Novel functionalized melamine-based nitroheterocycles: synthesis and activity against trypanosomatid parasites†‡

Alessandro Baliani,[a,b] Valerie Peal,[b] Ludovic Gros,[a] Reto Brun,[c] Marcel Kaiser,[c] Michael P. Barrett[d] and Ian H. Gilbert*[a,b]

Received 5th August 2008, Accepted 2nd December 2008
First published as an Advance Article on the web 28th January 2009
DOI: 10.1039/b813394h

Human African trypanosomiasis (HAT), caused by the protozoan parasite *Trypanosoma brucei spp.*, is a major health problem in sub-Saharan Africa. New drugs are urgently required for the disease. Selective uptake of toxic compounds into trypanosomes has been achieved by exploiting plasma membrane transporters. For example, the P2 aminopurine transporter, along with other transporters, selectively concentrates melamine and benzamidine moieties into trypanosomes. We have previously reported the use of the melamine motif to selectively target nitrofuran to the trypanosome. In this paper we report the further investigation of the structure activity relationships and the effect of the introduction of different functionalized substituents onto the melamine unit. Most of the compounds tested *in vitro* for their trypanocidal activity showed activities in the submicromolar range against *T. b. rhodesiense.*

Introduction

Parasitic organisms belonging to the order Kinetoplastida are responsible for several diseases affecting humans such as Human African Trypanosomiasis, Chagas' disease and leishmaniasis.[1] Human African trypanosomiasis (HAT) or sleeping sickness is a serious public health threat in 36 countries of sub-Saharan Africa where it is estimated that 60 million people are at risk for contracting the disease. HAT is caused by subspecies of *Trypanosoma brucei* (*T. b. gambiense* and *T. brucei rhodesiense*), a parasitic protozoan transmitted by the tsetse fly. Infection is fatal if untreated. Current drug therapy is inadequate due to serious side effects, poor efficacy, undesirable routes of administration and an increase of drug resistance.[2]

T. brucei lacks the ability to synthesise important nutrients for their survival. These include purines which they need to import from the host. A number of different nucleoside/nucleobase transporters have been identified in *T. brucei*.[3,4] One of these, called the P2 transporter, was characterised in *T. brucei* by Carter and Fairlamb in 1993.[5,6] In addition to uptake of adenosine and adenine, it also mediates the uptake of other motifs such as melamines (e.g. melamine-arsenical drugs) and benzamidines. The P2 transporter differs substantially from the human mammalian equilibrative nucleoside transporters.[7,8] Carter and Fairlamb[2] formulated a structure activity relationship for the binding of nucleosides/nucleobases to the P2 transporter which was later expanded using inhibition data collected from a series of potential substrates (De Koning and Jarvis).[9] The recognition motif for the P2 transporter is: an amidine moiety, an aromatic ring and an electronegative heteroatom, as found in the so-called melamine and benzamidine motifs.

Interestingly De Koning revealed the presence of two additional transporters specific for pentamidine whose physiological roles are still unknown: the high affinity pentamidine transporter (HAPT1) and the low affinity pentamidine transporter (LAPT1).[10,11] Furthermore, experiments indicate that melamine-arsenicals also have uptake routes in addition to the P2 transporter,[12] with HAPT1 being a likely candidate.[13] The novel diamidine, furamidine, also appears to enter cells *via* the P2 and other transporters.[14]

It is clear that both the melamine and benzamidine moieties are selectively concentrated in *T. brucei* and we have been trying to exploit this for selective delivery of compounds to *T. brucei* by coupling trypanotoxic agents to the P2 motif.[15] In our previous work we have described the trypanocidal activity of melamine compounds linked to nitroheterocycles such as nitrofuran. Nitrofurans were chosen because they are known for potent antimicrobial/trypanocidal activities.[16–18] Benznidazole (a nitroimidazole) and nifurtimox (a nitrofuran) are used for the treatment of Chagas' disease and nifurtimox is also under clinical trial for the treatment of HAT.[19,20]

Previous work had identified some melamine-nitroheterocycles which showed good selectivity and caused potent inhibition of growth of *T. b. rhodesiense*.[21] The lead compound was compound **5a** (Fig. 1). This compound and several analogues were also able to cure the rodent STIB795 model of human African trypanosomiasis. These structure activity relationship (SAR) studies indicated that it was possible to derivatise one of the melamine amino groups with a methyl and retain *in vivo* activity. Interestingly, multiple derivatisation of the melamine amino groups with methyls led to increased *in vitro* activity, but reduced *in vivo* activity. In this paper we report further SAR studies on the lead compound; in particular we decided to investigate the substituent on the amino group and

[a]*School of Life Sciences, Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee, DD1 5EH, UK. Fax: +44 (0) 1382 386 373; Tel: +44 (0) 1382 386 240*
[b]*Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3XF, UK*
[c]*Swiss Tropical Institute, Socinstrasse 57, CH-4002, Basel, Switzerland*
[d]*University of Glasgow, Glasgow Biomedical Research Centre, Institute of Biomedical and Life Sciences, Glasgow, G12 8TA, UK*

† This paper is dedicated to Professor Andrew Holmes on the occasion of his 65th birthday.

‡ Electronic supplementary information (ESI) available: Further experimental details and NMR spectra. See DOI: 10.1039/b813394h

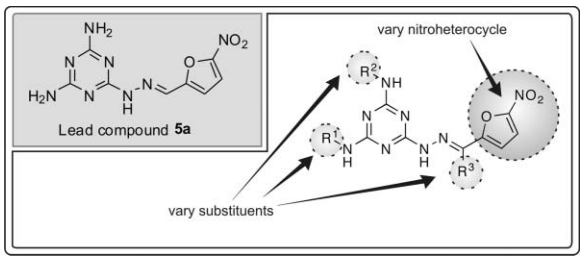


Fig. 1 Structure of the lead compound **5a** and planned modifications.

also the effect of varying the nitroheterocycle. The aim of these studies was to alter the physicochemical properties of the lead (especially solubility) and potency, thus increasing the spectrum of pharmacological properties associated with these trypanotoxins.

