [(M + H)]. Anal. ($C_{23}H_{30}N_3O_4Cl$/H_2O) Calcd: C, 59.29; H, 6.92; N, 9.02. Found: C, 59.27; H, 7.26; N, 9.02.

Acetic acid 2-[(2-Benzyloxycarbonylaminoethyl)(3-benzyloxycarbonylaminopropyl)amino]ethyl ester (18): 223 mg, 16%; ^{1}H NMR ($CDCl_3$) δ 7.28 (br s, 10H), 5.68 (br, NH), 5.52 (br, NH), 5.04 (s, 2H), 5.02 (s, 2H), 4.03 (t, 2H, J = 5.6 Hz), 3.25−3.05 (m, 4H), 2.60 (t, 2H, J = 5.6 Hz), 2.55−2.35 (m, 4H), 1.90 (s, 3H), 1.55 (quint, 2H); ^{13}C NMR ($CDCl_3$) δ 171.5, 157.0, 156.9, 137.1, 128.9, 128.5, 128.4, 66.95, 66.87, 62.4, 54.0, 52.7, 43.5, 39.9, 39.2, 27.3, 21.2; LRMS (ES^+) *m*/*z* 472 [(M + H), 100]. Anal. ($C_{25}H_{33}N_3O_6$) C, H, N.

{3-[(2-Benzyloxycarbonylaminoethyl)(2-hydroxyethyl)amino]propyl}carbamic Acid Benzyl Ester (20). A solution of bromoethanol (0.03 mL, 0.42 mmol) in CH_3CN (1 mL) was added to a solution of **5d** (208 mg, 0.54 mmol) in CH_3CN (4 mL). The reaction mixture was refluxed for 12 h and the solvent was removed by reduce pressure. The crude product was purified by chromatography with EtOAc/MeOH (80:20), and the resulting compound dissolved in a little CH_2Cl_2 was filtered on a path of Celite, affording the pure product **20** (124

mg, 69%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (br s, 10H), 5.63 (br, 1H), 5.46 (br, 1H), 4.97 (s, 4H), 3.44 (t, 2H, J = 5 Hz), 3.1 (m, 5H), 2.5−2.3 (m, 6H), 1.49 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.76, 156.57, 136.5, 128.3, 127.9, 66.4, 59.1, 55.8, 53.7, 51.3, 38.9, 38.6, 27.0; IR (neat) ν 3500−3300 (br), 2905, 1680, 1515, 1235, 715, 675 cm^{-1}; LRMS (ES$^+$) m/z 430 [(M + H)], 542 [(M + Na), 100]. Anal. ($C_{23}H_{31}N_3O_5 \cdot H_2O$) Calcd: C, 61.73; H, 7.43; N, 9.39. Found: C, 61.83; H, 6.98; N, 8.82.

3-(3-Benzyloxycarbonylaminopropyl)imidazolidine-1-carboxylic Acid Benzyl Ester (25). To a solution of amine **5d** (1.06 g, 2.7 mmol) and formaldehyde (37% in H_2O, 1.5 mL, 13.5 mmol) in CH_3CN was added $NaBH_3CN$ (226 mg, 3.6 mmol). After 10 min, a few drops of AcOH were added to the cloudy solution to adjust the pH to 6−7. The reaction was stirred for 17 h at room temperature and the solvent was removed by reduce pressure. The crude residue was treated with water and 1 M NaOH was added to adjust the pH to 10−11. The aqueous phase was extracted three times with CH_2Cl_2, and the organic extracts were dried (Na_2SO_4) and concentrated by reduce pressure. The crude oil was chromatographed (40M cartridge) with CH_2Cl_2/MeOH (98:2). The six-membered heterocycle **26** was eluted first, followed by **25**. Compound **25**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 10H), 5.3 (br, 1H, NH), 5.04 (s, 2H), 4.99 (s, 2H), 3.94 (s, 1H), 3.89 (s, 1H), 3.37 (td, 2H, J = 8.9 and 9.3 Hz), 3.17 (td, 2H, J = 9.0 and 9.6 Hz), 2.70 (m, 2H), 2.43 (br t, 2H, J = 9 Hz), 1.58 (quint, 2H, J = 9.7 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.0, 154.4, 137.2, 129.0, 128.6, 68.8, 68.5, 67.4, 67.1, 53.4, 52.6, 52.2, 44.9, 44.7, 43.6, 40.2, 28.8; LRMS (ES$^+$) m/z 398 [(M + H), 100].

