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Molecular modeling study and synthesis of novel dicationic flexible triaryl guanidines and imidamides as antiprotozoal agents

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ABSTRACT

A new series of fourteen dicationic flexible triaryl bis-guanidines **3a,b**, bis-N-substituted guanidines **7a,b** and **8a,b** as well as bis-imidamides **9**–**12a,b** having a 1,3- or 1,4-diphenoxybenzene scaffold backbone were synthesized. The *in vitro* activity of the novel dications as antiprotozoal agents against *Trypanosoma brucei rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P.f.*) was assessed. Interestingly, six of the newly synthesized dications *viz* **3a,b**, **7a,b** and **8a,b** were more active against *P.f.* than the reference drug pentamidine. Also, some of the dications showed moderate antitrypanosomal activity. Thermal melting analysis of the novel dications was performed to determine their ligand-DNA relative binding affinities. Finally, docking of the dications with an AT rich DNA oligonucleotide was executed to understand their binding mode with the minor groove.

1. Introduction

Protozoal manifestations caused by pathogenic parasites, and more specifically by those hemoflagellates predisposing to human African trypanosomiasis (HAT), leishmaniasis and malaria have always afflicted mankind. For the management of such infections, many therapeutic strategies have been explored including the use of aryl or heteroaryl dicationic minor groove binders. Up till now, pentamidine (I) Fig. 1), currently designated by FDA as an orphan drug, is the only aryl dicationic minor groove binder clinically available for control of first stage HAT, antimony-resistant leishmaniasis and also as a secondary drug for AIDS-related *Pneumocystis jirovecii* pneumonia (PCP) [1,2]. Furamidine (II) (Fig. 1), a diphenyl furan diamidinium analogue of pentamidine, has demonstrated better potency and toxicity profiles in murine models of trypanosomiasis [3]. On the other hand, many investigations have shown that introduction of other cationic centers *viz* guanidine and imidamides, formerly known as "reversed amidines", to classical groove binding frameworks, furamidine like, can lead to effective antimicrobial agents [4,5]. Also, benzguanidines connected by alkyl or fused heteroaryl linkers have displayed promising antiprotozoal activity [6,7]. We have recently reported linear triaryl diguanidiniums and N-substituted guanidiniums (III) Fig. 1) as promising antiprotozoal agents against *Trypanosoma brucei rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P. f.*) [8]. The mode of action of such compounds, though still not certain, is believed to involve binding to DNA minor groove at AT rich sites with subsequent inhibition of DNA dependent enzymes and direct inhibition of transcription [9,10].

It was earlier believed that activity resides in crescent shaped dications that match the curvature of the minor groove [1,11]. Yet, it has been proven that linear analogues like CGP40215 (IV) (Fig. 1) and linear triaryl dications (e.g. amidines, guanidines and imidamides) exhibit strong DNA minor groove binding by virtue of integration of water molecule(s) with the drug-DNA complex [12–14].

Based on the previous findings, it was deemed of interest to explore the effect of introducing some flexibility to the triaryl spacer moiety between the dicationic centers on the DNA binding affinity and the antiprotozoal activity of such potential drug molecules. For that, fourteen novel dications comprising bis-guanidines **3a,b**, bis-N-substituted guanidines **7a,b** and **8a,b** as well as bis-imidamides **9**–**12a,b** having a 1,3- or 1,4-diphenoxybenzene backbone were synthesized. The novel compounds were screened against *T.b.r.* and *P. f.* to determine their *in vitro* antiprotozoal activity. Furthermore, a thermal melting study

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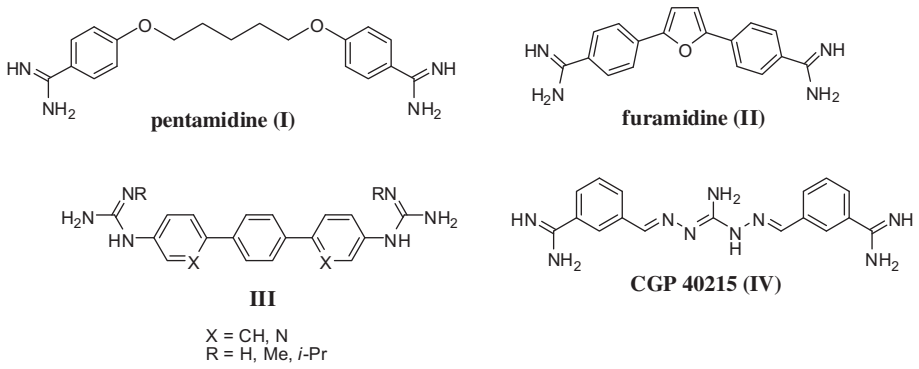


Fig. 1. Structures of key dicationic antiprotozoan agents.

of all the new compounds was performed to assess their ligand-DNA relative binding affinities. Finally, minor groove binding abilities of the synthesized compounds was assessed by evaluation of their binding modes and energies with a short AT rich DNA oligomer by utilizing docking techniques.

2. Results and discussion

2.1. Chemistry

The target flexible triaryl unsubstituted bis-guanidines **3a,b** were synthesized in two steps as depicted in Scheme 1 starting from the corresponding commercially available 4,4′-(1,3- or 1,4-phenylenebis(oxy))dianiline derivatives **1a** and **1b**, respectively. The first step comprises the preparation of the intermediate bis-Boc-protected guanidines **2a,b** by the use of bis-Boc-protected *N*-methylpseudothiourea and $HgCl_2$. Subsequent treatment of **2a,b** with ethanolic HCl provided the twofold purpose of guanidine deprotection, as well as formation of the bis-guanidine dihydrochloride salts **3a,b** [4–7].

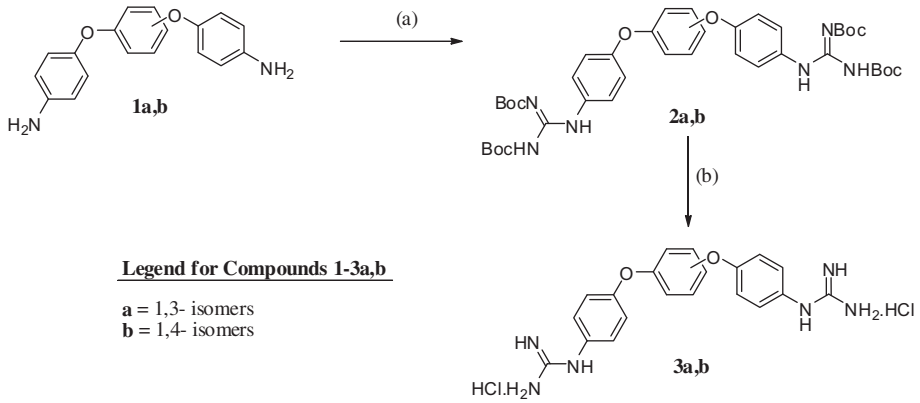
Scheme 2 outlines the synthesis of flexible triaryl N-substituted bis-guanidines **7a,b** and **8a,b**. Starting with the appropriate diamine (**1a** or **1b**), reaction with ethyl isothiocyanatoformate gave the carbamoyl thioureas (**4a, b**), which were further reacted with methylamine or *i*-propylamine in the presence of EDCI to furnish the carbamoyl guanidines **5a,b** and **6a,b**, respectively [15–17]. These synthetic intermediates were decarbamoylated through base catalyzed hydrolysis of the ester moiety with simultaneous decarboxylation to obtain the *N*-alkylguanidines **7a,b** and **8a,b**.

Finally, flexible triaryl imidamides were prepared as depicted in Scheme 3 where the diamines (**1a** or **1b**) were reacted with cyclohexyl-, phenyl-, 4-*t*-butylphenyl- or biphenyl-*S*-(2-naphthylmethyl) thioimidate to provide the target compounds (**9**–**12a,b**), respectively [5,6,18].

