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

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$X = \text{CH}_2, \text{NH}, \text{CO}, \text{SO}_2$
Abstract

The *in vitro* screening for trypanocidal activity against *T.b. rhodesiense* of an in-house library of 62 compounds [i.e. alkane, diphenyl and aza-alkane bisguanidines and bis(2-aminoimidazolines)] which were chosen for their structural similarity to the trypanocidal agents synthalin (1,10-decanediguanidine) and 4,4’-diguanidinodiphenylmethane, and the polyamine \(N^1\)-(3-amino-propyl)-propane-1,3-diamine respectively, is reported. The original synthetic procedure for the preparation of 21 of these compounds is also reported.

Most compounds displayed low micromolar anti-trypanosomal activity with five of them presenting a nanomolar inhibitory action on the parasite: 1,9-nonanediguanidine (1c), 1,12-dodecanediguanidine (1d), 4,4’-bis[1,3-di(tert-butoxycarbonyl)-2-imidazolidinylimino]diphenylamine (28a), 4,4’-bis(4,5-dihydro-1H-2-imidazolylamino)diphenylamine (28b), 4,4’-diguanidinodiphenylamine (32b) and 1,4-bis[4-(4,5-dihydro-1H-2-imidazolylamino)phenyl]piperazine (41b). Those molecules, which showed an excellent *in vitro* activity as well as high selectivity for the parasite [e.g. 1c (IC\(_{50}\) = 49 nM; SI > 5294), 28b (IC\(_{50}\) = 69 nM; SI = 3072), 32b (IC\(_{50}\) = 22 nM; SI = 29.5), 41b (IC\(_{50}\) = 118 nM; SI = 881)], represent new anti-trypanosomal lead compounds.

**Keywords**: polyamine, guanidine, 2-aminoimidazole, *Trypanosoma brucei*, antiprotozoal, chemotherapy
Introduction

Sleeping sickness (Human African Trypanosomiasis, HAT) is caused by two subspecies of African trypanosomes, *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*, responsible for the chronic and acute form of the disease respectively. Only four drugs are licensed for the treatment of HAT (DFMO, suramin, pentamidine and melarsoprol) although other drugs such as berenil (usually used against animal trypanosomiasis) or the nitrofuran nifurtimox (registered for use against Chagas’ disease) have also proved useful in some limited cases. The actual situation of re-emergence of sleeping sickness in sub-Saharan Africa, with prevalence of an estimated 500,000 infected individuals, and the drawbacks of the current HAT chemotherapy (e.g. toxicity, increasing resistance, parenteral mode of administration, price) make the search for new trypanocidal drugs urgently needed.

Many diamidine, diguanidine and polyamine compounds have been investigated for their antitrypanosomal activity as far as 65 years ago, giving rise, for instance, to the aromatic diamidine drug pentamidine which is still used nowadays for the treatment of early stage *T. b. gambiense* infections. However, this drug is unable to cross the blood-brain barrier in sufficient quantity to treat late-stage cases of HAT. Other aromatic diamidines such as propamidine or berenil are used as antiprotozoal chemotherapy in cattle and the trypanocidal activity of dicationic derivatives related to pentamidine have been reported as well (Figure 1). The polyamine metabolic pathway of trypanosomatid parasites has attracted much attention as drug target for the last 15 years. This research led to the synthesis and evaluation of many polyamine analogues as chemotherapeutic agents against parasitic infections, being the trypanothione reductase a particularly targeted enzyme (Figure 1).
There is a clear lack of research investment in the field of tropical diseases in comparison to the number of affected population.\textsuperscript{24,25} Thus, a reasonable approach for the discovery of new anti-trypanosomal lead compounds at a lesser cost is the screening for anti-parasitic activity of already available molecules. Hence, a rapid look at the structure of the polyamine, diguanidine and diamidine compounds reported as antiprotozoal agents in the literature put into evidence the potential as possible trypanocides of a series of bisguanidine and bis(2-aminoimidazoline) compounds previously synthesized in our laboratory for other purposes.\textsuperscript{26-28} Moreover, some of the alkanediguanidine [1,8-octanediguanidine (1b),\textsuperscript{29} 1,12-dodecanediguanidine (1d),\textsuperscript{6,30,31} bis(guanidinopropyl)amine (6a)\textsuperscript{32}] and diphenyl compounds [4,4’-diguanidinodiphenylmethane (31b), 4,4’-diguanidinodiphenylsulfone (33b)]\textsuperscript{8} available in our in-house library had been previously reported for their use as either trypanocide, microbicides or fungicides. Of particular interest was the recent report on \textit{N,N’}-bis(4-amidinophenyl)piperazine (Figure 1) which proved to be a very effective anti-trypanosomal agent \textit{in vivo}.\textsuperscript{15} Hence, in the search for new HAT chemotherapy, we decided to carry out an \textit{in vitro} screening against the parasite \textit{T. brucei rhodesiense} of a total of 62 compounds (Tables 1-5) taken from our in-house library and structurally related to the trypanocidal agents synthalin (1,10-decanediguanidine) and 4,4’-diguanidinodiphenylmethane (31b), and the polyamine \textit{N’}-(3-amino-propyl)-propane-1,3-diamine respectively. We also describe here the original synthesis of 21 molecules which had not been previously reported.
Results

Chemistry

Synthesis of the aliphatic compounds 1a-d, 2a-d, 3b-d, 4a-b, 5a, 5b, 5d, 6a-b, 7, 8, 9, 11-13, 21-24 (Tables 1, 2, 3 and 4) as well as the diphenyl derivatives 27a-33a, 27b-33b and 34 (Table 5, entry 1-15) has been previously reported by us.26,27,28

Synthesis of the 3-aza-1,6-hexane diamine derivatives (Scheme 1). Guanylation of 3-aza-1,6-hexanediamine with an excess of DCC in CH₃CN afforded a mixture of the di-substituted dicyclohexylguanidine derivative 5e and the tri-substituted compound 17. These products were separated by preparative reverse phase HPLC.
3-Aza-1,6-hexane diamine selectively protected on the primary amino group with benzylcyanoformate\textsuperscript{28,33} (5d) was used as starting material for the preparation of the 3-substituted derivatives 8-10, 14-16, 18-20 and 25-26 (Scheme 1). Alkylation of 5d with bromoethylbenzene or 2-bromoethanol yielded 8 (83\%) and 20 (69\%) respectively. Hydrogenolysis of 8 with 10\% Pd-C/1M HCl/MeOH afforded the amine 9 which was subsequently refluxed with S-methylisothiouronium sulphate in CH\textsubscript{3}CN, yielding the bis-guanidine 10 (46\%). When 5d was treated with chloroacetaldehyde in excess under reductive conditions (NaBH(OAc)\textsubscript{3}/AcOH/CH\textsubscript{3}CN) at room temperature, the chloroethyl derivative 19 (35\%) was obtained together with the acetate side-product 18 (16\%). In this reaction, the nucleophilic substitution of chloroacetaldehyde (or 2-chloroethanol derived from the reduction \textit{in situ} of chloroacetaldehyde) by the secondary amine 5d, leading to a hydroxyethyl intermediate that can react with AcOH present in the reaction medium, is a potential competitive reaction. Working at low temperature (0 °C) was unfavourable to the competitive nucleophilic substitution and 19 could be obtained in 69\% in these conditions.

