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Design, synthesis and evaluation of 2,4-diaminoquinazolines as inhibitors of trypanosomal and leishmanial dihydrofolate reductase

Soghra Khabnadideh,[a,b,†] Didier Pez,[a,†] Alexander Musso,[c] Reto Brun,[d] Luis M. Ruiz Pérez,[c] Dolores González-Pacanowska[c] and Ian H. Gilbert[a,*]

[a]*Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF, UK*
[b]*Department of Pharmacy, University of Shiraz, Shiraz, Iran*
[c]*Instituto de Parasitología y Biomedicina 'López-Neyra', CSIC, Avda. del Conocimiento s/n, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain*
[d]*Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland*

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Abstract—This paper describes the design, synthesis and evaluation of a series of 2,4-diaminoquinazolines as inhibitors of leishmanial and trypanosomal dihydrofolate reductase. Compounds were designed by a generating virtual library of compounds and docking them into the enzyme active site. Following their synthesis, they were found to be potent and selective inhibitors of leishmanial dihydrofolate reductase. The compounds were also found to have potent activity against *Trypanosoma brucei rhodesiense*, a causative organism of African trypanosomiasis and also against *Trypanosoma cruzi*, the causative organism of Chagas disease. There was significantly lower activity against *Leishmania donovani*, one of the causative organisms of leishmaniasis.

1. Introduction

Parasitic protozoa cause a number of diseases, including leishmaniasis (caused by species of *Leishmania*), Chagas disease (caused by *Trypanosoma cruzi*) and African trypanosomiasis (caused by *Trypanosoma brucei rhodesiense* and *T. b. gambiense*). These diseases are found predominantly in the developing world, where they cause significant rates of morbidity and mortality. The current drugs available to treat these diseases suffer from a number of disadvantages, including toxic side effects, poor clinical efficacy, parenteral administration and increasing problems with resistance. Therefore there is an urgent need for new drugs acting at new therapeutic targets to tackle these diseases.

We have been interested in dihydrofolate reductase as a drug target in these organisms.[1–8] This enzyme, which reduces dihydrofolate to tetrahydrofolate (Fig. 1) has successfully been used as a drug target in other diseases; however relatively little has been done to develop this as a drug target in leishmania and trypanosomes. Most clinically used DHFR inhibitors (for example, pyrimethamine, cycloguanil, methotrexate and trimethoprim) are not selective or only show poor selectivity for the trypanosomal and leishmanial enzymes. Relatively few selective inhibitors for leishmanial or trypanosomal DHFR have been reported in the literature.[1] However, Sirawaraporn et al.[9] described some substituted 5-benzyl-2,4-diaminopyrimidines as selective inhibitors of the leishmanial enzyme. In particular the 3′-octyloxy derivative (**1**, Fig. 1) showed promise.

We have previously reported a detailed structure–activity relationship study of 2,4-diaminopyrimidines,[2,7,8] varying substituents at the 6-, 3′- and 4′-positions (Structure **2**, Fig. 1). These studies have led to some potent inhibitors of the enzymes. In particular alkoxy substituents at the 3′- or 4′-position gave selective inhibition of the parasite enzyme, with maximum potency and selectivity when the chain length was 2–6 carbon atoms. Compounds also showed activity against *T. brucei* and *T. cruzi*, parasites with maximum activity with longer chain lengths (6–10 carbons atoms).

* Corresponding author. Fax: +44 29 2087 4149; e-mail: gilbertih@cardiff.ac.uk
† These authors contributed equally to the work published.

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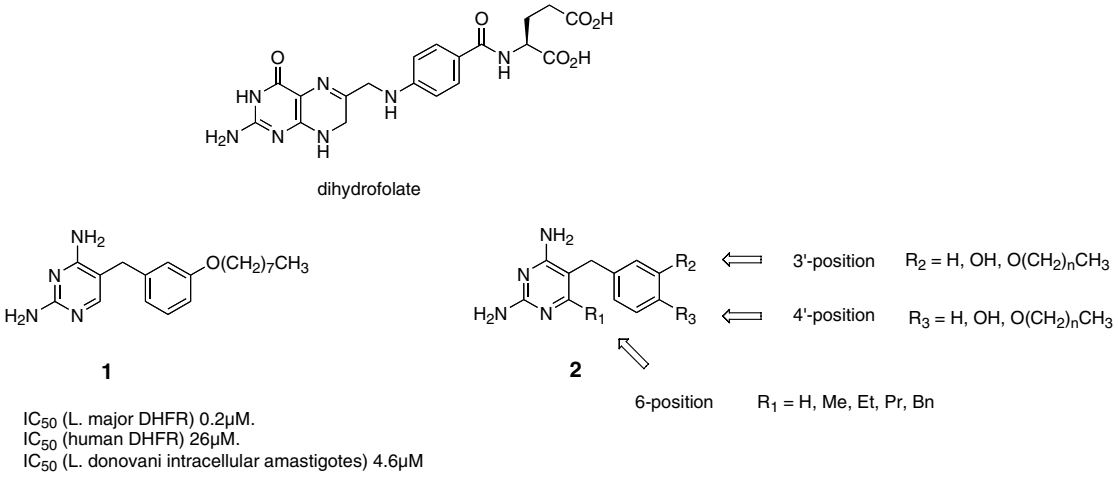


Figure 1.

We have carried out some modelling studies to try and explain the activity and selectivity of our compounds. We compared the active sites of *L. major*, *T. cruzi* and *T. brucei* DHFR and compared them with the human enzyme.[2,4,8] This data suggests that the active sites of the protozoal enzymes are relatively similar to one another and have significant differences compared to the human enzyme. Further the protozoal enzymes have more lipophilic residues than the human enzyme in the active site. In particular the parasitic enzymes have a phenylalanine residue, which interacts with the glutamate part of the substrate, whilst the human enzyme has an asparagine residue. Our modelling studies indicate that compounds with a lipophilic substituent (compound **2**, R_2 or R_3 = O-Alkyl) interact with this phenylalanine residue, in the case of the *Leishmania* enzyme.

In addition we investigated the active site of the *L. major* and human enzymes with the programme GRID,[10] using a hydrophobic probe. In the leishmanial enzyme active site, the regions where the hydrophobic probe interacts favourably with the enzyme active site has a larger volume and surface, compared to the human active site. This shows that there are more regions of the leishmanial active site, which can form thermodynamically favourable interactions with hydrophobic substituents than in the case of the human enzyme. Thus appropriately sized hydrophobic substituents will undergo favourable interactions with the leishmanial enzyme, but unfavourable interactions with the human enzyme.[8] This is consistent with the experimental results.

Many of the 2,4-diaminopyridimine derivatives that we have prepared are potent and selective inhibitors of the leishmanial and trypanosomal enzymes and some of the compounds show potent growth inhibition of the clinically relevant life-cycle stage of the intact parasite.[2,7,8] However the compounds that we prepared have shown very limited in vivo activity in rodent models of infection. We wanted to see if we could prepare a library of more potent inhibitors and to see if we could eventually derive inhibitors with in vivo activity.

2. Modelling

Literature precedence from the work of Berman's group and others suggested that the 2,4-diaminoquinazoline may be a good starting point for design of potent antitrypanosomal or anti-leishmanial activity.[11] Some 2,4-diaminoquinazolines have been shown to have in vivo activity in a rodent model of Chagas disease.[12–14] We wanted to combine the 2,4-diaminoquinazoline or related functionality with a lipophilic side chain, as we have shown compounds with a lipophilic side chain to give rise to selectivity for the leishmanial and trypanosomal enzymes. We decided to use modelling to optimise the design of these new compounds. From our previous modelling studies (Fig. 2),[2,4,15] the key interactions in the active site are: (i) hydrogen bonds between the diaminopyrimidine moiety and an acidic residue in the active site (Glu30, Asp52 or Asp48 in the human, *L. major* and *T. cruzi* enzymes, respectively); (ii) the lipophilic side chain which interacts with a phenylalanine residue in the case of *L. major*, *T. cruzi* or *T. brucei*. In the human enzyme this phenylalanine is replaced by an asparagine, which does not undergo a favourable interaction with the lipophilic side chain.