2.2. Biology

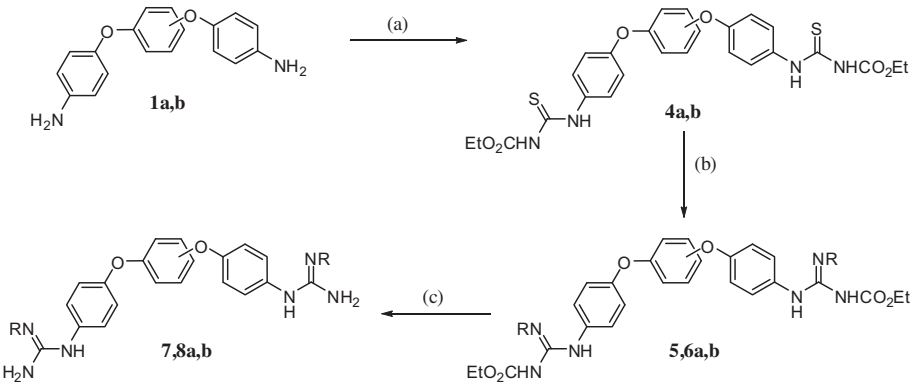
In vitro activities. The *in vitro* antiprotozoal activity against *T.b.r.* and *P.f.* as well as the cytotoxicity of all the newly synthesized flexible triaryl dications was assessed (data listed in Table 1). For comparative purposes the analogous data for pentamidine are also included.

Even as none of the new dications displayed better anti-trypanosomal activity against *T.b.r.* compared to the reference drug pentamidine (IC_{50} = 2.2 nM), most of these novel compounds exhibited much better antimalarial potencies against *P.f.* compared to pentamidine (IC_{50} = 46.4 nM). All the guanidines **3a,b**, *N*-methyl guanidines **7a,b** and *N*-isopropyl guanidines **8a,b** displayed the highest potency in this series with IC_{50} values between 14 and 38 nM being 3.2 to 1.2 times as active than pentamidine. In general, the 1,4-phenylenebis(oxy)dianiline derivatives showed higher potencies against *P.f.* than the corresponding 1,3- isomers. Also, the N-substituted guanidines were more potent than the parent unsubstituted ones with the *N*-methyl analogues displaying higher antimalarial activity than the *N*-isopropyl counterparts. The bis(*N*-methyl)guanidine (1,4-phenylenebis(oxy)dianiline derivative) **7b** not only exhibited the highest antimalarial activity in the series with an IC_{50} value of 14 nM but also demonstrated the second highest



Reagents and condtions: (a) Bis-Boc protected S-methylpseudothiourea, $HgCl_2$, TEA, DMF, r.t., overnight; (b) HCl (g), EtOH, CH_2Cl_2, r.t., 3 days

Scheme 1. Synthesis of flexible triaryl unsubstituted bis-guanidines.



Legend for Compounds 1a,b and 4-8a,b

5,7 R = Me
6,8 R = *i*-Pr
a = 1,3- isomers
b = 1,4- isomers

Reagents and condtions: (a) ethyl isothiocyanatoformate, CH_2Cl_2, r.t., overnight; (b) RNH_2, DIPEA, EDCI, CH_2Cl_2, r.t., overnight; (c) aq. alc KOH, 55 °C, 10 h

Scheme 2. Synthesis of flexible triaryl n-substituted bis-guanidines.

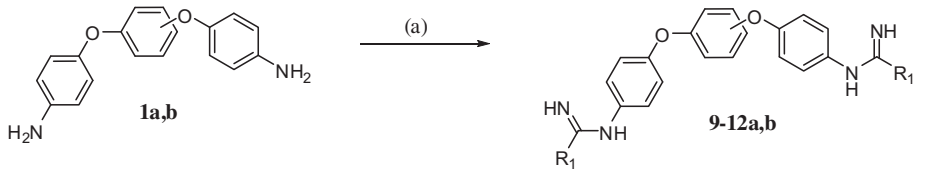
P.f. compared to *T.b.r.* selectivity amongst the tested guanidines (SI = 11) with compound **7a** possessing the highest selectivity (SI = 14.8).

On the other hand, three of the imidamides class of dications showed moderate potency against *P.f.* with IC_{50} values 60–75 nM. Interestingly, it was the 1,4-phenylenebis(oxy)dianiline derivatives that demonstrated antimalarial activity while the 1,3- isomers were devoid of such activity potentially due to their unfavorable geometry and lack of complementarity with the minor groove curvature causing steric clashing with the minor groove floor. Also, whilst the arylimidamides **10b**, **11b** and **12b** displayed moderate antimalarial potency, the cyclohexylimidamide **9b** displayed none. This may suggest that the presence of an aromatic ring, even if large in size (c.f. biphenyl in **12b**), is well tolerated and probably required due to the potential formation of π–π stacking interactions with the minor groove. However, the presence of the non-aromatic non-planar cycloalkyl (c.f. **9b**) does not help the biological activity due to lack of such an interaction.

Furthermore, all the tested guanidines and N-substituted guanidines were 5 to 39 fold less cytotoxic when compared to pentamidine with the most active analogue against *P.f.*, **7b**, having 27 folds lower cytotoxicity. Also, the biologically active members of the imidamides displayed similar to better cytotoxicity profiles when compared to pentamidine with the biphenyl derivative **12b** being almost 60 fold less cytotoxic.

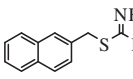
2.3. Relative binding affinity: ΔTm measurements

Dicationic compounds have been known to target the DNA minor groove. *Tm* increases for ligand-DNA complexes relative to uncomplexed DNA (Δ*Tm*) at specific DNA sequences provide an excellent method for ranking compound binding affinities. In this study, *Tm* values for the prepared flexible dications were determined by their addition to an AT polymer and data obtained are listed in Table 1. Unexpectedly, our charged compounds displayed Δ*Tm* values in the range of 5 to 0 °C reflecting poor to absolutely no minor groove binding affinities (Table 1). These surprising findings are suggested to be a consequence of an over-acute curvature of the dicationic linker 1,3- or 1,4-diphenoxyphenyl backbone which reduces complementarity with the curvature of the DNA minor groove and hence reducing the possibility of formation of the non-covalent binding interactions necessary for formation of the ligand-DNA complex.



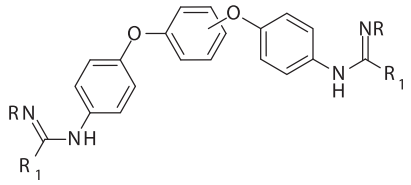
Legend for Compounds 1a,b and 9-12a,b

9 R_1 = cyclohexyl **10** R_1 = phenyl
11 R_1 = 4-*t*-butylphenyl **12** R_1 = biphenyl
a = 1,3- isomers
b = 1,4- isomers

Reagents and condtions: (a)  EtOH, MeCN, r.t., overnight;

Scheme 3. Synthesis of flexible triaryl bis-imidamides.

Table 1
In vitro antiprotozoal activity and cytotoxicity for flexible dicationic triaryl guanidines and imidamides.[a]



Code	Substitution	R	R_1	ΔTm[a] poly dA-dT	*T.b.r.* IC_{50} (nM)[b]	*P.f.* IC_{50} (nM)[b]	SI[c]	Cytotoxicity IC_{50} (μM)[d]
Pentamidine I	–	–	–	12.6	2.2	46.4	0.05	2.1
3a	1,3- (meta)	H	NH_2	1.1	171	27.0	6.3	10.6
3b	1,4- (para)	H	NH_2	2.1	151	26.5	5.7	11.6
7a	1,3- (meta)	Me	NH_2	0.1	318	21.5	14.8	45.7
7b	1,4- (para)	Me	NH_2	0.8	155	14.1	11	57.3
8a	1,3- (meta)	*i*-Pr	NH_2	0.0	155	38.2	4.1	82.1
8b	1,4- (para)	*i*-Pr	NH_2	0.0	160	17.2	9.3	19.6
9a	1,3- (meta)	H	cyclohexyl	0.0	395	307	1.3	3.9
9b	1,4- (para)	H	cyclohexyl	2.1	427	259	1.6	3.1
10a	1,3- (meta)	H	Phenyl	2.0	1042	251	4.2	0.9
10b	1,4- (para)	H	Phenyl	5.0	863	71.5	12.1	2.8
11a	1,3- (meta)	H	4-*t*-butylphenyl	0.0	2779	627	4.4	81.4
11b	1,4- (para)	H	4-*t*-butylphenyl	0.0	400	60.0	6.7	1.5
12a	1,3- (meta)	H	Biphenyl	0.0	82,300	4698	17	9.1
12b	1,4- (para)	H	Biphenyl	0.1	612	63.3	9.6	>120

[a] Buffer: MES10; ratio (compound/DNA): 0.3.
[b] *T.b.r.* strain used was STIB900 and the *P. f.* strain was K1, values are of duplicate determinations; see Ref. [21].
[c] Selectivity Index (IC_{50} *T.b.r.*/IC_{50} *P.f.*).
[d] Cytotoxicity was evaluated using cultured L-6 rat myoblast cells using an Alamar Blue assay; see Ref. [21].