3-(2-Benzyloxycarbonylaminoethyl)tetrahydropyrimidine-1-carboxylic acid benzyl ester (26): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 (br s, 10H, aro), 5.5 (br, 1H, NH), 5.1 [s, 2H, PhCH_2OC(O)NH], 5.06 [s, 2H, PhCH_2OC(O)N], 4.12 (s, 2H, NCH_2N), 3.48 (br t, 2H, J = 8.2 Hz, CbzNCH_2), 3.22 (br m, 2H, CH_2NHCbz), 2.67 (br t, 2H, NCH_2CH$_2$), 2.49 (m, 2H, NCH_2CH$_2$NHCbz), 1.53 (m, 2H, CH$_2$$CH_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.7 [s, C(=O)NH], 155.28 [s, C(=O)N], 136.94 (s, aro), 136.86 (s, aro), 128.7 (d, aro), 128.3 (d, aro), 128.1 (d, aro), 67.4 [t, PhCH_2OC(O)NH], 66.7 [t, PhCH_2OC(O)N], 65.1 (t, NCH_2N), 51.9 (t, NCH_2CH$_2$), 44.1 (t, CbzNCH_2), 38.3 (t, CH_2NHCbz), 22.5 (t, CH$_2$$CH_2CH_2$); LRMS (ES$^+$) m/z 398 [(M + H), 100].

4,4′-Bis[*N*^{3}-(2,2-diethoxyethyl)guanidino]diphenylmethane (31c). A 1 M ether solution of 2,2-diethoxyethylcarbodiimide (11.1 mL, 11.1 mmol) was added to a solution of 4,4′-diaminodiphenylmethane (1.05 g, 5.3 mmol) in dry EtOH under N_2. Methanesulfonic acid (0.69 mL, 10.6 mmol) was added dropwise to the clear reaction mixture and a white precipitate formed immediately. The reaction was refluxed for 44 h and then poured into 0.5 M aqueous NaOH solution. The aqueous phase was extracted (3 × CH_2Cl_2). Organic extracts were dried (Na_2SO_4) and concentrated by reduce pressure. The guanidine **31c** was crystallized with CH_2Cl_2 and washed with Et_2O. Some more compound was obtained by precipitation of the mother liquor with Et_2O: colorless solid (926 mg, 34%); mp 188−190 °C; ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.0 (d, 4H, J = 7.5 Hz), 6.73 (d, 4H, J = 7.5 Hz), 4.44 (t, 2H, J = 4.8 Hz), 3.75 (s, 2H), 3.8−3.4 (m, 8H), 3.19 (d, 4H, J = 4.8 Hz), 1.12 (t, 12 H, J = 7 Hz); ^{13}C ($CDCl_3$/CD_3OD) δ 154.5, 146.6, 136.1, 130.2, 124.2, 102.6, 63.8, 45.1, 41.2, 15.6; LRMS (EI) m/z 514 [(M$^{+\bullet}$), 70], 485 [(M − 29), 100]. Anal. ($C_{27}H_{42}N_6O_4$) C, H, N.

4,4′-Bis[*N*^{3}-(2,2-diethoxyethyl)guanidino]diphenylamine (32c). The same procedure as for **31c** starting from 4,4′-diaminodiphenylamine (421 mg, 2.1 mmol), 2,2-diethoxyethylcarbodiimide (4.6 mL, 4.6 mmol), and methanesulfonic acid (0.27 mL, 4.2 mmol) was used. The crude product dissolved in EtOH was treated with Et_2O. The solid precipitate was filtered off and the mother liquor was concentrated by reduce pressure. The residue was dissolved in MeOH and **32c** was isolated by Et_2O-mediated precipitation (707 mg, 65%). Methanesulfonate salt of **32c**: purple solid; IR (KBr) ν 1625, 1600, 1480, 1175, 1160, 1025, 1015, 750, 737 cm^{-1}; ^{1}H NMR (D_2O) δ 7.11 (s, 8H), 4.65 (m, 2H), 3.8−3.4 (m, 8H), 3.33 (m, 4H), 1.11 (t, 12H, J = 7.1 Hz); ^{13}C NMR (D_2O) δ 156.7, 143.1, 127.8, 126.9, 118.9, 101.3, 65.0, 44.9, 15.2; LRMS (ES$^+$) m/z 516 [(M + H), 100]. Anal. ($C_{28}H_{49}N_7O_{10}S_2 \cdot 2H_2O$) Calcd: C, 45.21; H, 7.18; N, 13.18; S, 8.62. Found: C, 44.80; H, 7.02; N, 12.70; S, 9.08.