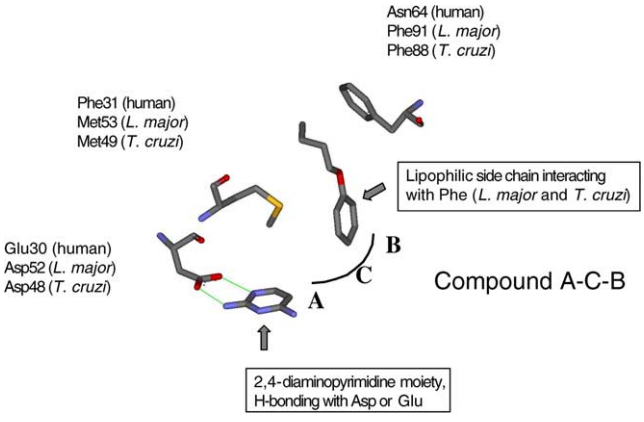


Figure 2. Important components in the compound structure.

To facilitate the modelling, we divided the potential inhibitors into three components:

- Component A is the 2,4-diaminoquinazoline or 2,4-diaminoquinazoline derivative, which makes hydrogen bonds with an acidic residue in the active site (Glu30, Asp52 or Asp48 in the human, *L. major* and *T. cruzi* enzymes, respectively).
- Component B is a lipophilic substituent, which gives selectivity for the leishmanial and trypanosomal enzyme over the human enzyme.
- Component C is the linker between components A and B (Fig. 2).

We then chose a number of different possibilities for components A, B and C and combined these. The fragments chosen are shown in Table 1.

Component A was a heterocycle based on the 2,4-diaminopyrimidine moiety fused to a five- or six-member ring with or without nitrogen atoms. These structures would be analogous to other known DHFR inhibitors. In total, nine different structures were considered including 2,4-diaminoquinazoline, pteridine, pyridopyrimidine and pyrrolopyrimidine structures.

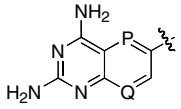
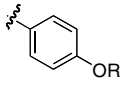
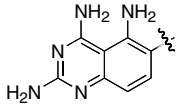
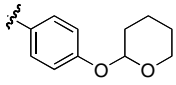
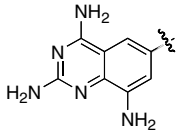
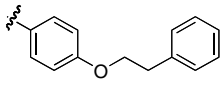
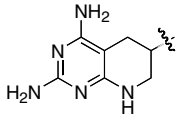
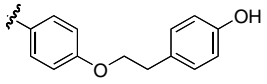
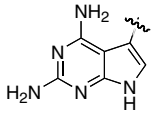
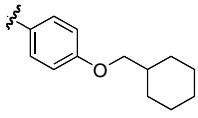
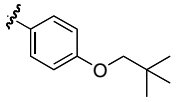
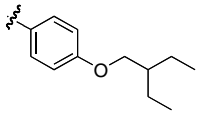
Component C is a spacer. It plays an important role because it places the lipophilic side chain in optimal position relative to Met53 and Phe91 of the *L. major* active site or relative to Met49 and Phe88 of the *T. cruzi* active site. Different potential spacers were used, including compounds with either one or two carbon atoms. In some cases oxygen or nitrogen were included to provide potential hydrogen bond donors and acceptors. In total, eight different linkers were designed including four linkers with one atom and fours linker with two atoms.

Component B is the lipophilic side chain. Different lipophilic functions were tested such as a linear or substituted alkyl chain or aromatic rings with or without hydroxyl group to see a possible difference in the conformation after the docking. In total, thirteen different side chains were designed.

It is also important to select compounds which have 'drug-like' properties.[16] One criteria that can be used are Lipinski's rules for oral bioavailability.[17]

By combination of the different components, A, B and C, a library of 936 molecules was generated. All these compounds have a molecular weight ranging from 285

Table 1. Fragments used for generating compounds for docking

Entry	Component A	Component C	Component B
1			
	P, Q = CH or N	X = CH_2, O, NH, NMe	R = H, Et, Bu, Hex, Octyl, Benzyl
2			
		X = CH_2, O, NH, NMe	
3			
4			
5			
6			
7			

to 409, between 2 and 5 hydrogen bond donors, and between 5 and 9 hydrogen bond acceptors, as indicated by Lipinski's rules. The Clog *P* range of the molecules was between −0.72 and 7.12. However, this parameter was not considered for the filtering protocol.

The compounds were drawn under the Sybyl programme. Their formal charges computed using the Gastinger method and their conformation energy minimised with the Powell method. The compounds were then docked into the human, *L. major* and *T. cruzi* DHFR active sites using FlexX programme. The compounds were docked into the human enzyme, in order to estimate the possibility of selectivity.

There was a lack of correlation between the FlexX scoring function and what was observed visually; therefore visual inspection was used to assess binding of ligands. Firstly, the heterocyclic moiety was evaluated for its ability to bind Glu30, Asp52 or Asp48 of, respectively, the human, *L. major* and *T. cruzi* enzymes. Secondly, the spacer units were assessed for their ability to position the aromatic ring of the side chain in a conformation to give a lipophilic contact with Met53 or Met49 of, respectively, the *L. major* and *T. cruzi* enzymes. Finally, the side chain was evaluated for its capacity to offer the maximal lipophilic contact with Phe91 or Phe88 of, respectively, the *L. major* and *T. cruzi* enzymes.

In general, all the heterocyclic rings (subunits A) gave a satisfactory binding with Glu30, Asp52 or Asp48. However, the heterocyclic rings (entries 2–5) were eliminated because they did not offer any binding improvement. These fragments contained extra hydrogen bond donors, but no additional hydrogen bonds could be detected with the protein active site.

The two-unit linkers (component C, entry 2) showed that they could place the aromatic ring of the side chain in a better position than the one-unit linkers (component C, entry 1). The heteroatoms did not contribute to increase the number of hydrogen bonds whether the compound had a one- or two-unit linker. One of the best and simplest spacer would therefore be the ethylene linker.

All the lipophilic side chains gave a satisfactory conformation. The lipophilic side chains were found in areas of the active site, where GRID predicted energetically

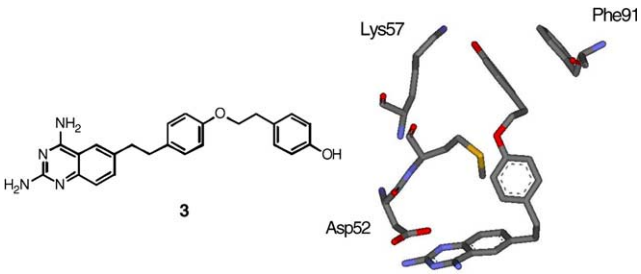


Figure 3. The predicted binding of one of the designed inhibitors (compound **3**) in the active site of the *L. major* enzyme.

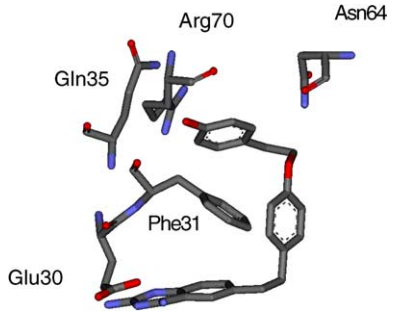


Figure 4. The predicted binding of one of the designed inhibitors (compound **3**) in the active site of the human enzyme.

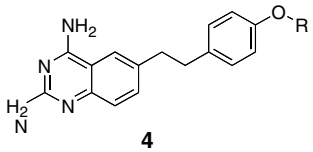


Figure 5. General structure of designed compounds.

favourable interactions of a hydrophobic probe with the active site.

The predicted binding of one of the compounds, compounds **3**, is shown in the active site of the *L. major* enzyme in Figure 3. The 2,4-diaminoquinazoline formed hydrogen bonds with Asp52, one of the aromatic rings had a lipophilic interaction with Met53 and the terminal aromatic ring had a good lipophilic interaction with Phe91. Moreover, the terminal (phenolic) hydroxyl group showed a strong interaction with the side chain amino of Lys57 (2.93 Å), which could either be a hydrogen bond, or an electrostatic interaction.