2.4. Molecular modeling

Dications are well reported to bind to DNA minor groove and in-silico screening has proved to be efficient in displaying and explaining such interactions [19]. In an attempt to better understand the possible reason behind the poor DNA binding of this class of dications with the DNA minor groove, docking study was performed using MOE software Version 2008.10. The X-ray crystal structure of the selective antiprotozoal agent pentamidine bound to the minor groove of DNA dodecamer d(CGCGAATTCGCG)$_2$ was obtained from the RCSB Protein Data Bank (PDB ID: 1d64) [20] and employed for this study. The docking study was initiated by a validation step through docking of pentamidine in its active site, and comparing the results with the binding mode determined experimentally through X-ray crystallography with the docked ligand. MOE was successful in reproducing the binding position for pentamidine, showing a RMS deviation of 0.51Å (Fig. 2). According to the crystal structure [20], pentamidine establishes several hydrogen bonding interactions with neighboring A and T base-pairs on both strands of the DNA minor groove a couple being mediated through a water bridge as shown in Fig. 2.

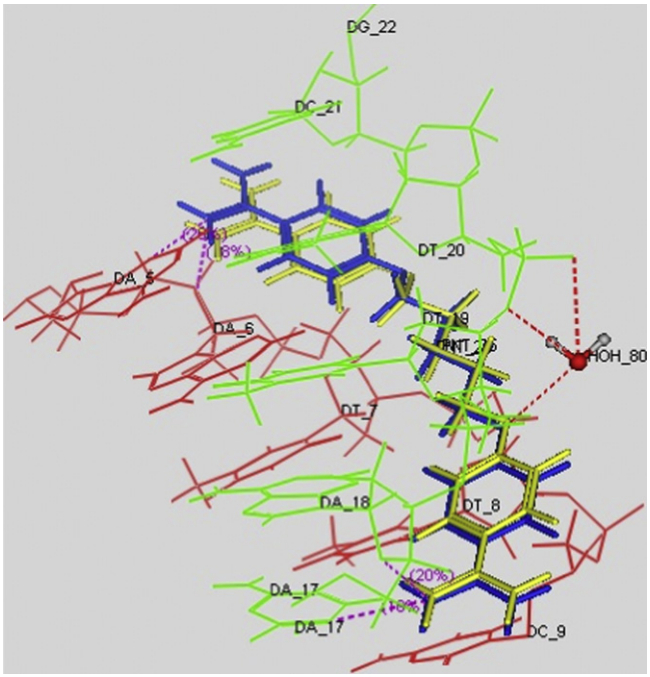


Fig. 2. Docking validation and binding mode of pentamidine in the minor groove of the polydA.polydT DNA oligonucleotide: crystal structure ligand (blue) docked ligand (yellow); RMSD 0.51 Å. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

After removing pentamidine from the complex, virtual screening of each of the new dications was done through docking with the short DNA dodecamer d(CGCGAATTCGCG)$_2$ to predict the mode of drug-DNA binding using MOE default parameters for all the variables adopting flexible-ligand rigid-receptor approach. The most stable docking solutions of the flexible guanidines and imidamides complexed with the DNA oligonucleotide along were determined.

The binding profile of the synthesized dications was compared with that of pentamidine molecule. Unexpectedly, the ligands were found to mainly interact with the DNA oligonucleotide through the formation of hydrogen bond(s) between only one of the dicationic centres and the DNA base pair(s) while the other dicationic group points out of the minor groove. An exemplary illustration for this binding mode is displayed for the dication with the highest anti-plasmodial activity in this study, **7b**, in Fig. 3 showing the 3D (panel a) and 2D (panel b) ligand binding interactions of this compound with the receptor DNA oligomer in comparison with the X-ray

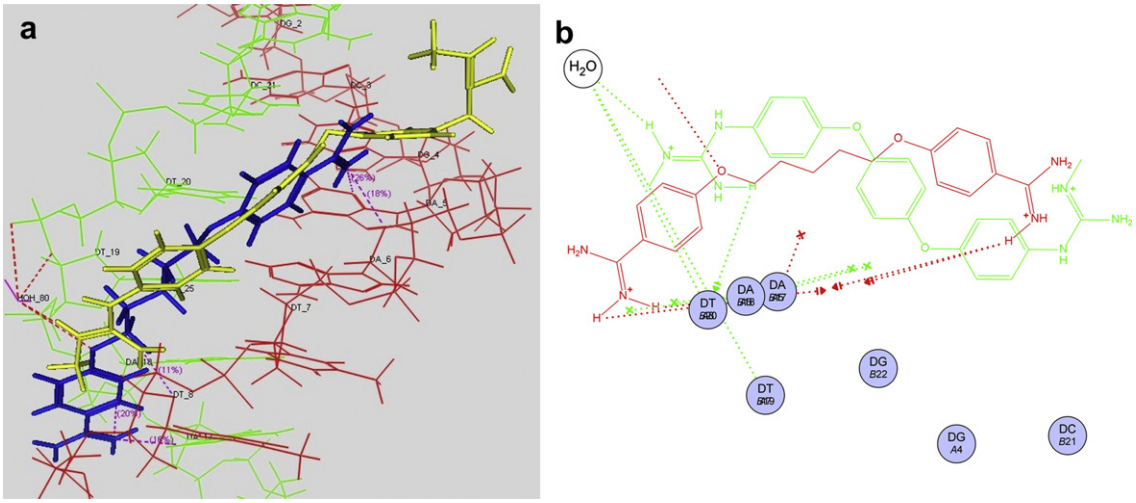


Fig. 3. Panel (a): 3D Binding interaction of **7b** (yellow) in the minor groove of the polydA.polydT DNA compared to that of pentamidine (blue); panel (b): overlay of **7b** (green) and pentamidine (red) showing their 2D binding interaction in the minor groove of the polydA.polydT DNA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

crystal ligand pentamidine. This behaviour of this class of dications can be possibly explained in terms of the comparatively over-acute curvature of these compounds, especially the 1,3-analogues, compared to pentamidine. Docking studies showed that for these derivatives to engage both dicationic moieties in their binding interactions they have to over arch and twist in an energetically unfavourable conformation with the triaryl backbone protruding out of the DNA minor groove (data not shown). This positioning is apparently not thermodynamically preferred by the molecules as reflected in the high conformational energy of such orientations (E_{conf} in MOE) leading to overall instability of the ligand-DNA complex.

Thus, docking studies were useful in showing that some of these dications prefer to bind to DNA as monocations and attain a more thermodynamically relaxed conformation which rationalizes the unexpected experimental findings of the thermal melting analysis that revealed poor to no DNA interactions.

3. Conclusion

The current study portrays the synthesis of fourteen novel dicationic flexible triaryl guanidines and imidamides. The *in vitro* antiprotozoal testing of the compounds against *T.b.r.* and *P.f.* showed that the new compounds had poor antitrypanosomal activity. However, the dicationic guanidiniums **3a,b**; **7a,b** and **8a,b** showed high antiprotozoal activity profiles against *P.f.* Also, the imdamides with a 1,4-diphenoxyphenyl dicationic groups bearing scaffold displayed reasonable antiplasmodial activity. On the other hand, thermal melting analysis experiments surprisingly showed that this series of dications bind poorly to the minor groove of DNA. For that, docking techniques were adopted in a trial to predict the binding mode of the tested compounds with the DNA minor groove. When the results of the thermal melting and docking studies are considered together with the biological screening data, it can be suggested that: (i) the dications especially those with the 1,3-diphenoxyphenyl backbone bind very poorly to DNA, as reflected in their Δ*Tm* values and docking contours, probably due to over-acute curvature and hence inadequate complementarity with the minor groove; (ii) poor activity of these dications against *T.b.r.* can possibly be explained in terms of lack of minor groove binding affinity; finally (iii) the activity of the guanidiniums and those imidamides with a 1,4-diphenoxyphenyl backbone against *P.f.*, some being even more active than the reference drug pentamidine, suggests that antiplasmodial activity may be only partly dependent on minor groove binding abilities and there may exist another mode of action through which these cations display their activity that is yet unknown and needs to be further explored.