The modifications can be summarised as follows:

- introduction of alkyl substituents to the melamine to alter the lipophilicity of the molecule;
- introduction of hydroxyl and carboxylic acid and ester derivatives to the melamine to obtain a series of derivatives with a wide range of partition coefficient values;
- introduction of small substituents on the hydrazone bridge and effect of the modification of the geometry of the hydrazone;
- replacement of 5-nitrofuran moiety with nitro-imidazoles;
- effect of the replacement of the nitro group.

Chemistry

The chemical start point was cyanuric chloride (**1**) which was converted to the key intermediate 2-amino-4,6-dichloro[1,3,5]triazine (**2**) (Scheme 1). The first amino group was introduced by adding ammonia in water solution at 0 °C which displaced the first chlorine affording (**2**).[21]

Displacement of one of the chloro groups of **2** with a variety of amines allowed introduction of different substituents. Thus, 2,4-bis-substituted-6-chloro-1,3,5-triazines **3c** and **3d** were obtained by reaction of **2** with propylamine and butylamine in the presence of sodium hydroxide. The insertion of the hydroxyl-alkyl chain was found to be more problematic. When ethanolamine was used with the conditions above, the outcome of the reaction was not clear; hence we decided to protect the hydroxyl group with TBDPSiCl to facilitate regioselectivity and to increase the lipophilicity to aid with purification. Ethanolamine and propanolamine were protected and then successfully reacted with **2** to afford **3e** and **3f** using the same conditions. The insertion of unprotected propanolamine to give **3g** was less problematic than observed for ethanolamine in water/acetone. The original yield of **3g** (31%) was improved to 91% by replacing the solvent acetone/water with DMF at –20 °C and using DIPEA as the base. The carboxylic acid substituted triazine products **3h–3j** were obtained in good yields by displacement with the relevant amino acids.

The displacement of the last chlorine is more difficult to achieve and requires more harsh conditions. Hydrazine-triazines **4a–4j** were achieved in good yields by refluxing the triazines **3a–3j** with hydrazine hydrate in water/ethanol. For the triazine derivative **4g**, DMF was used at 85 °C. Hydrazones **5b–5k** were obtained by reaction of the hydrazine triazines **4a–4k** with 5-nitrofuraldehyde. Hydrazones **5a–5d** showed low solubility in most organic solvents and were purified by recrystallisation from ethanolic or methanolic water solutions. Cleavage of the silyl protecting group in hydrazones **5e–5f** could not be achieved as decomposition occurred. The solubility of hydrazone **5g** in organic solvents was found to be dramatically improved compared to the hydrazone lead compound **5a**. The compound was purified by flash column chromatography (CH_2Cl_2:MeOH gradient to 95/5) and this represents a big advance as previously the solubility of some hydrazones made their purification problematic.

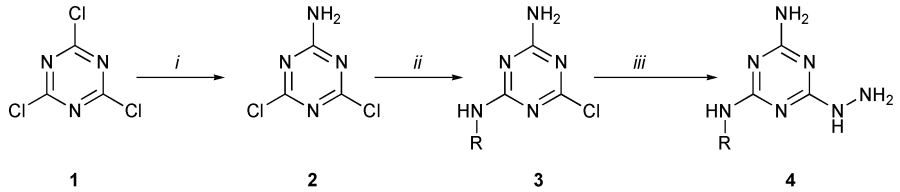
The purification of **5h–5j** was difficult to achieve and ion exchange chromatography was found to be the best approach using Dowex 50WX8.200 resin. Water/HCl/methanol mixtures as eluent gave rise to formation of the methyl ester of **5h**. Replacing methanol with acetonitrile avoided this problem; however further by-product formation was observed using larger amounts of acetonitrile. The first fractions off of the column contained pure product with the unwanted impurities eluting later and from this we can conclude that although the method is time consuming, with care it can work. Compound **5k** was purchased from Aldrich. In this compound both the amino groups of the triazine moiety bear one ethyl substituent.

Further information could be obtained by generating ester derivatives (**6a–6h**) in order to determine their effects on uptake and activity. The acetyl ester of **5g** was obtained in good yield with acetic anhydride and DMAP in pyridine. For the esterification of compounds **5h–5j** it was envisaged this could be cleanly carried out using HCl(g) bubbled through the carboxylic acid starting material and the desired alcohol. Although this certainly proved to be the case for the first compound prepared (**6b**), our subsequent attempt to prepare compound **6c** *via* this route met with considerable problems due to the difficulties in removing the large volume of alcohol used. With these problems in mind, subsequent esterifications (**6c–6h**) were carried out in alcohol using $SOCl_2$ (Scheme 1).

Another series of modifications we carried out on our lead structure involved the nitrofuran moiety. The hydrazones synthesised (**9a–9f**) are shown on Scheme 3. The nitro group was replaced with a carboxylic acid function (**9a**). The carboxylate should be an isostere for the nitro group and should remove any possible problems connected with toxicity of a nitroheterocycle. We also were interested to see the effect of a substituent on the hydrazone—this is a point of substitution that we have not investigated, that might be useful as a handle for altering the physicochemical properties of the molecule (**9b–d**). Finally we wanted to see the effect of changing a nitrofuran to a nitroimidazole (**9e–f**); this should have an effect on the redox potential of the nitroheterocycle.

Hydrazone **9a** was obtained by treatment of **4a** with the commercially available 5-formyl-2-furancarboxylic acid (**8a**, Scheme 3). The other nitroheterocycles were prepared as shown in Scheme 2 (**8b–8d**).

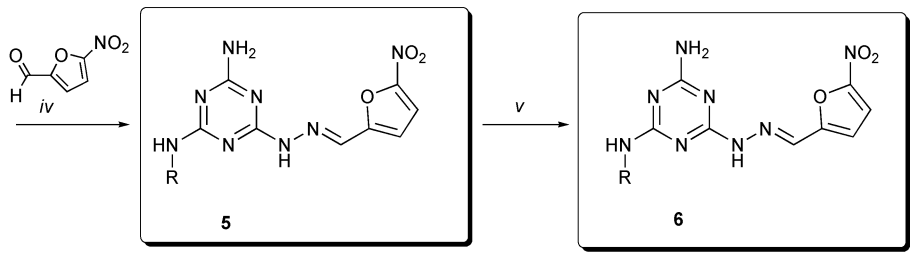
Nitration of 2-acetylfuran (**7b**) after different attempts, was carried out with concentrated nitric acid in acetic anhydride as a solvent, and a base such as triethylamine.[22,23] After the coupling with **4a** using methanol and molecular sieves overnight at room temperature two stereoisomers *E* (**9c**) and *Z* (**9b**) were generated, with an overall yield of 50%. The imidazole-carboxaldehyde derivatives (**7d**, **7e** Scheme 2) were also nitrated using HNO_3 70% at room temperature for a short period of time (30 minutes) giving the corresponding nitrated products (**8d**, **8e**) in quantitative yields.



