J. Med. Chem. 2008, 51, 909–923

New Bis(2-aminoimidazoline) and Bisguanidine DNA Minor Groove Binders with Potent in Vivo Antitrypanosomal and Antiplasmodial Activity

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Received October 17, 2007

A series of 75 guanidine and 2-aminoimidazoline analogue molecules were assayed in vitro against *Trypanosoma brucei rhodesiense* STIB900 and *Plasmodium falciparum* K1. The dicationic diphenyl compounds exhibited the best activities with IC_{50} values against *T. b. rhodesiense* and *P. falciparum* in the nanomolar range. Five compounds (**7b**, **9a**, **9b**, **10b**, and **14b**) cured 100% of treated mice upon ip administration at 20 mg/kg in the difficult to cure *T. b. rhodesiense* STIB900 mouse model. Overall, the compounds that bear the 2-aminoimidazoline cations benefit from better safety profiles than the guanidine counterparts. The observation of a correlation between DNA binding affinity at AT sites and trypanocidal activity for three series of compounds supported the view of a mechanism of antitrypanosomal action due in part to the formation of a DNA complex. No correlation between antiplasmodial activity and in vitro inhibition of ferriprotoporphyrin IX biomineralisation was observed, suggesting that additional mechanism of action is likely to be involved.

Introduction

Infectious diseases caused by protozoan parasites are responsible for great morbidity and mortality mainly in the least developed countries. Despite the lack of significant research investment on tropical diseases, rich countries recently started to pay attention to malaria because this disease also represents a potential threat for the developed world. Another tropical disease, human African trypanosomiasis (HAT[a] or sleeping sickness) is only present in sub-Saharan Africa and affects between 50 000 and 70 000 people.[1] HAT belongs to the most neglected diseases as defined by a World Health Organization/Industry working group.[2]

Drugs available for HAT are obsolete and present unacceptable adverse effects, as well as increasing treatment failures due to emergence of drug resistance or other reasons.[3–5] On the other hand, the chemotherapy of malaria is principally impaired by the appearance of drug resistant strains of *Plasmodium* spp. Hence, chloroquine, which was the most common antimalarial drug for decades, is now practically ineffective and emergence of resistance to other drugs such as mefloquine, halofantrine, or artemisinin is beginning to appear.[6] For those reasons, WHO now recommends the use of antimalarial drug combinations (e.g., artesunate/mefloquine, artesunate/amodiaquine) in order to delay the development of resistant strains.[5] Thus, the discovery of new safe and efficient antiprotozoal agents to treat HAT and malaria is a priority in international health.

Chart 1. General Structures of the Compounds Studied Highlighting the Skeleton Common to the Different Scaffolds

Scaffold: A, B, C; Cation =

Recent findings by our group have shown that bisguanidine and especially bis(2-aminoimidazoline)diphenyl compounds displayed potent antitrypanosomal activity in vitro and vivo against *T. b. rhodesiense*, the causative agent of acute HAT.[7,8] These studies revealed that compounds bearing 2-aminoimidazoline cations (scaffold A, Chart 1) had higher selectivity for the parasite and similar activities with respect to their guanidine counterparts. In addition, a correlation between antitrypanosomal activity and DNA binding affinity was observed, suggesting a possible mechanism of action for these compounds.[7] Finally, we showed that this class of compounds (i.e., **1a** and **1b**) entered into trypanosomes via different transporters in addition to P2, indicating that parasites that have lost the P2 transporter in

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[a] Abbreviations: CQ, chloroquine; FACS, fluorescence-activated cell sorting; FPIX, ferriprotoporphyrin IX; HAT, human African trypanosomiasis; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; MEM, minimum essential medium; MOA, mechanism of action; μCi, microcurie; Pip, piperidine; SI, selectivity index.

10.1021/jm7013088 CCC: $40.75 © 2008 American Chemical Society
Published on Web 02/05/2008

Table 1. In Vitro Antitrypanosomal and Antiplasmodial Activity of Diphenyl Dicationic Compounds (Scaffold A)

1-12 **13-14**

Cpd	R[a]	X	Y	IC_{50} (μM) *T.b.r.*[b]	*P.f.*[c]	Cytotoxicity L6-cells
1a	Gua	NH	-	0.022[d]	0.018	0.65
1b	Imi	NH	-	0.069[d]	0.0088	212
1c	$(EtO)_2CHCH_2$-Gua	NH	-	0.228[d]	0.113	> 175
1d	(Boc)Gua	NH	-	0.470[d]	0.077	3.7
1e	(Boc)Imi	NH	-	0.048[d]	0.059	9.8
2a	Gua	CH_2	-	0.161[d]	0.032	2.8
2b	Imi	CH_2	-	0.897[d]	0.0157	63.6
2c	$(EtO)_2CHCH_2$-Gua	CH_2	-	0.316[d]	0.036	> 175
3a	Gua	O	-	0.196	0.046	1.64
3b	Imi	O	-	0.467	0.038	41
4a	Gua	S	-	0.102	0.035	2.66
4b	Imi	S	-	0.386	0.025	23.3
5a	Gua	C=O	-	0.206[d]	0.068	2.7
5b	Imi	C=O	-	2.05[d]	0.129	> 214
5f	$CH_2P^+(n\text{-pentyl})_3$	C=O	-	0.414[d]	0.053	11.8
6a	Gua	SO_2	-	4.3[d]	0.444	> 222
6b	Imi	SO_2	-	32.4[d]	5.6	> 196
6e	(Boc)Imi	SO_2	-	2.6[d]	0.055	56.4
7a	Gua	NHC(O)NH	-	0.187	0.096	> 235
7b	Imi	NHC(O)NH	-	0.122	0.028	104
8a	Gua	NHC(S)NH	-	0.538	0.607	15.5
9a	Gua	NHC(O)	-	0.036	0.055	11.5
9b	Imi	NHC(O)	-	0.025	0.028	193
10a	Gua	CH_2CH_2	-	0.045	0.019	1.09
10b	Imi	CH_2CH_2	-	0.054	0.016	34.9
11a	Gua	piperidine	-	0.217	0.041	24
11b	Imi	piperidine	-	0.038	0.011	132
12a	Gua	piperazine	-	0.270[d]	0.0152	46.4
12b	Imi	piperazine	-	0.118[d]	0.0123	104
13a	4,4'-Gua	nil	CH_2	0.024[e]	0.0023[e]	4.7[e]
13b	4,4'-Imi	nil	CH_2	0.0049	0.0115	83.4
14a	5,4'-Gua	CH_2	CH_2	0.050	0.0088	0.73
14b	5,4'-Imi	CH_2	CH_2	0.060	0.0186	37.2

[a] Gua = $-NH-C(=NH)NH_2$. Imi = 2-imidazolinylamino ($-NH-$, N, NH). (Boc)Gua = $-NH-C(=N-CO_2{}^tBu)-NH-CO_2{}^tBu$. (Boc)Imi = $-N=C$ (imidazolidine, $N-CO_2{}^tBu$, $^tBuO_2C-N$). [b] *T. brucei rhodesiense* STIB900 strain. Control: melarsoprol, IC_{50} = 5.5 nM. [c] *P. falciparum* K1 strain. Control: chloroquine, IC_{50} = 0.278 μM. [d] Data previously reported in ref 8 and included here for comparison purposes [e] Data taken from ref 13.

selection of resistance to other drugs will not show cross-resistance to this class of compounds. Encouraged by these promising results, another series of 16 dicationic analogues (**3–4**, **7–11**, **13b**, and **14**, Table 1) were evaluated against *T. b. rhodesiense*, and their DNA binding affinity at AT-rich sites was estimated by ΔT_m measurements with a nonalternating AT sequence DNA polymer.[9]

Others have described excellent antiplasmodial activity of related aromatic dicationic structures such as pentamidine or DB75.[10–13] For example, DB289, the neutral prodrug of DB75,

Scheme 1[a]

X = NHCONH (**7**), NHCO (**9**), piperidin-4-yl (**11**)

X = NHCONH (**7**), NHCSNH (**8**), NHCO (**9**), piperidin-4-yl (**11**)

7d, **8d**, **9d**, **11d**: Z = nil

7e, **9e**, **11e**: Z = CH_2CH_2

TFA salt

HCl salt

7a, **8a**, **9a**, **11a**: Z = nil

7b, **9b**, **11b**: Z = CH_2CH_2

8: Z = nil

8b: Z = CH_2CH_2

X = PhCO- (**19**), PhNHCONH- (**20**), PhNHCO- (**22**), PhCONH- (**23**), CH_3CO- (**30**)

X = PhCO- (**19**), PhNHCONH- (**20**), PhNHCSNH- (**21**), PhNHCO- (**22**), PhCONH- (**23**), CH_3CO- (**30**)

19d, **20d**, **21d**, **22d**, **23d**, **30d**: Z = nil

19e, **20e**, **21e**, **22e**, **23e**, **30e**: Z = CH_2CH_2

TFA salt

20a, **21a**: Z = nil

20b, **21b**: Z = CH_2CH_2

HCl salt

19a, **22a**, **23a**, **30a**: Z = nil

19b, **22b**, **23b**, **30b**: Z = CH_2CH_2

[a] Reagents and conditions: (a) *N,N′*-di(*tert*-butoxycarbonyl)thiourea or *N,N′*-di(*tert*-butoxycarbonyl)imidazoline-2-thione, $HgCl_2$, Et_3N, solvent; (b) CH_2Cl_2/TFA (1:1); (c) IRA_{400} (Cl^-) Amberlyte anion exchange resin; (d) CS_2, CH_2Cl_2, 50 °C; (e) phenyl isothiocyanate (**21d**) or phenyl isocyanate (**20d**) or benzoyl chloride (**23d**), CH_2Cl_2, 0 °C and then room temp, 18 h.

has been used to treat uncomplicated *P. vivax* and *P. falciparum* malaria.[14] Stead et al. demonstrated that uptake of pentamidine into infected erythrocytes proceeds via a route similar to the new permeability pathway (NPP). In addition, it has been proposed that these diamidines share a common MOA with chloroquine by binding to ferriprotoporphyrin IX and inhibiting the formation of hemozoin.[15] These findings prompted us to test the antiplasmodial potential of our dicationic diphenyl compounds (Chart 1, scaffold A).[9] The capacity of the compounds to inhibit the formation of hemozoin as a possible mechanism of antiplasmodial activity was also evaluated in vitro with the ferriprotoporphyrin IX binding inhibition test (FBIT).[16]

Moreover, in order to extend our understanding of the SAR of this class of antiprotozoal agents two new series of cationic analogues, namely, 2-aminoimidazolinium compounds and their guanidinium counterparts (Chart 1, scaffolds B and C) were selected for in vitro screening on *T. b. rhodesiense* and *P. falciparum*. As can be shown in Chart 1, the three analogous series are strictly structurally related to one another; scaffold A represents the "full" dicationic diphenyl model compound. Scaffold B is analogous to A but devoid of one cationic group, whereas scaffold C has "lost" one cationic moiety and one phenyl ring. With these series in hand, we intend to demonstrate the significance of each part of the molecule for the overall activity of the dicationic diphenyl compounds and gain insight into the SAR of this series of antiprotozoal compounds.

Finally, to check whether the activity was maintained in vivo, the most active and selective compounds in vitro were assayed in an acute HAT mouse model (*T. b. rhodesiense* STIB900) or rodent malaria model (*P. berghei* GFP ANKA). Several lead compounds with excellent in vivo activity emerged from this screening.