4. Experimental

4.1. Synthetic protocols

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets and detected under UV light. ^1H and ^{13}C NMR spectra were recorded employing a Varian Unity Plus 300 spectrometer, and chemical shifts (δ) are in ppm relative to TMS as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer (EI) or a Thermo-Finningan LCQ MSD (ESI). Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA). Some compounds were analyzed correctly for fractional moles of water and/or ethanol of solvation. In each case ^1H NMR showed the presence of the indicated solvent(s). All chemicals and solvents (including anhydrous solvents) were purchased from Aldrich Chemical Co. or Lancaster Synthesis and used as purchased. Acetonitrile and triethylamine were distilled from CaH_2. Substituted *S*-(2-naphthylmethyl)thioimidates were prepared adopting the reported procedure [4].

4.1.1. Preparation of Bis(N′,N″-di-bocguanidino) derivatives (general procedure)

1,1′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))-*N′*,*N″*-di-Bocguanidine (2a). To a solution of 4,4′-(1,3-phenylenebis(oxy)) dianiline (**1a**) (0.5 g, 1.71 mmol) in anhydrous DMF (10 mL) was added 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea (1.05 g, 3.62 mmol), triethylamine (1.08 g, 10.74 mmol) and mercury(II) chloride (1.07 g, 3.96 mmol). The suspension was kept stirring at room-temperature overnight. The reaction mixture was diluted with CH_2Cl_2, washed with Na_2CO_3 solution, and filtered through a pad of Celite. The organic layer was washed with water (3×) followed by brine and then dried over anhydrous Na_2SO_4. After evaporating the solvent the obtained residue was crystallized from CH_2Cl_2/MeOH giving a white solid (1.19 g, 89%), mp >300 °C; ^1H NMR ($CDCl_3$) δ 1.50,

1.54 (2s, 36H, 4× C(CH_3)$_3$), 6.67–6.70 (m, 3H, Ar-H), 6.98 (d, J = 9 Hz, 4H, Ar-H), 7.22 (t, J = 8.1 Hz, 1H, Ar-H), 7.57 (d, J = 9 Hz, 4H, Ar-H), 10.29 (br s, 2H, 2× NH), 11.64 (br s, 2H, 2× NH). MS (EI) *m/z* (rel. int.) 777 (M^+ + 1, 92), 277 (100). Anal. Calcd. for $C_{40}H_{52}N_6O_{10}$: C, 61.82; H, 6.75; N, 10.82. Found: C, 61.66; H, 6.66; N, 10.73.

4.1.2. 1,1′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene))-N′,N″-di-Bocguanidine (**2b**)

The general procedure was adopted using 4,4′-(1,4-phenylenebis(oxy))dianiline (**1b**) to furnish **2b** as a white solid (1.27 g, 95%), mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 1.50, 1.55 (2s, 36H, 4× C(CH_3)$_3$), 6.95–6.98 (m, 8H, Ar-H), 7.56 (d, J = 8.7 Hz, 4H, Ar-H), 10.28 (br s, 2H, 2× NH), 11.65 (br s, 2H, 2× NH). ^{13}C NMR ($CDCl_3$) δ 163.78, 154.72, 153.79, 153.56, 153.06, 132.21, 124.01, 120.36, 118.98, 83.90, 79.81, 28.36. MS (EI) *m/z* (rel. int.) 777 (M^+ + 1, 28), 677 (100). Anal. Calcd. for $C_{40}H_{52}N_6O_{10}$: C, 61.82; H, 6.75; N, 10.82. Found: C, 61.53; H, 6.80; N, 10.76.

4.1.3. Deprotection of N′,N″-di-bocguanidines (general procedure) (Scheme 1)

1,1′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))diguanidine (3a). The N′,N″-di-Bocguanidine (**2a**) (0.75 g, 0.96 mmol) was dissolved in CH_2Cl_2 (10 mL), diluted with dry EtOH (15 mL) and the chilled solution was saturated with dry HCl. The reaction mixture was then kept stirring at room temperature for 3 days (drying tube), where a precipitate of the product started forming overtime. After evaporating the solvent to dryness, the residue was washed with ether multiple times and was dried under reduced pressure at 50–60 °C overnight to give white solid of the bis-guanidine dihydrochloride (0.13 g), mp 146–8 °C; ^{1}H NMR (DMSO-d_6) δ 6.68 (t, J = 2.4 Hz, 1H, Ar-H), 6.69 (dd, J = 2.4, 8.1 Hz, 2H, Ar-H), 7.11 (d, J = 8.7 Hz, 4H, Ar-H), 7.25 (d, J = 8.7 Hz, 4H, Ar-H), 7.38 (t, J = 8.1 Hz, 1H, Ar-H), 7.51 (br s, 8H, 2× NH_2 + 4× NH), 10.01 (br s, 2H, 2× HCl); ^{13}C NMR (DMSO-d_6) δ 157.97, 156.43, 154.35, 130.95, 130.63, 126.89, 119.96, 113.05, 108.46. HRMS *m/z* 377.1730 (M^+ + 1) (calcd. for $C_{20}H_{21}N_6O_2$: 377.1726). Anal. Calcd. for $C_{20}H_{20}N_6O_2$-2HCl-0.5H_2O-0.25C_2H_5OH: C, 52.40; H, 5.25; N, 17.88. Found: C, 52.41; H, 5.24; N, 17.60.

4.1.4. 1,1′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene)) diguanidine (**3b**)

The general procedure was adopted starting with **2b,** mp 250-2 °C; ^{1}H NMR (DMSO-d_6) δ 7.04–7.09 (m, 8H, Ar-H), 7.25 (d, J = 8.7 Hz, 4H, Ar-H), 7.58 (br s, 8H, 2× NH_2 + 4× NH), 10.02 (br s, 2H, 2× HCl); ^{13}C NMR (DMSO-d_6) δ 156.36, 155.68, 152.26, 130.36, 127.23, 120.60, 119.18. HRMS *m/z* 377.1720 (M^+ + 1) (calcd. for $C_{20}H_{21}N_6O_2$: 377.1726). Anal. Calcd. for $C_{20}H_{20}N_6O_2$-2HCl-0.4H_2O: C, 52.61; H, 5.03; N, 18.40. Found: C, 52.64; H, 5.03; N, 18.11.

4.1.5. Preparation of Bis(N″-Substitutedguanidino)Derivatives (general procedure)

1,1′-(4,4′-(1,3-Phenylenebis(oxy))bis(4,1-phenylene))bis(N′-ethoxycarbonylthiourea) (4a). Ethyl isothiocyanatoformate (1.86 g, 15.04 mmol) was added to a solution of **1a** (2 g, 6.84 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at room-temperature for 24 h. The reaction was diluted with hexane and the precipitate formed was collected and dried to yield the bis-carbamoylthiourea as a white solid in quantitative yield; m.p. 174-6 °C; ^{1}H NMR (DMSO-d_6): δ 1.25 (t, J = 7.2 Hz, 6H, 2× $CH_2\underline{CH_3}$), 4.20 (q, J = 7.2 Hz, 4H, 2× $\underline{CH_2}CH_3$), 6.66 (t, J = 2.4 Hz, 1H, Ar-H), 6.75 (dd, J = 2.4, 8.4 Hz, 2H, Ar-H), 7.06 (d, J = 8.7 Hz, 4H, Ar-H), 7.38 (t, J = 8.4 Hz, 1H, Ar-H), 7.57 (d, J = 8.7 Hz, 4H, Ar-H), 11.22 (br s, 2H, 2× NH), 11.45 (br s, 2H, 2× NH). MS (ESI) m/e (rel. int.): 555 (M^++1, 100), 360 (17). HRMS *m/z* 555.1352 (M^+ + 1) (calcd. for $C_{26}H_{27}N_4O_6S_2$: 555.1372). Anal. Calcd. for $C_{26}H_{26}N_4O_6S_2$: C, 56.30; H, 4.72; N, 10.10. Found: C, 56.08; H, 4.54; N, 9.78.