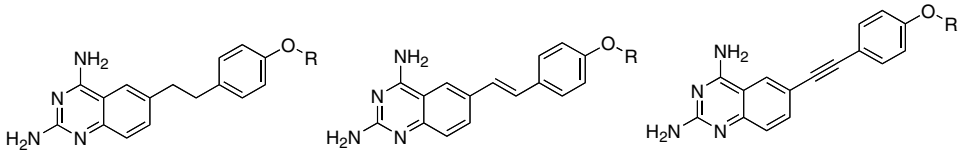
The result of the docking of compound **3** into the human DHFR active site was also analysed. In this case, it was observed that the 2,4-diaminoquinazoline ring and the middle aromatic ring exhibited the same kind of interactions with Glu30 and Phe31 as those in the *L. major* DHFR active site with Asp52 and Met53. However, the aromatic ring at the end of chain C was more bent due to a hydrogen bond between the terminal hydroxyl group of the molecule and Arg70 (2.89 Å, Fig. 4).

The docking of compound **3** into the *T. cruzi* DHFR active site did not give a satisfactory conformation. This failure could not be explained.

The analysis of the docked conformations of all the compounds of the new library showed that compounds of general structure **4** (Fig. 5), where R is a lipophilic group, could be potentially new DHFR inhibitors. Their potential selectivity for the parasite enzymes over the human enzyme has also been illustrated.

3. Chemistry

The compounds prepared are shown in Figure 6. The synthetic route to these compounds are shown in



5, R = H
6, R = Et
7, R = Bu
8, R = Hex
9, R = Octyl
10, R = Decyl
11, R = CH_2Ph

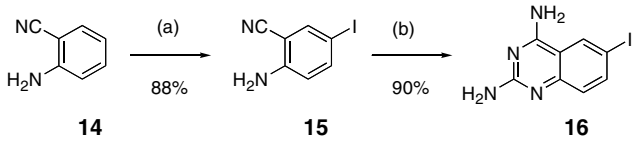
12, R = CH_2Ph

13, R = CH_2Ph

Figure 6. Compounds prepared for evaluation.

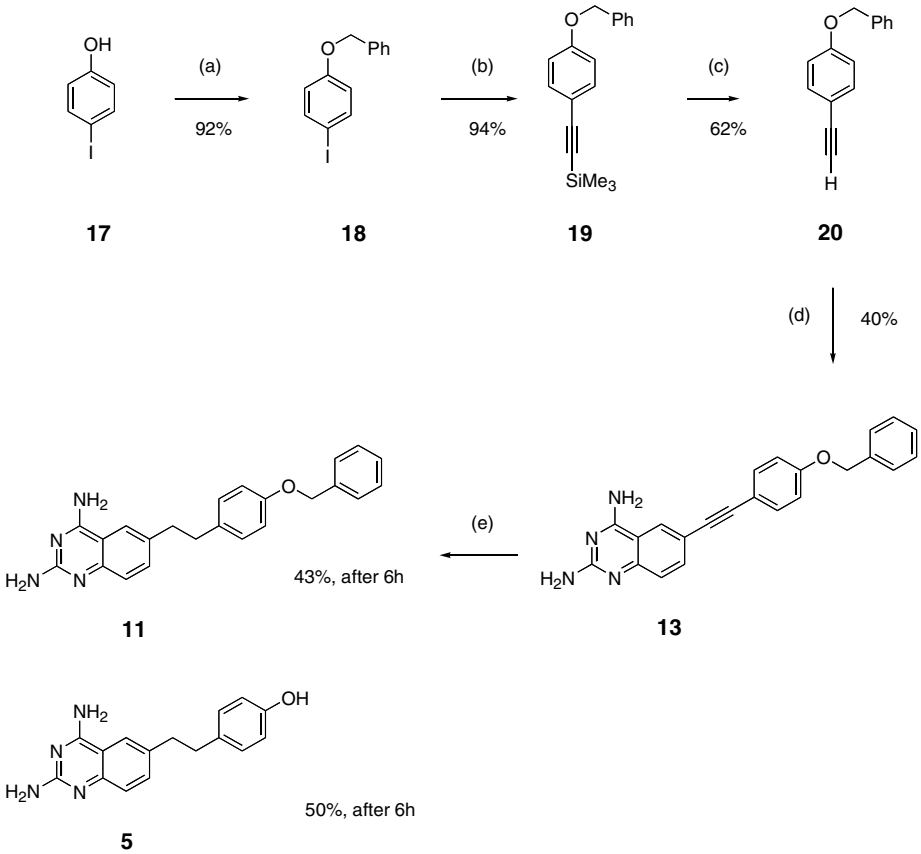
Schemes 1–3. Our strategy is a modification of methodology developed by Harris et al.[18,19] Essentially, the molecules were prepared by preparation of a 2,4-diaminoquinazoline (Scheme 1) and then coupling it to a phenoxy derivative by either a Sonogoshira (Scheme 2) or Heck coupling (Scheme 3).

The 2,4-diaminoquinazoline moiety was prepared in two steps.[18] Firstly 2-aminobenzonitrile (**14**) was iodinated with iodine monochloride to give the iodide **15**. The next step was quinazoline formation by cyclisation with guanidine. Initially this step proved to be problematic in terms of reproducibility and yield. However by carrying out the reaction in the absence of solvent, it was possible to form the product **16** in reliably in high yield.



14 **15** **16**

Scheme 1. Reagents and conditions: (a) ICl, AcOH, rt; (b) guanidine, 150 °C, 2 h.

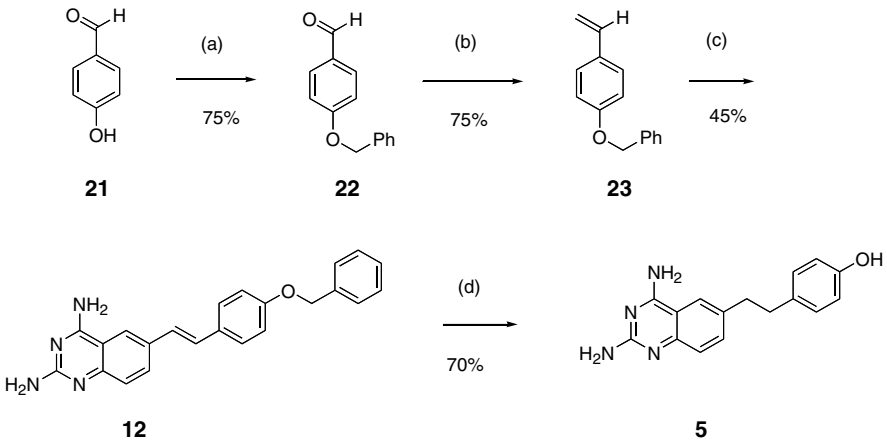


17 **18** **19** **20**

11 **13**

5

Scheme 2. Reagents and conditions: (a) BnBr, K_2CO_3, 2-butanone; (b) $Me_3SiC{\equiv}CH$, $(Ph_3P)_2PdCl_2$, CuI, Et_3N; (c) K_2CO_3, MeOH; (d) **16**, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, DMF, 50 °C; (e) H_2, $Pd(OH)_2$, DMF.



Scheme 3. Reagents and conditions: (a) BnBr, K_2CO_3, 2-butanone, reflux; (b) $Ph_3PMe^+Br^-$, K_2CO_3, dioxane, water, reflux; (c) **16**, $Pd(OAc)_2$, $(MePh)_3P$, piperidine, DMF, 120 °C; (d) H_2, $Pd(OH)_2$, DMF.

The next stage in the synthesis is to add on a phenoxy derivative. This was done using two methods. In the first method, the 2,4-diaminoquinazoline **16** was coupled to a phenoxy derivative using a Sonogoshira coupling.[20] In order to perform a Sonogoshira coupling, an acetylene derivative was required. The starting point was iodophenol (**17**) (Scheme 2). The phenol group was protected as the benzyl ether (**18**), followed by replacement of the iodo-group with a trimethylacetylene functionality (**19**). The trimethylsilyl group was removed using potassium carbonate in methanol (**20**), followed by coupling to the 6-iodo-2,4-diaminoquinazoline using Sonogoshira conditions (**13**). The final step was a simultaneous hydrogenation of the acetylene functionality and hydrogenolysis of the benzyl group. If the reaction was not left to go to completion, it was possible to isolate an intermediate compound, in which the acetylene group had been reduced, but the benzyl group had not been removed. Leaving the reaction for 24 h, led to increasing levels of the required product (**5**) compared to partially reduced product (**11**). Careful choice of conditions was necessary; the solvent used was DMF and the catalyst Pearlman's catalyst.[18] Use of other catalysts or solvents failed to yield the desired product.