1 2 3 4

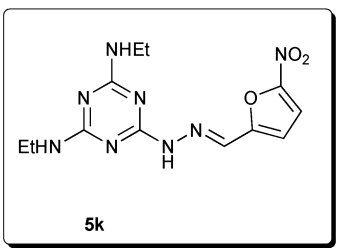
3a, R = H;
3b, R = *i*Pr;
3c, R = Pr (76%);
3d, R = Bu (94%);
3e, R = -$(CH_2)_2$-OTBDP (35%);
3f, R = -$(CH_2)_3$-OTBDP (45%);
3g, R = -$(CH_2)_3$-OH (91%);
3h, R = -$(CH_2)_2$-COOH (75%);
3i, R = -$(CH_2)_3$-COOH (78%);
3j, R = -$(CH_2)_5$-COOH (88%).

4a, R = H (72%);
4b, R = *i*Pr (49%);
4c, R = Pr (71%);
4d, R = Bu (52%);
4e, R = -$(CH_2)_2$-OTBDP (86%);
4f, R = -$(CH_2)_3$-OTBDP (52%);
4g, R = -$(CH_2)_3$-OH
4h, R = -$(CH_2)_2$-COOH
4i, R = -$(CH_2)_3$-COOH (92%);
4j. R = -$(CH_2)_5$-COOH (100%);



5a, R = H (36%)
5b, R = Pr (64%)
5c, R = *i*Pr (25%)
5d, R = Bu (73%)
5e, R = -$(CH_2)_2$-OTBDP (9%)
5f, R = -$(CH_2)_3$-OTBDP (84%)
5g, R = -$(CH_2)_3$-OH (17%)
5h, R = -$(CH_2)_2$-COOH (32%)
5i, R = -$(CH_2)_3$-COOH (79%)
5j, R = -$(CH_2)_5$-COOH (56%)

6a, R = -$(CH_2)_3$-OAc
6b, R = -$(CH_2)_2$-COOEt (20%)
6c, R = -$(CH_2)_2$-COOBn (57%)
6d, R = -$(CH_2)_3$-COOEt (88%)
6e, R = -$(CH_2)_3$-COOBn (54%)
6f, R = -$(CH_2)_5$-COOEt (67%)
6g, R = -$(CH_2)_5$-COOBn (97%)
6h, R = -$(CH_2)_5$-COOMe (65%)



Scheme 1 Reagents and conditions: (i) NH_4OH, acetone, 0 °C to rt; (ii) **3b**–**3f**. RNH_2, NaOH 2 N, acetone/H_2O, rt; **3g**. R_1NH_2, –20 °C to rt, DIPEA, DMF; **3h**–**3j**. R_1NH_2, 2 °C to rt, $NaHCO_3$, EtOH; (iii) **4a**–**4e**. NH_2NH_2, H_2O, reflux; **4f**, **4a**–**4j**, NH_2NH_2, EtOH, reflux; **4g**.NH_2NH_2, DMF; (iv) **5a**–**5k**, nitrofuraldehyde, MeOH, rt; (v) **6a**. Ac_2O, Py, DMAP, 0 °C to rt; **6b**, **6d**, **6f**. $SOCl_2$, EtOH, rt; **6c**, **6e**, **6g**. $SOCl_2$, BnOH, rt; 6 h. $SOCl_2$, BnOH, rt. The preparation of compound **5a** has been reported previously.[21]

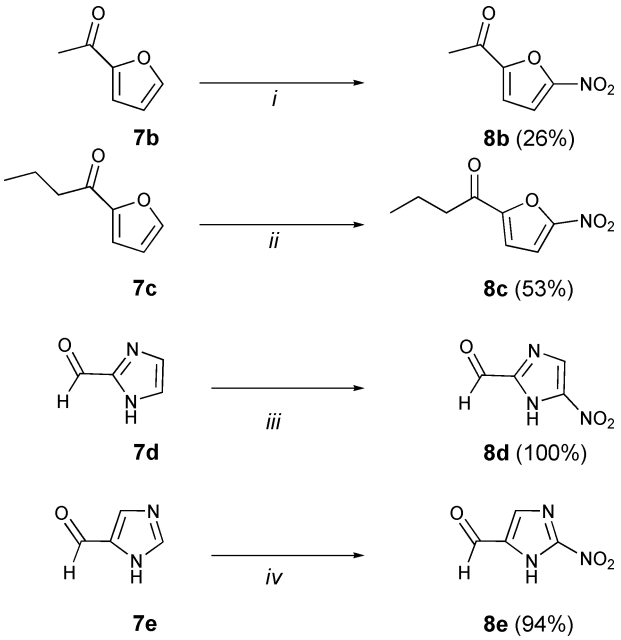
Hydrazones corresponding compounds **9e** and **9f** were obtained in methanol with the conditions described above and the purification was achieved by reverse phase chromatography.

Biology

Activities against *T. brucei in vitro*

Compounds were evaluated for their *in vitro* activities against *T. brucei rhodesiense* lines, with a counterscreen against L6 cells (rat skeletal myoblast cells) to give an indication of selectivity of the compound for the parasite (Table 1). The following comments can be made on the structure activity relationships, with comparison to the lead compound **5a**.

Alkyl groups on one of the amines (**5b–d**) did not affect the trypanocidal activity compared to the lead compound **5a**, although there was a drop in selectivity with substantially increased toxicity to the mammalian L6 cell line apparent. Substitution of ethyl onto two of the amines (**5k**) led to drop in activity of about 10 fold.