4.1.6. 1,1′-(4,4′-(1,4-Phenylenebis(oxy))bis(4,1-phenylene))bis(N′-ethoxycarbonylthiourea) (**4b**)

The procedure described for **4a** was adopted starting with **1b** (1 g, 3.42 mmol) to furnish the bis-carbamoylthiourea as a yellow solid in quantitative yield; m.p. 236-8 °C; ^{1}H NMR (DMSO-d_6): δ 1.27 (t, J = 7.2 Hz, 6H, 2 X $CH_2\underline{CH_3}$), 4.22 (q, J = 7.2 Hz, 4H, 2× $\underline{CH_2}CH_3$), 7.02 (d, J = 9 Hz, 4H, Ar-H), 7.07 (s, 4H, Ar-H), 7.56 (d, J = 9 Hz, 4H, Ar-H), 10.98 (br s, 2H, 2× NH), 11.40 (br s, 2H, 2× NH). ^{13}C NMR (DMSO-d_6) δ 178.58, 154.89, 153.26, 152.05, 133.13, 126.01, 120.31, 117.73, 61.74, 13.80. MS (ESI) m/e (rel. int.): 555 (M^++1, 41), 509 (100). Anal. Calcd. for $C_{26}H_{26}N_4O_6S_2$: C, 56.30; H, 4.72; N, 10.10. Found: C, 55.91; H, 4.52; N, 9.86.

4.1.7. 1,1′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-ethoxycarbonyl-N″-methylguanidine) (**5a**)

A stirred solution of carbamoyl-thiourea **4a** (1.25 g, 2.25 mmol), methylamine hydrochloride (0.73 mgl, 9 mmol), and diisopropylethylamine (1.73 g, 15 mmol) in anhydrous DMF (10 mL) was cooled to 0 °C. EDCI (1.73 g, 9 mmol) was added, and the solution was stirred at room-temperature over night. The reaction mixture was poured onto ice/water. The solid obtained was filtered, washed with water (3 × 100 mL) and dried. The residue remaining after removal of the solvent was crystallized from DMF/water, yield 86%; mp 97-8 °C; ^{1}H NMR (DMSO-d_6): δ 1.13 (t, J = 7.2 Hz, 6H, 2× $CH_2\underline{CH_3}$), 2.81 (s, 6H, 2× CH_3), 3.92 (q, J = 7.2 Hz, 4H, 2× $\underline{CH_2}CH_3$), 6.62 (t, J = 2.4 Hz, 1H, Ar-H), 6.71 (dd, J = 2.4, 8.4 Hz, 2H, Ar-H), 7.03 (d, J = 8.7 Hz, 4H, Ar-H), 7.32–7.37 (m, 5H, Ar-H), 11.20 (br s, 2H, 2× NH), 11.40 (br s, 2H, 2× NH). MS (ESI) m/e (rel. int.): 459 (M^++1, 100), 275 (34). Anal. Calcd. for $C_{28}H_{32}N_6O_6$: C, 61.28; H, 5.88; N, 15.32. Found: C, 61.07; H, 5.95; N, 15.10.

4.1.8. 1,1′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-ethoxycarbonyl-N″-methylguanidine) (**5b**)

The procedure used for preparation of **5a** was adopted starting with **4b** (1 g, 1.80 mmol), yield 83%; mp 91-2 °C; ^{1}H NMR (DMSO-d_6): δ 1.13 (t, J = 7.2 Hz, 6H, 2× $CH_2\underline{CH_3}$), 2.81 (s, 6H, 2× CH_3), 3.92 (q, J = 7.2 Hz, 4H, 2× $\underline{CH_2}CH_3$), 6.97 (d, J = 8.7 Hz, 4H, Ar-H), 7.05 (s, 4H, Ar-H), 7.31 (d, J = 8.7 Hz, 4H, Ar-H), 10.82 (br s, 2H, 2× NH), 11.33 (br s, 2H, 2× NH). MS (ESI) m/e (rel. int.): 459 (M^++1, 100), 275 (30). HRMS *m/z* 549.2455 (M^+ + 1) (calcd. for $C_{28}H_{33}N_6O_6$: 549.2462). Anal. Calcd. for $C_{28}H_{32}N_6O_6$: C, 61.28; H, 5.88; N, 15.32. Found: C, 61.08; H, 5.61; N, 15.54.

4.1.9. 1,1′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-ethoxycarbonyl-N″-isopropylguanidine) (**6a**)

The procedure used for preparation of **5a** was adopted starting with **4a** and using isoprpylamine, yield 88%; mp 98-9 °C; ^{1}H NMR (DMSO-d_6): δ 1.09–1.15 (m, 18H, 2× $CH_2\underline{CH_3}$ + 2× $CH(\underline{CH_3})_2$), 3.90 (q, J = 7.2 Hz, 4H, 2× $\underline{CH_2}CH_3$), 4.01–4.09 (m, 2H, 2× $\underline{CH}(CH_3)_2$), 6.60 (t, J = 2.4 Hz, 1H, Ar-H), 6.71 (dd, J = 2.4, 8.4 Hz, 2H, Ar-H), 7.02 (d, J = 8.7 Hz, 4H, Ar-H), 7.32–7.34 (m, 5H, Ar-H), 11.20 (br s, 2H, 2× NH), 11.40 (br s, 2H, 2× NH). MS (ESI) m/e (rel. int.): 605 (M^++1, 100). HRMS *m/z* 605.3080 (M^+ + 1) (calcd. for $C_{32}H_{41}N_6O_6$: 605.3088). Anal. Calcd. for $C_{32}H_{40}N_6O_6$: C, 63.54; H, 6.67; N, 13.90. Found: C, 63.14; H, 6.57; N, 13.61.

4.1.10. 1,1′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-ethoxycarbonyl-N‴-isopropylguanidine) (**6b**)

The procedure used for preparation of **5a** was adopted starting with **4b** and using isoprpylamine, yield 89%; mp 93-5 °C; ^{1}H NMR (DMSO-d_6): δ 1.09–1.15 (m, 18H, 2× $CH_2\underline{CH_3}$ + 2× $CH(\underline{CH_3})_2$), 3.90 (q, J = 7.2 Hz, 4H, 2× $\underline{CH_2}CH_3$), 4.01–4.09 (m, 2H, Ar-H), 6.97 (d,

J = 8.4 Hz, 4H, Ar-H), 7.04 (s, 4H, Ar-H), 7.31 (d, J = 8.4 Hz, 4H, Ar-H), 10.84 (br s, 2H, 2× NH), 11.30 (br s, 2H, 2× NH). MS (ESI) m/e (rel. int.): 605 (M^++1, 100). HRMS *m/z* 605.3097 (M^+ + 1) (calcd. for $C_{32}H_{41}N_6O_6$: 605.3088). Anal. Calcd. for $C_{32}H_{40}N_6O_6$: C, 63.54; H, 6.67; N, 13.90. Found: C, 63.48; H, 6.41; N, 13.54.

4.1.11. 1,1′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-methylguanidine) (**7a**)

The bis substituted carbamoyl-guanidine **5a** (0.80 g, 1.45 mmol) was suspended in EtOH (20 mL). 1N KOH (5 mL, 11.66 mmol) was then added and the reaction mixture was kept stirring over night maintaining the temperature at 55–60 °C. The reaction mixture was diluted with water and the solid formed was collected by filtration, washed multiple times with water and recrystallized from aqueous EtOH to give a beige solid, yield 93%; m.p. 209-11 °C; ^{1}H NMR (DMSO-d_6): δ 2.64 (s, 6H, 2× CH_3), 5.10 (br s, 6H, 2× NH_2 + 2× NH), 6.48 (t, J = 1.5 Hz, 1H, Ar-H), 6.56 (dd, J = 1.5, 8.1 Hz, 2H, Ar-H), 6.75 (d, J = 8.4 Hz, 4H, Ar-H), 6.87 (d, J = 8.4 Hz, 4H, Ar-H), 7.21 (t, J = 8.1 Hz, 1H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 159.70, 152.39, 148.59, 147.67, 130.46, 124.05, 120.45, 110.49, 105.88, 27.69.