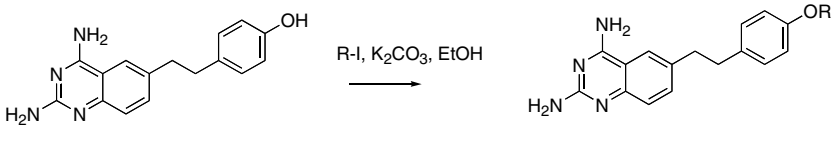
In order to carry out a Heck coupling, the ethylene derivative of the phenoxy compound was required. The starting point was 4-hydroxybenzaldehyde, which was protected as the benzyl ether (**22**) (Scheme 3). A Wittig reaction was then performed to give the required ethylene derivative **23**. A Heck coupling was then carried out with 6-iodo-2,4-diaminoquinazoline (**16**) to give **12**, similarly to the conditions described by Graffner-Nordberg et al.[21] This double bond could then be reduced and the benzyl group removed using hydrogen in the presence of Pearlman's catalyst.

The final step of the synthetic scheme was alkylating the free hydroxyl analogue **5**. This was carried out using the required alkyl iodide with potassium carbonate in ethanol (Scheme 4).

4. Enzyme assays

Compounds were assayed against recombinant *L. major* and human DHFR. The results of these assays are presented in Table 2. There was no clear trend for the alkyl derivatives, all showing potent inhibition against the *L. major* enzyme. The decyl derivative (**10**) showed the weakest inhibition. Comparing compounds **11** and **12**, which both have a benzyl group at the terminal position, **11** is the most potent. This may be due to the alkene group in **12** preventing the compound from adopting an optimal conformation, in particular preventing the terminal benzyl group from forming a π-stacking interaction with Phe91.

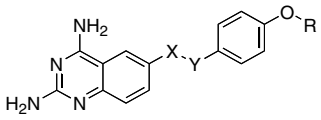
Compounds gave a weaker inhibition of the human enzyme than of the *L. major* enzyme, but followed similar



6, R = Et, 56%
7, R = Bu, 64%
8, R = Hex, 75%
9, R = Oct, 70%
10, R = Decyl, 45%

Scheme 4.

Table 2. Inhibition of recombinant *L. major* and human DHFR



Compound	X–Y	R	*L. major*		Human		Selectivity
			K_i (μM)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	
5	CH_2–CH_2	H	0.0014	0.034	0.034	1.65	24.2
6	CH_2–CH_2	Ethyl	0.0078	0.18	0.048	2.34	6.15
7	CH_2–CH_2	Butyl	0.0019	0.044	0.042	2.04	22.1
8	CH_2–CH_2	Hexyl	0.0076	0.177	0.072	3.47	9.47
9	CH_2–CH_2	Octyl	0.0053	0.123	0.269	12.89	50.7
10	CH_2–CH_2	Decyl	0.17	3.97	1.288	61.7	7.57
11	CH_2–CH_2	CH_2Ph	0.0052	0.12	0.038	1.85	7.3
12	CH=CH	CH_2Ph	0.212	4.89	0.908	43.5	4.28

trends. Thus the alkyl derivatives (**5**–**10**) gave similar levels of inhibition, although the octyl (**9**) and decyl (**10**) derivatives were slightly less active. For those compounds that have a benzyl group at the terminal position, **12** with an alkene was less effective than the reduced analogue **11**.

The compounds were all moderately selective for the parasite enzyme; the highest selectivity index obtained was 51.

5. Anti-parasite assays

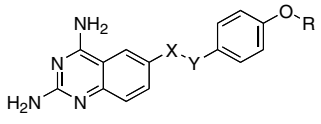
Compounds were evaluated against axenic *L. donovani* amastigotes. *T. cruzi* and *T. brucei* are parasites related to *Leishmania*. An analysis of the homology models of the *T. cruzi* and *T. brucei* DHFRs, revealed a great similarity between the enzyme active sites. Therefore compounds were also screened against intracellular *T. cruzi* amastigotes and *T. b. rhodesiense* trypomastigotes (Table 3). The stages of the life-cycle studied here are the clinically relevant stages of the life-cycle.

Against *L. donovani*, the alkyl derivatives (**5**–**10**) showed inhibition of growth at the micromolar level. All the compounds showed similar growth inhibition, except the hydroxyl analogue (**5**), which showed low activity. The benzyl analogue **11** was poorly active, although interestingly the conformationally constrained analogues of this compound showed more potent inhibition of growth, despite the fact that **12** was much less active against the enzyme compared to **11**. With the exception of these conformationally constrained analogues (**12**, **13**) none of the compounds were selective for the leishmania parasite.

All compounds were potent inhibitors in the nanomolar range of the growth of *T. b. rhodesiense*. No clear trend is observable. Of the alkyl derivatives, the unsubstituted analogue (**5**) shows the most potent inhibition. The benzyl derivative (**11**) also shows potent growth inhibition, along with the alkene derivative, **12**. The alkyne analogue (**13**) shows weaker inhibition. The compounds show a 4–16-fold selectivity for the parasite over the mammalian cells.

The compounds also showed potent inhibition of the growth of *T. cruzi*, with IC_{50} value in the nanomolar range. The activity seems to increase as the alkyl chain is lengthened, with the ethyl (**6**) and butyl (**7**) derivatives

Table 3. The effect of the compounds on the growth of axenic *L. donovani* amastigotes, intracellular *T. cruzi* amastigotes and *T. b. rhodesiense* trypomastigotes



Compound	X–Y	R	*T. b. rhodesiense* IC_{50} (μM)	*T. cruzi* IC_{50} (μM)	*L. donovani* IC_{50} (μM)	L6-cells IC_{50} (μM)
5	CH_2–CH_2	H	0.054 (12)[a]	0.078 (8)	79.9 (0.01)	0.82
6	CH_2–CH_2	Ethyl	0.081 (10)	0.041 (20)	2.0 (0.4)	0.84
7	CH_2–CH_2	Butyl	0.23 (5)	0.042 (26)	1.7 (0.6)	1.1
8	CH_2–CH_2	Hexyl	0.095 (16)	0.11 (14)	2.1 (0.7)	1.5
9	CH_2–CH_2	Octyl	0.35 (4)	0.22 (6)	2.0 (0.6)	1.2
10	CH_2–CH_2	Decyl	0.67 (6)	0.15 (25)	5.5 (0.7)	3.8
11	CH_2–CH_2	CH_2Ph	0.10 (12)	0.059 (20)	13.8 (0.1)	1.2
12	CH=CH	CH_2Ph	0.17 (9)	0.18 (9)	0.53 (3)	1.6
13	C≡C	CH_2Ph	0.42 (12)	0.50 (10)	1.6 (3)	4.9

Standard drugs: *T. b. rhodesiense*, melarsoprol, IC_{50} 0.004 μM; *T. cruzi*, benznidazole, IC_{50} 1.5 μM; *L. donovani*, miltefosine, IC_{50} 0.47 μM; L6-cells, podophyllotoxin, IC_{50} 0.005 μM.

[a] Selectivity data is shown in parentheses, where selectivity is defined: IC_{50} (L-6 cells)/IC_{50} (parasite).

showing the most potent inhibition. As the chain length is increased, the growth inhibition decreased (**8**–**10**). The compounds showed a selectivity of 6–26-fold compared to mammalian cells.

The compounds appear to have similar levels of toxicity against mammalian cells, with IC_{50} values at about 1 μM. For *L. donovani*, the compounds are less active against the parasites than the mammalian cells.

6. Discussion

DHFR is a potential drug target against Leishmania and trypanosomes. However, it is necessary to find inhibitors, which are sufficiently potent and selective for the enzyme from these organisms, and which show activity against the parasites in vitro and in vivo. In previous papers we have reported a number of compounds based on 2,4-diaminopyrimidines, which are potent inhibitors of the enzyme and show activity in vitro, but only show limited activity in vivo. The use of a lipophilic substituent appears to give selectivity for the parasite enzyme over the human enzyme.