Results

Chemistry. Many of the compounds presented here were previously synthesized by us for other purposes. The synthesis of **1a**–**e**, **2a**–**c**, **5a**–**f**, **6a**–**e**, and **12a**,**b** was described earlier.[8,17] The synthesis of compounds **3a**,**b**, **4a**,**b**, **15a**,**b**, **17a**,**b**, **18b**, **24–26a**,**b**, **29a**,**b**, **31a**,**b**, **33–35a**,**b**, and their Boc-protected precursors was described in a recent paper of Rodriguez et al.[18] The synthesis of **10a**,**b**, **13b**, **14a**,**b**, **16a**,**b**, **27a**,**b**, **28a**, **32a**,**b**, **36a**,**b**, **37b**, and their Boc-protected precurors will be reported elsewhere.[19] The synthesis of **7a**,**b**, **8a**, **9a**,**b**, **11a**,**b**, **19–23a**,**b** and **30a**,**b** is described in Schemes 1 and 2. Among these derivatives, **7a**, **11a**,**b**, **19a**,**b**, **20a**,**b**, **21a**,**b**, **22b**, and **23b** (in addition to all the Boc-protected precursors) are new, whereas **7b**,[20] **8a**,[21] **9a**,[22] **9b**,[23] **22a**,[24,25] and **23a**[22] have been previously described in the literature using different synthetic strategies. Briefly, our synthetic approach to introduce the guanidine and 2-aminoimidazoline groups relied on the reaction between primary amines or diamines and *N,N′*-bis(*tert*-butoxycarbonyl)thiourea/$HgCl_2$/Et_3N[26] or *N,N′*-bis(*tert*-butoxycarbonyl)imidazoline-2-thione/$HgCl_2$/Et_3N,[17] affording the Boc-protected guanidines (**7d**, **9d**, **11d**, **19d**, **20d**, **22d**, **23d**, and **30d**) and 2-aminoimidazolines (**7e**, **9e**, **11e**, **19e**, **20e**, **22e**, **23e**, and **30e**), respectively. Removal of the Boc protecting groups with TFA followed by anion exchange chromatography afforded the hydrochloride salts of the compounds **7a**,**b**, **8a**, **9a**,**b**, **11a**,**b**, **19a**,**b**, **22a**,**b**, **23a**,**b**, and **30a**,**b**. Compounds **20a**, **20b**, **21a**, and **21b** were studied as their trifluoroacetate salts (Scheme 1). In the case of the thiourea derivatives **8d**, **21d**, and **21e**, an alternative strategy was employed to avoid the competitive reaction occurring between the thiourea linker of the starting material (i.e., **8** and **21**) and the thiourea guanidine precursors for the $HgCl_2$ catalyst (Scheme 1). Hence, **8d** was obtained by condensation of the monomer **8**[18] in an excess of carbon disulfide. Alternatively, the reaction of **8** with 1 equiv of phenyl isothiocyanate in CH_2Cl_2 afforded the guanidine derivative **21d** (68%). Compounds **20d** and **23d** could also be synthesized in a similar way by reaction of **8** with phenyl isocyanate and benzoyl chloride, respectively. A similar approach was used for the synthesis of the 2-aminoimidazoline analogue **21e** starting from **8b**. Thus, 1,4-phenylenediamine was reacted with 1 equiv of *N,N′*-bis(*tert*-butoxycarbonyl)imidazoline-2-thione/$HgCl_2$/

Scheme 2[a]

[a] Reagents and conditions: (a) H_2, 5% Pd−C, MeOH, room temp; (b) HNO_3, H_2SO_4; (c) 1-fluoro-4-nitrobenzene, DMF, 100 °C, 72 h; (d) phenyl isocyanate, CH_2Cl_2, 0 °C; (e) aniline, Et_3N, TBTU, CH_2Cl_2; (f) TFA, CH_2Cl_2.

Table 2. In Vitro Antitrypanosomal and Antiplasmodial Activity of Diphenyl Monocationic Compounds (Scaffold B)

			IC_{50} (μM)		
compd	R[a]	X	*T.b.r.*[b]	*P.f.*[c]	cytotoxicity L6-cells
15a	Gua	NH	4.8	1.6	49.5
15b	Imi	NH	1.2	0.549	90.7
16a	Gua	CH_2	15.9	3.8	43.9
16b	Imi	CH_2	4.9	1.7	73.0
17a	Gua	O	18.8	7.9	59.9
17b	Imi	O	5.0	5.7	166.7
18b	Imi	S	1.9	1.2	28.5
19a	Gua	CO	14.4	>18	118.1
19b	Imi	CO	9.4	5.2	225.8
20a	Gua	NH-CO-NH	32.9	4.1	>234
20b	Imi	NH-CO-NH	45.4	3.4	>219
21a	Gua	NH-CS-NH	1.3	8.7	>225
21b	Imi	NH-CS-NH	2.6	3.1	91.1
22a	Gua	NH-CO	87.4	13.3	>309
22b	Imi	NH-CO	42.9	>15	>284
23a	Gua	CO-NH	121.7	10.4	>309
23b	Imi	CO-NH	94.7	>15	>284

[a] Gua = . Imi = . [b] *T. brucei rhodesiense* STIB900 strain. Control: melarsoprol, IC_{50} = 5.5 nM. [c] *P. falciparum* K1 strain. Control: chloroquine, IC_{50} = 0.278 μM.

Et_3N[17] to afford the monomer **8b**. The crude product **8b** was reacted without further purification with 1 equiv of phenylisothiocyanate, yielding **21e** after chromatography on neutral alumina (22% for two steps). Four of the starting material amines (**7**,[27] **11**,[28,29] **20**, and **22**) were obtained in a straightforward manner as depicted in Scheme 2.

Biological Results. In Vitro Activity. Structure–Activity Relationships. The results of the in vitro antitrypanosomal and antiplasmodial activity are presented in Tables 1−3. In order to gain insights into the mechanism of antiplasmodial and antitrypanosomal activity, the most active compounds (i.e., diphenyldicationic derivatives, scaffold A) were also tested as inhibitors of β-hematin formation[16] and as DNA minor groove binders.[30]

Antitrypanosomal Activity. In general, the presence of two cations (scaffold A) was essential to get nanomolar anti-*T. brucei* activity. Accordingly, compounds with only one cation and no phenyl ring in the para position (scaffold C) showed micromolar IC_{50} values. The 1-(2,3-dihydro-1*H*-inden-5-yl)guanidine derivative **32a** (IC_{50} = 0.99 and 0.95 μM against *T. b. rhodesiense* and *P. falciparum*, respectively) was the most active molecule of this series. In contrast, scaffold A compounds displayed nanomolar activities with the exception of the compounds with an electron-attracting group such as SO_2 (**6a**, **6b**, and **6e**) or CO (**5b**) linking both phenyl rings. Removal of one cationic moiety (scaffold B) or one phenyl cationic moiety (scaffold C) led to a great loss of activity (e.g., compare **1a/15a/24a**, **2a/16a/27a**, **2b/16b/27b**). Interestingly, the decrease in activity observed in a homologous series with the removal of one cationic moiety (i.e., scaffold A → scaffold B) was less pronounced for the bis(2-aminoimidazolinium) vs bisguanidinium compounds [e.g., compare **1a/15a** (×218) vs **1b/15b** (×17), **2a/16a** (×98) vs **2b/16b** (×5.5), **3a/17a** (×96) vs **3b/17b** (×10)]. Another remarkable effect of the 2-aminoimidazolinium cation was the higher selectivity for the parasite observed in all the cases compared with the guanidinium analogues [e.g., **3a** (SI = 8) vs **3b** (SI = 88), **9a** (SI = 319) vs **9b** (SI = 7731), **14a** (SI = 15) vs **14b** (SI = 624)]. This observation was also true for the antiplasmodial activity of the dicationic compounds.

Regarding the bridge linking both phenyl rings, the same range of activity (IC_{50} in the low micromolar range) was observed for electron donating groups: NH (**1**) ~ CH_2CH_2 (**10**) ≫ piperidine (**11**) ~ piperazine (**12**). Isosteric replacement of the CH_2 (**2**) by a sulfur (**3**) or oxygen atom (**4**) hardly changed the activity. The best activity was observed with an amide (**9a**, **9b**) or ethane bridge (**10a**, **10b**). Fused ring dicationic compounds **13** (fluorene) and **14** (dihydroanthracene) also gave excellent antitrypanosomal activity. The bis(2-aminoimidazoline)fluorene derivative **13b** was the best anti-*T. brucei* agent in vitro (IC_{50} = 4.9 nM, SI = 17 000). This outstanding value is to be compared with the guanidine analogue **13a** (IC_{50} = 24 nM, SI = 196) previously described by Boykin and coworkers.[13] Hence, replacement of the guanidine cations by 2-aminoimidazoline cations led to a 5-fold increase in activity and 86-fold increase in selectivity for this scaffold.

Dicationic guanidine compounds (scaffold A) with NH, CH_2, O, S, CO, or SO_2 bridge linking both phenyl rings were 2- to 10-fold more potent than their 2-aminoimidazoline counterparts (compare **1a/1b**, **2a/2b**, **3a/3b**, **4a/4b**, **5a/5b**, and **6a/6b**). The opposite effect was observed for monocationic compounds (scaffold B). However, no significant difference in antitrypanosomal activities was observed between the bisguanidine and bis(2-aminoimidazoline) counterparts for the compounds with the urea (**7a**, **7b**), amide (**9a**, **9b**), or ethane bridge (**10a**, **10b**).

Antiplasmodial Activity. In most cases, the best activities for scaffolds A−C were observed for compounds bearing the 2-aminoimidazolinium cations except for the fluorene analogue **13b** (IC_{50} = 11.5 nM), the activity of which was 5-fold lower than that reported by Boykin and co-workers for the guanidine counterpart **13a** (2.3 nM).[13] Among 20 dicationic compounds

Table 3. In Vitro Antitrypanosomal and Antiplasmodial Activity of Phenyl Monocationic Compounds (Scaffold C)

compd	R[a]	X	Y	IC$_{50}$ (μM) *T.b.r.*[b]	*P.f.*[c]	cytotoxicity L6-cells
24a	Gua	NH$_2$	H	311.2	>26	131.8
25b	Imi	NH$_2$	H	nd	15.0	>423
25a	Gua	(CH$_3$)$_2$NH	H	20.1	>19	>358
25b	Imi	(CH$_3$)$_2$NH	H	46.6	>18	>324
26a	Gua	Et$_2$NH	H	2.4	5.7	78.1
26b	Imi	Et$_2$NH	H	40.1	15.0	>444
27a[d]	Gua	CH$_3$	H	163.7	>27	>484
27b	Imi	CH$_3$	H	262.4	>23	>425
28a	Gua	Et	H	45.7	9.0	309.6
29a	Gua	CH$_3$S	H	5.9	1.3	239.7
29b	Imi	CH$_3$S	H	7.3	1.7	173.9
30a[d]	Gua	CH$_3$CO	H	155.4	>23	>421
30b	Imi	CH$_3$CO	H	54.6	5.7	>375
31a	Gua	4-piperidin-1-yl	H	48.6	7.9	231.8
31b	Imi	4-piperidin-1-yl	H	50.4	12.0	>283
32a	Gua	fused cyclopentane		0.99	0.95	73.7
32b	Imi	fused cyclopentane		9.2	5.3	>378
33a	Gua	Fused 1,4-dioxane		149.8	9.0	>391
33b	Imi	Fused 1,4-dioxane		68.4	>19	>351
34a[d]	Gua	fused 1,3-dioxolane		146.0	>23	>417
34b	Imi	fused 1,3-dioxolane		93.5	>20	>372
35a[d]	Gua	CH$_3$O	CH$_3$O	237.0	14.8	>388
35b	Imi	CH$_3$O	CH$_3$O	84.2	14.8	>349
36a	Gua	CH$_3$	CH$_3$	39.9	12.2	189.8
36b	Imi	CH$_3$	CH$_3$	49.8	17.1	>398
37b	Imi	H	H	181.8	>25	>455

[a] Gua = (HN)(NH$_2$)C–NH–. Imi = 4,5-dihydro-1H-imidazol-2-yl–NH–. [b] *T. brucei rhodesiense* STIB900 strain. Control: melarsoprol, IC$_{50}$ = 5.5 nM; [c] *P. falciparum* K1 strain. Control: chloroquine, IC$_{50}$ = 0.278 μM; [d] the antiplasmodial action against *P. gallinaceum* in chicks had been studied before by King, H. and Tonkin, I. M. *J. Chem. Soc.* **1946**, 1063–1069.

that displayed IC$_{50}$ values less than 50 nM, diphenylamine derivative **1b** is the best antiplasmodial agent of the series (IC$_{50}$ = 8.8 nM, SI = 24 000). Most of the dicationic compounds displayed excellent antimalarial activities regardless of the linker X. However, compounds with an electron withdrawing linker [SO$_2$ (**6**), CO (**5**), NHCSNH (**8**)] exhibited somewhat lower activity. The remarkable in vitro antiplasmodial activities of the piperazine derivatives **12a** and **12b** (IC$_{50}$ = 15.2 and 12.3 nM, respectively) were comparable to that of their diamidine homologue reported by Mayence et al. (IC$_{50}$ = 4 nM).[10] As observed for the antitrypanosomal activity, the removal of one cationic moiety from scaffold A compounds produced a dramatic loss of activity [Tables 1 and 2; compare **1b/15b** (×62), **2b/16b** (×108), **3a/17a** (×171), **3b/17b** (×150), **4b/18b** (×48), **5b/19b** (×40), **7a/20a** (×42), **7b/20b** (×121), **8a/21a** (×14), **9a/22a** (×241), **9b/22b** (>535)].

The nanomolar activity observed for the uncharged Boc-substituted guanidine (**1d**) and imidazoline (**1e**, **6e**) derivatives is worth noting. Interestingly, the bis(*n*-pentylphosphonium) dicationic analogue **5f** (IC$_{50}$ = 53 nM, SI = 222) produced a 2-fold increase in activity together with a loss of selectivity with respect to its imidazoline counterpart **5b** (IC$_{50}$ = 129 nM, SI > 1658). Altogether, the data seem to indicate that lipophilic groups, either cationic (phosphonium derivative **5f**) or uncharged (Boc-protected compounds **1d** and **1e**), are allowed for good in vitro antiplasmodial activity of this class of symmetric diphenyl compounds.