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

![Scheme 1](image)

**Synthesis of the diphenyl derivatives** (Scheme 2). Guanidines 31c and 32c were
obtained by reaction of an ether solution of $N$-(2,2'-diethoxyethyl)carbodiimide
(prepared from BrCN and aminoacetaldehyde-diethylacetal) with 4,4'-
diaminodiphenylamine and 4,4'-diaminodiphenymethane in the presence of CH$_3$SO$_3$H,
respectively. Preparation of the 2-aminoimidazole 35 was carried out by base-catalysed
cyclisation of the guanidine precursor 31c following the methodology of Munk et al..
In this reaction, two cyclisation products could potentially form: (1H-imidazol-2-yl)aryl-amine (“endocyclic” amino group) and 1-aryl-1H-imidazol-2-ylamine (“exocyclic”
amino group). $^1$H NMR spectrum of 35 showed a unique broad singlet for 4-H and 5-H
imidazol protons at 6.76 ppm whereas decoupled $^{13}$C NMR spectrum showed a unique
signal for both 4- and 5- imidazole carbons indicating the magnetic equivalence of these atoms. These data demonstrated that the expected product 35 with the endocyclic amino group was obtained (e.g. if the isomer with exocyclic amino group were obtained, a typical AB system would be observed for the 4- and 5-H imidazol protons).

![Chemical structure diagram]

**Scheme 2.** Reagents and conditions. (a) N-(2,2'-diethoxyethyl)carbodiimide (2.2 equiv.); CH$_3$SO$_3$H (2 equiv.); EtOH, reflux, 23h; (b) 1) 6M HCl, rt, 3h, 2) 10% NaOH, rt, 1h; (c) NBS, t-BuOOH, CCl$_4$, reflux; (d) tri-n-pentylphosphine (4 equiv.); PhMe, reflux, 24h.

Compound 37 has been previously described$^{37}$ as a B$_2$ bradykinin receptor antagonist. The reported procedure was quite lengthy so we designed a three steps synthesis starting from 4,4'-dimethylbenzophenone. Radical bromination of 4,4'-dimethylbenzophenone with NBS/t-BuOOH/CCl$_4$ allowed the formation of the dibromo-derivative compound 36 which was isolated by crystallisation from the reaction mixture (22 % yield). The low yield obtained could be explained by the formation of a mixture of mono- and poly-halogenated derivatives (e.g. three spots were observed by TLC of the crude reaction mixture).$^{38}$ The bis-phosphonium compound 37 was obtained by nucleophilic substitution of the bromine atoms of 36 with an excess of tri-n-pentylphosphine in refluxing toluene. Tri-n-pentylphosphine was prepared by a
modification of the procedure described by Davies et al. The reaction of an excess of bromopentane Grignard’s reagent with phosphorous trichloride working at -78 ºC afforded the tri-\textit{n}-pentylphosphine which was purified by fractional distillation.

Synthetic approach for the preparation of the piperazine-based bisguanidine and bis(2-aminimidazoline) compounds is depicted in scheme 3. Aromatic nucleophilic substitution of 1-fluoro-4-nitrobenzene with the commercially available 1-(4-nitrophenyl)-piperazine in DMSO at 100 ºC afforded 38. Nitro groups were reduced by catalytic hydrogenation (10 % Pd-C/HCl/MeOH) affording the amine 39. Introduction of the Boc-protected guanidine and imidazoline moieties (compounds 40a and 41a respectively) was carried out in good yield with \(N,N'\)-di(\textit{tert}-butoxycarbonyl)thiourea and \(N,N'\)-di(\textit{tert}-butoxycarbonyl)imidazoline-2-thione respectively. Removal of the Boc-protecting groups was accomplished by treatment with TFA, affording 40b and 41b as their trifluoroacetate salts.

![Scheme 3](image-url)
Biological results. *In vitro* anti-trypanosomal activity

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

**Alkane and aza-alkane derivatives** (Tables 1 and 2). The most potent compound within the alkane (Table 1) and aza-alkane (Table 2) derivative series was 1,9-nonanenediguanidine (*1c*) with IC$_{50}$ = 49 nM and a remarkable selectivity for the parasite (SI > 5294). In these series, the guanidinium cation gave in general more active compounds (about 2 to 4-fold) than the 2-aminoimidazolinium counterpart (compare *1a*-d / *2a*-d). This was also true for the aza-alkane series (Table 2, *5a*/*5b* and *6a*/*6b*). Increasing the chain length of the methylene spacer (n = 6, 8, 9, 12) between either guanidinium or 2-aminoimidazolinium cations tended to increase the activity with the following order: n = 6 < n = 8 (*ca* 30-fold) < n = 9 ~ n = 12 (Table 1). Regarding the selectivity, the nine-methylene spacer (*1c* and *2c*) gave the best SI (5294 and 71) in both series. Noteworthy was the greater activity displayed by the dicationic derivatives *1b*-d and *2b*-d with respect to their monocationic counterparts (Table 1). This behaviour was also observed for the aza-alkane compound *6b* which was 3.5-fold more active than *7* (Table 2).
Introduction of an unsubstituted nitrogen atom in the methylene chain (e.g. in the aza-alkane series, Table 2) tended to reduce the activity compared to alkyl spacer. This is exemplified by the activity of compounds 1a and 2a (8 and 19.3 µM respectively) and their aza-analogues 5a and 5b (21.4 and 69.1 µM respectively). Another interesting result was that of the dicyclohexylguanidine 5c (IC$_{50}$ = 2.4 µM) which was 9-fold more active than the guanidine analogue 5a (21.4 µM). This result might reflect better pharmacokinetic properties of the more lipophilic derivative 5c (i.e. to cross biological membranes).

The dicyclohexylguanidine compound 17 (IC$_{50}$ = 0.98 µM) displayed the best activity and selectivity (SI > 82) of all the 3-aza-1,6-hexanediamine derivatives (Table 3). Again, it appeared that lipophilicity was an important factor for good activity. In this series, substitution of the secondary amino group with a phenethyl, 3(2-ethyl)indole or methyl group afforded molecules slightly more active than the parent compound (compare the activities of 10/5a, 11/5b and 16/5b). In addition, the amines protected with a carbobenzyloxy group (Cbz) were more active than their free amino counterparts (compare 8/9, 12/13 and 14/15. Regarding the effect of the substituent on the secondary nitrogen in the Cbz-protected series, the following results, in order of decreasing activity, were obtained: indole (13: 1 µM) > methyl (14: 3.1 µM) ~ phenethyl (8: 3.88 µM) > CH$_2$CH$_2$OAc (18: 7.1 µM) > CH$_2$CH$_2$OH (20: 14 µM).
Worth mentioning is the result obtained for the cyclic analogues 25 and 26 (Table 4), which showed the same range of activity as the parent compound 14 (IC\textsubscript{50} = 3.1 µM, Table 3) but a lower selectivity (SI = 27 and 18.6 respectively, compared to 41 for 14). This behaviour was also observed with the cyclic analogue 23 (71.2 µM) which displayed the same activity as the aliphatic parent 5b (69.1 µM).

Table 4 around here

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

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

Table 5 around here

Regarding the bridge linking both phenyl rings, the same behaviour was observed for the guanidinium and 2-aminimidazolinium series, i.e. NH >> CH$_2$ > CO > SO$_2$ in order of decreasing activity (compare 27b-30b and 31b-33b, 34). When a piperazine moiety was used as bridge between both phenyl rings (Table 5, entry 21-25), the 2-aminimidazolinium compound 41b showed the best activity (0.118 µM) and also a 5 times higher selectivity (SI = 881) with respect to the guanidinium analogue (SI = 172).

Discussion

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

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

For short methylene chains (n = 5, 6, 7), introduction of a secondary nitrogen atom into the alkyl spacer afforded less active molecules although further substitution of this nitrogen could increase slightly the activity (compounds 8-20). Conformational restriction of the aza-alkane molecules did not affect nor increase the trypanocidal action compared to their linear analogues (compounds 23, 25 and 26). The importance of lipophilicy of these molecules, facilitating drug uptake by the parasite by passive diffusion, was exemplified by the higher anti-trypanosomal activity of 5c with respect to 5a.