2,4-Diaminoquinazoline-based compounds have been shown to have in vivo activity in a rodent model of Chagas disease, extending the life-span of infected animals, although not giving radical cure (Davoll et al., Elslager et al. and Thompson et al.).[12–14] We hypothesised that coupling our lipophilic substituents to the 2,4-diaminoquinazoline or a related moiety may give rise to compounds with good enzyme selectivity and in vivo activity.

In this paper we report the use of a combinatorial docking routine to design a series of potential inhibitors of DHFR based on 2,4-diaminoquinazolines. We report the development of methodology to prepare these compounds. The compounds prepared showed potent activity against *L. major* DHFR. Surprisingly there was relatively weak inhibition of the intact *L. donovani* axenic amastigotes, and little correlation between inhibition of the enzyme and growth of *L. donovani*. We cannot explain the lack of activity against the *L. donovani*. Access of compounds to the parasite should not be a problem, as in this model the parasite is cultured axenically. However the low pH of the medium (pH 5.4) may reduce diffusion of these basic compounds into the parasites.

Structurally the enzymes from *T. brucei* and *T. cruzi* are very similar to those from *L. major*. Therefore if a compound inhibits the *L. major* enzyme it will almost certainly inhibit the *T. brucei* or *T. cruzi* enzymes. Thus compounds were evaluated against the parasites *T. b. rhodesiense* and *T. cruzi* and showed potent growth inhibition. There is some correlation between inhibition of the enzyme and growth inhibition of *T. b. rhodesiense* and growth inhibition of *T. cruzi*. Thus, for example, compound **5** shows potent inhibition of the enzyme and potent growth inhibition of *T. b. rhodesiense* and *T. cruzi*; and compounds **10** and **12** show relatively weak inhibition of the enzyme and also show relatively weak growth inhibition of *T. b. rhodesiense* and *T. cruzi*. This implies that the compounds may be killing the parasites by inhibition of DHFR. In other compounds there was a lower correlation between inhibition of enzyme and growth inhibition. However, it is unlikely that there would be a direct correlation between enzyme inhibition and growth inhibition in all cases, as in cellular assays there are factors other than enzyme inhibition, which may be important, such as penetration of compounds into the cell, metabolism of compounds, etc.

These compounds represent new leads in the search for anti-parasitic agents, and this class of compound merits further work for development as potential anti-trypanosomal compounds.

7. Experimental

All solvents and chemicals were purchased from Aldrich Chemical Co. Reactions were monitored by TLC using pre-coated silica gel 60 F254 plates. Chromatography was performed with silica gel (220–240 mesh) prepacked columns or by flash master chromatography. 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 300 and 75 MHz, respectively.

The numbering system used in Figure 7 is used in interpreting the NMR spectra.

7.1. 2-Amino-5-iodobenzonitrile (15)[19]

A solution of iodine monochloride (1.374 g, 8.46 mmol, 1 equiv) in acetic acid (10 mL) was added to a solution of **14** (1 g, 8.46 mmol, 1 equiv) in acetic acid (10 mL). The mixture was stirred at room temperature for 5 h. Then the reaction mixture was poured into 50 mL cold water. The solid was filtered off, washed with cold water and dried. A recrystallisation with toluene and hexane gave a light brown powder (1.82 g, 88%, mp = 85 °C). R_f = 0.45 (40% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 7.71 (s, 1H, H6), 7.62 (d, 1H, *J* 8.7 Hz, H4), 6.6 (d, 1H, *J* 8.8 Hz, H3), 4.6 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$) δ 149.51 (C2), 142.95 (C6), 140.41 (C4), 117.56 (C3), 116.51 (CN), 98.69 (C1), 98.68 (C5); MS (EI^+) *m/z* 244.0 [M, 90%].

7.2. 6-Iodo-2,4-quinazolinediamine (16)[19]

Sodium ethoxide (4.858 g, 71.4 mmol, 3 equiv) was added to a solution of guanidine hydrochloride

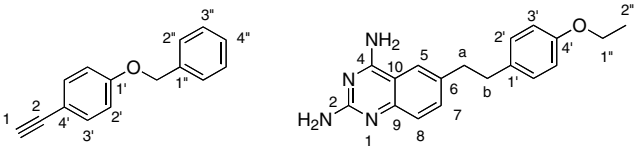


Figure 7. The numbering system used in interpreting NMR spectra.

(6.82 g, 71.4 mmol, 3 equiv) in dry ethanol (30 mL) and stirred for 20 min at room temperature. The suspension was filtered through Celite and the solid washed with ethanol. The filtrate evaporate in reduced pressure and the viscose oil was heated for 2 h at 150 °C with compound **15** (5.8 g, 23.8 mmol, 1 equiv). The resulting solid was cooled to 80 °C, ethanol (30 mL) was added and refluxed for 24 h. The flask was kept in the refrigerator overnight. The precipitate was filtered off, washed with cold ethanol and dried by high vacuum (6.12 g, 90%, mp = 265 °C); R_f = 0.2 (50% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H, H5), 7.72 (d, 1H, *J* 8.8 Hz, H7), 7.36 (s, 2H, NH_2), 6.98 (d, 1H, *J* 8.8 Hz, H8), 6.17 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$) δ 167.79 (4), 161.35 (2), 152.19 (9), 140.82 (7), 132.33 (5), 126.98 (8), 112.95 (10), 82.57 (6); MS (ES^+ and CI^+) *m*/*z* 287 [M+1, 100%].

7.3. 1-Benzyloxy-4-iodobenzene (18)

A solution of iodobenzene **17** (6 g, 27.3 mmol, 1 equiv), anhydrous potassium carbonate (11.320 g, 81.9 mmol, 3 equiv), benzyl bromide (14 g, 9.8 mL, 81.9 mmol, 3 equiv) in 2-butanone (50 mL) was stirred under reflux for 48 h. Then the reaction mixture was extracted with ethyl acetate and washed with water. The organic layer was dried by Na_2SO_4, evaporated in low pressure and dried by high vacuum. A white solid was obtained (7.78 g, 92%); mp = 58 °C (lit. 61[22]); R_f = 0.8 (10% EtOAC/hexane); ^{1}H NMR ($CDCl_3$) δ 7.52 (d, 2H, *J* 8.9 Hz, H3 + H5), 7.4–7.2 (m, 5H, H2′–H6′), 6.7 (d, 2H, *J* 8.9 Hz, H2 + H6), 5.0 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 159.11 (C1), 138.74 (C3 + C5), 137 (C1′), 129.14 (C2′ + C6′), 128.61 (C4′), 127.94 (C3′ + C5′), 117.79 (C2 + C6), 83.59 (C4), 70.54 (CH_2); MS (ES^+) *m*/*z* 311.4 [M+1, 6%].

7.4. 2-[4-(Benzyloxy)phenyl]-1-ethynyl(trimethyl)silane (19)

To a solution of **18** (7 g, 22.6 mmol, 1 equiv) in triethylamine (50 mL), copper iodide (43 mg, 0.226 mmol, 0.01 equiv), bis(triphenylphosphine)palladium chloride (159 mg, 0.226 mmol, 0.01 equiv) and trimethylsilylacetylene (4.44 g, 6.4 mL, 45.2 mmol, 2 equiv) were added and the reaction mixture was stirred at room temperature for 6 h. Then the solution was filtered off and concentrated under reduced pressure. The crude product was purified by flash master chromatography with hexane and ethylacetate. A yellow solid product was obtained (5.95 g, 94%, mp = 50 °C); R_f = 0.5 (10% EtOAC/hexane); ^{1}H NMR ($CDCl_3$) δ 7.5–7.3 (m, 7H, H3′ + H5′, H2″–H6″), 6.85 (d, 2H, *J* 8.9 Hz, H2′ + H6′), 5.10 (s, 2H, CH_2), 0.3 (s, 9H, $(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 158.53 (C1′), 138.16 (C3′ + C5′), 133.4 (C1″), 128.56 (C2″ + C6″), 128.03 (C4″), 127.39 (C3″ + C5″), 117.21 (C2′ + C6′), 114.65 (C4′), 105.06 (C2), 82.98 (C1), 69.97 (CH_2), 0.0 ($(CH_3)_3$); MS (ES^+) *m*/*z* 281.1 [M+1, 9%].