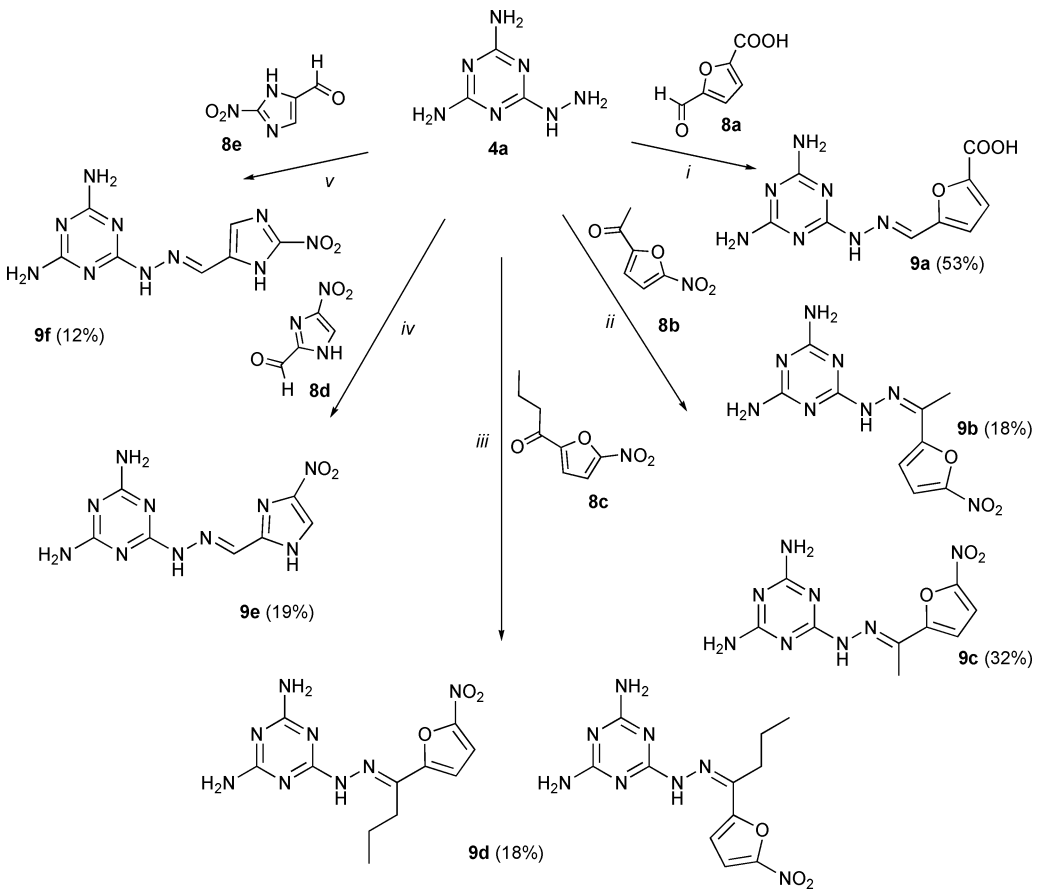


Scheme 2 Reagents and conditions: (i) and (ii): 1) HNO_3 70%, Ac_2O 0 °C, 2) TEA, rt 3 h; (iii), (iv): HNO_3, 70%, 30 min, rt.

The introduction of a hydroxypropyl chain (compound **5g**) maintained the activity against *T. b. rhodesiense* in the nanomolar range (IC_{50} 49 nM) together with a good selectivity compared to the mammalian cells (1700 fold). When the hydroxyl function was acetylated (**6a**) both activity and the selectivity were retained. It is possible that in the conditions of the cellular assay the acetate was rapidly cleaved by esterases. Derivatisation of the hydroxypropyl with a *tert*-butyl diphenylsilyl group also led to retention of activity. It is conceivable that the silyl group is also labile under the assay conditions. Alternatively, the molecular target is tolerant of hydrophobic substituents at this position. Interestingly, the silylated hydroxyethyl derivative (**5e**) was about 5-fold less active that the corresponding silylated hydroxypropyl derivative.

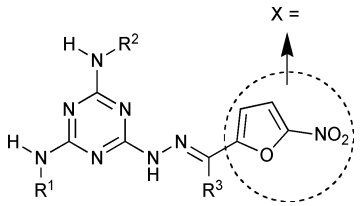
Compounds with a free carboxylic acid (**5h–j**) were markedly less active than their alkyl substituted counterparts (IC_{50} 28 μM, 8 μM, 0.8 μM). Interestingly as the alkyl chain length was increased, the potency increased, with the hexanoyl derivative being approximately 30x more active than the propionyl derivative.

Esterification of the propionyl derivative (**5h**) to give the ethyl ester (**6b**) led to a significant (400 fold) increase in potency. This was also found for the ethyl ester (**6d**) of the butanoyl derivative (**5i**), where there was a 17 fold increase in activity. However for the hexanoyl derivative there was no effect on converting the free acid (**5j**) to the ethyl ester (**6f**). Interestingly, the benzyl esters of the propionyl (**6c**) and butanoyl (**6e**) derivatives were significantly less active than the corresponding ethyl esters (**6b** and **6d**). With the hexanoyl series, the free acid (**5j**), the methyl ester (**6h**), the ethyl ester (**6f**) and the benzyl ester (**6g**) were all equipotent and had broadly similar levels of selectivity compared to L6 cells. Interestingly these 4 compounds are predicted to have



Scheme 3 Reagents and conditions: (i) MeOH, rt, 6 h; (ii–v) MeOH, mol. sieves, overnight.