The most interesting results probably came from the diphenyl series with a NH bridge. The bis(2-aminoimidazoline) derivative 28b was extremely potent (IC₅₀ = 69
nM) and also highly selective for the parasite (SI = 3072). The Boc-protected counterpart 28a had the same range of activity but a lower selectivity index (SI = 202). This result in particular might be relevant because of the higher lipophilicity of the Boc-protected compound 28a. Late-stage cases of HAT involve CNS infection and hence, require drugs able to cross the blood-brain barrier. However, the Boc-protecting group is probably stable in the conditions of the in vitro assay but potentially could be metabolised in vivo to afford the unprotected derivative 28b.

Changing the 2-aminoimidazolinium cations for guanidinium ones led to the most active compound of this screening: 32b (22 nM, SI = 29.5). The nature of the bridge linking both phenyl rings had a clear influence on the trypanocidal action of these compounds. Electron-donating groups such as NH, piperazine or CH₂ afforded better trypanocides than electron-withdrawing groups such as C=O or SO₂. Such behaviour was consistent with the findings of Donkor et al. in the pentamidine congener series where electron-rich phenyl groups (e.g. phenoxy) afforded better trypanocides than electron-poor phenyl rings (e.g. acetylated aniline or pyridine).

If we compare the different cationic species studied (i.e. guanidinium, 2-aminoimidazolinium, phosphonium), the good activity and selectivity displayed by the bis-phosphonium derivative 37 (IC₅₀ = 0.414 μM, SI = 28.5) is of particular interest. These results suggest that lipophilic bis-phosphonium diphenyl derivatives might be a good alternative (with potentially better pharmacokinetic properties) to the guanidine or 2-aminoimidazoline derivatives. With respect to the guanidine cation, N-substitution with a diethoxyethane moiety afforded highly selective anti-trypanosomal agents (SI > 754 and 767 for 31c and 32c respectively).

Anti-trypanocidal efficacy of a drug depends on its effective uptake by the parasite. It is known that diamidines such as pentamidine, which have very slow rate of diffusion
across biological membranes, can be transported into the cell by a P2-amino-purine transporter that specifically recognises the main \( \text{H}_2\text{N-C}(\text{R}_1) = \text{NR}_2 \) motif.\textsuperscript{46,47} The guanidine molecules reported here also present this recognition motif. In the case of the diphenyl derivatives, most of the Boc-protected molecules (i.e. the most lipophilic) showed a weaker activity than the charged, unprotected, guanidinium analogues. This might account for a more efficient transport of the unprotected derivatives through the P2 transporter, although affinity assays for this transporter remain to be done.

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

Several compounds presented in this paper were first and foremost studied for different activities on the CNS (i.e. \( \alpha_1\)-adrenergic antagonism, \( I_2\)-imidazoline binding site affinity or analgesic properties). The knowledge of these interactions (i.e. possible
side effects) is of importance because useful anti-trypanocidal agents are expected to penetrate the CNS to cure late-stage cases of HAT. The diphenyl compounds (Table 5, entries 5-8 and 12-15) present $\alpha_1$-adrenergic antagonist activity in various tissues.\(^{26,51}\) In particular, the blood pressure and heart rate responses of two compounds (29b and 31b) had been tested on rats \textit{in vivo}, suggesting a smaller magnitude of cardiovascular effects than the $\alpha_1$-adrenergic antagonist Doxazosin at the same dose.\(^{52}\) On the other hand, the alkane derivatives (Table 1, 1a-d and 2a-d) showed a moderate to good affinity for the I$_2$-imidazoline binding sites and $\alpha_2$-adrenoceptors in human brain membranes.\(^{27}\) Finally, several aza-alkane derivatives (Tables 2 and 3: 4a-b, 5a-b, 6a-b, 7, 10, 11, 23) were tested for analgesic activity in mice.\(^{28}\) These data are relevant and should be taken into consideration when choosing possible lead compounds for in vivo assays.

**Conclusion**

We have reported here the screening for trypanocidal activity against \textit{T.b. rhodesiense} of an in-house library of 62 compounds [i.e. alkane, diphenyl and aza-alkane bisguanidine and bis(2-aminoimidazoline)] which were chosen for their structural similarity to the trypanocidal agents synthalin (1,10-decanediguanidine), and 4,4’-diguanidinodiphenylmethane, and the polyamine $N^1$-(3-amino-propyl)-propane-1,3-diamine respectively. The original synthetic procedure for the preparation of 21 of these compounds was also reported.

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

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

**Experimental Section**

**Chemistry.** All reaction solvents were purchased anhydrous and used as received. Other solvents used were reagent grade. Reactions were monitored by TLC using pre-coated silica gel 60 F254 plates. Chromatography was performed either with silica gel 60 PF$_{254}$ (particle size 40-63 µm) or with a medium pressure chromatography system using KP-Sil™ 40S or 40M cartridges (particle size 32-63µm, 60Å). All reactions requiring anhydrous conditions or an inert atmosphere were performed under a positive pressure of N$_2$. $^1$H NMR and $^{13}$C NMR spectra were recorded at 200 and 50 MHz respectively, unless otherwise noted. Chemical shifts of the $^1$H NMR spectra were internally referenced to the residual proton resonance of the deuterated solvents: CDCl$_3$ (7.26 ppm), D$_2$O ($\delta$ 4.6 ppm), CD$_3$OD (3.49 ppm) and DMSO ($\delta$ 2.49 ppm). Chemical shifts of $^{13}$C NMR and $^{31}$P spectra were referenced with a capilar of DMSO-d$_6$ ($\delta$ 39.5 ppm) and H$_3$PO$_4$ ($\delta$ 0 ppm) respectively. IR spectra were recorded as KBr pellets or neat. Melting points were determined with a Reichert-Jung Thermovar apparatus and
are uncorrected. Mass spectra were recorded on a Hewlett Packard Series 1100 MSD spectrometer (ES, APCI) and on a VG Autospec spectrometer (FAB). Elemental analysis was performed on a Heraeus CHN-O Rapid analyser. Analytical results were within ± 0.4 % of the theoretical values unless otherwise noted. Analytical HPLC was run on a Beckman LC-168 HPLC with either a Waters Delta Pak 5µ-C18-100Å (3.9x150 mm) (column I) or a Varian Microsorb-MV-C18-100Å column (column II) using the following conditions: gradient time = 40 min and 15 min for columns I and II respectively, H₂O/CH₃CN (100:0→0:100) (TFA 0.1 %), flow rate = 1 mL/min, λ = 214 and 254 nm. Preparative HPLC (compounds 5c and 17) was carried out using a Waters Deltaprep apparatus with a Waters prepak®-RCM Base column and detection at 214 nm.

Compounds 1a, 1b-1d, 2a, 2b, 2c, 2d, 3b, 3c, 3d, 4a- 6a,53 4b, 5b, 5d, 6b, 7, 8, 9, 11, 12, 13, 21, 22, 23, 24, 27a-33a, 27b-33b and 34 were prepared as previously reported.26-28, 53

**N-{3-[(2-Guanidino-ethyl)-phenethyl-amino]-propyl}-guanidine (10).** A solution of 9 (0.5mmol) and S-methylisothiouronium sulfate (148 mg, 0.53 mmol) in dry MeOH (7 mL) was heated for 12 h at reflux. The solvent was removed by reduce pressure and the crude product dissolved in a mixture of H₂O/EtOH was treated with a few drops of 5 % H₂SO₄. The solution was allowed to stand 3 days in the fridge and the supernatant was discarded. Acetone was added and the oily residue was triturated with a spatula until the product crystallised. The solid was dried *in vacuo* affording 10 as a highly hygroscopic colorless solid (104 mg, 46 %). ^H NMR (D₂O) δ 7.3-7.0 (m, 5H);
3.6-2.8 (m, 12H); 1.87 (m, 2H); $^{13}$C NMR (D$_2$O) $\delta$ 155.9 (br); 135.0; 128.1; 127.7; 126.4; 53.0; 50.6; 49.9; 37.1; 35.4; 28.2; 22.7. LRMS (ES$^+$) $m/e$: 307 [(M+H)]; 100 %.