7.5. 1-(Benzyloxy)-4-(1-ethynyl)benzene (20)

A solution of **19** (5.9 g, 21 mmol, 1 equiv) and anhydrous potassium carbonate (10.16 g, 73.5 mmol, 3.5 equiv) in dry methanol (50 mL) was stirred at room temperature for 6 h. The reaction mixture filtered off and the filtrate evaporate under reduced pressure. The crude product extracted by ethyl acetate and washed with water. The organic layer dried by Na_2SO_4 and evaporate in vacuum to get a brown solid (2.72 g, 62%, mp = 65 °C); R_f = 0.4 (10% EtOAC/hexane); ^{1}H NMR ($CDCl_3$) δ 7.6–7.4 (m, 7H, H3′ + H5′, H2″–H6″), 6.95 (d, 2H, *J* 8.9 Hz, H2′ + H6′), 5.10 (s, 2H, CH_2), 3.05 (s, 1H, ≡CH); ^{13}C NMR ($CDCl_3$) δ 159.06 (C1′), 138.69 (C3′ + C5′), 134.07 (C1″), 129.09 (C2″ + C6″), 128.56 (C4″), 127.88 (C3″ + C5″), 117.74 (C2′ + C6′), 115.29 (C4′), 83.50 (C2), 76.34 (C1), 70.46, (CH_2); MS (ES^-) *m*/*z* 209 [M+1, 9%].

7.6. 6-{2-[4-(Benzyloxy)phenyl]-1-ethynyl}-2,4-quinazolinediamine (13)

7.6.1. Method 1—Sonogoshira reaction. A solution of **16** (1.75 g, 6.12 mmol, 1 equiv), **20** (1.78 g, 8.56 mmol, 1.4 equiv), copper iodide (234 mg, 1.22 mmol, 0.2 equiv), bis(triphenylphosphine)palladium chloride (430 mg, 0.61 mmol, 0.1 equiv) and triethylamine (4.99 g, 7.9 mL, 49 mmol, 8 equiv) in DMF (20 mL) stirred at 50 °C for 48 h. Solvent removed in vacuum and the residue stirred in dilute aqueous ammonia (30 mL) and ether (30 mL) for 30 min. The mixture filtrated and purified by flash chromatography. A yellow solid obtained (0.9 g, 40%, mp = 260 °C).

7.6.2. Method 2—Heck coupling. A solution of **16** (0.418 g, 1.46 mmol, 1 equiv), **20** (0.458 g, 2.2 mmol, 1.5 equiv), copper iodide (57 mg, 0.3 mmol, 0.2 equiv), palladium acetate (33 mg, 0.15 mmol, 0.1 equiv), tri-*o*-tolylphosphine (0.09 g, 0.3 mmol, 0.2 equiv) and piperidine (1.02 g, 1.2 mL, 12 mmol, 8 equiv) in DMF (10 mL) stirred at 90 °C for 24 h. Solvent removed in vacuum and purified by flash chromatography. A yellow solid obtained (0.24 g, 45%, mp = 260 °C); R_f = 0.5 (25% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H, H5), 7.50 (d, 1H, *J* 8.23 Hz, H7), 7.46–7.35 (m, 8H, H8, H2 + H6′, H2″–H6″), 7.09 (d, 2H, *J* 8.8 Hz, H3′ + H5′), 5.15 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 162.42 (C10), 161.70 (C4′), 158.76 (C4 + C2), 152.83 (C9), 137.07 (C5), 135.15 (C7), 133.03 (C2′ + C6′), 128.84 (C2″ + C6″), 128.32 (C4″), 128.15 (C3″ + C5″), 127.39 (C1″), 124.99 (C8), 115.62 (C3′ + C5′), 115.21 (C6), 113.65 (C1′), 89.06 (b), 88.42 (a), 69.71 (CH_2); MS (ES^+) *m*/*z* 367.1 [M+1, 100%], HRMS (ES^+) $(M+H)^+$ $C_{23}H_{19}N_4O^+$ requires 367.1553, found 367.1552.

7.7. 4[2(2,4-Diamino-6-quinazolinyl)ethyl]phenol (5), and 6-[4-(benzyloxy)phenethyl]2,4-quinazolindiamine (11)

Compound **13** (300 mg, 0.82 mmol) and palladium hydroxide (300 mg) stirred in dry DMF (20 mL) under H_2 gas for 6 h at room temperature. The reaction mixture filtrate on Celite, concentrated in vacuum and purified by column chromatography. Two distinctive compounds were proved by spectroscopic methods: (**5**, 115 mg, 50%, mp = 275 °C and **11**, 130 mg, 43%, mp = 215 °C). We can increase the ratio of compound

5 up to 65% by increasing the time of reaction until 24 h.

Compound **5**: R_f = 0.1 (40% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.9 (s, 1H, H5), 7.45 (d, 1H, *J* 8.42 Hz, H8), 7.18 (d, 1H, *J* 8.42 Hz, H7), 7.05 (d, 2H, *J* 8.6 Hz, H2′ + H6′), 6.7 (d, 2H, *J* 8.42 Hz, H3′ + H5′), 2.8 (s, 2H, a), 2.5 (s, 2H, b); ^{13}C NMR ($CDCl_3$) δ 162.82 (C9), 159.14 (C2), 155.72 (C4 + C4′), 134.92 (C6), 134.50 (C1′), 131.86 (C5), 129.52 (C2′ + C6′), 123.05 (C7), 122.32 (C8), 115.40 (C3′ + C5′ + C10), 37.53 (a), 36.53 (b); MS (ES^+) *m*/*z* 281.2 [M+1, 100%], HRMS (ES^+) $(M+H)^+$ $C_{16}H_{17}N_4O^+$ requires 281.1397, found 281.1404.

Compound **11**: TLC R_f = 0.2 (40% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.85 (s, 1H, H5), 7.5–7.3 (m, 6H, H7, H2″–H6″), 7.2–7.1 (m, 4H, H2′–H6′), 6.9 (d, 1H, *J* 8.8 Hz, H8), 5.1 (s, 2H, c), 2.9 (s, 2H, a), 2.5 (s, 2H, b); ^{13}C NMR ($CDCl_3$) δ 162.65 (C9), 160.55 (C4), 156.87 (C2 + C4′), 137.59 (C5), 134.08 (C1″), 133.87 (C6), 133.66 (C1′), 129.65 (C2′ + C6′), 128.76 (C2″ + C6″), 128.11 (C4″), 127.99 (C3″ + C5″), 124.22 (C7), 122.67 (C8), 114.94 (C3′ + C5′ + C10), 69.48 (c) 37.47(a), 36.46 (b); MS (ES^+) *m*/*z* 371.2 [M+1, 100%], HRMS (ES^+) $(M+H)^+$ $C_{23}H_{23}N_4O^+$ requires 371.1866, found 371.1863.

7.8. 4-(Benzyloxy)benzaldehyde (22)

A solution of 4-hydroxybenzaldehyde **21** (5 g, 41 mmol, 1 equiv), anhydrous potassium carbonate (17 g, 123 mmol, 3 equiv), benzyl bromide (21.04 g, 14.6 mL, 123 mmol, 3 equiv) in 2-butanone (50 mL) were stirred under reflux for 24 h. Then the reaction mixture was extracted with ethyl acetate and washed with water. The organic layer dried by Na_2SO_4, evaporated in low pressure and purified by flash chromatography. A yellow solid was obtained (6.52 g, 75%, mp = 60 °C); R_f = 0.4 (10% EtOAC/hexane); ^{1}H NMR ($CDCl_3$) δ 9.95 (s, 1H, CHO), 7.9 (d, 2H, *J* 8.6 Hz, H2 + H6), 7.49–7.47 (m, 5H, H2′–H6′), 7.15 (d, 2H, *J* 8.8 Hz, H3 + H5), 5.21 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 191.21 (CHO), 164.17 (C4), 136.38 (C1′), 132.44 (C2 + C6), 130.58 (C1), 129.18 (C2′ + C6′), 128.78 (C4′), 127.93 (C3′ + C5′), 115.59 (C3 + C5), 70.71 (CH_2); MS (CI^+) *m*/*z* 213.1 [M+1, 100%].