Table 1 *In vitro* activities of compounds against bloodstream *T. brucei*, and mammalian cells as a measure of toxicity



	Substituents	*T. brucei rhodesiense* (IC_{50} [μM])	L6 cells (IC_{50} [μM])	Selectivity	LogD (pH 7.4)
5a	R_1, R_2, R_3=H	0.025	183	7320	–1.9
5b	R_1= -Pr	0.023	17	752	–0.5
5c	R_1= -i-Pr	0.065	5.5	84	–0.7
5d	R_1= -Bu	0.025	18	733	0.05
5e	R_1= -$(CH_2)_2OSiPh_2{}^tBu$	0.17	0.49	3	4.9
5f	R_1= -$(CH_2)_3OSiPh_2{}^tBu$	0.037	7.4	199	5.1
5g	R_1= -$(CH_2)_3OH$	0.049	84	1710	–2.0
5h	R_1= -$(CH_2)_2CO_2H$	27.7	268	10	–4.6
5i	R_1= -$(CH_2)_3CO_2H$	8.0	257	32	–4.2
5j	R_1= -$(CH_2)_5CO_2H$	0.81	>238	294	–3.6
5k	R_1= Et, R_2= Et	0.19	36	187	–0.2
6a	R_1= -$(CH_2)_3OAc$	0.049	75	1523	–1.1
6b	R_1= -$(CH_2)_2CO_2Et$	0.07	110	1567	–0.8
6c	R_1= -$(CH_2)_2CO_2Bn$	0.4	133	332	0.5
6d	R_1= -$(CH_2)_3CO_2Et$	0.46	75	162	–0.5
6e	R_1= -$(CH_2)_3CO_2Bn$	6.7	116	17	0.7
6f	R_1= -$(CH_2)_5CO_2Et$	0.93	155	167	0.2
6g	R_1= -$(CH_2)_5CO_2Bn$	0.86	>192	>223	1.2
6h	R_1= -$(CH_2)_5CO_2Me$	1.3	187	145	–0.5
9a	X= 2-carboxyl-furan-5-yl-	285	>342	>1	–5.0
9b	R_3= -Me (*Z*)	61	>324	>5	–1.9
9c	R_3= -Me (*E*)	96	>324	>3	–1.9
9d	R_3= -n-Pr (*E*, *Z*)	0.075	96.9	1292	–0.8
9e	X= 4-nitro-imidazol-2-yl-	118.2	>341	>3	NA
9f	X= 2-nitro-imidazol-5-yl-	16.32	>341	>21	NA
10	NA	0.68	40	59	–0.2
11	NA	2.3	20	9	0.3
Melarsoprol	NA	0.006	7.8	1300	
Nifurtimox	NA	1.5	68	45	

NA = not applicable, X = 5-nitrofuran-2-yl when not specified, R_1, R_2 and R_3 are H when not specified. LogD values were calculated using the ACD software. Data for compound **5a** have been reported previously—references 21 and 24.

widely varying degrees of lipophilicity; assuming the same mode of action, their equipotency is consistent with some kind of active transport.

The SAR of this series of compounds does not lend itself to simple explanation. For the carboxylate derivatives (**5h–j**) as the chain length was increased, the activity increased. One possible explanation for this is that the carboxylate group makes a specific interaction with a molecular target. Another possibility is that an increase in chain length could give more lipophilic compounds with greater cellular permeability. However, interestingly as the chain length was increased, the calculated logD value did not increase significantly, suggesting that the increase in activity was probably not due to a significant increase in passive diffusion. Indeed the logD values suggested that uptake was probably not primarily by passive diffusion, but by active transport. When considering the esters of the carboxylate series, (**6b–h**) ethyl esters were generally more potent that the more lipophilic benzyl esters. The lack of clear correlations probably relates to the fact that overall activity is multi factorial, involving uptake at the cellular membrane, possible partitioning into different cellular compartments, thus crossing additional membranes, and interaction with cellular targets.

Replacement of the nitro group with the carboxylic acid (**9a**) led to a large reduction of activity. The carboxylate should be an isostere for the nitro group, in terms of its size, steric properties and H-bond acceptor properties. However, like other replacements that we have investigated previously (hydrogen and nitrile)[21] it was inactive. This implies that the redox properties of the nitro(heterocycle) are critical for anti-trypanosomal activity.

Attachment of a substituent to the hydrazone linker may provide another point for variation of the activity, selectivity and physicochemical properties of the compounds. The methyl substituent was isolated as the two stereoisomers **9b** and **9c**. The stereochemistries of these were assigned by NOESY experiments. With the propyl derivative, the stereoisomers were much more difficult to separate, and the compound was investigated as the mixture of stereoisomers (60/40). Compounds **9b** and **9c** showed very little activity, although it was noted that they were both very insoluble. The propyl derivatives, however, were potent trypanocides (IC_{50} of 75 nM).

Replacing the nitrofuran with a nitroimidazole (**9e** and **9f**) led to a drastic loss in activity. Low activity was also observed when nitroimidazoles were linked to a benzamidine moiety.[24] In this case, the nitroimidazole is conjugated to the melamine ring in a

Table 2 *In vivo* activities

Compound	STIB 795 (*T. b. brucei*)			STIB 900 (*T. b. rhodesiense*)		
	Dose (mg/Kg) ip	Cured/infected	Survival average (days)	Dose (mg/Kg) ip	Cured/infected	Survival average (days)
Control	—	0/4	6	—	0/4	8
5a	4 × 20	4/4	>60	4 × 20	1/4	35.25
5b	4 × 50	4/4	>30	4 × 50	0/4	16.75
5c	4 × 50	4/4	>30	4 × 50	1/4	>26.75
5d	4 × 50	3/4	>30	4 × 50	1/4	>40.75
5f	4 × 50	0/4	>27.25	ND	ND	ND
5g	4 × 50	4/4	>30	ND	ND	ND
5j	4 × 40	0/4	5.75	ND	ND	ND
6a	4 × 50	4/4	>30	ND	ND	ND
6d	ND	ND	ND	4 × 50	0/4	8.75

similar fashion to the active nitrofurans. One possible explanation for the loss in activity is the probable change in redox potential with nitroimidazoles being more difficult to reduce.

To summarise the SAR, simple substituents led to high cellular potency. The carboxylic acid derivatives reduced activity. Esterification of the carboxylic acid improved potency, but this was not as significant when the chain length of the carboxylic acid increased. There was no marked correlation between lipophilicity and activity.

Given their potent in vitro activity against *T. brucei*, some selected compounds were tested in two different rodent models of infection: STIB 795 *T. brucei brucei* infection model and the more stringent STIB 900 *T. brucei rhodesiense* model. Table 2 shows a summary of the *in vivo* activities for the tested compounds. Previously published data[21,24] for the lead compound **5a** are shown, for comparative purposes. Compounds **5b**, **5c**, **5g** and **6a** were able to cure all the mice infected with the STIB 795 model of infection when treated at a dose of 50 mg/Kg intraperitoneally for 4 days. The mice were considered cured when no infection was found after 30 days. No overt sign of toxicity was found in these mice. Compound **5d** also cured 3 of the 4 mice infected with the same model of infections. Compounds **5c** and **5d** were also able to cure one of the 4 mice infected with the more stringent model of infection STIB 900 and **5b** showed a significant increase in survival time.