Hydrochloride salt of 7a. The free base was dissolved in dry EtOH (20 mL) and the solution was chilled in an ice-bath. After passing HCl gas for 10 min, the reaction was concentrated under reduced pressure and then diluted with ether. The precipitate formed was collected by filtration; m.p. 156-8 °C; ^{1}H NMR (DMSO-d_6): δ 2.79, 2.81 (2 s, 6H, 2× CH_3), 6.68 (t, J = 2.4 Hz, 1H, Ar-H), 6.76 (dd, J = 2.4, 8.4 Hz, 2H, Ar-H), 7.11 (d, J = 8.7 Hz, 4H, Ar-H), 7.25 (d, J = 8.7 Hz, 4H, Ar-H), 7.38 (t, J = 8.4 Hz, 1H, Ar-H), 7.68 (br s, 4H, 2× NH_2), 7.80 (br s, 2H, 2× NH), 9.87 (br s, 2H, 2× HCl). HRMS *m/z* 405.2033 (M^+ + 1) (calcd. for $C_{22}H_{25}N_6O_2$: 405.2039). Anal. Calc. for $C_{22}H_{24}N_6O_2$-2.0HCl-0.5H_2O-0.25C_2H_5OH: C, 54.27; H, 5.76; N, 16.87. Found: C, 54.09; H, 5.73; N, 16.64.

4.1.12. 1,1′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-methylguanidine) (**7b**)

The procedure described for **7a** was adopted starting with **5b**, yield 73%; mp 224-5 °C; ^{1}H NMR (DMSO-d_6): δ 2.63 (s, 6H, 2× CH_3), 3.35 (br s, 2H, 2× NH), 5.15 (br s, 4H, 2× NH_2), 6.74 (d, J = 8.7 Hz, 4H, Ar-H), 6.83 (d, J = 8.7 Hz, 4H, Ar-H), 6.92 (s, 4H, Ar-H). MS (ESI) m/e (rel. int.): 605 (M^+ + 1, 100).

Hydrochloride salt of 7b. m.p. 162-4 °C ^{1}H NMR (DMSO-d_6): δ 2.79, 2.81 (2 s, 6H, 2× CH_3), 7.06 (d, J = 8.7 Hz, 4H, Ar-H), 7.09 (s, 4H, Ar-H), 7.24 (d, J = 8.7 Hz, 4H, Ar-H), 7.63 (br s, 4H, 2× NH_2), 7.72 (br s, 2H, 2× NH), 9.70 (br s, 2H, 2× HCl). Anal. Calc. for $C_{22}H_{24}N_6O_2$-2.0HCl-1.2H_2O-0.5C_2H_5OH: C, 52.94; H, 6.06; N, 16.09. Found: C, 53.34; H, 5.92; N, 15.70.

4.1.13. 1,1′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-isopropylguanidine) (**8a**)

The procedure described for **7a** was adopted starting with **6a**, yield 85%; mp 220-2 °C; ^{1}H NMR (DMSO-d_6): δ 1.08 (d, J = 6.3 Hz, 12H, 2× CH($\underline{CH_3}$)$_2$), 3.78–3.86 (m, 2H, 2× $\underline{CH}$(CH_3)$_2$), 4.82 (br s, 4H, 2× NH_2), 5.22 (br s, 2H, 2× NH), 6.48 (t, J = 2.4 Hz, 1H, Ar-H), 6.55 (dd, J = 2.4, 8.1 Hz, 2H, Ar-H), 6.73 (d, J = 8.4 Hz, 4H, Ar-H), 6.85 (d, J = 8.4 Hz, 4H, Ar-H), 7.22 (t, J = 8.1 Hz, 1H, Ar-H).

Hydrochloride salt of 7a. m.p. 131-3 °C; ^{1}H NMR (DMSO-d_6): δ 1.16 (d, J = 6.3 Hz, 12H, 2× CH(CH_3)$_2$), 3.89–3.97 (m, 2H, 2× CH(CH_3)$_2$), 6.67 (t, J = 2.4 Hz, 1H, Ar-H), 6.76 (dd, J = 2.4, 8.4 Hz, 2H, Ar-H), 7.10 (d, J = 8.7 Hz, 4H, Ar-H), 7.25 (d, J = 8.7 Hz, 4H, Ar-H), 7.38 (t, J = 8.4 Hz, 1H, Ar-H), 7.68 (br s, 4H, 2× NH_2), 8.13 (br s, 2H, 2× NH), 9.90 (br s, 2H, 2× HCl). HRMS *m/z* 461.2646 (M^+ + 1) (calcd. for $C_{26}H_{33}N_6O_2$: 461.2665). Anal. Calc. for $C_{26}H_{32}N_6O_2$-2.0HCl-0.5H_2O-C_2H_5OH: C, 57.13; H, 7.02; N, 14.27. Found: C, 57.13; H, 7.11; N, 13.99.

4.1.14. 1,1′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene))bis(N′-isopropylguanidine) (**8b**)

The procedure described for **7a** was adopted starting with **6b**, yield 96%; mp 203-5 °C; ^{1}H NMR (DMSO-d_6): δ 1.08 (d, J = 6.3 Hz, 12H, 2× CH($\underline{CH_3}$)$_2$), 3.78–3.86 (m, 2H, 2× $\underline{CH}$(CH_3)$_2$), 3.35 (br s, 2H, 2× NH), 5.15 (br s, 4H, 2× NH_2), 6.73 (d, J = 8.4 Hz, 4H, Ar-H), 6.83 (d, J = 8.4 Hz, 4H, Ar-H), 6.92 (s, 4H, Ar-H).

Hydrochloride salt of 7a. m.p. 158-60 °C; ^{1}H NMR (DMSO-d_6): δ 1.16 (d, J = 6.3 Hz, 12H, 2× CH(CH_3)$_2$), 3.86–3.92 (m, 2H, 2× CH(CH_3)$_2$), 7.05 (d, J = 8.7 Hz, 4H, Ar-H), 7.08 (s, 4H, Ar-H), 7.23 (d, J = 8.7 Hz, 4H, Ar-H), 7.60 (br s, 4H, 2× NH_2), 7.99 (br s, 2H, 2× NH), 9.72 (br s, 2H, 2× HCl). Anal. Calc. for $C_{26}H_{32}N_6O_2$-2.0HCl-0.75C_2H_5OH: C, 58.14; H, 6.83; N, 14.79. Found: C, 58.24; H, 6.48; N, 14.59.

4.1.15. Preparation of arylimidamides (general procedure) (Scheme 3). N,N′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene)) dicyclohexanecarboximidamide (**9a**)

A chilled solution of the **1a** (0.5 g, 1.71 mmol) in dry MeCN (10 mL) and dry EtOH (15 mL) was treated with *S*-(2-naphthylcyclohexyl) thiobenzimidate hydrobromide (1.31 g, 3.59 mmol). After stirring at r.t. for 24 h, the solvent was evaporated to dryness leaving an oily residue. Treatment with ether gave the reversed amidine as the hydrobromide salt, which was dissolved in EtOH, basified with 1N NaOH, extracted with EtOAc, dried over Na_2SO_4 and finally the solvent was evaporated to dryness giving the free base of the reversed amidine in an analytically pure form, yield 57%, mp 156-8 °C; ^{1}H NMR (DMSO-d_6): δ 1.13–1.30 (m, 6H, cyclohex.), 1.44 (q, J = 11.4 Hz, 4H, cyclohex.), 1.64 (t, J = 11.4 Hz, 2H, cyclohex.), 1.72–1.84 (m, 8H, cyclohex.), 2.11 (d, J = 11.4 Hz, 2H, cyclohex.), 5.72 (br s, 4H, 4× NH), 6.48 (t, J = 2.4 Hz, 1H, Ar-H), 6.60 (dd, J = 2.4, 8.1 Hz, 2H, Ar-H), 6.74 (d, J = 8.7 Hz, 4H, Ar-H), 6.91 (d, J = 8.7 Hz, 4H, Ar-H), 7.24 (t, J = 8.4 Hz, 1H, Ar-H).

Hydrochloride salt of 9a. An ice-bath cold solution of the free base in dry EtOH was treated with HCl gas for 5–10 min, and the reaction mixture was kept stirring for 5 h; mp 200-2 °C; ^{1}H NMR (DMSO-d_6): δ 1.22–1.30 (m, 6H, cyclohex.), 1.68–1.90 (m, 14H, cyclohex.), 2.74 (t, J = 11.4 Hz, 2H, cyclohex.), 6.74 (t, J = 2.1 Hz, 1H, Ar-H), 6.84 (dd, J = 2.1, 8.4 Hz, 2H, Ar-H), 7.17 (d, J = 8.7 Hz, 4H, Ar-H), 7.32 (d, J = 8.7 Hz, 4H, Ar-H), 7.42 (t, J = 8.4 Hz, 1H, Ar-H), 8.47 (br s, 2H, 2× NH), 9.37 (br s, 2H, 2× NH), 11.46 (br s, 2H, 2× HCl). Anal. Calc. for $C_{32}H_{38}N_4O_2$-2.0HCl-H_2O: C, 63.88; H, 7.03; N, 9.31. Found: C, 63.82; H, 6.85; N, 9.17.