Table 4. In Vivo Antitrypanosomal Activity in the *T. b. rhodesiense* (STIB900) Mouse Model[a]

compd	dosage route[b]	dosage (mg/kg)	cured[c]/infected	survival (days)[d]
control			0/4	7.5
7b	ip	4×20	4/4	>60
9a	ip	4×20	4/4	>60
9b	ip	4×20	4/4	>60
10b	ip	4×20	4/4	>60
11b	ip	4×20	2/4	>47.25
14b	ip	4×20	4/4	>60

[a] See Experimental Section for details of STIB 900 (*T. b. rhodesiense*) model. [b] ip = intraperitoneal. [c] Number of mice that survive and are parasite free for 60 days. [d] Average days of survival.

Table 5. In Vivo Antiplasmodial Activity in the *P. Berghei* (ANKA GFP) Mouse Model[a]

compd	dosage route[b]	dosage (mg/kg)	cured[c]/infected	% of activity[d]	survival (days)[e]
control			0/4		6.2
chloroquine	ip	4 × 5	0/4	99.6	9
	ip	4 × 10	0/4	99.6	20
1b	ip	4 × 20	0/4	65.51	7
2b	ip	4 × 20	0/4	8.9	6.7
3b	ip	4 × 30	0/4	44.1	7
4b	ip	4 × 50	0/4	97.5	11.3
7a	ip	4 × 50	T[f]	T	
10a	ip	4 × 50	T	T	
11a	ip	4 × 50	T	T	
12a	ip	4 × 20	0/4	42.48	7
12b	ip	4 × 20	0/4	0	7
14a	ip	4 × 50	T	T	

[a] See Experimental Section for details of ANKA GFP (*P. berghei*) models. [b] ip = intraperitoneal. [c] Number of mice that survive and are parasite free for 60 days. [d] % of reduction of parasitemia. [e] Average days of survival. [f] Toxic at the dose tested.

In Vivo Activity (Tables 4 and 5). The compounds displaying the best in vitro antitrypanosomal activities and selectivity were administered intraperitoneally to mice infected with *T. b. rhodesiense* STIB900. Five compounds (**7b**, **9a**, **9b**, **10b**, and **14b**) cured all mice at 20 mg/kg, whereas **11b** only cured 2/4 mice at this dose (Table 4). These data were somehow surprising because the in vitro activity and selectivity of **11b** and the other compounds tested were similar. Another interesting finding was the enhanced antitrypanosomal activity and reduced toxicity of the piperidine compound **11b** compared to the piperazine molecule **12a** (i.e., **12a** was toxic at 20 mg/kg in this model[7]). Apparently, the presence of tertiary amino groups in **12a** and **11b** is not favorable for good in vivo activity, which may possibly relate to unfavorable pharmacokinetic properties of the compounds.

Hence, the in vivo activity of the new compounds in the difficult to cure STIB900 mouse model was comparable to that of the lead compound **1b** reported earlier.[7] Additional experiments to determine the minimum curative dose, oral bioavailability, and potential activity in the chronic CNS mouse model of sleeping sickness are ongoing.

In addition, 10 compounds were tested in the *P. berghei* mouse model to check whether the excellent in vitro antiplasmodial activity of the dicationic compounds was retained in vivo. Four compounds (**1b**, **3b**, **4b**, and **12a**) reduced significantly the parasitaemia upon ip treatment at 20–50 mg/kg, but none of them was curative (Table 5). However, the bis(2-aminoimidazoline) derivative **4b** reduced parasitaemia drastically (97.5%) at 50 mg/kg and was able to increase mice survival almost 2-fold compared to control animals. This activity compared favorably with that of the control drug chloroquine at 5 mg/kg. Besides, the ip administration of **3b** (30 mg/kg)

Table 6. DNA Binding Affinity and Selectivity Index of the Diphenyl Dicationic Compounds

1-12 13-14

Cpd	R	X	Y	ΔT_m (°C)[a] poly(dA•dT)$_2$	Selectivity index[c] (SI)
1a	Gua		-	29.6[b]	30
1b	Imi	NH	-	38.5[b]	3072
1c	$(EtO)_2CHCH_2$-Gua		-	13.6[b]	> 767
2a	Gua		-	15.0[b]	17
2b	Imi	CH_2	-	18.8[b]	71
2c	$(EtO)_2CHCH_2$-Gua		-	5.3[b]	> 553
3a	Gua	O	-	22.1	8
3b	Imi		-	27.1	88
4a	Gua	S	-	20.1	26
4b	Imi		-	24.1	60
5a	Gua	C(=O)	-	27.6[b]	13
5f	(*n*-pentyl)$_3$P$^+$		-	1.2[b]	29
6a	Gua	SO_2	-	12.8[b]	> 51
6b	Imi		-	12.1[b]	> 6
7a	Gua	–NH–C(=O)–NH–	-	24.1	> 1256
7b	Imi		-	31.0	852
8a	Gua	–NH–C(=S)–NH–	-	23.0	29
9a	Gua	–NH–C(=O)–	-	40.1	319
9b	Imi		-	47.1	7720
10a	Gua	–CH_2CH_2–	-	26.1	24
10b	Imi		-	31.2	646
11a	Gua	piperidine-4,1-diyl	-	30.0	111
11b	Imi		-	35.0	3474
12a	Gua	piperazine-1,4-diyl	-	27.8[b]	172
12b	Imi		-	38.8[b]	881
13b	4,4’-Imi	nil	CH_2	44.1	17020
14a	5,4’-Gua	CH_2	CH_2	31.1	15
14b	5,4’-Imi	CH_2	CH_2	33.2	620

[a] Pentamidine: ΔT_m = 32.3 °C (see ref 7). [b] Data taken from ref 7. [c] Selectivity index = (IC_{50} L_6-cells)/(IC_{50} *T. b. rhodesiense*).

produced an activity of 44.1% similar to its diamidine counterpart administered subcutaneously at 40 mg/kg.[31] Altogether, the data confirm the antimalarial potential of the diphenyl amine, diphenyl ether, and diphenyl sulfide scaffolds.

The rest of the compounds were either toxic at the tested dose of 50 mg/kg (**7a**, **10a**, **11a**, and **14a**) or inactive (**12b**). Taken together, the in vivo results suggest that in this series guanidine derivatives are more toxic than their cyclic congeners, in agreement with the higher SI observed in vitro for the 2-aminoimidazoline analogues.

Insights into the Mechanism of Action. (1) DNA Binding Affinity. Many aromatic diamidines and diguanidines are strong DNA minor groove binders.[11,32,33] Some evidence suggests that this interaction is responsible to some extent for the antiprotozoal activity frequently displayed by this class of compounds. We previously reported the existence of a correlation between DNA binding affinity to AT-rich sites and in vitro antitrypanosomal activity of some of the dicationic compounds presented here (**1a**, **1c**, **2a**, **2c**, **5f** and **1b**, **2b**, **5a**, **12a**, **12b**).[7] In order to extend our knowledge of the structural requirements responsible for high-quality DNA minor groove binding, and possibly good antiprotozoal activity, the thermal melting curves (ΔT_m) of the new set of compounds (**3a**,**b**, **4a**,**b**, **7–11a**,**b**, **13b**, and **14a**,**b**) were determined using a nonalternating AT sequence DNA polymer. We used the same experimental conditions (i.e., low salt buffer) as reported before,[7] thus allowing the comparison of the ΔT_m values of the entire series. The results of DNA binding of the previous set of compounds (**1**, **2**, **5**, and **12**) and the new series (**3**, **4**, **7–11**, **13**, **14**) are presented in Table 6. Overall, strong DNA binding was observed for this new series of dicationic compounds with ΔT_m values ranging from 20.1 to 47.1 °C. The 2-aminoimidazoline derivatives consistently

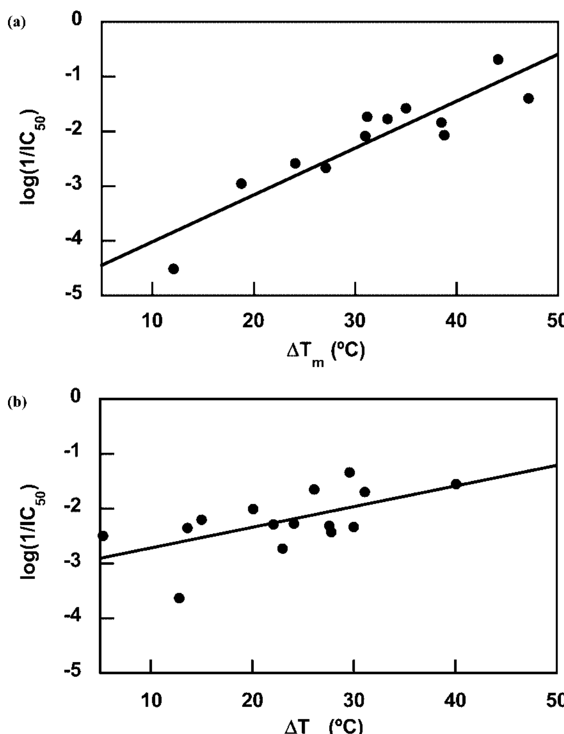

Figure 1. Plot of $\log(1/IC_{50})$ vs ΔT_m showing the correlation between in vitro antitrypanosomal activity and T_m increase (a) for the set of bis(2-aminoimidazoline) compounds, $Y = 0.0857x - 4.8776$, $R = 0.9056$, and (b) for the set of bisguanidine derivatives, $Y = 0.03767x - 3.0968$, $R = 0.5994$.

displayed higher ΔT_m values (2−7 °C) than the guanidine counterparts, indicating that the ethylene bridge of the 2-aminoimidazoline group confers some special features, possibly extra hydrophobic contacts with the walls of the groove that contribute positively to the binding of these compounds.[34] Curiously, the opposite effect is usually observed when comparing series of dicationic benzamidines and their imidazolines analogues, the amidine group conferring habitually better minor groove binders than the imidazoline counterparts.[35–38]

Most interesting was the correlation observed between in vitro antiprotozoal activity and DNA binding affinity for the set of 2-aminoimidazoline compounds. Hence, plotting ΔT_m versus IC_{50} against trypanosomes revealed the two to be correlated as shown in Figure 1a. In addition, a clear trend showing an increase in activity with an increase in ΔT_m was observed for the guanidine set of compounds (Figure 1b). The most exciting finding from this DNA binding affinity study was observed with the 2-aminoimidazoline set of compounds, the SI of which correlated in a fairly good manner with the T_m increase (Figure 2). In contrast, no correlation was observed for the guanidine set of analogues.

(2) Inhibition of *β*-Hematin Formation. Earlier studies have shown that several diphenyldiamidine compounds displaying antiplasmodial activity bind effectively to FPIX in vitro, inhibiting the crystallization of toxic FPIX into nontoxic hemozoin. The result of this interaction would be responsible for the antimalarial activity of that class of compounds.[10,15] We decided to test whether our bisguanidine and bis(2-aminoimidazoline) derivatives might work in the same way to evaluate this hypothesis. A simple in vitro assay,[16] the detection by optical density measurement of solubilized *β*-hematin remaining after contact with drugs, was used to assess the capacity of our compounds to inhibit hemozoin biomineralization (Table 7).

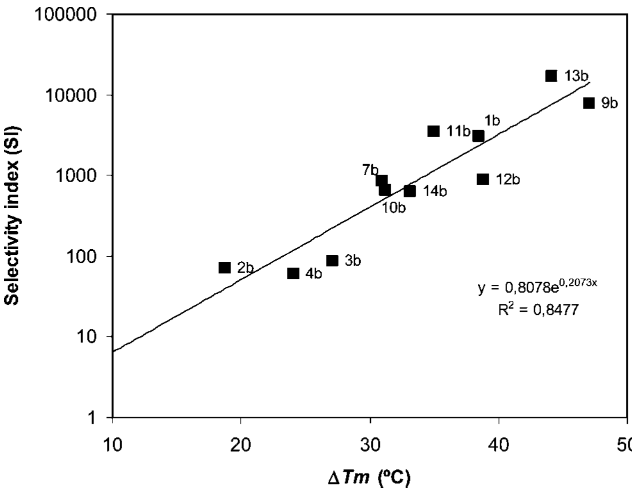

Figure 2. Plot of SI vs T_m for the bis(2-aminoimidazoline) compounds showing a good correlation between in vitro selectivity (SI = (IC_{50} L_6-cells)/(IC_{50} *T. brucei*)) and T_m increase: (■) **1b**, **2b**, **3b**, **4b**, **7b**, **9b**, **10b**, **11b**, **12b**, **13b**, and **14b**.