{3-[(2-Benzyloxy carbonylamino-ethyl)-methyl- amino- propyl]-carbamic acid benzyl ester (14).} NaBH$_3$CN (100 mg, 1.49 mmol) was added to a solution of amine 5d (443 mg, 1.15 mmol) and 37 % aqueous formaldehyde (0.4 mL, 4.6 mmol). The reaction was stirred 4 h at room temperature and the solvents were removed by reduce pressure. The crude residue was partitioned between CHCl$_3$ and water. The organic phase was collected and the aqueous phase was extracted 3 times with CHCl$_3$ . Organic extracts were washed with brine, dried (Na$_2$SO$_4$) and concentrated by reduce pressure. Flash chromatography (40S cartridge) with CH$_2$Cl$_2$/MeOH (95:5) afforded the methylated amine 14 as a colorless solid (236 mg, 51 %); mp 60-62 °C; IR (KBr) $\nu$ 3300, 2900, 2725, 1665, 1515, 1250, 1120, 960, 720, 685, 670 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 7.4-7.2 (m, 10H); 5.6 (br, NH); 5.4 (br, NH); 5.07 (s, 2H); 5.05 (s, 2H); 3.3-3.1 (m, 4H); 2.5-2.3 (m, 4H); 2.17 (s, 3H); 1.62 (quint, 2H, $J = 6$ Hz); $^{13}$C NMR (CDCl$_3$) $\delta$ 157.1; 137.3; 137.2; 129.0; 128.6; 128.5; 67.1; 67.0; 57.3; 55.9; 42.2; 40.1; 38.9; 27.4; LRMS (ES$^+$) $m/e$: 400 [(M+H), 100 %]; Anal. (C$_{22}$H$_{29}$N$_3$O$_4$) C, H, N.

$N^1$-(2-Amino-ethyl)-$N^4$-methyl-propane-1,3-diamine (15).} Catalytic hydrogenation of a suspension of 14 (230 mg, 0.57mmol), 10 % Pd-C (23 mg) and 1M HCl (1 mL) in MeOH (30 mL) under a 36 Psi hydrogen pressure for 24 h at room temperature afforded the HCl salt of 15 as a colorless oil (118 mg, quantitative). $^1$H NMR (D$_2$O) $\delta$ 3.0-2.8 (m, 4H); 2.58 (t, 2H, $J = 6.8$ Hz); 2.46 (t, 2H, $J = 7.6$ Hz); 2.16 (s, 3H); 1.74 (quint, 2H, $J = 7.6$ Hz); $^{13}$C NMR (D$_2$O) $\delta$ 54.8 (t); 54.5 (t); 41.0 (q); 38.5 (t); 37.0 (t); 24.5 (t); LRMS (APCI$^+$) $m/e$ 132 [(M+H), 100 %]. Anal. (C$_6$H$_{20}$N$_3$Cl$_3$ / 0.3 H$_2$O) calcd: C, 29.47; H, 8.49; N, 17.19; found: C, 29.35; H, 8.40; N, 17.00.
N,N’-di[2-(4,5-dihydro-1H-imidazo-2-ylamino)-ethyl]-N’-methyl-propane-
1,3-diamine (16). A solution of 15 (110 mg, 0.84 mmol), 2-methylmercapto-4,5-
dihydro-1H-imidazole iodide (410 mg, 1.76 mmol) in EtOH (10 mL) was heated 24 h at 
reflux (CAUTION: the noxious gas CH$_3$SH is evolved during the reaction and it should 
be trapped with a concentrated aqueous NaOH solution). The solvent was removed by 
reduce pressure and the crude compound was purified by formation of its picrate salt: a 
hot solution of picric acid (400 mg in 5 mL H$_2$O) was added to the hot reaction mixture 
and the flask was allowed to stand in the fridge for one week. The crystals were 
collected by filtration and rinsed successively with water, hexane and Et$_2$O. Picrate of 
16: yellow solid (302 mg, 53 %); mp 81-83 °C; $^1$H NMR (400MHz, DMSO-d$_6$) $\delta$ 9.94 
(br s, 1H); 9.25 (br s, 1H); 8.54 (s, 4H); 8.18 (t, 1H, $J = 5.7$ Hz); 8.13 (t, 1H, $J = 4.8$
 Hz); 7.65 (br, 1H); 3.80 (s, 3H); 3.58 (s, 4H); 3.55 (s, 4H); 3.1 (m, 4H); 2.8-2.7 (m, 4H);  
1.8 (br, 2H); $^{13}$C NMR (50 MHz, DMSO-d$_6$) $\delta$ 170.5; 160.9 (s); 159.4 (s); 141.5 (s);  
125.3 (d); 124.8 (s); 53.6 (br, t); 53.0 (t); 45.2 (t); 42.6 (t); 40.2 (q); 37.2 (t); LRMS  
(FAB$^+$) $m/e$ 268 [(M+H)]. Anal. (C$_{24}$H$_{31}$N$_{13}$O$_{14}$) C, H, N.

3-Azahexane-1,7-(N,N’-dicyclohexyl) diguanidine (5c). A solution of 3-(2-
aminoethylamino)propylamine (1 mL, 8.5 mmol) and DCC (3.7 g, 17.9 mmol) in dry 
CH$_3$CN (25 mL) was stirred 4 days at room temperature under argon atmosphere. The 
solvent was removed by reduce pressure and the crude oil was dissolved in Et$_2$O. A 
current of HCl$_8$ was bubbled into the solution for 2 min. The white precipitate was 
collected, rinsed with Et$_2$O and dried in vacuo affording a mixture of the di- and tri-
substituted compounds 5c and 17 which were separated by preparative HPLC using the 
following eluent system: H$_2$O/CH$_3$CN (100:0$\rightarrow$0:100) (TFA 0.1 %). Trifluoroacetate of 
5c: white solid; mp 88-93 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.45 (t, 2H, $J = 6.3$ Hz);  
3.6-3.25 (m, 10H); 3.18 (t, 2H, $J = 7$ Hz); 2.95 (br t, 2H); 1.82 (q, 2H, $J = 7.8$ Hz); 1.69
(br s, 8H); 1.60 (br d, 8H); 1.46 (br d, 4H); 1.16 (t, 16H, J = 10 Hz); 1.0 (br m, 5H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 163.7 (TFA); 154.0 (s); 154.0 (s); 117.6 (TFA); 52.6 (d); 52.5 (d); 47.3 (t); 46.5 (t); 39.3 (t); 38.9 (t); 33.4 (t); 33.4 (t); 26.5 (t); 25.8 (t); 25.7 (t); LRMS (ES$^+$) m/e 265.9 [(M+2H), 100 %]; 644.6 [(M+TFA)]; 758.6 [(M+2TFA)]; HPLC (column II): $R_t = 9.37$ min (100 %).