4,4′-Bis(2-imidazolylamino)diphenylamine (35). In a flask cooled to 0 °C, was dissolved the guanidine **32c** (350 mg, 0.68 mmol) in 6 M HCl (5 mL). After stirring for 3 h at room temperature, 10% NaOH was added until a precipitate formed (pH > 11). The reaction mixture was stirred for 75 min and was poured into a 1 M NaOH solution. The aqueous phase was extracted with CH_2Cl_2. The crude product was collected by filtration of the aqueous phase. The crude solid was dissolved in boiling water (10 mL) and the flask was allowed to stand overnight at room temperature. The product was collected by filtration, washed several times with H_2O, and dried in vacuo at 50 °C, affording the free base of **35** as a purple solid (82 mg). The hydrochloride salt was prepared in the following manner: to **35** dissolved in H_2O was added 3 N HCl until pH 2 was reached. The compound was lyophilized, dissolved in MeOH, and purified by Et_2O-mediated precipitation: purple solid (51 mg, 19%); mp >200 °C (dec); IR (KBr) ν 1650, 1590, 1500, 1310, 815, 670 cm^{-1}; ^{1}H NMR (D_2O) δ 7.15 (br s, 8H), 6.76 (br s, 4H); ^{13}C NMR (D_2O) δ 145.9 (s), 142.5 (s), 130.2 (s), 125.4 (d), 119.8 (d), 114.0 (d); LRMS (ES$^+$) m/z 332 [(M + H), 100], 166.6 [(M + 2H)]. Anal. ($C_{18}H_{20}Cl_3N_7$) Calcd: C, 49.05; H, 4.57; N, 22.25. Found: C, 49.71; H, 4.45; N, 21.66.

Bis(4-bromomethylphenyl)methanone (36). A solution of 4,4′-dimethylbenzophenone (1 g, 4.8 mmol), NBS (1.71 g, 9.6 mmol), and four drops of *t*-BuOOH in CCl_4 (15 mL) was heated at reflux for 18 h under argon atmosphere. The insoluble succinimide was filterered off and the solvent was removed by reduce pressure. The pure product was obtained by crystallization from CH_2Cl_2 as colorless needles (387 mg, 22%): mp 135−137 °C; IR (KBr) ν 1630, 1585, 1390, 1255, 1155, 905, 665 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.78 (d, 4H, J = 8.4 Hz), 7.51 (d, 4H, J = 8.4 Hz), 4.57 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 195.8, 142.9, 137.8, 131.1, 129.6, 32.9. Anal. ($C_{15}H_{12}Br_2O \cdot 0.5H_2O$) Calcd: C, 47.78; H, 3.47. Found: C, 47.87; H, 3.08.

Tri-*n*-pentylphosphine. To a suspension of magnesium (1.96 g) in dry THF (50 mL) under argon was added a solution of 1-bromopentane (10 mL, 80.7 mmol) in THF (20 mL). The resulting reaction mixture was heated at reflux for 20 min. Then, the reaction was cooled to −78 °C and a solution of phosphorus trichloride (1.74 mL, 20 mmol) in THF (10 mL) was added dropwise. The reaction was stirred 30 min at −78 °C and the cold bath was removed. The reaction was allowed to warm to room temperature and was then heated at reflux for 30 min. The reaction was quenched with saturated NH_4Br solution (20 mL). The precipitate was filtered off under argon atmosphere and the crude product was distilled under vacuum, affording the tri-*n*-pentylphosphine as a colorless oil (1.305 g, 27%). The product was conserved under argon in the refrigerator: bp (3 mmHg) 115−125 °C; ^{1}H NMR ($CDCl_3$) δ 1.61 (m, 6H), 1.36 (m, 18H), 0.88 (m, 9H); ^{13}C NMR (D_2O) δ 33.9 (d, $J_{31P-13C}$ = 13.7 Hz), 29.1, 27.8, 22.8, 22.0, 14.5; ^{31}P NMR ($CDCl_3$) δ 50.23.