Table 7. Ferriprotoporphyrin IX Biomineralization Inhibition and Selectivity Index of the Diphenyl Dicationic Compounds

compound	inhibition FPIX[a] IC_{50} (μM)[b]	selectivity[c] SI
1a	0%	36
1b	429.2	24091
1c	161.8	>1548
1d	45%	48
1e	170.7	166
2a	0%	88
2b	NT[d]	4051
2c	15%	>4861
3a	0%	36
3b	0%	1079
4a	0%	76
4b	952.1	932
5a	NT	40
5b	117.2	>1658
5f	219.2	223
6a	42%	>500
6b	NT	>35
6e	27%	1025
7a	0%	>2547
7b	393.3	3714
8a	686.3	26
9a	0%	209
9b	704.7	6893
10a	0%	57
10b	0%	2181
11a	0%	585
11b	0%	12000
12a	0%	3053
12b	127.5	8455
13a	NT	2043
13b	491	7252
14a	0%	83
14b	161	2000

[a] Chloroquine: $IC_{50} = 17.9$ μM. [b] The percentage of inhibition at 1 mg/mL (highest concentration tested) is given when the IC_{50} could not be determined. [c] Selectivity index = IC_{50}(L_6-cells)/IC_{50} (*P. falciparum*). [d] Not tested.

Some of the compounds inhibited hemozoin formation at micromolar concentrations comparable to that of quinine (IC_{50} = 324 μM)[16] though higher than chloroquine (IC_{50} = 17.9 μM). However, no correlation between in vitro antiplasmodial activity and hemozoin inhibition was observed.

Figure 3. SAR against *T. b. rhodesiense* and *P. falciparum*.

Discussion

Encouraged by the excellent in vitro and in vivo trypanocidal activity of a series of dicationic diphenyl compounds previously discovered by our group,[7] we report a continued investigation of 2-aminoimidazoline and guanidine analogues as potential antiplasmodial and antitrypanosomal agents. In vitro and in vivo data from three new series of derivatives revealed some important trends for the SAR of these compounds. In all the cases, the diphenyl dicationic compounds (scaffold A) were the most active antitrypanosomal and antiplasmodial compounds with IC_{50} values in the nanomolar range. These compounds were more efficient than their monocationic analogues which showed IC_{50} in the micromolar range (scaffold B), highlighting once more that the presence of two cationic groups is essential for consistent activity in this series. The same trend was reported earlier with amidine containing diphenyl ureas active against *P. falciparum*,[39] guanidine diphenyl derivatives active against *T. equiperdum*,[21,40] or furamidine analogues.[33,41] In addition, some basic SAR emerged relating to the best linker between both phenyl rings (i.e., NH, NHCO, and CH_2CH_2) and the best cation (i.e., 2-aminoimidazoline) to get selective compounds with good in vivo antitrypanosomal activity (Figure 3). Fused ring dicationic compounds (**13** and **14**) also exhibited excellent activity in agreement with the results reported earlier by Arafa et al.[13] Interestingly, with regard to the linker between the two phenyl rings, our results agree to some extent with the findings of Turner[27] for a series of bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines]. Thus, it appears that the 1,2-diphenylethane and fluorene moieties should be considered as good scaffolds for the design of symmetric dicationic antitrypanosomal drugs regardless of the cationic moiety present in the molecule.

We do not have a clear explanation concerning the extra selectivity and lower in vivo toxicity observed in all the cases with the 2-aminoimidazoline cations in comparison with the guanidine one. One may speculate whether the higher lipophilicity and/or a reduced H-bond donating capacity plays a role in the reduced toxicity of the imidazoline derivatives. On the other hand, the observation that the 2-aminoimidazoline derivatives consistently displayed higher DNA binding affinity than the guanidine counterparts and the correlation observed between selectivity and T_m increase for this series suggest that the formation of a DNA complex may be an important issue to explain the MOA and selectivity profile of these dicationic derivatives. This new and quite exciting observation indicates that more investigations on 2-aminoimidazolines as DNA minor groove binders and antiprotozoal agents are warranted.

Of note was the excellent in vitro antiplasmodial activity of the urea dicationic derivatives **7a** and **7b** (IC_{50} = 96 and 28 nM, respectively). Similar activities had been reported for amidine-containing diphenylureas.[39] Interestingly, Jiang and coworkers have also reported micromolar in vitro anti-*P. falciparum* activity of a monocationic diphenylurea compound. That compound, WR268961, the structure of which is related to **20** (i.e., scaffold B), was an inhibitor of the recombinant *P. falciparum* aspartic protease plasmepsin II.[42] This is relevant because plasmepsins are validated targets for antimalarial therapy[43–45] and the activity of **7**, **20**, and **21** might be related to plasmepsin inhibition, even though this would need experimental confirmation.

Another exciting finding was the nanomolar activity exhibited by the Boc-protected compounds (**1d**, **1e**, and **6e**) against *P. falciparum* K1. The fair FPIX biomineralization inhibition displayed by **1e** in the FBIT assay (IC_{50} = 170.7 μM) suggests that this compound could interfere in the heme detoxification process of the parasite. However, since these highly lipophilic precursors are uncharged molecules, one might expect their pharmacodynamics to be different from the dicationic derivatives.

Despite the lack of correlation between FPIX biomineralization inhibition and in vitro activity against *P. falciparum*, the binding to FPIX might be responsible to some extent for the observed antiplasmodial activity and/or for the accumulation of the compounds into the parasites as shown previously for some diamidine drugs.[15] However, care should be taken in the interpretation of these results because we have no evidence at this time that these inhibitors are able to reach the food vacuole of the parasite to target the heme detoxification pathway. Indeed, additional MOA is likely to be involved because our dicationic compounds were less potent FPIX inhibitors than CQ, whereas they showed nanomolar range activity about 1 order of magnitude superior to CQ against the *P. falciparum* CQ-resistant strain K1.

The diphenyl dicationic compounds retained only moderate activity in the *P. berghei* murine model, in contrast to CQ, which may possibly indicate unfavorable pharmacokinetic properties or a different MOA. On the other hand, the modest in vivo activity observed after ip administration in the *P. berghei* model does not necessarily mean that these diphenyldiguanidine compounds are poor antimalarials. In fact, some recent evidence suggests that the *P. berghei* mouse model might not be optimal to predict the activity of aromatic diamidines against human malaria.[11] For instance, DB289, which showed only modest activity in the *P. berghei* mouse model, has shown remarkable activity in a human trial against *P. falciparum*.[14] For that reason, we believe that these families of compounds deserve more thorough investigations of their in vivo antimalarial action.

Conclusion

In order to extend our understanding of the SAR of the bis(2-aminoimidazoline) lead compounds reported earlier,[7,8] we have screened a new series of symmetric dicationic guanidine and 2-aminoimidazoline aromatic analogues against *T. b. rhodesiense* and *P. falciparum*. We have shown that in this series the 2-aminoimidazoline derivatives were safer (higher SI) and more potent in vivo against *T. b. rhodesiense* than their guanidine counterparts. Moreover, a correlation between DNA binding affinity and selectivity toward the parasite was observed indicating that high affinity binding to the minor groove of DNA may be part of their mechanism of antitrypanosomal action. This view[7] was also supported by the observation of a correlation between DNA binding affinity and trypanocidal activity of two series of compounds.

Five new dicationic lead compounds (**7b**, **9a**, **9b**, **10b**, and **14b**), upon ip administration of 20 (mg/kg)/day, cured 100% of treated mice in the *T. b. rhodesiense* STIB900 model. In

contrast, despite their excellent in vitro antiplasmodial activity and capacity for reducing the parasitaemia of mice infected with *P. berghei*, the dicationic compounds **1b**, **2b**, **3b**, **4b**, and **12a** did not cure the animals in this model. However, the *P. berghei* mouse model may not be the best predictive model for this kind of dicationic compounds as we have pointed out before.

In this study we also described the excellent in vitro antiplasmodial activity of noncationic Boc-protected guanidines and imidazolines as well as phosphonium derivatives, the in vivo antimalarial efficacy of which is currently being evaluated. Hence, more thorough investigation on the antiplasmodial activity of this class of compounds is warranted.

In light of these promising results, we believe that bis(2-aminoimidazoline) derivatives deserve more investigation as antiprotozoal agents and DNA minor groove binders. The synthesis and study of new derivatives and prodrugs of our lead compounds is ongoing and will be reported in due course.

Experimental Section

Chemistry. All the commercial chemicals were obtained from Sigma-Aldrich, Fluka, or Lancaster and were used without further purification. Deuterated solvents for NMR use were purchased from Apollo. Dry solvents were prepared using standard procedures according to Vogel,[46] with distillation prior to use. Chromatographic columns were run using silica gel 60 (230–400 mesh ASTM) or aluminum oxide (activated, Neutral Brockman I STD grade 150 mesh). Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F_{254} silica gel plates or Polygram Alox N/UV_{254} aluminum oxide plates. Visualization was by UV light (254 nm). NMR spectra were recorded in a Bruker DPX-400 Avance spectrometer operating at 400.13 and 600.1 MHz for ^{1}H NMR and 100.6 and 150.9 MHz for ^{13}C NMR. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker Win-NMR 5.0 software. Electrospray mass spectra were recorded on a Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with methanol, water, or ethanol as carrier solvents. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrometer equipped with a Gateway 2000 4DX2-66 workstation and on a PerkinElmer Spectrum One FT-IR spectrometer equipped with Universal ATR sampling accessory. Sample analysis was carried out in Nujol using NaCl plates. Elemental analysis was carried out at the Microanalysis Laboratory, School of Chemistry and Chemical Biology, University College Dublin. Analytical results were within ±0.4% of the theoretical (calcd) values unless otherwise noted.

(1) Synthesis of the Starting Material Amines. 1,3-Bis(4-aminophenyl)urea (7).[27] A suspension of 5 g (16.5 mmol) of 1,3-bis(4-nitrophenyl)urea in methanol in the presence of 5% Pd−C (480 mg) was hydrogenated at atmospheric pressure and room temperature for 22 h. Afterward, the catalyst was filtered through a pad of Celite. The filter cake was rinsed with MeOH/DMF (1:1) and the solvent removed in vacuo to afford 3.9 g (97%) of 1,3-bis(4-aminophenyl)urea **7** as a white solid. ^{1}H NMR (DMSO-d_6) δ 4.73 (broad s, 4H, NH_2), 6.51 (d, 4H, J = 8.0 Hz, Ar), 7.07 (d, 4H, J = 8.0 Hz, Ar), 8.08 (broad s, 2H, NH); ^{13}C NMR (DMSO-d_6) δ 115.6, 121.8, 130.6, 145.0 (Ar), 154.8 (CO).

1,4-Bis(4-nitrophenyl)piperidine (11f). A solution of 2.5 g (12.1 mmol) of 4-(4-nitrophenyl)piperidine[28,29] and 1.712 g (12.1 mmol) of 1-fluoro-4-nitrobenzene in DMF (8 mL) was heated at 100 °C for 72 h. Afterward, the solvent was removed in vacuo and the residue obtained was recrystallized from CH_3CN to yield 2.95 g (74%) of 1,4-bis(4-nitrophenyl)piperidine **11f** as a yellowish solid: mp 170–172 °C; MS (ESI$^+$) m/z 350.1105 [M + Na]$^+$; IR (Nujol) ν 1597, 1516 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.62–1.80 (m, 2H, H_3Pip), 1.83–1.97 (m, 2H, H_3Pip), 2.99–3.19 (m, 3H, H_{2e}Pip + H_4Pip), 4.16–4.31 (m, 2H, H_{2a}Pip), 7.08 (d, 2H, J = 8.8 Hz, Ar), 7.56 (d, 2H, J = 7.8 Hz, Ar), 8.06 (d, 2H, J = 8.8 Hz, Ar), 8.17 (d, 2H, J = 7.8 Hz, Ar); ^{13}C NMR (DMSO-d_6) δ 33.0 (C_3Pip), 42.7 (C_4Pip), 48.4 (C_2Pip), 113.9, 124.9, 127.2, 129.5, 137.6, 147.3, 154.8, 155.7 (Ar).

1,4-Bis(4-aminophenyl)piperidine (11). A suspension of 1.5 g (4.6 mmol) of 1,4-bis(4-nitrophenyl)piperidine **11f** in methanol in the presence of 5% Pd−C (185 mg) was hydrogenated at 3 bar and room temperature for 22 h. Afterward, the catalyst was filtered off and the solvent removed in vacuo to afford 1.198 g (98%) of **11** as a navy-blue solid: mp 156–158 °C; MS (ESI$^+$) m/z 268.1802 [M + H]$^+$; IR (Nujol) ν 3365, 3297, 3201 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.63–1.80 (m, 4H, H_3Pip), 2.32–2.43 (m, 1H, H_4Pip), 2.53–2.61 (m, 2H, H_{2e}Pip), 3.38–3.49 (m, 2H, H_{2a}Pip), 4.58 (broad s, 2H, NH_2), 4.85 (broad s, 2H, NH_2), 6.46–6.54 (m, 4H, Ar), 6.73 (d, 2H, J = 8.8 Hz, Ar), 6.92 (d, 2H, J = 8.3 Hz, Ar); ^{13}C NMR (DMSO-d_6) δ 35.1 (C_3Pip), 42.1 (C_4Pip), 53.3 (C_2Pip), 115.4, 116.2, 120.0, 128.4, 134.9, 143.4, 144.5, 148.0 (Ar).