3-Azahexane-1,3,7-(N,N'-dicyclohexyl)triguanidine (17). Trifluoroacetate of 17: white flocculent solid. $^1$H NMR (500 MHz, CD$_3$OD) δ 3.73-3.60 (m, 8H); 3.56-3.44 (m, 6H); 2.14-1.8 (m, 30H); 1.7-1.3 (m, 32H); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 160.1 (s); 154.2 (s); 56.0 (d); 52.6 (d); 52.6 (d); 49.8 (t); 47.7 (t); 39.9 (t); 39.7 (t); 34.5 (t); 33.9 (t); 33.9 (t); 28.2 (t); 26.2 (t); 26.2 (t); 26.1 (t); 26.1 (t); 26.0 (t); LRMS (ES$^+$) m/e 369 [(M+2H), 100 %]; 246.5 [(M+3H)]; HPLC (column I): $R_t = 30.55$ min (99.58 %).

{3-[(2-Benzoxycarbonylamino-ethyl)-(2-chloro-ethyl)-amino]-propyl}-carbamic acid benzyl ester (19). Chloroacetaldehyde (50 % in water, 1.5 mL, 11.6 mmol) was added to a solution of 5d (1.11 g, 2.9 mmol) in CH$_3$CN (20 mL). After a few minutes, AcOH (0.5 mL, 8.5 mmol) was added, followed 5 min later by NaBH(OAc)$_3$ (1.24g, 5.8 mmol). The reaction mixture was stirred for 4 h at room temperature adjusting the pH to 5-6 with AcOH during the course of the reaction. The reaction was quenched by careful addition of 5 % NaHCO$_3$ and diluted with CH$_2$Cl$_2$. The organic phase was separated and the aqueous phase was extracted 3 times with CH$_2$Cl$_2$. Combined organic extracts were washed with brine, dried (Na$_2$SO$_4$) and concentrated by reduce pressure. Chromatography (40M cartridge) with petroleum ether/acetone (80:20) yielded the acetyl side-product 18 (16 %) and the expected chloro-derivative 19 as an oil that solidified as a yellowish pasty residue (450 mg, 35 %); IR (KBr): ν: 1680; 1415; 1240; 755; 713; 675 cm$^{-1}$; $^1$H NMR (CDCl$_3$) δ 7.29 (br s, 10H); 5.67 (br, NH); 5.60 (br, NH); 5.09 (s, 2H); 5.08 (s, 2H); 3.43 (t, 2H, J = 6.1 Hz); 3.3-3.1
(m, 4H); 2.68 (t, 2H, J = 6.3 Hz); 2.6-2.4 (m, 4H); 1.56 (quint, 2H, J = 6.4 Hz); 13C NMR (CDCl₃) δ 157.0 (2C); 137.1; 128.8; 128.3; 66.8; 56.0; 54.0; 52.4; 42.5; 39.7; 39.3; 27.5; LRMS (ES⁺) m/e 448.5 [(M+HCl), 100 %]; 412 [(M+H)]; Anal. (C₂₃H₃₀N₃O₄Cl / H₂O) calcd: C, 59.29; H, 6.92; N, 9.02; found: C, 59.27; H, 7.26; N, 9.02.

**Acetic acid 2-[(2-benzyloxycarbonylamino-ethyl)-(3-benzyloxycarbonylamino-propyl)-amino]-ethyl ester (18).** (223 mg, 16 %); ¹H NMR (CDCl₃) δ 7.28 (br s, 10H); 5.68 (br, NH); 5.52 (br, NH); 5.04 (s, 2H); 5.02 (s, 2H); 4.03 (t, 2H, J = 5.6 Hz); 3.25-3.05 (m, 4H); 2.60 (t, 2H, J = 5.6 Hz); 2.55-2.35 (m, 4H); 1.90 (s, 3H); 1.55 (quint, 2H); ¹³C NMR (CDCl₃) δ 171.5; 157.0; 156.9; 137.1; 128.9; 128.5; 128.4; 66.95; 66.87; 62.4; 54.0; 52.7; 43.5; 39.9; 39.2; 27.3; 21.2; LRMS (ES⁺) m/e 472 [(M+H), 100%]. Anal. (C₂₅H₃₃N₃O₆) C, H, N.

**{3-[(2-Benzyloxycarbonylamino-ethyl)-(2-hydroxy-ethyl)-amino]-propyl}-carbamic acid benzyl ester (20).** A solution of bromoethanol (0.03 mL, 0.42 mmol) in CH₃CN (1 mL) was added to a solution of 5d (208 mg, 0.54 mmol) in CH₃CN (4 mL). The reaction mixture was refluxed 12 h and the solvent was removed by reduce pressure. The crude product was purified by chromatography with EtOAc/MeOH (80:20) and the resulting compound dissolved in a little CH₂Cl₂ was filtered on a path of Celite affording the pure product 20 (124 mg, 69 %). ¹H NMR (300 MHz, CDCl₃) δ 7.22 (br s, 10H); 5.63 (br, 1H); 5.46 (br, 1H); 4.97 (s, 4H); 3.44 (t, 2H, J = 5 Hz); 3.1 (m, 5H); 2.5-2.3 (m, 6H); 1.49 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 156.76; 156.57; 136.5; 128.3; 127.9; 66.4; 59.1; 55.8; 53.7; 51.3; 38.9; 38.6; 27.0; IR (neat) ν 3500-3300 (br); 2905; 1680; 1515; 1235; 715; 675 cm⁻¹; LRMS (ES⁺) m/z 430 [(M+H)], 542 [(M+Na), 100%]; Anal. (C₂₃H₃₁N₃O₅ / 1 H₂O) calcd: C, 61.73; H, 7.43; N, 9.39; found: C, 61.83; H, 6.98; N, 8.82.
3-(3-Benzoyloxy carbonylamino-propyl)-imidazolidine-1-carboxylic acid benzyl ester (25). To a solution of amine 5d (1.06 g, 2.7 mmol) and formaldehyde (37 % in H₂O, 1.5 mL, 13.5 mmol) in CH₃CN was added NaBH₃CN (226 mg, 3.6 mmol). After 10 min, few drops of AcOH were added to the cloudy solution to adjust the pH to 6-7. The reaction was stirred 17 h at room temperature and the solvent was removed by reduce pressure. The crude residue was treated with water and 1M NaOH was added to adjust the pH to 10-11. The aqueous phase was extracted 3 times with CH₂Cl₂ and the organic extracts were dried (Na₂SO₄) and concentrated by reduce pressure. The crude oil was chromatographed (40M cartridge) with CH₂Cl₂/MeOH (98:2). The six-membered heterocycle 26 was eluted first followed by 25. Compound 25: ¹H NMR (300MHz, CDCl₃) δ 7.25 (m, 10H); 5.3 (br, 1H, NH); 5.04 (s, 2H); 4.99 (s, 2H); 3.94 (s, 1H); 3.89 (s, 1H); 3.37 (td, 2H, J = 8.9 and 9.3 Hz); 3.17 (td, 2H, J = 9.0 and 9.6 Hz); 2.70 (m, 2H); 2.43 (br t, 2H, J = 9 Hz); 1.58 (quint, 2H, J = 9.7 Hz); ¹³C NMR (300MHz, CDCl₃) δ 157.0; 154.4; 137.2; 129.0; 128.6; 68.8; 68.5; 67.4; 67.1; 53.4; 52.6; 52.2; 44.9; 44.7; 43.6; 40.2; 28.8; LRMS (ES⁺) m/z 398 [(M+H), 100 %].