4.1.16. N,N′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene)) dicyclohexanecarboximidamide (**9b**)

The procedure used for **9a** was adopted starting with **1b,** yield 55%; mp 201-3 °C; ^{1}H NMR (DMSO-d_6): δ 1.16–1.32 (m, 6H, cyclohex.), 1.47 (q, J = 11.4 Hz, 4H, cyclohex.), 1.62–1.85 (m, 10H, cyclohex.), 2.15 (t, J = 11.4 Hz, 2H, cyclohex.), 5.65 (br s, 4H, 4× NH), 6.74 (d, J = 8.4 Hz, 4H, Ar-H), 6.90 (d, J = 8.4 Hz, 4H, Ar-H), 6.96 (s, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 170.96, 156.58, 151.95, 128.85, 127.36, 120.43, 119.04, 55.72, 41.88, 28.62, 24.92, 24.47, 18.17.

Hydrochloride salt of 9b. m.p. 276-8 °C; ^{1}H NMR (DMSO-d_6): δ 1.22–1.33 (m, 6H, cyclohex.), 1.67–1.90 (m, 14H, cyclohex.), 2.71 (t, J = 11.7 Hz, 2H, cyclohex.), 7.09–7.13 (m, 8H, Ar-H), 7.31 (d, J = 8.7 Hz, 4H, Ar-H), 8.47 (br s, 2H, 2× NH), 9.31 (br s, 2H, 2× NH), 11.37 (br s, 2H, 2× HCl). Anal. Calc. for $C_{32}H_{38}N_4O_2$-2.0HCl-1.5H_2O-0.5C_2H_5OH: C, 62.55; H, 7.31; N, 8.84. Found: C, 62.91; H, 7.37; N, 8.26.

4.1.17. N,N′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene)) dibenzimidamide (**10a**)

The procedure used for **9a** was adopted starting with **1a,** yield 79%; mp 183-4 °C; ^{1}H NMR (DMSO-d_6): δ 6.32 (br s, 4H, 4× NH),

6.56 (t, $J = 2.4$ Hz, 1H, Ar-H), 6.65 (dd, $J = 2.4$, 8.1 Hz, 2H, Ar-H), 6.87 (d, $J = 8.7$ Hz, 4H, Ar-H), 6.99 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.28 (t, $J = 8.4$ Hz, 1H, Ar-H), 7.41–7.44 (m, 6H, Ar-H), 7.88 (d, $J = 8.4$ Hz, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 159.53, 154.28, 150.08, 147.24, 135.90, 130.65, 130.10, 128.03, 127.08, 123.00, 120.83, 11.04, 106.43.

Hydrochloride salt of 10a. m.p. 198–201 °C; ^{1}H NMR (DMSO-d_6): δ 6.81 (t, $J = 2.4$ Hz, 1H, Ar-H), 6.86 (dd, $J = 2.4$, 8.4 Hz, 2H, Ar-H), 7.24 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.42–7.52 (m, 5H, Ar-H), 7.66 (t, $J = 7.5$ Hz, 4H, Ar-H), 7.77 (t, $J = 7.5$ Hz, 2H, Ar-H), 7.94 (d, $J = 8.7$ Hz, 4H, Ar-H), 8.97 (br s, 2H, 2× NH), 9.87 (br s, 2H, 2× NH), 11.57 (br s, 2H, 2× HCl). HRMS *m/z* 499.2122 ($M^+ + 1$) (calcd. for $C_{32}H_{27}N_4O_2$: 499.2134). Anal. Calc. for $C_{32}H_{26}N_4O_2$-2.0HCl-1.2H_2O: C, 64.80; H, 5.16; N, 9.44. Found: C, 64.77; H, 5.07; N, 9.32.

4.1.18. N,N′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene)) dibenzimidamide (**10b**)

The procedure used for **9a** was adopted starting with **1b**, yield 66%; mp 236-8 °C; ^{1}H NMR (DMSO-d_6): δ 6.31 (br s, 4H, 4× NH), 6.85 (d, $J = 8.7$ Hz, 4H, Ar-H), 6.98 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.02 (s, 4H, Ar-H), 7.39, 7.46 (m, 6H, Ar-H), 7.95 (d, $J = 7.5$ Hz, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 154.15, 152.88, 151.50, 146.42, 135.90, 130.00, 127.95, 127.00, 122.84, 119.67, 119.33.

Hydrochloride salt of 10b. m.p. 287-9 °C; ^{1}H NMR (DMSO-d_6): 7.17–7.20 (m, 8H, Ar-H), 7.49 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.65 (t, $J = 7.5$ Hz, 4H, Ar-H), 7.77 (t, $J = 7.5$ Hz, 2H, Ar-H), 7.94 (d, $J = 7.5$ Hz, 4H, Ar-H), 8.96 (br s, 2H, 2× NH), 9.87 (br s, 2H, 2× NH), 11.59 (br s, 2H, 2× HCl). HRMS *m/z* 499.2130 ($M^+ + 1$) (calcd. for $C_{32}H_{27}N_4O_2$: 499.2134). Anal. Calc. for $C_{32}H_{26}N_4O_2$-2.0HCl-0.5H_2O: C, 66.20; H, 5.03; N, 9.65. Found: C, 66.13; H, 5.00; N, 9.65.

4.1.19. N,N′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(4-tert-butylbenzimidamide) (**11a**)

The procedure used for **9a** was adopted starting with **1a**, yield 48%; mp 286-8 °C; ^{1}H NMR (DMSO-d_6): δ 1.30 (s, 18H, 2× C(CH_3)$_3$), 6.24 (br s, 4H, 4× NH), 6.59 (t, $J = 2.4$ Hz, 1H, Ar-H), 6.66 (dd, $J = 2.4$, 8.4 Hz, 2H, Ar-H), 6.84 (d, $J = 8.1$ Hz, 4H, Ar-H), 7.01 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.28 (t, $J = 8.4$ Hz, 1H, Ar-H), 7.42 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.88 (d, $J = 8.1$ Hz, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 163.22, 157.93, 157.06, 155.71, 131.27, 130.27, 128.68, 127.81, 125.74, 125.66, 120.31, 113.53, 108.96, 34.98, 30.75.

Hydrochloride salt of 11a. m.p. >300 °C; ^{1}H NMR (DMSO-d_6): δ 1.33 (s, 18H, 2× C(CH_3)$_3$), 6.81 (t, $J = 2.4$ Hz, 1H, Ar-H), 6.86 (dd, $J = 2.4$, 8.4 Hz, 2H, Ar-H), 7.24 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.42–7.50 (m, 5H, Ar-H), 7.68 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.89 (d, $J = 8.4$ Hz, 4H, Ar-H), 8.92 (br s, 2H, 2× NH), 9.80 (br s, 2H, 2× NH), 11.47 (br s, 2H, 2× HCl). HRMS *m/z* 611.3367 ($M^+ + 1$) (calcd. for $C_{40}H_{43}N_4O_2$: 611.3386). Anal. Calc. for $C_{40}H_{42}N_4O_2$-2.0HCl-0.25H_2O: C, 69.80; H, 6.51; N, 8.14. Found: C, 69.75; H, 6.44; N, 8.02.

4.1.20. N,N′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene))bis(4-tert-butylbenzimidamide) (**11b**)

The procedure used for **9a** was adopted starting with **1b,** yield 57%; mp 280-2 °C; ^{1}H NMR (DMSO-d_6): δ 1.32 (s, 18H, 2× C(CH_3)$_3$), 6.23 (br s, 4H, 4× NH), 7.08–7.11 (m, 8H, Ar-H), 7.24 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.59 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.84 (d, $J = 7.8$ Hz, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 154.15, 152.88, 151.50, 146.42, 135.90, 130.00, 127.95, 127.00, 122.84, 119.67, 119.33.