1-(4-Aminophenyl)-3-phenylurea (20). An amount of 1.10 mL (10.0 mmol) of phenyl isocyanate was added under an inert atmosphere and at 0 °C over a solution of 1.082 g (10.0 mmol) of 1,4-phenylenediamine in dry CH_2Cl_2 (10 mL). The mixture was allowed to reach room temperature and was stirred for 10 min. The white solid precipitated was filtered and washed with cold CH_2Cl_2 to afford 2.15 g (95%) of the pure 1-(4-aminophenyl)-3-phenylurea: mp, decomposes at 212 °C (lit.,[47] 260 °C); IR (Nujol) ν 3353, 3292, 3181 (NH, NH_2), 1622 (CO, lit.,[48] 1630) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.80 (broad s, 2H, NH_2), 6.56 (d, 2H, J = 8.5 Hz, Ar), 6.88–6.99 (m, 1H, Ar), 7.12 (d, 2H, J = 8.5 Hz, Ar), 7.21–7.30 (m, 2H, Ar), 7.46 (d, 2H, J = 8.0 Hz, Ar), 8.17 (broad s, 1H, NH), 8.51 (broad s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 115.5, 119.3, 122.2, 122.7, 129.9, 130.1, 141.5, 145.4 (Ar), 154.3 (CO).

4-(*tert*-Butoxycarbonylamino)benzamide (22c). Aniline (930 mg, 10 mmol) and TBTU (3.211 g, 10 mmol) were added at 0 °C and under argon over a solution containing 4-[(*tert*-butoxycarbonyl)amino]benzoic acid (2.373 g, 10.0 mmol) and Et_3N (5.6 mL, 40 mmol) in 15 mL of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 7 h. After that time a white precipitate was formed. This precipitate turned out to be the desired compound and was filtered and washed with cold hexane to yield 2.3 g (74%) of 4-(*tert*-butoxycarbonylamino)benzamide as a white solid: mp 205–207 °C; IR (Nujol) ν 3352, 3330 (NH), 1704, 1646 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.51 (s, 9H, $(CH_3)_3$), 7.06–7.15 (m, 1H, Ar), 7.32–7.42 (m, 2H, Ar), 7.54–7.67 (m, 2H, Ar), 7.74–7.85 (m, 2H, Ar), 7.89–7.99 (m, 2H, Ar), 9.72 (broad s, 1H, NH), 10.09 (broad s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 29.4 ($(CH_3)_3$), 80.9 ($\underline{C}(CH_3)_3$), 118.5, 121.7, 124.8, 129.4, 129.9, 130.0, 140.7, 144.1 (Ar), 154.0 ($(CH_3)_3COC\underline{O}$), 166.3 (PhNH$\underline{C}$OPh).

4-Aminobenzanilide (22). A solution of 3.113 g (10.0 mmol) of **22c** in 35 mL of CH_2Cl_2/TFA (1:1) was stirred at room temperature for 6 h. After that time, the solvent was eliminated under vacuum to generate the trifluoroacetate salt. This salt was redissolved in 20 mL of CH_2Cl_2 and was washed with a 2 M NaOH solution (2 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered and the solvent was evaporated to afford 1.961 g (93%) of 4-aminobenzanilide as a white solid: mp 136–138 °C (lit.,[49] 138–140 °C); IR (Nujol) ν 3393, 3350, 3184 (NH, NH_2), 1644 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6)[50] δ 5.79 (broad s, 2H, NH_2), 6.64 (d, 2H, J = 8.8 Hz, Ar), 7.02–7.11 (m, 1H, Ar), 7.31–7.40 (m, 2H, Ar), 7.72–7.86 (m, 4H, Ar), 9.80 (broad s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 113.9, 121.5, 122.4, 124.2, 129.8, 130.7, 141.1, 153.5 (Ar), 166.7 (CO).

(2) Synthesis of Boc-Protected Precursors and New Tested Compounds. General Method A for the Synthesis of the Boc-Protected Guanidine and Boc-Protected 2-Aminoimidazoline Precursors. An amount of 6.6 mmol of $HgCl_2$ was added over a solution of 3.0 mmol of the corresponding diamine, 6.0 mmol of *N,N′*-di(*tert*-butoxycarbonyl)thiourea (for **7d**, **9d**, and **11d**), or *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione (for **7e**, **9e**, and **11e**) and 2.1 mL (15.0 mmol) of Et_3N in DMF (10 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake

was rinsed with EtOAc. The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by column chromatography as specified.

General Method B for the Removal of the Boc-Protecting Groups and Regeneration of the Hydrochloride Salts. A solution of the corresponding Boc-protected derivative (0.5 mmol) in 20 mL of CH_2Cl_2/TFA (1:1) was stirred at room temperature for the appropriate duration. After that time, the solvent was eliminated under vacuum to generate the trifluoroacetate salt. This salt was dissolved in 20 mL of water and treated for 24 h with IRA400 Amberlyte resin in its Cl^- form. Then the resin was removed by filtration and the aqueous solution washed with CH_2Cl_2 (2 × 10 mL). Evaporation of the water afforded the pure dihydrochloride salt as a hygroscopic solid. Absence of the trifluoroacetate salt was checked by ^{19}F NMR.

Alternative Method C for the Synthesis of the Boc-Protected Guanidines 20d, 21d, and 23d. A solution of 4-[2,3-di(*tert*-butoxycarbonyl)guanidino]aniline **8** in dry CH_2Cl_2 was treated under an inert atmosphere and at 0 °C with the corresponding electrophile (phenyl isocyanate for **20d**, phenyl isothiocyanate for **21d**, and benzoyl chloride for **23d**) and with Et_3N (only in the case of **23d**). After the reaction mixture was allowed to reach room temperature, it was stirred for the appropriate duration. Further workup followed by column cromatography as specified afforded the corresponding Boc-protected guanidine.

1,3-Bis(4-[2,3-di(*tert*-butoxycarbonyl)guanidino]phenyl)urea (7d). Following the general synthetic method A, the crude residue was purified by silica gel column chromatography, eluting with hexane/EtOAc (3:1) to yield 1.24 g (57%) of **7d** as a white solid: mp >300 °C; MS (ESI$^+$) *m/z* 727.3777 [M + H]$^+$; IR (Nujol) ν 3368, 3307, 3264 (NH), 1720, 1644, 1624, 1605 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 18H, $(CH_3)_3$), 1.58 (s, 18H, $(CH_3)_3$), 7.25 (d, 4H, J = 8.5 Hz, Ar), 7.29 (d, 4H, J = 8.5 Hz, Ar), 7.40 (broad s, 2H, NHCONH), 10.14 (broad s, 2H, NH_{Gu}), 11.64 (broad s, 2H, NH_{Gu}); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.1 ($(CH_3)_3$), 80.0, 83.8 ($\underline{C}(CH_3)_3$), 118.8, 124.8, 129.9, 137.6 (Ar), 152.1, 153.1 ($(CH_3)_3CO\underline{C}O$), 155.3, 163.2 (NHCONH, CN).

1,3-Bis(4-[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]phenyl)urea (7e). Following the general synthetic method A, the crude residue was purified by neutral alumina column flash chromatography, eluting with CH_2Cl_2/EtOAc (1:5). The residue obtained after the column was precipitated with cold hexane to yield 1.25 g (63%) of **7e** as a white solid: mp 180–182 °C; IR (Nujol) ν 3357 (NH), 1757, 1704, 1648 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 36H, $(CH_3)_3$), 3.83 (s, 8H, CH_2), 6.90 (d, 4H, J = 7.0 Hz, Ar), 7.14–7.26 (m, 6H, 4Ar + 2NH); ^{13}C NMR ($CDCl_3$) δ 27.8 ($(CH_3)_3$), 43.1 (CH_2), 82.9 ($\underline{C}(CH_3)_3$), 120.6, 121.8, 133.9, 139.3 (Ar), 143.4 ($(CH_3)_3CO\underline{C}O$), 150.2, 153.5 (NHCONH, CN).

Dihydrochloride Salt of 1,3-Bis(4-guanidinophenyl)urea (7a). Following general method B, an amount of 210 mg (95%) of the pure dihydrochloride salt of **7a** was obtained as a white solid: mp, decomposes at 248 °C; MS (ESI$^+$) *m/z* 327.1928 [M + H]$^+$; ^{1}H NMR (D_2O) δ 7.27 (d, 4H, J = 8.5 Hz, Ar), 7.43 (d, 4H, J = 8.5 Hz, Ar); ^{13}C NMR (D_2O) δ 121.6, 126.4, 129.1, 137.0 (Ar), 155.0, 156.0 (CO, CN). Anal. ($C_{15}H_{20}Cl_2N_8O \cdot 2.3H_2O$) C, H, N.

1,3-Bis(4-[2,3-di(*tert*-butoxycarbonyl)guanidino]phenyl)thiourea-(8d). A solution of 701 mg (2.0 mmol) of 4-[2,3-di(*tert*-butoxycarbonyl)guanidino]aniline **8** and 3.0 mL (50.0 mmol) of carbon disulfide in 20 mL of CH_2Cl_2 was stirred at reflux for 54 h. Afterward, the solvent was evaporated to give a residue that was purified by silica gel column chromatography, eluting with hexane/EtOAc (3:2) to yield 240 mg (32%) of **8d** as a white solid: mp >300 °C; MS (ESI$^+$) *m/z* 743.3412 [M + H]$^+$; IR (Nujol) ν 3396, 3291, 3169 (NH), 1720, 1646, 1630, 1152 (CO, CN, CS) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 18H, $(CH_3)_3$), 1.55 (s, 18H, $(CH_3)_3$), 7.34 (d, 4H, J = 8.0 Hz, Ar), 7.61 (d, 4H, J = 8.0 Hz, Ar), 7.98 (broad s, 2H, NHCSNH), 10.36 (broad s, 2H, NH_{Gu}), 11.63 (broad s, 2H, NH_{Gu}); ^{13}C NMR ($CDCl_3$) δ 27.8, 27.9 ($(CH_3)_3$), 79.6, 83.6 ($\underline{C}(CH_3)_3$), 123.4, 124.9, 134.0, 134.8 (Ar), 152.9, 153.8 (CO), 163.1 (CN), 179.4 (CS).

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]benzanilide (9d). Following the general synthetic method A, the crude residue was purified by silica gel column chromatography, eluting with hexane/EtOAc (2:1) to yield 1.3 g (61%) of **9d** as a white solid: mp >300 °C; MS (ESI$^+$) *m/z* 712.3635 [M + H]$^+$; IR (Nujol) ν 3368, 3291, 3261 (NH), 1738, 1720, 1645, 1627, 1608 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H, $(CH_3)_3$), 1.51 (s, 9H, $(CH_3)_3$), 1.56 (s, 18H, $(CH_3)_3$), 7.54 (d, 2H, J = 8.6 Hz, Ar), 7.64 (d, 2H, J = 8.0 Hz, Ar), 7.68 (d, 2H, J = 8.0 Hz, Ar), 7.82 (d, 2H, J = 8.6 Hz, Ar), 8.19 (broad s, 1H, PhNHCOPh), 10.30 (broad s, 1H, NH_{Gu}), 10.51 (broad s, 1H, NH_{Gu}), 11.63 (broad s, 2H, NH_{Gu}); ^{13}C NMR ($CDCl_3$) δ 27.9, 28.0 ($(CH_3)_3$), 79.6, 80.0, 83.6, 84.0 ($\underline{C}(CH_3)_3$), 120.6, 122.1, 123.4, 128.1, 131.2, 132.1, 135.9, 139.3 (Ar), 153.2, 153.7, 154.0 ($(CH_3)_3CO\underline{C}O$), 163.1, 163.4 (CN), 165.2 (PhNH$\underline{C}$OPh).