3-(2-Benzoyloxy carbonylamino-ethyl)-tetrahydro-pyrimidine-1-carboxylic acid benzyl ester (26). ¹H NMR (300 MHz, CDCl₃) δ 7.3 (br s, 10H, aro); 5.5 (br, 1H, NH); 5.1 [s, 2H, PhCH₂OC(O)NH]; 5.06 [s, 2H, PhCH₂OC(O)N]; 4.12 (s, 2H, NCH₂N); 3.48 (br t, 2H, J = 8.2 Hz, CbzNCH₂); 3.22 (br m, 2H, CH₂NHCbz); 2.67 (br t, 2H, NCH₂CH₂); 2.49 (m, 2H, NCH₂CH₂NHCbz); 1.53 (m, 2H, CH₂CH₂CH₂); ¹³C NMR (75 MHz, CDCl₃) δ 156.7 [s, C(=O)NH]; 155.28 [s, C(=O)N]; 136.94 (s, aro); 136.86 (s, aro); 128.7 (d, aro); 128.3 (d, aro); 128.1 (d, aro); 67.4 [t, PhCH₂OC(O)NH]; 66.7 [t, PhCH₂OC(O)N]; 65.1 (t, NCH₂N); 51.9 (t, NCH₂CH₂); 44.1 (t, CbzNCH₂); 38.3 (t, CH₂NHCbz); 22.5 (t, CH₂CH₂CH₂); LRMS (ES⁺) m/z 398 [(M+H), 100 %].

4,4'-Bis[¹³N³-(2,2-diethoxyethyl)guanidino]diphenylmethane (31c). A 1M ether
solution of 2,2-diethoxy-ethyl-carbodiimide (11.1 mL, 11.1 mmol) was added to a solution of 4,4’-diaminodiphenylmethane (1.05 g, 5.3 mmol) in dry EtOH under N₂. Methanesulfonic acid (0.69 mL, 10.6 mmol) was added drop wise to the clear reaction mixture and a white precipitate formed immediately. The reaction was refluxed 44 h and then poured into 0.5 M aqueous NaOH solution. The aqueous phase was extracted (3×CH₂Cl₂). Organic extracts were dried (Na₂SO₄) and concentrated by reduce pressure. The guanidine 31c was crystallised with CH₂Cl₂ and washed with Et₂O. Some more compound was obtained by precipitation of the mother liquor with Et₂O. Colorless solid (926 mg, 34 %); mp 188-190 °C; ¹H NMR (CDCl₃/CD₃OD) δ 7.0 (d, 4H, J = 7.5 Hz); 6.73 (d, 4H, J = 7.5 Hz); 4.44 (t, 2H, J = 4.8 Hz); 3.75 (s, 2H); 3.8-3.4 (m, 8H); 3.19 (d, 4H, J = 4.8 Hz); 1.12 (t, 12 H, J = 7 Hz); ¹³C (CDCl₃/CD₃OD) δ 154.5; 146.6; 136.1; 130.2; 124.2; 102.6; 63.8; 45.1; 41.2; 15.6; LRMS (EI) m/z 514 [(M⁺), 70 %], 485 [(M-29), 100 %]; Anal. (C₂₇H₄₂N₆O₄): C, H, N.

4,4’-Bis[³-(2,2-diethoxyethyl)guanidino]diphenylamine (32c). Same procedure as 31c starting from 4,4’-diaminodiphenylamine (421 mg, 2.1 mmol), 2,2-diethoxy-ethyl-carbodiimide (4.6 mL, 4.6 mmol) and methanesulfonic acid (0.27 mL, 4.2 mmol). The crude product dissolved in EtOH was treated with Et₂O. The solid precipitate was filtered off and the mother liquor was concentrated by reduce pressure. The residue was dissolved in MeOH and 32c was isolated by Et₂O mediated precipitation (707 mg, 65 %); Methanesulfonate salt of 32c: purple solid; IR (KBr) ν 1625; 1600; 1480; 1175; 1160; 1025; 1015; 750; 737 cm⁻¹; ¹H NMR (D₂O) δ 7.11 (s, 8H); 4.65 (m, 2H); 3.8-3.4 (m, 8H); 3.33 (m, 4H); 1.11 (t, 12H, J = 7.1 Hz); ¹³C NMR (D₂O) δ 156.7; 143.1; 127.8; 126.9; 118.9; 101.3; 65.0; 44.9; 15.2; LRMS (ES⁺) m/e 516 [(M+H), 100 %]. Anal. (C₂₈H₄₀N₇O₁₀S₂ / 2 H₂O) calcd: C, 45.21; H, 7.18; N, 13.18; S, 8.62; found: C, 44.80; H, 7.02; N, 12.70; S, 9.08.
4,4’-Bis(2-imidazolylamino)diphenylamine (35). In a flask cooled to 0 °C, was dissolved the guanidine 32c (350mg, 0.68 mmol) in 6 M HCl (5 mL). After stirring 3 h at room temperature, 10 % NaOH was added until a precipitate formed (pH > 11). The reaction mixture was stirred 75 min and was poured into a 1M NaOH solution. The aqueous phase was extracted with CH_2Cl_2. The crude product was collected by filtration of the aqueous phase. The crude solid was dissolved in boiling water (10 mL) and the flask was allowed to stand overnight at room temperature. The product was collected by filtration, washed several times with H_2O and dried in vacuo at 50 °C affording the free base of 35 as a purple solid (82 mg). The hydrochloride salt was prepared in the following manner: to 35 dissolved in H_2O was added 3 N HCl until pH 2 was reached. The compound was lyophilised, dissolved in MeOH and purified by Et_2O mediated precipitation. Purple solid (51 mg, 19 %); mp > 200 °C (dec.); IR (KBr) ν 1650; 1590; 1500; 1310; 815; 670 cm⁻¹; ¹H NMR (D_2O) δ 7.15 (br s, 8H); 6.76 (br s, 4H); ¹³C NMR (D_2O) δ 145.9 (s); 142.5 (s); 130.2 (s); 125.4 (d); 119.8 (d); 114.0 (d); LRMS (ES⁺) m/e 332 [(M+H), 100 %]; 166.6 [(M+2H)]; Anal. (C_{18}H_{20}Cl_{3}N_{7}) calcd: C, 49.05; H, 4.57; N, 22.25; found: C, 49.71; H, 4.45; N, 21.66.

Bis-(4-bromomethyl-phenyl)-methanone (36). A solution of 4,4’-dimethylbenzophenone (1 g, 4.8 mmol), NBS (1.71 g, 9.6 mmol) and four drops of t-BuOOH in CCl_4 (15 mL) was heated at reflux for 18 h under argon atmosphere. The insoluble succinimide was filtered off and the solvent was removed by reduce pressure. The pure product was obtained by crystallisation from CH_2Cl_2 as colorless needles (387 mg, 22 %); mp 135-137 °C; IR (KBr) ν 1630, 1585, 1390, 1255, 1155, 905, 665 cm⁻¹; ¹H NMR (CDCl_3) δ 7.78 (d, 4H, J = 8.4 Hz); 7.51 (d, 4H, J = 8.4 Hz); 4.57 (s, 4H); ¹³C NMR (CDCl_3) δ 195.8; 142.9, 137.8, 131.1, 129.6, 32.9; Anal. (C_{15}H_{12}Br_2O / 0.5 H_2O) calcd: C, 47.78; H, 3.47; found: C, 47.87; H, 3.08.
**Tri-n-pentylphosphine.** To a suspension of magnesium (1.96 g) in dry THF (50 mL) under argon, was added a solution of 1-bromopentane (10 mL, 80.7 mmol) in THF (20 mL). The resulting reaction mixture was heated at reflux for 20 min. Then, the reaction was cooled to -78 °C and a solution of phosphorous trichloride (1.74 mL, 20 mmol) in THF (10 mL) was added drop wise. The reaction was stirred 30 min at – 78 °C and the cold bath was removed. The reaction was allowed to warm up to room temperature and was then heated at reflux for 30 min. The reaction was quenched with saturated NH₄Br solution (20 mL). The precipitate was filtered off under argon atmosphere and the crude product was distilled under vacuum, affording the tri-n-pentylphosphine as a colorless oil (1.305 g, 27 %). The product was conserved under argon in the fridge. Bp (3 mm Hg) 115-125 °C; ¹H NMR (CDCl₃) δ 1.61 (m, 6H); 1.36 (m, 18H); 0.88 (m, 9H); ¹³C NMR (D₂O) δ 33.9 (d, J²P-¹³C = 13.7 Hz), 29.1, 27.8, 22.8, 22.0, 14.5; ³¹P NMR (CDCl₃) δ 50.23.