The derivatives with one alkyl group retained *in vivo* activity, indicating that there is some room for variation, if there is one substituent on just one of the melamine amino groups. The silyl derivative was slightly less active. However the acid (**5j**) and ester (**6d**) derivatives lost activity. This is probably due to a combination of slightly lower activity against the parasites (as shown by the cellular assays) and pharmacokinetic reasons.

Compounds were also tested for their activities against the other kinetoplastid parasites *T. cruzi*, *L. donovani* and the apicomplexan *Plasmodium falciparum* (Table 3). In general the activities were lower than against *T. b. rhodesiense.* This is not surprising, as the compounds were designed for selective uptake into *T. brucei* and are quite polar and may not cross cell membranes readily by passive diffusion. However, several of the compounds showed submicromolar activity against *T. cruzi*; **5d**, **5f** and **6d** were particularly potent (IC_{50} <0.26 μM). This is significant, given the intracellular location of the parasites, which makes it more difficult to access and kill them. Compound **5f** was also active against *L. donovani* and *P. falciparum.* Given the relatively low IC_{50} against L6 cells, this suggests that this compound may have a general cytotoxic effect and, moreover, be generally cell permeant due to increased hydrophobicity.

Conclusions

We have reported the synthesis of new functionalised melamine derivatives as novel potential drugs for the treatment of human African trypanosomiasis. We have also investigated the structure activity relationship and the effect of the introduction of functionalised groups onto the melamine moiety of the compounds. Derivatisation at one of the amino groups of the melamine is possible, creating compounds that retain anti-parasite activity in cellular and rodent models. Complex structure activity relationships exist, indicative of the multifactorial nature of drug action (uptake at the plasma membrane, possible crossing of organellar membranes and interaction with molecular targets all playing roles). Compounds **5c** and **5d** are particularly interesting, since they were potently anti-trypanosomal in cell culture and also in rodent models of infection, indicating that pharmacological properties were sufficiently good to allow the compounds to remain present at trypanocidal concentrations long enough to be effective *in vivo.* In one case, substitution of the hydrazone led to a compound with significant activity *in vitro*, which indicates a possible point for modification of the physicochemical properties of the molecule.

Attempts to replace the nitro group have, to date, failed to yield compounds with substantial trypanocidal activity, indicating that the nitro group is necessary for activity. Moreover, we investigated the possibility of replacing the nitrofuran trypanocidal moiety with other nitroheterocycles. Straight replacement of the nitrofuran with a nitroimidazole appears to cause a significant drop in activity. This may well be due to a significant change in the redox potential of the nitroheterocycle.

Experimental

Cell assays and *in vivo* assays

The protocols for these assays have been described previously.[21]

General

Normal phase tlc was carried out on pre-coated silica plates (Kieselgel 60 F_{254}, BDH), whilst reverse phase tlc was carried on Merck RP-18F reverse phase silica coated aluminium plates with visualisation *via* U.V. light and/or ninhydrin solution. ^{1}H-NMR

Table 3 Activities against *T.cruzi*, *Leishmania donovani* and *Plasmodium falciparum*

Compound	MW	*T. cruzi* (IC_{50} [μM])	*Leishmania donovani* (IC_{50} [μM])	*P. falciparum* (IC_{50} [μM])	*L6 cells* (IC_{50} [μM])
5a	264.28	2.1	>12.5	2.9	183
5b	306.28	22.17	2.38	2.96	17.33
5c	306.28	0.98	2.63	3.59	5.45
5d	320.31	0.26	1.25	2.47	18.32
5e	546.65	36.76	1.64	1.45	0.49
5f	560.68	0.13	0.54	0.24	7.38
5g	322.28	64.85	18.37	4.06	83.77
5h	336.26	>89.2	41.9	>14.9	267.6
5i	350.29	85.6	38.3	>14.3	256.9
5j	378.34	12.2	22.6	>13.2	>238
5k	320.31	0.43	2.18	6.58	35.6
6a	364.32	70.54	11.31	4.72	74.65
6b	364.32	0.8	9.6	9.2	109.7
6c	426.39	0.2	6.3	3.8	132.97
6d	378.34	0.59	6.66	2.60	74.53
6e	440.41	1.04	4.08	1.03	116.02
6f	406.4	1.42	7.52	5.61	155.02
6g	468.19	12.3	7.19	4.27	>192
6h	392.37	2.42	5.63	8.66	186.8
9a	263.21	>114.0	>114.0	>19	>341.9
9b	278.23	>107.8	>107.8	>18	>323.5
9c	278.23	>107.8	28.75	>18	>323.5
9d	306.28	ND	5.97	ND	96.9
9e	264.20	>113.5	>113.5	>18.9	>340.6
9f	264.20	>113.5	23.8	18.9	>340.6
10	156.1	ND	Tox	4.5	40.4
11	155.1	ND	Tox	18.0	20