7.9. 1-Benzyloxy-4-vinylbenzene (23)

A solution of **22** (3.5 g, 16.5 mmol, 1 equiv) in dioxane (20 mL) was added to a solution of methyltriphenylphosphonium bromide (8.85 g, 24.8 mmol, 1.5 equiv), anhydrous potassium carbonate (3.43 g, 24.8 mmol, 1.5 equiv) in dioxane (30 mL) and water (2 mL). The reaction mixture was stirred under reflux for 48 h. The crude product was extracted with DCM and washed with water. The organic layer dried by Na_2SO_4, concentrated in vacuum and purified by flash chromatography to get a white solid (2.6 g, 75%, mp = 65 °C); R_f = 0.8 (10% EtOAC/hexane); ^{1}H NMR ($CDCl_3$) δ 7.46–7.35 (m, 7H, H3 + H5, H2′–H6′), 6.96 (d, 2H, *J* 8.8 Hz, H2 + H6), 6.69 (q, 1H, *J* 10.98 Hz, *J* 6.59 Hz, =CH), 5.66 (d, 1H, *J* 17.56 Hz, =C*H*H), 5.16 (d, 1H, *J* 10.98 Hz, =CH*H*), 5.08 (s, 2H, –CH_2–); ^{13}C NMR ($CDCl_3$) δ 159 (C1), 137.37 (C1′), 136.63 (=CH), 131.12 (C4), 129.04 (C2′ + C6′), 128.42 (C4′), 127.90 (C3′ + C5′), 127.84 (C3 + C5), 115.31 (C2 + C6), 112.16 (=CH_2), 70.46 (–CH_2–); MS (CI^+) *m*/*z* 211.1 [M+1, 100%], (EI^+) *m*/*z* 210.1 [M, 9%].

7.10. 6-{2-[4-(Benzyloxy)phenyl]-1-ethenyl}-2,4-quinazolinediamine (12)

Compound **16** (1.96 g, 6.85 mmol, 1 equiv), compound **23** (2.16 g, 10.26 mmol, 1.5 equiv) palladium acetate (34 mg, 0.14 mmol, 0.02 equiv), tri-*o*-tolyl phosphine (84 mg, 0.27 mmol, 0.04 equiv), piperidine (5.5 mL) in dry DMF (9 mL) were stirred at 120 °C for 60 h. Solvent was removed in vacuo and the dark residue stirred with 10% KOH for 1 h. The precipitate filtrated, washed with water and purified by column chromatography. A yellow solid was obtained (1.13 g, 45%, mp = 275 °C); R_f = 0.1 (20% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.15 (s, 1H, H5), 7.75 (d, 1H, *J* 8.8 Hz, H8), 7.50–7.30 (m, 8H, H7 + Ha + Hb + H2′ + H6′, H3″–H5″), 7.2 (d, 2H, *J* 8.23 Hz, 2″ + 6″), 7 (d, 2H, *J* 8.9, H3′ + H5′), 5.15 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 167.82 (C4), 162.83 (C4′), 161.19 (C9), 158.16 (C2), 137.40 (C1″), 131.11 (C5), 130.58 (C7), 130.22 (a), 129.26 (C2′ + C6′), 128.8 (b), 128.56 (C2″ + C6″), 128.13 (C3″ + C5″), 127.71 (C8), 126.44 (C1′), 121.44 (C4″), 115.52 (C3′ + C5′), 114.93 (C6), 110.68 (C10), 69.60 (CH_2); MS (ES^+) *m*/*z* 369.0 [M+1, 98%], HRMS (ES^+) $(M+H)^+$ $C_{23}H_{21}N_4O^+$ requires 369.1710, found 369.1712.

7.11. 4[2(2,4-Diamino-6-quinazolinyl)ethyl]phenol (5). {Alternate method}

Compound **12** (300 mg, 0.82 mmol) and palladium hydroxide (300 mg) stirred in dry DMF (20 mL) under H_2 gas for 24 h at room temperature. The reaction mixture filtrate on Celite, concentrated in vacuum and purified by column chromatography to get the product (160 mg, 70%, mp = 278 °C).

7.12. 6[4-(Ethoxy)phenethyl]-2,4-quinazolinediamine (6)

Compound **5** (130 mg, 0.46 mmol, 1 equiv), 1-iodoethane (217 mg, 0.12 mL, 1.39 mmol, 3 equiv) and potassium carbonate (160 mg, 1.16 mmol, 2.5 equiv) in ethanol (10 mL) refluxed for 24 h. Solvent removed under reduced pressure and purified by column chromatography to get the product (80 mg, 56%, mp = 230 °C); R_f = 0.2 (25% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.9 (s, 1H, H5), 7.4 (d, 1H, *J* 8.23 Hz, H7), 7.2 (d, 1H, *J* 8.23 Hz, H8), 7.1 (d, 2H, *J* 8.6 Hz, H2′ + H6′), 6.8 (d, 2H, *J* 8.6 Hz, H3′ + H5′), 3.98 (q, 2H, *J* 6.9 Hz, H1″), 2.85 (s, 2H, a), 2.5 (s, 2H, b), 1.3 (t, 3H, *J* 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 162.74 (C4′ + C9), 159.74 (C2), 157.06 (C4), 134.24 (C5 + C6), 133.59 (C1′), 129.60 (C2′ + C6′), 123.18 (C7), 122.90 (C8), 114.52 (C3′ + C5′), 110.30 (C10), 63.17 (C1″), 37.45 (a), 36.44 (b), 15.07 (CH_3); MS (ES^+) *m*/*z* 309.1 [M+1 = 100%], HRMS (ES^+) $(M+H)^+$ $C_{18}H_{21}N_4O^+$ requires 309.1710, found 309.1722.

7.13. 6[4-(Butyloxy)phenethyl]-2,4-quinazolinediamine (7)

Compound **5** (130 mg, 0.46 mmol, 1 equiv), 1-iodobutane (256 mg, 0.16 mL, 1.39 mmol, 3 equiv) and potassium carbonate (160 mg, 1.16 mmol, 2.5 equiv) in ethanol (10 mL) refluxed for 24 h. Solvent removed under reduced pressure and purified by column chromatography to get the product (100 mg, 64%, mp = 180 °C); R_f = 0.4 (30% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.85 (s, 1H, H5), 7.4 (d, 1H, J 8.4 Hz, H7), 7.2–7.1 (m, 3H, H8 + H2′ + H6′), 6.85 (d, 2H, J 8.4 Hz, H3′ + H5′), 3.9 (t, 2H, J 6.5 Hz, H1″), 2.85 (s, 2H, a), 2.5 (s, 2H, b), 1.7 (m, 2H, H2″), 1.4 (m, 2H, H3″), 0.9 (t, 3H, J 7.3 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 162.65 (C9), 160.49 (C2), 157.22 (C4 + C4′), 133.85 (C5), 133.65 (C6 + C1′), 129.59 (C2′ + C6′), 124.09 (C7), 122.70 (C8), 114.56 (C3′ + C5′), 110.43 (C10), 67.34 (C1″), 37.52 (a), 36.51 (b), 31.17 (C2″), 19.12 (C3″), 14.07 (CH_3); MS (ES^+) m/z 337.2 [M+1 = 100%], HRMS (ES^+) $(M+H)^+$ $C_{20}H_{25}N_4O^+$ requires 337.2023, found 337.2027.