**4,4’-Bis(tri-n-pentylphosphonium)benzophenone bromide (37).** A solution of 36 (344 mg, 0.93 mmol) and tri-n-pentylphosphine (1.02 g, 4.2 mmol) in dry toluene (10 mL) was heated at reflux for 24 h. The precipitate that had formed while cooling the reaction mixture was triturated with a spatula until a solid formed. The solid was collected by filtration, rinsed with dry toluene and dried in vacuo at 70 °C. Colorless hygroscopic solid (748 mg, 94 %); Spectroscopic data are in agreement with the literature.³⁷ LRMS (FAB⁺) m/e 695.5 [(M⁺); 100%]; Anal. (C₄₅H₇₈OP₂Br₂) C, H.

**1,4-Bis-(4-nitro-phenyl)-piperazine (38).** A solution of 1-(4-nitro-phenyl)-piperazine (2.58 g, 12.5 mmol) and 1-fluoro-4-nitrobenzene (599 mg, 4.16 mmol) in DMSO (15 mL) was heated 60 h at 100 °C. The cool reaction was poured into water (50 mL). The precipitate was collected by filtration and rinsed with a small quantity of water. The product was first crystallised with PhMe/EtOH and rinsed with cold toluene
and cold EtOH respectively. The pure compound was obtained as a red solid by crystallisation with CH$_3$CN (1g, 73 %). Mp 265-266 °C [Lit.$^{54}$ 261 °C, PhNO$_2$]; $^1$H NMR (DMSO-d$_6$) δ 8.1 (d, 4H, $J = 9$ Hz); 7.0 (d, 4H, $J = 9$ Hz); 3.71 (s, 8H); $^{13}$C NMR (DMSO-d$_6$) δ 153.9; 136.8; 125.6; 111.9; 45.1. Anal. (C$_{16}$H$_{16}$N$_4$O$_4$ / 0.8 H$_2$O) calcd: C, 56.10; H, 5.18; N, 16.36; found: C, 56.12; H, 5.35; N, 16.24.

1,4-Bis-(4-amino-phenyl)-piperazine (39).$^{42}$ The nitro compound 38 ((705 mg, 2.1 mmol) was dissolved in HCl saturated methanolic solution (70 mL). The solution was hydrogenated (40 Psi H$_2$) in the presence of 10 % Pd-C (165 mg) for 14 h at room temperature. The catalyst was filtered off and the solvent was removed by reduce pressure affording the crude hydrochloride of 39. Recrystallisation with EtOH afforded the pure HCl salt of 39 (300 mg, 41%); mp > 350 °C; $^1$H NMR (D$_2$O) δ 7.24 (s, 8H); 3.49 (s, 8H); LRMS (ES$^+$) m/z 269 [(M+H), 100 %], 135 [(M+2H)]. Anal. (C$_{16}$H$_{24}$Cl$_4$N$_4$ / 1.7 H$_2$O) calcd: C, 43.40; H, 6.24; N, 12.65; found: C, 43.08; 5.49; N, 12.83.

1,4-Bis-[4-(N$_2$,N$_3$-di(tert-butoxycarbonyl)guanidino)-phenyl]-piperazine (40a). To a solution of 39 (54 mg, 0.2 mmol), N,N'-di(tert-butoxycarbonyl)thiourea (122 mg, 0.44 mmol), and Et$_3$N (0.14 mL, 1 mmol) in DMF (2 mL) at 0 °C under N$_2$ was added HgCl$_2$ (119 mg, 0.44 mmol) at once. A precipitate formed immediately. The resulting dark reaction mixture was stirred 30 min at 0 °C and 2.5 days at room temperature. The reaction was diluted with CH$_2$Cl$_2$ and filtered through a path of Celite. The filter cake was rinsed with CH$_2$Cl$_2$. the organic phase was washed with brine, dried (MgSO$_4$) and concentrated. Non-mobile impurities were removed by a short flash chromatography on silica with Hexane/EtOAc (75:25). The pure product was obtained by crystallisation from hexane. Yellowish solid (110 mg, 73 %); mp > 300 °C dec.; $^1$H NMR (CDCl$_3$) δ 11.64 (br, 2H); 10.17 (br, 2H); 7.47 (d, 4H, $J = 8.9$ Hz); 6.92 (d, 4H, $J = 8.9$ Hz); 3.27 (s, 8H); 1.5 (br s, 36H); $^{13}$C NMR (CDCl$_3$) δ 164.2; 154.1; 153.9; 149.2;
130.0; 124.1; 117.4; 84.0; 79.9; 50.3; 28.7; LRMS (ES\(^+\)) m/z 753 [(M+H)]; Anal. (C\(_{38}\)H\(_{56}\)N\(_8\)O\(_8\) / 0.7 C\(_6\)H\(_{14}\)) calcd: C, 62.37; H, 8.16; N, 13.79; found: C, 62.23; H, 8.80; N, 14.11.

**1,4-Bis-(4-guanidino-phenyl)-piperazine (40b).** TFA (2 mL) was added to a stirred solution of 40a (37 mg, 0.049 mmol) in CH\(_2\)Cl\(_2\) (3 mL). After 2 days, the volatiles were removed by reduce pressure and the product was precipitated by addition of Et\(_2\)O. The compound was dried *in vacuo* affording 40b as a greenish hygroscopic solid (25 mg, 88%). Trifluoroacetic salt of 40b. \(^1\)H NMR (D\(_2\)O) \(\delta\) 7.19 (m, 8H); 3.36 (s, 8H); \(^{13}\)C NMR (D\(_2\)O) \(\delta\) 156.2; 148.4; 128.2; 127.1; 118.7; 49.4; LRMS (ES\(^+\)) m/z 353 [(M+H)], 177 [(M+2H), 100%]. Anal. (C\(_{24}\)H\(_{27}\)F\(_9\)N\(_8\)O\(_6\)) calcd: C, 41.51; H, 3.92; N, 16.13; found: C, 41.52; H, 4.33; N, 17.04.

**Di(tert-butyl) 2-(4-[4-(4-[1,3-di(tert-butyl)oxycarbonyl]tetrahydro-1\(H\)-2-imidazolyliden]aminophenyl)piperazino]phenylimino)-1,3-imidazolidinedicarboxylate (41a).** Same procedure as 40a starting from the HCl salt of 39 (111 mg, 0.27 mmol), Et\(_3\)N (0.37 mL, 2.7 mmol), HgCl\(_2\) (160 mg, 0.59 mmol) and using \(N,N'\)-di(tert-butoxycarbonyl)imidazoline-2-thione (178 mg, 0.59 mmol) as reagent for the introduction of the imidazoline nucleus. Flash chromatography with Hexane/EtOAc (50:50) afforded the product as a colorless solid (143 mg, 66%). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 6.9 (m, 8H); 3.79 (s, 8H); 3.20 (s, 8H); 1.31 (s, 36H); \(^{13}\)C NMR (50MHz, CDCl\(_3\)) \(\delta\) 150.3 (s); 147.2 (s); 141.5 (s); 138.5 (s); 122.1 (d); 117.3 (d); 82.4 (s); 50.4 (t); 42.9 (t); 27.7 (q); Anal. (C\(_{42}\)H\(_{60}\)N\(_8\)O\(_8\)) C, H, N.