Hydrochloride salt of 11b. m.p. >300 °C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 18H, 2× C(CH_3)$_3$), 7.17–7.19 (m, 8H, Ar-H), 7.47 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.67 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.89 (d, $J = 8.1$ Hz, 4H, Ar-H), 8.91 (br s, 2H, 2× NH), 9.80 (br s, 2H, 2× NH), 11.48 (br s, 2H, 2× HCl). HRMS *m/z* 611.3362 ($M^+ + 1$) (calcd. for $C_{40}H_{43}N_4O_2$: 611.3386). Anal. Calc. for $C_{40}H_{42}N_4O_2$-2.0HCl-1.25H_2O: C, 68.02; H, 6.63; N, 7.93. Found: C, 68.02; H, 6.27; N, 7.87.

4.1.21. N,N′-(4,4′-(1,3-phenylenebis(oxy))bis(4,1-phenylene)) dibiphenyl-4-carboximidamide (**12a**)

The procedure used for **9a** was adopted starting with **1a**, yield 72%; mp 267-9 °C; ^{1}H NMR (DMSO-d_6): δ 6.20 (br s, 4H, 4× NH), 6.59 (t, $J = 2.4$ Hz, 1H, Ar-H), 6.70 (dd, $J = 2.4$, 8.4 Hz, 2H, Ar-H), 6.93 (d, $J = 8.1$ Hz, 4H, Ar-H), 7.00 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.318 (t, $J = 8.4$ Hz, 1H, Ar-H), 7.40 (t, $J = 7.2$ Hz, 2H, Ar-H), 7.47 (t, $J = 7.2$ Hz, 4H, Ar-H), 7.69–7.71 (m, 8H, Ar-H), 8.00 (d, $J = 8.1$ Hz, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 163.04, 157.97, 155.76, 145.17, 138.33, 131.29, 130.28, 129.55, 129.18, 128.71, 127.89, 127.18, 127.03, 126.88, 120.35, 113.55, 108.97.

Hydrochloride salt of 12a. m.p. 275-7 °C; ^{1}H NMR (DMSO-d_6): δ 6.83 (t, $J = 2.4$ Hz, 1H, Ar-H), 6.86 (dd, $J = 2.4$, 8.4 Hz, 2H, Ar-H), 7.24 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.43–7.56 (m, 11H, Ar-H), 7.81 (d, $J = 7.2$ Hz, 4H, Ar-H), 7.98 (d, $J = 8.4$ Hz, 4H, Ar-H), 8.06 (d, $J = 8.4$ Hz, 4H, Ar-H), 8.98 (br s, 2H, 2× NH), 9.93 (br s, 2H, 2× NH), 11.65 (br s, 2H, 2× HCl). Anal. Calc. for $C_{44}H_{34}N_4O_2$-2.0HCl-0.75H_2O: C, 71.68; H, 5.12; N, 7.59. Found: C, 71.69; H, 5.05; N, 7.54.

4.1.22. N,N′-(4,4′-(1,4-phenylenebis(oxy))bis(4,1-phenylene)) dibiphenyl-4-carboximidamide (**12b**)

The procedure used for **9a** was adopted starting with **1b,** yield 82%; mp 298–300 °C; ^{1}H NMR (DMSO-d_6): δ 6.23 (br s, 4H, 4× NH), 6.60 (d, $J = 8.4$ Hz, 2H, Ar-H), 6.75 (d $J = 8.4$ Hz, 2H, Ar-H), 6.87–7.34 (m, 10H, Ar-H), 7.36–7.50 (m, 6H, Ar-H), 7.69–6.72 (m, 6H, Ar-H), 8.00 (d, $J = 7.8$ Hz, 4H, Ar-H).

Hydrochloride salt of 12b. m.p. 222-4 °C; ^{1}H NMR (DMSO-d_6): 7.19–7.21 (m, 8H, Ar-H), 7.46–7.56 (m, 10H, Ar-H), 7.81 (d, $J = 7.8$ Hz, 4H, Ar-H), 7.98 (d, $J = 8.4$ Hz, 4H, Ar-H), 8.04 (d, $J = 8.4$ Hz, 4H, Ar-H), 8.97 (br s, 2H, 2× NH), 9.89 (br s, 2H, 2× NH), 11.58 (br s, 2H, 2× HCl). Anal. Calc. for $C_{44}H_{34}N_4O_2$-2.0HCl-1.25H_2O: C, 70.82; H, 5.20; N, 7.50. Found: C, 71.04; H, 5.22; N, 7.31.

4.1.23. S-(2-Naphthylmethyl)-4-cyclohexylthioimidate.HBr

Yield 73%, mp 187-9 °C; ^{1}H NMR (DMSO-d_6) δ 1.15–1.31 (m, 3H, cyclohex.), 1.45 (q, $J = 11.4$ Hz, 2H, cyclohex.), 1.63 (t, $J = 11.4$ Hz, 1H, cyclohex.), 1.72–1.84 (m, 4H, cyclohex.), 2.10 (d, $J = 11.4$ Hz, 1H, cyclohex.), 4.91 (s, 2H, CH_2), 7.52–7.61 (m, 3H, Ar-H), 7.90–7.99 (m, 3H, Ar-H), 8.06 (s, 1H, Ar-H), 10.32 (br s, 1H, HBr). MS (ESI) m/e (rel. int.): 365 ($M^+ + 2$, 10), 364 ($M^+ + 1$, 100). Anal. Calc. for $C_{18}H_{21}NS$.HBr: C, 59.34; H, 6.09; N, 3.84. Found C, 59.29; H, 6.28; N, 3.49.

4.2. Biology

4.2.1. In vitro assay for T.b.r.

Minimum essential medium (50 μl) supplemented according to Baltz *et al* [21]. with 2-mercaptoethanol and 15% heat-inactivated horse serum were added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 μL of trypanosome suspension (*T.b.r.* STIB 900) 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 more 2–4 h. The plate was read in a microplate fluorometer system (Spectramax Gemini by Molecular Devi-ces) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm [21]. Fluorescence development was expre-ssed as percentage of the control, and IC_{50} values determined. Assays were carried out twice independently and in duplicates.

4.2.2. In vitro assay for P. f.

Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]-hypoxanthine incorporation assay was used [21]. Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in

microtiter plates for 48 h. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by liquid scintillation counting 24 h after the addition of the radiolabel. The counts were expressed as percentage of the control cultures, sigmoidal inhibition curves were drawn and IC_{50} values calculated. Assays were carried out twice independently and in duplicates.

4.2.3. Cytotoxicity studies

Cytotoxicity was evaluated using cultured L-6 rat myoblast cells using the same assay procedure as for *T.b.r.* [21].

4.3. Absorbance spectroscopy and thermal melting (T_m) experiments

Experiments were done in MES10 buffer (0.01M MES (2-(N-morpholino)ethanesulfonic acid), 0.001M ethylenediaminetetraacetic acid (EDTA), 0.1M NaCl with the pH was adjusted to 6.25). DNA polymers were purchased from Pharmacia and characterized by their melting curves. Absorbance and thermal melting (T_m) experiments were done using a Cary 300 Bio spectrophotometer with the software supplied with the instrument. For absorbance measurements, the buffer was scanned from 400 to 250 nm in 1 cm quartz cuvettes and aliquots of concentrated stock solutions of the compounds were titrated into the buffer and the solutions were rescanned. Cuvettes were mounted in a thermal block, and the solution temperatures were monitored by a thermistor in a reference cuvette with a computer-controlled heating rate of 0.5 °C/min. Experiments were conducted at a concentration of 2×10^{-5} M base pair for polydA·polydT. For experiments with complexes a ratio of 0.3 compound per base pair for polydA·polydT was generally used.

4.4. Molecular modeling and docking studies

Docking studies were performed using MOE software Version 2008.10. The X-ray crystal structure of the selective antiprotozoal agent pentamidine bound to the minor groove of DNA dodecamer $d(CGCGAATTCGCG)_2$ was obtained from the RCSB Protein Data Bank (PDB ID: 1d64). The enzyme was prepared for docking as follows: (a) pentamidine interactions were determined to reveal the different types of interaction as a validation for the docking is to be performed. (b) Pentamidine and solvent molecules were removed from the binding site. (c) The hydrogens were added with their standard geometry. (d) The newly synthesized dications were constructed and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol. (e) Docking of the energy minimized ligand molecules was carried out using the default MOE-dock parameters. The docking aims at searching for favorable binding configuration between the flexible dicationic ligands and the DNA minor groove and to assess the strength of the binding interactions of the novel dications as compared to the classical minor groove binder pentamidine. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand-DNA assembly dG calculated in Kcal/mol (S value in MOE) [22].

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