7.14. 6[4-(Hexyloxy)phenethyl]-2,4-quinazolinediamine (8)

Compound **5** (100 mg, 0.36 mmol, 1 equiv), 1-iodohexane (227 mg, 0.16 mL, 1.07 mmol, 3 equiv) and potassium carbonate (125 mg, 0.89 mmol, 2.5 equiv) in ethanol (10 mL) refluxed for 24 h. Solvent removed under reduced pressure and purified by column chromatography to get the product (98 mg, 75%, mp = 175 °C); R_f = 0.4 (30% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.9 (s, 1H, H5), 7.35 (d, 1H, J 8.23 Hz, H7), 7.2–7.1 (m, 3H, H8 + H2′ + H6′), 6.8 (d, 2H, J 8.4 Hz, H3′ + H5′), 3.95 (t, 2H, J 6.4 Hz, H1″), 2.85 (s, 2H, a), 2.5 (s, 2H, b), 1.7 (m, 2H, H2″), 1.5–1.3 (m, 6H, H3″–H5″), 0.9 (t, 3H, J 6.6 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 162.66 (C4), 160.35 (C9), 157.22 (C2 + C4′), 133.90 (C5), 133.75 (C7), 133.64 (C1′ + C6), 129.59 (C2′ + C6′), 123.94 (C8), 122.74 (C10), 114.56 (C3′ + C5′), 67.64 (C1″), 37.51 (a), 36.50 (b), 31.38 (C4″), 29.07 (C2″), 25.58 (C3″), 22.44 (C5″), 14.27 (CH_3); MS (ES^+) m/z 365.2 [M+1 = 100%], HRMS (ES^+) $(M+H)^+$ $C_{22}H_{29}N_4O^+$ requires 365.2336, found 365.2348.

7.15. 6[4-(Octyloxy)phenethyl]-2,4-quinazolinediamine (9)

Compound **5** (100 mg, 0.36 mmol, 1 equiv), 1-iodooctane (260 mg, 0.2 mL, 1.07 mmol, 3 equiv) and potassium carbonate (125 mg, 0.89 mmol, 2.5 equiv) in ethanol (10 mL) refluxed for 24 h. Solvent removed under reduced pressure and purified by column chromatography to get the product (98 mg, 70%, mp = 230 °C); R_f = 0.4 (30% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.95 (s, 1H, H5), 7.50 (d, 1H, J 8.23 Hz, H7), 7.25 (d, 1H, J 8.23 Hz, H8), 7.15 (d, 2H, J 8.4 Hz, H2′ + H6′), 6.8 (d, 2H, J 8.4 Hz, H3′ + H5′), 3.90 (t, 2H, H1″), 2.9 (s, 2H, a), 2.5 (s, 2H, b), 1.7 (m, 2H, H2″), 1.45–1.25 (m, 10H, H3″–H7″), 0.9 (t, 3H, J 6.68 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 162.97 (C2 + C10), 157.25 (C4 + C4′), 131.50 (C6), 133.39 (C7 + C1′), 129.60 (C8 + C2′ + C6′), 123.32 (C9), 120.92 (C5), 114.58 (C3′ + C5′), 67.65 (C1″), 38.31 (a), 37.31 (b), 31.59 (C6″), 29.10 (C4″ + C5″), 29.02 (C2″), 25.90 (C3″), 22.44 (C7″), 14.31 (CH_3); MS (CI^+) m/z 393.4 [M+1 = 100%], HRMS (ES^+) $(M+H)^+$ $C_{24}H_{33}N_4O^+$ requires 393.2649, found 393.2653.

7.16. 6[4-(Decyloxy)phenethyl]-2,4-quinazolinediamine (10)

Compound **5** (100 mg, 0.36 mmol, 1 equiv), 1-iododecane (290 mg, 0.23 mL, 1.07 mmol, 3 equiv) and potassium carbonate (125 mg, 0.89 mmol, 2.5 equiv) in ethanol (10 mL) refluxed for 24 h. Solvent removed under reduced pressure and purified by column chromatography to get the product (67 mg, 45%, mp = 300 °C); R_f = 0.45 (25% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8 (s, 1H, H5), 7.5 (d, 1H, J 8.23 Hz, H7), 7.2 (d, 1H, J 8.05 Hz, H8), 7.05 (d, 2H, J 8.6 Hz, H2′ + H6′), 6.8 (d, 2H, J 8.6 Hz, H3′ + H5′), 3.85 (t, 2H, J 6.4 Hz, H1″), 2.8 (s, 2H, a), 2.45 (s, 2H, b), 1.55 (m, 2H, H2″), 1.4–1.1 (m, 14H, H3″–H9″), 0.8 (t, 3H, J 6.4 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.19 (C9), 157.29 (C2), 156 (C4), 140.74 (C4′), 137.2 (C5), 135.81 (C6), 133.21 (C1′), 129.6 (C2′ + C6′), 123.75 (C7), 118.68 (C8), 114.6 (C3′ + C5′), 109.71 (C10), 67.65 (C1″), 37.19 (a), 36.13 (b), 31.65 (C8″), 29.36 (C6″), 29.30 (C7″), 29.13 (C3″), 29.08 (C5″), 29.05 (C9″), 25.89 (C4″), 22.45 (C2″), 14.32 (CH_3); MS (ES^+) m/z 421.1 (M+1, 22%), HRMS (ES^+) $(M+H)^+$ $C_{26}H_{37}N_4O^+$ requires 421.2962, found 421.2974.

7.17. Modelling studies

Modelling was performed on Silicon Graphics O2 workstations using Sybyl 6.7 and GRID19. For docking, water molecules were removed from the protein. For FlexX the active site was defined as all residues within a radius of 6.5 Å from the folate ligand (in the case of the human enzyme) or 6.5 Å from the methotrexate ligand (in case of the *L. major* enzyme). The compounds for docking were drawn in Sybyl. Before docking, the compounds were subjected to energy minimisation using the Powell method using Sybyl with a gradient cut-off of 0.01 kcal/mol or a limit of 1000 iterations.

7.18. Enzyme assays

DHFR catalyses the NADPH dependant reduction of dihydrofolate to tetrahydrofolate. Assays in the absence and presence of inhibitor were performed spectrophotometrically by measuring the rate of decrease in NADPH absorbance at 340 nm at 25 °C. The inhibition constant (K_i) was calculated from K_m and IC_{50} values assuming competitive inhibition. Standard reactions contained dihydrofolate (DHF) 30 μM, NADPH 100 μM, bovine serum albumin (BSA) (1 mg/mL) and enzyme in 50 mM TES pH 7.0, 75 mM β-mercaptoethanol, 1 mM EDTA. Enzyme concentrations used were 2.72 nM for *L. major* DHFR and 8.5 nM for the human enzyme. Routinely, a range of at least five different inhibitor concentrations was tested for IC_{50} calculations. Absorbance

was recorded in a Hewlett–Packard model 8543 spectrophotometer. Dihydrofolate was prepared from folic acid by reduction with sodium dithionite according to the method of Futterman.[23] The following K_m values were used for calculation of K_i values: *L. major* DHFR, 1.3 μM; human DHFR, 0.64 μM. NADPH and BSA were purchased from SIGMA. Once prepared, dihydrofolate was stored at −80 °C until use. The concentration of dihydrofolate was measured spectrophotometrically at 282 nm.

7.19. Anti-parasite assays

7.19.1. *Trypanosoma b. rhodesiense*. Minimum essential medium (50 μL) supplemented according to Baltz et al.[24] with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtitre plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Then 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μL were added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μL, 12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2–4 h. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm.[25] Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices), which calculated IC_{50} values. Cytotoxicity was assessed using the same assay and rat skeletal myoblasts (L-6 cells).

7.19.2. *Trypanosoma cruzi*. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 μL RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h the medium was removed and replaced by 100 μL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene. After 48 h the medium was removed from the wells and replaced by 100 μL fresh medium with or without a serial drug dilution. Seven 3-fold dilutions were used covering a range from 90 to 0.123 μg/mL. Each drug was tested in duplicate. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then the substrate CPRG/Nonidet (50 μL) was added to all wells. A colour reaction developed within 2–6 h and could be read photometrically at 540 nm. Data were transferred into the graphic programme Softmax Pro (Molecular Devices), which calculated IC_{50} values.

7.19.3. *Leishmania donovani*. Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37 °C in SM medium[26] at pH 5.4 supplemented with 10% heat-inactivated foetal bovine serum under an atmosphere of 5% CO_2 in air. Hundred microlitres of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtitre plates. Seven 3-fold dilutions were used covering a range from 30 to 0.041 μg/mL. Each drug was tested in duplicate and each assay was repeated at least once. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μL of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and the plates incubated for another 2 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 were analysed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (= inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC_{50} values were calculated.

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