**1,4-bis[4-(4,5-dihydro-1\(H\)-2-imidazolylamino)phenyl]piperazine (41b).** TFA (2 mL) was added to a stirred solution of 41a (65 mg, 0.08 mmol) in CH\(_2\)Cl\(_2\) (3 mL). After 12 h, the volatiles were removed by reduce pressure and the product dissolved in water was extracted with CH\(_2\)Cl\(_2\) to remove organic soluble impurities. The water was
evaporated and the product was dried in vacuo to afford 41b as a greenish hygroscopic solid. $^1$H NMR (D$_2$O) δ 7.3-7.0 (br m, 8H); 3.63 (s, 8H); 3.59 (s, 8H); $^{13}$C NMR (CD$_3$OD, 75 MHz) δ 161.1; 152.5; 128.7; 127.4; 118.6; 50.6; 44.5; LRMS (ES$^+$) m/z 405 [(M+H)]; 203.2 [(M+2H), 100%]; Anal. (C$_{26}$H$_{30}$F$_6$N$_8$O$_4$) C, H, N.

**Biological tests**

*In Vitro antitrypanosomal activity against Trypanosoma brucei rhodesiense.*

Minimum Essential Medium (50 µl) supplemented with 25 mM HEPES, 1g/l additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 2mM Na-pyruvate, 0.1mM hypoxanthine and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. 3-fold serial drug dilutions were prepared in duplicate in the columns covering a range from 90 µg/ml to 0.123 µg/ml. Then 10$^4$ bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 µl was added to each well and the plate incubated at 37 °C under a 5 % CO$_2$ atmosphere for 72 h. Alamar Blue (10 µl) was then added to each well and incubation continued for a further 2-4 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. Data are analysed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA).

*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 (200mM) and 10% fetal bovine serum, and 4 x 10$^4$ L-6 cells (rat skeletal myoblasts) with or without a serial drug dilution columns covering a range from 90 µg/ml to 0.123 µg/ml. Each compound was tested in duplicate. After 72 hours of
incubation the plates were inspected under an inverted microscope to assure growth of
the controls and sterile conditions. 10µl of Alamar Blue was then added to each well
and the plates incubated for another 2 hours. Then the plates were read with a
Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation,
Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave
length of 588 nm. Data were analysed using the microplate reader software Softmax Pro
(Molecular Devices Cooperation, Sunnyvale, CA, USA).

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Research Training grant, category 20), the Spanish MECD (SB2001-0174) and the
UNDP/World Bank/WHO Special Programme for Research and Training in Tropical
Diseases (TDR). The excellent technical assistance of Elke Gobright at the Swiss
Tropical Institute is highly acknowledged.
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(32) Ueda, T. Bis(guanidinopropyl)amine derivatives: Japan, 1963; pp 177.


(45) Note: the derivatised nitrogen atoms are presumably not protonated in the conditions of the assay.


**Table 1.** Structure, in vitro trypanocidal activity and cytotoxicity of guanidine and 2-aminoimidazoline alkane derivatives 1a-3d.

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<th>Compound</th>
<th>n</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th><strong>T. brucei rhodesiense</strong> IC$_{50}$ (µM)$^a$</th>
<th><strong>Cytotoxicity</strong> L6-cells IC$_{50}$ (µM)</th>
<th><strong>Selectivity</strong>$^b$</th>
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$^a$ Controls: melarsoprol IC$_{50}$ = 5.5 nM (SI= 3456); diminazene diacetate IC$_{50}$ = 8.9 nM; CGP 40215 IC$_{50}$ = 4.5 nM [ref 43].

$^b$ Selectivity index (SI) expressed as the ratio [IC$_{50}$ L6-cells / IC$_{50}$ T.b. rhodesiense]
Table 2. Structure, in vitro trypanocidal activity and cytotoxicity of guanidine and 2-aminoimidazoline aza-alkane derivatives 4a-7.

<table>
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<th>Compd</th>
<th>R</th>
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<th>T. brucei rhodesiense IC₅₀ (µM)ᵃ</th>
<th>Cytotoxicity L6-cells IC₅₀ (µM)</th>
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</thead>
<tbody>
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ᵃ Controls: melarsoprol IC₅₀ = 5.5 nM (SI= 3456); diminazene diaceturate IC₅₀= 8.9 nM; CGP 40215 IC₅₀ = 4.5 nM [ref 43]. ᵇ Selectivity index (SI) expressed as the ratio [IC₅₀ L6-cells / IC₅₀ T.b. rhodesiense].
Table 3. Structure, in vitro trypanocidal activity and cytotoxicity of 3-aza-1,6-hexanediamine derivatives 8-20.

![Chemical Structures](image)

| Compd | R     | R'    | T. brucei rhodesiense IC$_{50}$ (µM)$^a$ | Cytotoxicity L6-cells IC$_{50}$ (µM) | Selectivity$^b$ |
|-------|-------|-------|---------------------------------------|-------------------------------------|----------------|--|
| 8     | Cbz   |       | 3.88                                  | 24.7                                | 6.4            |
| 9     | H     |       | 17.4                                  | 214                                 | 12             |
| 10    | Gua   |       | 4.5                                   | >199                                 | > 43           |
| 11    | Imi   |       | 27.6                                  | -                                    | -              |
| 12    | H     |       | 13.8                                  | -                                    | -              |
| 13    | Cbz   |       | 1.0                                   | 7.8                                  | 7.8            |
| 14    | Cbz   |       | 3.1                                   | 130                                  | 41             |
| 15    | H     | CH$_3$| 61.8                                  | -                                    | -              |
| 16    | Imi   |       | 46.6                                  | -                                    | -              |
| 17    | Gua-C$_3$H$_{11}$ | Gua-C$_3$H$_{11}$ | 0.98                                  | >83                                  | > 82           |
| 18    | Cbz   | OAc   | 7.1                                   | >191                                 | > 26           |
| 20    | Cbz   | OH    | 14.0                                  | 116                                  | 8.3            |

$^a$ Controls: melarsoprol IC$_{50}$ = 5.5 nM (SI= 3456); diminazene diacetate IC$_{50}$= 8.9 nM; CGP 40215 IC$_{50}$ = 4.5 nM [ref 43]. $^b$ Selectivity index (SI) expressed as the ratio [IC$_{50}$ L6-cells / IC$_{50}$ T.b. rhodesiense]
Table 4. Structure, in vitro trypanocidal activity and cytotoxicity of 3-aza-1,6-hexanedi­amine cyclic derivatives 21-26.

<table>
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<tr>
<th>Compd</th>
<th>Structure</th>
<th>R</th>
<th>T. brucei rhodesiense IC_{50} (µM)</th>
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^a Controls: melarsoprol IC_{50} = 5.5 nM (SI= 3456); diminazene diaceturate IC_{50} = 8.9 nM; CGP 40215 IC_{50} = 4.5 nM [ref 43].

^b Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells / IC_{50} T. b. rhodesiense]
Table 5. Structure, in vitro trypanocidal activity and cytotoxicity of diphenyl derivatives 27a-41b.

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^{a} Controls: melarsoprol IC_{50} = 5.5 nM (SI= 3456); diminazene diaceturate IC_{50}= 8.9 nM; CGP40215 IC_{50} = 4.5 nM [ref 43]. ^{b} Selectivity index (SI) expressed as the ratio [IC_{50} L6-cells / IC_{50} T. b. rhodesiense]