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]benzanilide (9e). Following the general synthetic method A, the crude residue was purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc (1:4). The residue obtained after the column was recrystallized from Et_2O to yield 1.040 g (45%) of **9e** as a white solid: mp 198–200 °C; IR (Nujol) ν 3346 (NH), 1733, 1709, 1689, 1663, 1598 (CO, CN) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 36H, $(CH_3)_3$), 3.77 (s, 4H, CH_2), 3.80 (s, 4H, CH_2), 6.83 (d, 2H, J = 8.5 Hz, Ar), 6.92 (d, 2H, J = 8.5 Hz, Ar), 7.66 (d, 2H, J = 8.5 Hz, Ar), 7.89 (d, 2H, J = 8.5 Hz, Ar), 9.92 (broad s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 28.4, 28.5 ($(CH_3)_3$), 43.9, 44.0 (CH_2), 82.4, 82.6 ($\underline{C}(CH_3)_3$), 121.3, 121.6, 121.7, 128.6, 129.3, 134.8, 139.8, 141.0 (Ar), 145.1, 150.4, 150.6, 152.9 ($(CH_3)_3CO\underline{C}O$), CN), 165.4 (PhNH$\underline{C}$OPh).

1,4-Bis[4-(N^2,N^3-bis(*tert*-butyloxycarbonyl)guanidino)phenyl]piperidine (11d). General method A was used, with 598 mg (2.2 mmol) of $HgCl_2$, 268 mg (1.0 mmol) of **11**, 553 mg (2.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)thiourea, and 0.9 mL (6.4 mmol) of Et_3N in CH_2Cl_2/DMF (1:2) (8 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 36 h more at room temperature. After general workup, the residue was purified by silica gel column chromatography, eluting with hexane/EtOAc (3:1) to yield 483 mg (64%) of **11d** as a white solid: mp, decomposes at 220 °C; MS (ESI$^+$) *m/z* 752.4326 [M + H]$^+$; IR (Nujol) ν 3291, 3266 (NH), 1728, 1638, 1608 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 18H, $(CH_3)_3$), 1.55 (s, 18H, $(CH_3)_3$), 1.82–2.00 (m, 4H, H_3Pip), 2.56–2.69 (m, 1H, H_4Pip), 2.71–2.89 (m, 2H, H_{2e}Pip), 3.71–3.82 (m, 2H, H_{2a}Pip), 6.95 (d, 2H, J = 8.0 Hz, Ar), 7.22 (d, 2H, J = 7.0 Hz, Ar), 7.48 (d, 2H, J = 8.0 Hz, Ar), 7.54 (d, 2H, J = 7.0 Hz, Ar), 10.19 (broad s, 1H, NH), 10.29 (broad s, 1H, NH), 11.68 (broad s, 2H, NH); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.1, 28.2 ($(CH_3)_3$), 33.1 (C_3Pip), 41.8 (C_4Pip), 50.8 (C_2Pip), 79.3, 79.4, 83.3, 83.5 ($\underline{C}(CH_3)_3$), 117.0, 122.4, 123.3, 127.2, 128.7, 134.7, 142.5, 149.0 (Ar), 153.2, 153.3, 153.4, 153.5 (CO), 163.5, 163.6 (CN).

Di-*tert*-butyl-2-(4-[4-(4-[1,3-bis(*tert*-butyloxycarbonyl)tetrahydro-1*H*-2-imidazolyliden]aminophenyl)piperidino]phenylimino)-1,3-imidazolidinedicarboxylate (11e). Method A was used, with 598 mg (2.2 mmol) of $HgCl_2$, 268 mg (1.0 mmol) of **11**, 605 mg (2.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 0.9 mL (6.4 mmol) of Et_3N in CH_2Cl_2/DMF (1:2) (8 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 41 h more at room temperature. The reaction was worked up and purified by silica gel column chromatography, eluting with hexane/EtOAc (1:1). The residue obtained after the column was recrystallized from hexane/Et_2O (1:1) to yield 523 mg (65%) of **11e** as a white solid: mp 191–193 °C; MS (ESI$^+$) *m/z* 804.4681 [M + H]$^+$; IR (Nujol) ν 1753, 1704, 1662, 1606 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 18H, $(CH_3)_3$), 1.34 (s, 18H, $(CH_3)_3$), 1.83–1.98 (m, 4H, H_3Pip), 2.49–2.61 (m, 1H, H_4Pip), 2.66–2.79 (m, 2H, H_{2e}Pip), 3.60–3.71 (m, 2H, H_{2a}Pip), 3.83 (s, 8H, CH_2), 6.85–7.0 (m, 6H, Ar), 7.11 (d, 2H, J = 8.0 Hz, Ar); ^{13}C NMR ($CDCl_3$) δ 27.8, 27.9 ($(CH_3)_3$), 33.4 (C_3Pip), 41.8 (C_4Pip), 43.0 (CH_2), 51.8 (C_2Pip), 82.5, 82.7 ($\underline{C}(CH_3)_3$), 117.8, 121.4, 122.2, 126.9, 138.4, 139.0, 140.4, 141.1 (Ar), 146.4, 148.0 (CO); 150.3, 150.4 (CN).

Trihydrochloride Salt of 1,4-Bis(4-guanidinophenyl)piperidine (11a). Following method B, an amount of 224 mg (90%) of the pure hydrochloride salt of **11a** was obtained as a brown solid:

mp, decomposes at 220 °C; ^{1}H NMR (D_2O) δ 2.19–2.33 (m, 4H, H_3Pip), 3.10–3.24 (m, 1H, H_4Pip), 3.80–3.93 (m, 4H, H_2Pip), 7.32 (d, 2H, J = 8.0 Hz, Ar), 7.48 (d, 2H, J = 8.0 Hz, Ar), 7.54 (d, 2H, J = 8.5 Hz, Ar), 7.78 (d, 2H, J = 8.5 Hz, Ar); ^{13}C NMR (D_2O) δ 29.9 (C_3Pip) 37.4 (C_4Pip), 56.3 (C_2Pip) 122.3, 125.8, 126.6, 127.8, 132.3, 135.9, 139.4, 142.9 (Ar), 155.6, 155.9 (CN). Anal. ($C_{19}H_{28}Cl_3N_7 \cdot 2H_2O$) C, H, N.

Trihydrochloride Salt of 1,4-Bis[4-(4,5-dihydro-1*H*-2-imidazolylamino)phenyl]piperidine (11b). Following method B, an amount of 279 mg (94%) of the pure hydrochloride salt of **11b** was obtained as a brown solid: mp 101–103 °C; MS (ESI$^+$) *m/z* 404.2578 [M + H]$^+$; ^{1}H NMR (D_2O) δ 2.14–2.29 (m, 4H, H_3Pip), 3.07–3.19 (m, 1H, H_4Pip), 3.72 (s, 4H, CH_2), 3.76 (s, 4H, CH_2), 3.74–3.86 (m, 4H, H_2Pip), 7.26 (d, 2H, J = 8.6 Hz, Ar), 7.43 (d, 2H, J = 8.0 Hz, Ar), 7.49 (d, 2H, J = 8.6 Hz, Ar), 7.75 (d, 2H, J = 8.0 Hz, Ar); ^{13}C NMR (D_2O) δ 29.8 (C_3Pip) 37.3 (C_4Pip), 42.2, 42.3 (CH_2), 56.3 (C_2Pip) 122.2, 124.0, 125.0, 127.7, 133.2, 136.5, 138.9, 142.3 (Ar), 157.8, 158.2 (CN). Anal. ($C_{23}H_{32}Cl_3N_7 \cdot 2.3H_2O$) C, H, N.

4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]benzophenone (19d). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 592 mg (3.0 mmol) of 4-aminobenzophenone, 830 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of Et_3N in DMF (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 48 h more at room temperature. The reaction was worked up and purified by silica gel column chromatography, eluting with hexane/EtOAc (2:1) to yield 976 mg (74%) of **19d** as a white solid: mp 151–153 °C; IR (Nujol) ν 3197, 3154 (NH), 1718, 1656, 1643, 1595 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 9H, $(CH_3)_3$), 1.56 (s, 9H, $(CH_3)_3$), 7.44–7.51 (m, 2H, Ar), 7.54–7.60 (m, 1H, Ar), 7.75–7.86 (m, 6H, Ar), 10.63 (broad s, 1H, NH), 11.66 (broad s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.1 ($(CH_3)_3$), 80.0, 84.1 ($\underline{C}(CH_3)_3$), 120.9, 128.1, 129.8, 131.3, 132.1, 133.2, 137.8, 140.8 (Ar), 153.1, 153.2 ($(CH_3)_3COC\underline{O}$), 163.2 (CN), 195.6 (PhCOPh).

4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]benzophenone (19e). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 592 mg (3.0 mmol) of 4-aminobenzophenone, 907 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of Et_3N in DMF (5 mL). The resulting mixture was stirred at 0 °C for 1 and 48 h more at room temperature. The reaction was worked up and purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc (2:3). The residue obtained after the column was precipitated and washed with cold hexane/Et_2O (1:1) to yield 600 mg (43%) of **19e** as a white solid: mp 49–51 °C; IR (Nujol) ν 1750, 1719, 1644 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 18H, $(CH_3)_3$), 3.78 (s, 4H, CH_2), 6.96 (d, 2H, J = 8.5 Hz, Ar), 7.33–7.39 (m, 2H, Ar), 7.41–7.48 (m, 1H, Ar), 7.61–7.70 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$) δ 27.5 ($(CH_3)_3$), 42.9 (CH_2), 82.6 ($\underline{C}(CH_3)_3$), 120.6, 127.7, 129.2, 130.7, 131.1, 131.4, 138.1, 140.1 (Ar), 149.5 ($(CH_3)_3COCO$), 152.9 (CN), 195.4 (PhCOPh).

Hydrochloride Salt of 4-Guanidinobenzophenone (19a). Following method B, 145 mg (97%) of the pure hydrochloride salt of **19a** was obtained as a white solid: mp 187–189 °C; MS (ESI$^+$) *m/z* 240.1208 [M + H]$^+$; ^{1}H NMR (D_2O) δ 7.15 (d, 2H, J = 8.0 Hz, Ar), 7.23–7.31 (m, 2H, Ar), 7.40 (d, 2H, J = 8.0 Hz, Ar), 7.44–7.52 (m, 3H, Ar); ^{13}C NMR (D_2O) δ 122.7, 127.9, 129.5, 131.4, 132.9, 133.7, 135.6, 138.7 (Ar), 154.9 (CN), 197.6 (CO).

Hydrochloride Salt of 4-(2-Imidazolidinylimino)benzophenone (19b). Following method B, 156 mg (92%) of the pure hydrochloride salt of **19b** were obtained as a yellow solid: mp 64–66 °C; MS (ESI$^+$) *m/z* 266.1324 [M + H]$^+$; ^{1}H NMR (D_2O) δ 3.64 (s, 4H, CH_2), 7.01 (d, 2H, J = 8.8 Hz, Ar), 7.18–7.25 (m, 2H, Ar), 7.31 (d, 2H, J = 7.2 Hz, Ar), 7.34–7.43 (m, 3H, Ar); ^{13}C NMR (D_2O) δ 42.2 (CH_2), 120.7, 127.9, 129.4, 131.4, 132.8, 133.0, 135.5, 139.2 (Ar), 156.5 (CN), 197.1 (CO). Anal. ($C_{16}H_{16}ClN_3O \cdot 2.0H_2O$) C, H, N.

1-(4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]phenyl)-3-phenylurea (20d). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 682 mg (3.0 mmol) of 1-(4-aminophenyl)-3-phenylurea **20**, 830 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of Et_3N in DMF (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 26 h more at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by silica gel column chromatography, eluting with hexane/EtOAc (1:1) to yield 940 mg (67%) of **20d** as a white solid.

Method C is as follows. An amount of 0.33 mL (3.0 mmol) of phenyl isocyanate was added under an inert atmosphere and at 0 °C over a solution of 1.052 g (3.0 mmol) of 4-[2,3-di(*tert*-butoxycarbonyl)guanidino]aniline **8** in dry CH_2Cl_2 (10 mL). The mixture was allowed to reach room temperature and was stirred for 10 h. Then the solvent was removed in vacuo and the residue obtained was purified by silica gel column chromatography, eluting with hexane/EtOAc (1:1). The pure compound **20d** was obtained as a white solid (1.034 g, 73%) by recrystallization from hexane/CH_2Cl_2: mp >300 °C; MS (ESI$^+$) *m/z* 470.2425 [M + H]$^+$; IR (Nujol) ν 3300 (NH), 1726, 1633, 1600, 1568 (CO, CN) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 9H, $(CH_3)_3$), 1.53 (s, 9H, $(CH_3)_3$), 6.94–7.02 (m, 1H, Ar), 7.24–7.33 (m, 2H, Ar), 7.38–7.53 (m, 6H, Ar), 8.69 (broad s, 1H, NHCONH), 8.71 (broad s, 1H, NHCONH), 9.96 (broad s, 1H, NH_{Gu}), 11.54 (broad s, 1H, NH_{Gu}); ^{13}C NMR (DMSO-d_6) δ 28.9, 29.2 ($(CH_3)_3$), 80.0, 84.6 ($\underline{C}(CH_3)_3$), 119.5, 119.6, 123.1, 125.1, 130.1, 131.8, 138.2, 141.0 (Ar), 153.6, 153.8, 154.5, 164.2 ($(CH_3)_3COC\underline{O}$, CN, NHCONH).