4,4′-Bis(tri-*n*-pentylphosphonium)benzophenone Bromide (37). A solution of **36** (344 mg, 0.93 mmol) and tri-*n*-pentylphosphine (1.02 g, 4.2 mmol) in dry toluene (10 mL) was heated at reflux for 24 h. The precipitate that had formed while the reaction mixture cooled was triturated with a spatula until a solid formed. The solid was collected by filtration, rinsed with dry toluene, and dried in vacuo at 70 °C: colorless hygroscopic solid (748 mg, 94%); spectroscopic data are in agreement with the literature;[37] LRMS (FAB$^+$) m/z 695.5 [(M$^+$); 100]. Anal. ($C_{45}H_{78}OP_2Br_2$) C, H.

1,4-Bis(4-nitrophenyl)piperazine (38).[54] A solution of 1-(4-nitrophenyl)piperazine (2.58 g, 12.5 mmol) and 1-fluoro-4-nitrobenzene (599 mg, 4.16 mmol) in DMSO (15 mL) was heated 60 h at 100 °C. The cool reaction was poured into water (50 mL). The precipitate was collected by filtration and rinsed with a small quantity of water. The product was first crystallized with PhMe/EtOH and rinsed with cold toluene and cold

EtOH, respectively. The pure compound was obtained as a red solid by crystallization with CH_3CN (1 g, 73%): mp 265−266 °C (lit.[54] mp 261 °C, $PhNO_2$); ^1H NMR (DMSO-d_6) δ 8.1 (d, 4H, J = 9 Hz), 7.0 (d, 4H, J = 9 Hz), 3.71 (s, 8H); ^{13}C NMR (DMSO-d_6) δ 153.9, 136.8, 125.6, 111.9, 45.1. Anal. ($C_{16}H_{16}N_4O_4 \cdot 0.8H_2O$) Calcd: C, 56.10; H, 5.18; N, 16.36. Found: C, 56.12; H, 5.35; N, 16.24.

1,4-Bis(4-aminophenyl)piperazine (39).[42] The nitro compound **38** (705 mg, 2.1 mmol) was dissolved in HCl-saturated methanolic solution (70 mL). The solution was hydrogenated (40 psi H_2) in the presence of 10% Pd−C (165 mg) for 14 h at room temperature. The catalyst was filtered off and the solvent was removed by reduce pressure, affording the crude hydrochloride of **39**. Recrystallization with EtOH afforded the pure HCl salt of **39** (300 mg, 41%): mp >350 °C; ^1H NMR (D_2O) δ 7.24 (s, 8H), 3.49 (s, 8H); LRMS (ES^+) m/z 269 [(M + H), 100], 135 [(M + 2H)]. Anal. ($C_{16}H_{24}Cl_4N_4 \cdot 1.7H_2O$) Calcd: C, 43.40; H, 6.24; N, 12.65. Found: C, 43.08; 5.49; N, 12.83.

1,4-Bis[4-(*N*^{2},*N*^{3}-bis(*tert*-butyloxycarbonyl)guanidino)-phenyl]piperazine (40a). To a solution of **39** (54 mg, 0.2 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (122 mg, 0.44 mmol), and Et_3N (0.14 mL, 1 mmol) in DMF (2 mL) at 0 °C under N_2 was added $HgCl_2$ (119 mg, 0.44 mmol) at once. A precipitate formed immediately. The resulting dark reaction mixture was stirred for 30 min at 0 °C and 2.5 days at room temperature. The reaction was diluted with CH_2Cl_2 and filtered through a path of Celite. The filter cake was rinsed with CH_2Cl_2. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Nonmobile impurities were removed by short flash chromatography on silica with hexane/EtOAc (75:25). The pure product was obtained by crystallization from hexane: yellowish solid (110 mg, 73%); mp >300 °C dec; ^1H NMR ($CDCl_3$) δ 11.64 (br, 2H), 10.17 (br, 2H), 7.47 (d, 4H, J = 8.9 Hz), 6.92 (d, 4H, J = 8.9 Hz), 3.27 (s, 8H), 1.5 (br s, 36H); ^{13}C NMR ($CDCl_3$) δ 164.2, 154.1, 153.9, 149.2, 130.0, 124.1, 117.4, 84.0, 79.9, 50.3, 28.7; LRMS (ES^+) m/z 753 [(M + H)]. Anal. ($C_{38}H_{56}N_8O_8 \cdot 0.7C_6H_{14}$) Calcd: C, 62.37; H, 8.16; N, 13.79. Found: C, 62.23; H, 8.80; N, 14.11.