spectra were recorded at a Bruker Avance DPX500 spectrometer 300 MHz and 500 MHz spectrometers using the applied solvent simultaneously as internal standard. Chemical shifts (δ) are given in ppm together with the relative frequency, assignment, the coupling constants (nJ(H,H)/Hz) and the multiplicity: singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet, quintuplet, sextet, septuplet, multiplet (m), broad multiplet (bm). ^{13}C-NMR spectra were recorded at a Bruker 300 MHz NMR spectrometer using the applied solvent simultaneously as internal standard. Chemical shifts (δ) are given in ppm. DMSO-d_6/TFA was used in some experiments as solvent for some insoluble compounds and represents a mixture of DMSO-d_6 with two drops of TFA. Extra peaks associated to tautomeric effects observed in some groups were indicated with an asterisk (*) after the formula. The ^{13}C signals for the triazine carbons are quaternary and hence very weak and this is exacerbated by tautomerism, hence not all triazine carbon signals are always visible. It is impossible with the information that we have to assign those that are detected. In some cases, the numbering and the nomenclature system used for the structures are not referring to IUPAC nomenclature in order to aid the NMR interpretation. Elemental analyses were carried out by MEDAC Ltd. IR spectra were recorded at a FT-IR spectrometer 1600 from Perkin Elmer using the Diffuse Reflectance Accessory from Spectra Tech. (Refl.), a potassium bromide pellet (KBr), or methylene chloride solution of the compound between potassium bromide single crystals (film). The band positions were characterised by their wave numbers (ν/cm^{-1}), intensity (strong, medium, weak, broad), and assignment. Mass spectra were recorded at a Platform II mass spectrometer (Micromass) from Fisons. High resolution mass spectra were performed respectively on a Waters ZQ4000 and a Finningan MAT 95XP at EPSRC National Mass Spectrometry Service centre in the Chemistry department, University of Wales Swansea, Swansea, Wales, UK. Purification by column chromatography was performed on Sorbosil C60A silica-gel-40–60 μm from Merck. In some cases higher flow rates were maintained by using a slight pressure. Qualitative thin-layer chromatography (TLC) was done on pre-coated aluminium sheets Silica gel 60 F254 from Merck. Compounds were detected either with iodine, ninhydrin or 254 nm UV-light. Purification by reversed phased was performed on silica gel 100 C18. In several experiments flash chromatography was performed using Combiflash Companion and prepacked column (silica gel and C18 reverse phase) purchased from Redisep e Presearch. Purification by ion exchange chromatography was performed on a Dowex 50WX8.200 resin (prewashed with MeOH, HCl 1 M and water) using HCl gradient (1 M to 2 M) and eluting first with water and then stepwise increasing amounts of HCl and MeCN. Melting points were determined with a Gallenkamp melting point apparatus and are not corrected. Solvents and reagents were purchased from chemical companies and used without further purification. Dry solvents were purchased in sure sealed bottles stored over molecular sieves. Compound **5k** was purchased from Aldrich. Experimental details for the preparation of compounds **2**, **3** and **4** are in the supplementary data.

5-Nitro-2-furaldehyde (N^4-propyl-4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (5b)

5-Nitrofuraldehyde (203.7 mg, 99%, 1.43 mmol) and N^4-propyl-4,6-diamino-1,3,5-triazin-2-yl-hydrazine (264 mg, 1.34 mmol) were suspended in MeOH (5 mL) and left stirring overnight at room temperature. The mixture was filtered and washed several times with MeOH. The yellow product was dried over high vacuum giving **5b** (282 mg, 64%) as a pure yellow product; mp 236–238 °C; (Found: C, 41.9; H, 4.5; N, 34.9; Cl, 0.6. $C_{11}H_{14}N_8O_3 \cdot 0.5\ H_2O \cdot 0.08$ HCl requires C, 41.5; H, 4.8; N, 35.2; Cl, 0.9%);

δ_H(500 MHz, DMSO-d_6) 0.86 (3 H, t, *J* 7.32, -CH_2CH_3), 1.49 (2 H, m, -CH_2CH_3), 3.18 (2 H, m, CH_2NH-), 6.41–7.13 (3 H, m, N*H*-, NH_2), 7.05 (1 H, d, *J* 3.45, furan-C*H*-), 7.76 (1 H, d, *J* 3.45, furan-C*H*-), 8.00–8.04 (1H, m, =C*H*-), 11.15 (1 H, br s, -N*H*=N-); δ_C(125 MHz, DMSO-d_6) 8.4 (CH_2CH_3), 22.3 ($CH_2CH_2CH_2$), 22.5 (CH_2C*H_2CH_2), 41.7 (CH_2NH-), 113.6 (furan-*C*H), 114.0 (furan-*C**H), 115.2 (furan-*C*H), 128.9 (=*C*H-), 129.2 (=*C**H-), 151.4 (furan-*C*), 152.9 (furan-*C*), 164.0 (triazine-*C*), 164.2 (triazine-*C**), 166.0 (triazine-*C*), 166.9 (triazine-*C*), 167.1 (triazine-*C**); *m/z* (EI$^+$) 306.2 (M^+, 10%); 307.1261 (M + H^+. $C_{11}H_{15}N_8O_3^+$ requires 307.1262).

5-Nitro-2-furaldehyde (N^4-isopropyl-4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (5c)

5-Nitrofuraldehyde (92.6 mg, 99%, 0.65 mmol) and N^4-isopropyl-4,6-diamino-[1,3,5]-triazin-2-yl-hydrazine (120 mg, 0.65 mmol) were suspended in MeOH (3 mL) and the suspension left stirring overnight at room temperature. The mixture was filtered and recrystallised by EtOH. The yellow product was dried over high vacuum giving **5c** (0.050 g, 25%) as a yellow solid; mp 178–180 °C; (Found: C, 38.6; H, 4.2; N, 29.6; Cl, 4.0. $C_{11}H_{14}N_8O_3{\cdot}2\,H_2O{\cdot}0.2\,CH_2Cl_2{\cdot}$EtOH requires C, 38.5, H, 5.7, N, 29.0, Cl, 3.7%); δ_H(500 MHz, DMSO-d_6) 1.17 (6 H, d, *J* 6.22, $CH(CH_3)_2$), 4.10 (1 H, m, C*H*(CH_3)$_2$), 7.15–9.18 (3 H, m, N*H*-, NH_2), 7.85 (1 H, m, furan-C*H*), 8.20 (1 H, s, furan-C*H*), 8.25–8.30 (1 H, s, =C*H*-), 13.0 (1 H, br s, -N*H*=N-); δ_C(125 MHz, DMSO-d_6) 22.0 (C(CH_3)$_2$), 42.7 (*C*(CH_3)$_2$), 114.4 (furan-*C*H), 115.5 (furan-*C*H), 134.4 (=*C*H-), 151.7 (furan-*C*-), 151.8 (furan-*C**-), 152.0 (furan-*C*-); *m/z* (ES$^+$) 307.11 ((M + H)$^+$, 100%), 329.2 ((M + Na)$^+$, 20%); 307.1258 (M + H^+. $C_{11}H_{15}N_8O_3^+$ requires 307.1262).