1-(4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]phenyl)-3-phenylurea (20e). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 682 mg (3.0 mmol) of **20**, 907 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of Et_3N in DMF (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 38 h more at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc/CH_2Cl_2 (6:4:1) to yield 924 mg (62%) of **20e** as a white solid: mp 162–164 °C; IR (Nujol) ν 3365 (NH), 1739, 1728, 1708, 1694 (CO, CN) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 18H, $(CH_3)_3$), 3.75 (s, 4H, CH_2), 6.79 (d, 2H, J = 7.0 Hz, Ar), 6.92–7.01 (m, 1H, Ar), 7.23–7.36 (m, 4H, Ar), 7.45 (d, 2H, J = 7.5 Hz, Ar), 8.51 (broad s, 1H, NH), 8.57 (broad s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 28.8 ($(CH_3)_3$), 44.2 (CH_2), 82.7 ($\underline{C}(CH_3)_3$), 119.4, 120.0, 122.5, 122.9, 130.1, 135.5, 140.0, 141.2 (Ar), 144.3, 151.0, 153.8 ($(CH_3)_3COC\underline{O}$, CN, NHCONH).

Trifluoroacetate Salt of 1-(4-Guanidinophenyl)-3-phenylurea (20a). A solution of 235 mg (0.5 mmol) of **20d** in 20 mL of CH_2Cl_2/TFA (1:1) was stirred at room temperature for 3 h. After that time, the solvent was eliminated under vacuum and cold water was added to generate a precipitate that was filtered, washed with an additional 100 mL of cold water, and dried to yield 176 mg (91%) of the pure trifluoroacetate salt as a purple solid: mp 116–118 °C; MS (ESI$^+$) *m/z* 270.1328 [M + H]$^+$; ^{1}H NMR (CD_3OD) δ 6.96–7.03 (m, 1H, Ar), 7.18 (d, 2H, J = 8.5 Hz, Ar), 7.23–7.31 (m, 2H, Ar), 7.36–7.45 (m, 2H, Ar), 7.43 (d, 2H, J = 8.0 Hz, Ar); ^{13}C NMR (CD_3OD) δ 120.5, 121.4, 123.8, 124.0, 127.7, 129.8, 140.3, 140.4 (Ar), 155.3, 158.3 (CN, CO); ^{19}F NMR (CD_3OD) δ −79.35. Anal. ($C_{16}H_{16}F_3N_5O_3 \cdot 0.2H_2O$) C, H, N.

Trifluoroacetate Salt of 1-[4-(2-imidazolidinylimino)phenyl]-3-phenylurea (20b). A solution of 248 mg (0.5 mmol) of **20e** in 20 mL of CH_2Cl_2/TFA (1:1) was stirred at room temperature for 3 h. After that time, the solvent was eliminated under vacuum and cold water was added to generate a precipitate that was filtered, washed with an additional 100 mL of cold water, and dried to yield 194 mg (89%) of the pure trifluoroacetate salt as a gray solid: mp 102–104 °C; MS (ESI$^+$) *m/z* 296.1522 [M + H]$^+$; ^{1}H NMR (CD_3OD) δ 3.73 (s, 4H, CH_2), 6.96–7.04 (m, 1H, Ar), 7.18 (d, 2H, J = 9.0 Hz, Ar), 7.22–7.30 (m, 2H, Ar), 7.44 (d, 2H, J = 7.6 Hz,

Ar), 7.53 (d, 2H, $J = 9.0$ Hz, Ar); ^{13}C NMR (CD_3OD) δ 44.1 (CH_2), 120.5, 121.2, 124.0, 126.3, 129.8, 130.9, 140.1, 140.3 (Ar), 155.3, 160.4 (CN, CO); ^{19}F NMR (CD_3OD) δ −78.90. Anal. ($C_{18}H_{18}F_3N_5O_3 \cdot 1.4H_2O$) C, H, N.

1-(4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]phenyl)-3-phenylthiourea (21d). Method C was used, with 0.37 mL (3.0 mmol) of phenyl isothiocyanate and 1.052 g (3.0 mmol) of 4-[2,3-di(*tert*-butoxycarbonyl)guanidine]aniline **8** in dry CH_2Cl_2 (10 mL). The mixture was allowed to reach room temperature and was stirred for 18 h. Then the solvent was removed in vacuo and the residue obtained was purified by silica gel column chromatography, eluting with hexane/EtOAc (1:1). The pure compound **21d** was obtained as a white solid (988 mg, 68%) by recrystallization from hexane/CH_2Cl_2: mp 120–122 °C; MS (ESI$^+$) *m/z* 486.2248 [M + H]$^+$; IR (Nujol) ν 3291, 3247, 3165, 3099 (NH), 1717, 1644, 1599, 1150 (CO, CN, CS) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.43 (s, 9H, $(CH_3)_3$), 1.54 (s, 9H, $(CH_3)_3$), 7.09–7.17 (m, 1H, Ar), 7.29–7.40 (m, 2H, Ar), 7.43–7.62 (m, 6H, Ar), 9.82 (broad s, 1H, NHCSNH), 9.83 (broad s, 1H, NHCSNH), 10.02 (broad s, 1H, NH_{Gu}), 11.50 (broad s, 1H, NH_{Gu}); ^{13}C NMR (DMSO-d_6) δ 29.0, 29.2 ($(CH_3)_3$), 80.1, 84.7 ($\underline{C}(CH_3)_3$), 124.2, 124.9, 125.2, 125.7, 129.7, 134.2, 137.6, 140.8 (Ar), 153.5, 154.2 (CO), 164.1 (CN), 180.8 (CS).

1-(4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]phenyl)-3-phenylthiourea (21e). An amount of 896 mg (3.3 mmol) of $HgCl_2$ was added over a solution of 325 mg (3.0 mmol) of 1,4-phenylenediamine, 907 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of Et_3N in DMF (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 22 h more at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a dark residue. This residue was dissolved in 10 mL of dry CH_2Cl_2, and 0.37 mL (3.0 mmol) of phenyl isothiocyanate was added under an inert atmosphere and at 0 °C. The mixture was allowed to reach room temperature and was stirred for 19 h. Then the solvent was removed in vacuo and the new residue obtained was purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:7) to yield 347 mg (22%) of **21e** as a white solid: mp 118–120 °C; IR (Nujol) ν 3311 (NH), 1756, 1716, 1151 (CO, CN, CS) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 18H, $(CH_3)_3$), 3.82 (s, 4H, CH_2), 6.99 (d, 2H, $J = 8.0$ Hz, Ar), 7.16–7.25 (m, 3H, Ar), 7.30–7.42 (m, 4H, Ar), 7.89 (broad s, 1H, NH), 8.16 (broad s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.8 ($(CH_3)_3$), 43.1 (CH_2), 82.9 ($\underline{C}(CH_3)_3$), 122.2, 124.7, 125.9, 126.1, 128.8, 130.8, 137.8, 140.0 (Ar), 147.5, 149.8 (CO, CN), 179.8 (CS).

Trifluoroacetate Salt of 1-(4-Guanidinophenyl)-3-phenylthiourea (21a). A solution of 243 mg (0.5 mmol) of **21d** in 20 mL of CH_2Cl_2/TFA (1:1) was stirred at room temperature for 3 h. After that time, the solvent was eliminated under vacuum and cold water was added to generate a precipitate that was filtered, washed with an additional 100 mL of cold water, and dried to yield 197 mg (94%) of the pure trifluoroacetate salt as a white solid: mp 166–168 °C; MS (ESI$^+$) *m/z* 286.1183 [M + H]$^+$; ^{1}H NMR (CD_3OD) δ 7.16–7.28 (m, 3H, Ar), 7.31–7.47 (m, 4H, Ar), 7.53 (d, 2H, $J = 7.5$ Hz, Ar); ^{13}C NMR (CD_3OD) δ 122.4, 123.6, 124.2, 124.6, 126.6, 129.9, 136.5, 136.9 (Ar), 158.4 (CN), 182.4 (CS); ^{19}F NMR (CD_3OD) δ −78.97. Anal. ($C_{16}H_{16}F_3N_5O_2S \cdot 1.0H_2O$) C, H, N.

Trifluoroacetate Salt of 1-[4-(2-Imidazolidinylimino)phenyl]-3-phenylthiourea (21b). A solution of 256 mg (0.5 mmol) of **21e** in 20 mL of CH_2Cl_2/TFA (1:1) was stirred at room temperature for 3 h. After that time, the solvent was eliminated under vacuum and cold water was added to generate a precipitate that was filtered, washed with an additional 100 mL of cold water, and dried to yield 221 mg (92%) of the pure trifluoroacetate salt as a yellow solid: mp 138–140 °C; MS (ESI$^+$) *m/z* 312.1379 [M + H]$^+$; ^{1}H NMR (D_2O) δ 3.72 (s, 4H, CH_2), 7.26–7.40 (m, 7H, Ar), 7.41–7.48 (m, 2H, Ar); ^{13}C NMR (D_2O) δ 42.2 (CH_2), 124.7, 125.8, 127.1, 127.4, 129.1, 133.3, 136.3, 136.6 (Ar), 158.2 (CN), 179.4 (CS); ^{19}F NMR (D_2O) δ −76.15. Anal. ($C_{18}H_{18}F_3N_5O_2S \cdot 3.0H_2O$) C, H, N.

4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]benzanilide (22d). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 637 mg (3.0 mmol) of 4-aminobenzanilide, 830 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of Et_3N in CH_2Cl_2/DMF (5:1) (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 22 h more at room temperature. The reaction was worked up and purified by silica gel column chromatography, eluting with hexane/EtOAc (2:1) to yield 1.015 g (74%) of **22d** as a white solid: mp >300 °C; MS (ESI$^+$) *m/z* 477.2269 [M + Na]$^+$; IR (Nujol, cm^{-1}) ν 3375, 3187 (NH), 1718, 1658, 1643, 1596 (CO, CN); ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 9H, $(CH_3)_3$), 1.57 (s, 9H, $(CH_3)_3$), 7.11–7.20 (m, 1H, Ar), 7.33–7.42 (m, 2H, Ar), 7.63–7.78 (m, 4H, Ar), 7.80–7.93 (m, 2H, Ar), 8.08 (broad s, 1H, PhNHCOPh), 10.55 (broad s, 1H, NH_{Gu}), 11.65 (broad s, 1H, NH_{Gu}); ^{13}C NMR ($CDCl_3$) δ 27.9, 28.0 ($(CH_3)_3$), 80.1, 84.1 ($\underline{C}(CH_3)_3$), 120.1, 122.4, 124.1, 128.0, 128.8, 131.3, 138.4, 139.3 (Ar), 153.1, 153.8 ($(CH_3)_3CO\underline{C}O$), 163.1 (CN), 165.6 (PhNH$\underline{C}$OPh).

4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]benzanilide (22e). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 637 mg (3.0 mmol) of 4-aminobenzanilide, 907 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of Et_3N in CH_2Cl_2/DMF (5:1) (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 25 h more at room temperature. The reaction was worked up and purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc (6:4). The residue obtained after the column was recrystallized from hexane/Et_2O (1:1) to yield 843 mg (58%) of **22e** as a white solid: mp 163–165 °C; IR (Nujol) ν 3338 (NH), 1737, 1707, 1670, 1637 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 18H, $(CH_3)_3$), 3.85 (s, 4H, CH_2), 7.03 (d, 2H, $J = 8.5$ Hz, Ar), 7.08–7.16 (m, 1H, Ar), 7.29–7.39 (m, 2H, Ar), 7.66 (d, 2H, $J = 8.0$ Hz, Ar), 7.80 (d, 2H, $J = 8.5$ Hz, Ar), 8.04 (broad s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.8 ($(CH_3)_3$), 43.2 (CH_2), 83.0 ($\underline{C}(CH_3)_3$), 120.0, 121.2, 123.9, 127.8, 128.1, 128.8, 138.3, 140.2 (Ar), 149.8 ($(CH_3)_3CO\underline{C}O$), 151.9 (CN), 165.3 (PhNH$\underline{C}$OPh).

Hydrochloride Salt of 4-(2-Imidazolidinylimino)benzanilide (22b). Following method B, an amount of 158 mg (94%) of the pure hydrochloride salt of **22b** was obtained as a white solid: mp, decomposes at 270 °C; MS (ESI$^+$) *m/z* 281.1369 [M + H]$^+$; ^{1}H NMR (D_2O) δ 3.71 (s, 4H, CH_2), 7.22–7.32 (m, 3H, Ar), 7.41–7.52 (m, 4H, Ar), 7.74–7.83 (m, 2H, Ar); ^{13}C NMR (D_2O) δ 42.1 (CH_2), 121.7, 121.8, 125.3, 128.6, 128.7, 130.7, 136.3, 138.4 (Ar), 157.0 (CN), 167.3 (CO). Anal. ($C_{16}H_{17}ClN_4O \cdot 1.0H_2O$) C, H, N.