1,4-Bis(4-guanidinophenyl)piperazine (40b). TFA (2 mL) was added to a stirred solution of **40a** (37 mg, 0.049 mmol) in CH_2Cl_2 (3 mL). After 2 days, the volatiles were removed by reduce pressure, and the product was precipitated by addition of Et_2O. The compound was dried in vacuo, affording **40b** as a greenish hygroscopic solid (25 mg, 88%). Trifluoroacetic salt of **40b**: ^1H NMR (D_2O) δ 7.19 (m, 8H), 3.36 (s, 8H); ^{13}C NMR (D_2O) δ 156.2, 148.4, 128.2, 127.1, 118.7, 49.4; LRMS (ES^+) m/z 353 [(M + H)], 177 [(M + 2H), 100]. Anal. ($C_{24}H_{27}F_6N_8O_6$) Calcd: C, 41.51; H, 3.92; N, 16.13. Found: C, 41.52; H, 4.33; N, 17.04.

Di-*tert*-butyl 2-(4-[4-(4-[1,3-bis(*tert*-butyloxycarbonyl)-tetrahydro-1*H*-2-imidazolyliden]aminophenyl)piperazino]-phenylimino)-1,3-imidazolidinedicarboxylate (41a). The same procedure as for 40a starting from the HCl salt of 39 (111 mg, 0.27 mmol), Et_3N (0.37 mL, 2.7 mmol), and $HgCl_2$ (160 mg, 0.59 mmol) and using *N*,*N*′-bis(*tert*-butoxycarbonyl)-imidazoline-2-thione (178 mg, 0.59 mmol) as reagent for the introduction of the imidazoline nucleus was used. Flash chromatography with hexane/EtOAc (50:50) afforded the product as a colorless solid (143 mg, 66%): ^1H NMR ($CDCl_3$) δ 6.9 (m, 8H), 3.79 (s, 8H), 3.20 (s, 8H), 1.31 (s, 36H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 150.3 (s), 147.2 (s), 141.5 (s), 138.5 (s), 122.1 (d), 117.3 (d), 82.4 (s), 50.4 (t), 42.9 (t), 27.7 (q). Anal. ($C_{42}H_{60}N_8O_8$) C, H, N.

1,4-Bis[4-(4,5-dihydro-1*H*-2-imidazolylamino)phenyl]-piperazine (41b). TFA (2 mL) was added to a stirred solution of **41a** (65 mg, 0.08 mmol) in CH_2Cl_2 (3 mL). After 12 h, the volatiles were removed by reduce pressure, and the product dissolved in water was extracted with CH_2Cl_2 to remove organic soluble impurities. The water was evaporated and the product was dried in vacuo to afford **41b** as a greenish hygroscopic solid: ^1H NMR (D_2O) δ 7.3−7.0 (br m, 8H), 3.63 (s, 8H), 3.59 (s, 8H); ^{13}C NMR (CD_3OD, 75 MHz) δ 161.1, 152.5, 128.7, 127.4, 118.6, 50.6, 44.5; LRMS (ES^+) m/z 405 [(M + H)], 203.2 [(M + 2H), 100]. Anal. ($C_{26}H_{30}F_6N_8O_4$) C, H, N.

Biological Tests. In Vitro Antitrypanosomal Activity against *Trypanosoma brucei rhodesiense*. Minimum essential medium (50 μL) supplemented with 25 mM HEPES, 1 g/L additional glucose, 1% MEM nonessential amino acids (100×), 0.2 mM 2-mercaptoethanol, 2 mM Na-pyruvate, 0.1 mM hypoxanthine, and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Three-fold serial drug dilutions were prepared in duplicate in the columns covering a range from 90 to 0.123 μg/mL. Then 10^4 bloodstream forms of *T.b. rhodesiense* STIB 900 in 50 μL was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μL) was then added to each well and incubation continued for a further 2−4 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data are analyzed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA).

In Vitro Cytotoxicity with L-6 Cells. Assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM), 10% fetal bovine serum, and 4×10^4 L-6 cells (rat skeletal myoblasts) with or without a serial drug dilution columns covering a range from 90 to 0.123 μg/mL. Each compound was tested in duplicate. After 72 h of incubation the plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. Then 10 μL of Alamar Blue was added to each well, and the plates were incubated for another 2 h. The plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA).

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