4′-[2,3-Di(*tert*-butoxycarbonyl)guanidino]benzanilide (23d). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 637 mg (3.0 mmol) of 4′-aminobenzanilide, 830 mg (3.0 mmol) of *N,N′*-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of Et_3N in CH_2Cl_2/DMF (5:1) (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 22 h more at room temperature. The reaction was worked up and purified by silica gel column chromatography, eluting with hexane/EtOAc (3:1) to yield 850 mg (62%) of **23d** as a white solid.

Method C is as follows. Amounts of 0.28 mL (2.0 mmol) of Et_3N and 0.23 mL (2.0 mmol) of benzoyl chloride were added under an inert atmosphere and at 0 °C to a solution of 701 mg (2.0 mmol) of 4-[2,3-di(*tert*-butoxycarbonyl)guanidino]aniline **8** in dry CH_2Cl_2 (10 mL). The reaction mixture was allowed to reach room temperature and stirred for 16 h. Then an additional 20 mL of CH_2Cl_2 was added and the organic phase was washed with water (2 × 15 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the residue obtained was purified by silica gel column chromatography, eluting with hexane/EtOAc (3:1) to yield 820 mg (90%) of **23d** as a white solid: mp >300 °C; MS (ESI$^+$) *m/z* 477.2126 [M + Na]$^+$; IR (Nujol) ν 3379, 3261, 3170 (NH), 1718, 1645, 1627, 1575 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9H, $(CH_3)_3$), 1.57 (s, 9H, $(CH_3)_3$), 7.25 (d, 2H, $J = 9.0$ Hz, Ar), 7.38–7.52 (m, 3H, Ar), 7.56 (d, 2H, $J = 8.5$ Hz, Ar), 7.81 (d, 2H, $J = 7.0$ Hz, Ar), 8.74 (broad s, 1H, PhNHCOPh), 10.03 (broad s, 1H, NH_{Gu}), 11.50 (broad s, 1H, NH_{Gu}); ^{13}C NMR ($CDCl_3$) δ 27.9, 28.0 ($(CH_3)_3$), 79.7, 83.7 ($\underline{C}(CH_3)_3$), 120.7, 124.5, 127.1,

128.2, 131.6, 131.2, 135.5, 136.5 (Ar), 153.0, 154.8 (($(CH_3)_3$COCO), 163.1 (CN), 166.0 (PhNH C OPh).

4′-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]benzanilide (23e). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 637 mg (3.0 mmol) of 4′-aminobenzanilide **23**, 907 mg (3.0 mmol) of *N,N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of Et_3N in CH_2Cl_2/DMF (5:1) (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 25 h more at room temperature. The reaction was worked up and purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:7). The residue obtained after the column was recrystallized from EtOAc to yield 697 mg (48%) of **23e** as a white solid: mp 115–117 °C; IR (Nujol) ν 3309 (NH), 1752, 1718, 1696 (CO, CN) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 18H, $(CH_3)_3$), 3.77 (s, 4H, CH_2), 6.85 (d, 2H, J = 8.5 Hz, Ar), 7.49–7.62 (m, 3H, Ar), 7.67 (d, 2H, J = 8.0 Hz, Ar), 7.98 (d, 2H, J = 7.0 Hz, Ar), 10.14 (broad s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 28.8 ($(CH_3)_3$), 44.2 (CH_2), 82.7 ($\underline{C}(CH_3)_3$), 122.0, 122.1, 128.9, 129.6, 132.6, 134.9, 136.4, 140.3 (Ar), 145.8 ($(CH_3)_3COC\underline{O}$), 151.0 (CN), 166.3 (PhNH$\underline{C}$OPh).

Hydrochloride Salt of 4′-(2-Imidazolidinylimino)benzanilide (23b). Following method B, 157 mg (96%) of the pure hydrochloride salt of **23b** were obtained as a white solid: mp, decomposes at 180 °C; MS (ESI$^+$) m/z 281.1369 [M + H]$^+$; ^{1}H NMR (D_2O) δ 3.70 (s, 4H, CH_2), 7.23 (d, 2H, J = 8.6 Hz, Ar), 7.48–7.58 (m, 4H, Ar), 7.60–7.65 (m, 1H, Ar), 7.80 (d, 2H, J = 7.0 Hz, Ar); ^{13}C NMR (D_2O) δ 42.1 (CH_2), 122.8, 124.2, 126.9, 128.3, 131.5, 132.0, 133.0, 135.4 (Ar), 158.0 (CN), 168.9 (CO). Anal. ($C_{16}H_{17}ClN_4O\cdot0.5H_2O$) C, H, N.

4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]acetophenone (30d). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 406 mg (3.0 mmol) of 4-aminoacetophenone **30**, 830 mg (3.0 mmol) of *N,N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of Et_3N in DMF (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 41 h more at room temperature. The reaction was worked up and purified by silica gel column chromatography, eluting with hexane/EtOAc (3:1) to yield 943 mg (83%) of **30d** as a white solid: mp, decomposes at 176 °C; IR (Nujol) ν 3278, 3146 (NH), 1718, 1686, 1637, 1598 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.51 (s, 9H, $(CH_3)_3$), 1.53 (s, 9H, $(CH_3)_3$), 2.55 (s, 3H, CH_3), 7.74 (d, 2H, J = 8.5 Hz, Ar), 7.92 (d, 2H, J = 8.0 Hz, Ar), 10.56 (broad s, 1H, NH), 11.61 (broad s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 26.3 (CH_3), 27.9, 28.0 ($(CH_3)_3$), 79.9, 84.0 ($\underline{C}(CH_3)_3$), 121.0, 129.3, 132.9, 141.2 (Ar), 153.1 ($(CH_3)_3COC\underline{O}$), 163.1 (CN), 196.8 ($CH_3\underline{C}O$).

4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]acetophenone (30e). Method A was used, with 896 mg (3.3 mmol) of $HgCl_2$, 406 mg (3.0 mmol) of 4-aminoacetophenone **30**, 907 mg (3.0 mmol) of *N,N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of Et_3N in DMF (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 25 h more at room temperature. The reaction was worked up and purified by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:2). The residue obtained after the column was precipitated with Et_2O and washed with cold hexane to yield 613 mg (50%) of **30e** as a white solid: mp 113–115 °C; IR (Nujol) ν 1744, 1705, 1670 (CO, CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 18H, $(CH_3)_3$), 2.53 (s, 3H, CH_3), 3.84 (s, 4H, CH_2), 6.99 (d, 2H, J = 8.0 Hz, Ar), 7.85 (d, 2H, J = 8.0 Hz, Ar); ^{13}C NMR ($CDCl_3$) δ 26.3 (CH_3), 27.8 ($(CH_3)_3$), 43.2 (CH_2), 83.0 ($C(CH_3)_3$), 121.0, 129.4, 131.2, 140.2 (Ar), 149.8 ($(CH_3)_3COCO$), 153.3 (CN), 197.1 (CH_3CO).

DNA Binding Assays. Thermal melting experiments were conducted with a Varian Cary 300 Bio spectrophotometer equipped with a 6 × 6 multicell temperature-controlled block. Temperature was monitored with a thermistor inserted into a 1 mL quartz cuvette containing the same volume of water as in the sample cells. Absorbance changes at 260 nm were monitored from 20 to 95 °C with a heating/cooling rate of 0.5 °C/min and a data collection rate of two points per °C. The poly(dA)−poly(dT) DNA polymer was purchased from Amersham Pharmacia Biotech Inc., NJ (extinction coefficient ϵ_{260}= 6000 cm^{-1} M^{-1} base). A quartz cell with a 1 cm path length was filled with a 1 mL solution of DNA polymer or DNA−compound complex. The DNA polymer (40 μM base) and the compound solution (12 μM) were prepared in a low salt buffer (0.01 M [2-(*N*-morpholino)ethanesulfonic acid], 0.001 M disodium EDTA, adjusted to pH 6.25) so that a compound to DNA base ratio of 0.3 was obtained. This effectively saturates all minor groove binding sites for these compounds. The thermal melting temperatures of the duplex or duplex−compound complex obtained from the first derivative of the melting curves are reported.

Ferriprotoporphyrin IX Biomineralization Inhibition Test (FBIT). The FBIT was performed according to Deharo et al.[16] In a 96-well plate was incubated at 37 °C for 18–24 h a mixture containing 50 μL of 0.5 mg/mL of hemin chloride (Sigma H 5533) freshly dissolved in DMSO, 100 μL of 0.5 M sodium acetate buffer (pH 4.4) and 50 μL of different concentrations of drug solution or 50 μL of solvent (for control). The plate was centrifuged at 1600g for 5 min, and the supernatant was discarded. The remaining pellet was resuspended with 200 μL of DMSO to eliminate unreacted hemin. Then the plate was centrifuged once again and the supernatant similarly discarded. The precipitate (β-hematin) was dissolved in 150 μL of 0.1 N NaOH, and the absorbance was read at 405 nm with an ELISA reader (ELX 800 Biotech Instruments). The percentage of inhibition of ferriprotoporphyrin IX biomineralization was calculated with the following equation:

$$\text{inhibition}(\%) = 100 \times \{[(\text{Abs of control}) - (\text{Abs of drug})]/(\text{Abs of control})\}$$

where Abs is absorbance. IC_{50} values were determined using the TENDANCE function of the Excel program.

In Vitro Activity against *T. brucei rhodesiense* STIB900. This trypanosoma strain was isolated in 1982 from a human patient in Tanzania. 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 sodium pyruvate, 0.1 mM hypoxanthine, and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. The 3-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 was incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. An amount of 10 μL of resazurin solution (12.5 mg of resazurin dissolved in 100 mL of distilled water) 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 were analyzed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA).

In Vitro Activity against *P. falciparum* K1. In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay, using the chloroquine and pyrimethamine resistant K1 strain and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), AlbuMax (5 g/L), and washed human red cells A^+ at 2.5% hematocrit (0.3% parasitemia). Serial doubling dilutions of each drug were prepared in 96-well microtiter plates and incubated in a humidified atmosphere at 37 °C, 4% CO_2, 3% O_2, 93% N_2. After 48 h, 50 μL of ^{3}H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate cell harvester (Wallac, Zurich, Switzerland) and the red blood cells transferred onto a glass fiber filter and then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid and counted in a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves using Microsoft Excel.

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) and 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. An amount of 10 μL of Alamar blue (12.5 mg of resazurin in 100 mL of phosphate buffered saline) was then added to each well, and the plates were incubated for another 2 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 were analyzed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA).

In Vivo Activity against *T. brucei*. All efficacy studies were approved by the institutional animal experimentation ethics committee. Female NMRI mice weighting 22–25 g were infected with cryopreserved stabilates of *T. brucei rhodesiense* STIB 900. Each mouse was infected intraperitoneally with 2×10^4 (STIB 900) bloodstream forms. Groups of four mice were treated intraperitoneally with the compounds on days 3−6. A control group remained untreated. The parasitemia of all animals was checked every second day up to day 14 postinfection and two times a week thereafter until 60 days. Death of animals was recorded to calculate the mean survival time. Surviving and aparasitemic mice were considered cured at 60 days and then euthanized.

In Vivo Antimalarial Efficacy Studies. All efficacy studies were approved by the institutional animal experimentation ethics committee. In vivo antimalarial activity was assessed basically as previously described.[51] Groups of three female NMRI mice (20–22 g) were intravenously infected with 2×10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA.[52] Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water, and administered intraperitoneally in a volume of 10 mL kg^{-1} on four consecutive days (4, 24, 48, and 72 h postinfection). Parasitemia was determined on day 4 postinfection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitaemia for the control ($n = 5$ mice) and treated groups expressed as a percent relative to the control group. The survival time in days was also recorded up to 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites.

Acknowledgment. We are grateful to the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (RB), the CSIC-I3 program (Grant PIE200680I121), and the Spanish Ministerio de Educación y Ciencia (Grant SAF2006-04698) for funding. F.R. thanks the Consejería de Educación Cultura y Deporte de la Comunidad Autónoma de La Rioja for his grant. Research on DNA interactions is supported by National Institutes of Health Grant AI064200 (W.D.W.). We thank J. C. Janse, Leiden University, for providing the GFP transfected *P. berghei* strain (RB).

Supporting Information Available: Synthesis and characterization of the compounds **7b**, **8a**, **9a**, **9b**, **22a**, **23a**, **30a**, and **30b** and combustion analysis data for compounds **7a**, **11a**, **11b**, **19b**, **20a**, **21a**, **21b**, **22b**, and **23b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM7013088