5-Nitro-2-furaldehyde (N^4-butyl-4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (5d)

5-Nitrofuraldehyde (191 mg, 99%, 1.34 mmol) and N^4-butyl-4,6-diamino-1,3,5-triazin-2-yl-hydrazine (264 mg, 1.34 mmol) were suspended in MeOH (5 mL) and left stirring overnight at room temperature. The mixture was filtered and washed several times with MeOH. The yellow product was dried over high vacuum giving **5d** (317 mg, 73%) as a pure yellow product; mp 226–228 °C; (Found: C, 43.9; H, 4.9; N, 33.5; Cl, 0.7. $C_{12}H_{16}N_8O_3{\cdot}0.5\,H_2O{\cdot}0.05$ HCl requires C, 43.5; H, 5.2; N, 33.8; Cl, 0.5%); δ_H(500 MHz, DMSO-d_6) 0.88 (3 H, t, *J* 7.28, -CH_2CH_3), 1.30 (2 H, m, -CH_2CH_3), 1.47 (2 H, m, $CH_2CH_2CH_2$), 3.22 (2 H, m, CH_2NH-), 6.58–7.13 (3 H, m, N*H*-, NH_2), 7.04 (1 H, d, *J* 3.20, furan-C*H*-), 7.77 (1 H, d, *J* 3.20, furan-C*H*-), 8.00–8.04 (1 H, m, =C*H*-), 11.06 (1 H, br s, N*H*=C-); δ_C(125 MHz, DMSO-d_6) 13.7 (CH_2CH_3), 19.6 (-CH_2CH_3), 30.6 ($CH_2CH_2CH_2$), 31.2 (CH_2C*H_2CH_2), 40.0 (CH_2-NH-), 113.6 (furan-*C*H), 114.0 (furan-*C**H), 115.2 (furan-*C*H), 128.9 (=*C*H-), 129.1 (=*C**H-), 151.4 (furan-*C*), 152.9 (furan-*C*), 164.0 (triazine-*C*), 164.2 (triazine-*C**), 166.0 (triazine-*C*), 166.9 (triazine-*C*), 167.1 (triazine-*C**); *m/z* (EI$^+$) 320.2 (M^+, 10%); 321.1415 (M + H^+. $C_{12}H_{17}N_8O_3^+$ requires 321.1418).

5-Nitro-2-furaldehyde {N^4-[3-(*t*-butyl-diphenylsilyl)-oxy]-ethyl}-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (5e)

5-Nitrofuraldehyde (57.0 mg, 99%, 0.40 mmol) and N^4-{[3-(*t*-butyl-diphenylsilyl)-oxy]-ethyl}-4,6-diamino-[1,3,5]-triazin-2-yl-hydrazine (170 mg, 0.40 mmol) were suspended in MeOH (5 mL) and left stirring overnight at room temperature. The mixture was filtered and washed with cold EtOH and with Et_2O. The product was dried over high vacuum giving **5e** (0.020 g, 9%) as a solid; mp 163–165 °C; δ_H(300 MHz, DMSO-d_6) 0.99 (9 H, s, 3 CCH_3), 3.48 (2 H, m, -CH_2-NH-), 3.73 (2 H, m, -CH_2-O-), 6.40–7.00 (3 H, 3 m, N*H*-, -NH_2), 7.43 (6 H, m, Ar-C-*H*), 7.63 (4 H, m, Ar-C-*H*), 7.76 (1 H, d, *J* 3.24, furan-C-*H*), 7.84 (1 H, d, *J* 3.24, furan-C-*H*), 8.02 (1 H, s, =C*H*-), 11.10 (1 H, br s, -N*H*=N-); δ_C(75 MHz, DMSO-d_6) 19.1 (*C*CH_3), 26.9 (*C*CH_3), 42.4 (*C*H_2NH-), 62.8 (-*C*H_2O-), 114.4 (furan-C*H*), 115.6 (furan-*C*H), 120.1 (=*C*H-), 128.2 (Ar-*C*H), 130.1 (Ar-*C*H), 133.5 (Ar-*C*H), 135.4 (Ar-*C*H), 151.8 (furan-*C*), 166.6 (triazine-*C*); *m/z* (ES$^+$) 547.5 ((M + H)$^+$, 100%); 569.2069 (M + Na^+. $C_{26}H_{30}N_8O_4SiNa^+$ requires 569.2057).

5-Nitro-2-furaldehyde {N^4-[3-(*t*-butyl-diphenylsilyl)-oxy]-propyl}-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (5f)

5-Nitrofuraldehyde (81.2 mg, 99%, 0.57 mmol) and N^4-{[3-(*t*-butyl-diphenylsilyl)-oxy]-propyl}-4,6-diamino-[1,3,5]-triazin-2-yl-hydrazine (250 mg, 0.57 mmol) were suspended in EtOH (5 mL) and left stirring at room temperature. The yellow colour turned into a dark orange colour after 30 min. The mixture was left stirring overnight at room temperature and then was filtered and washed with cold EtOH and with Et_2O. The product was dried over high vacuum giving **5f** (264 mg, 84%) as a solid; mp >320 °C; δ_H(500 MHz, DMSO-d_6) 0.99 (9 H, s, CCH_3), 1.81 (2 H, t, *J* 6.23, -CH_2NH-), 3.40 (2 H, m, $CH_2CH_2CH_2$), 3.72 (2 H, t, *J* 6.23, -CH_2O-), 5.95–6.85 (3 H, br m, 3H, N*H*-, -NH_2), 7.05 (1 H, d, *J* 3.59, furan-C*H*), 7.43 (6 H, m, Ar-C*H*), 7.62 (4 H, m, Ar-C*H*), 7.77 (1 H, d, *J* 3.59, furan-C*H*), 8.05–8.10 (1 H, m, =C*H*-), 11.10 (1 H, br s, -N*H*=N-); δ_C(125 MHz, DMSO-d_6) 18.7 (*C*CH_3), 19.6 (*C*H_2NH-), 26.6 (CCH_3), 36.9 ($CH_2CH_2CH_2$), 56.0 (*C*H_2-NH-), 61.5 (-*C*H_2-O-), 115.1 (furan-*C*H), 115.2 (furan-*C*H), 127.8 (Ar-*C*-H), 129.7 (=*C*H-), 133.2 (Ar-*C*-H), 134.9 (Ar-*C*), 151.4 (furan-*C*), 152.9 (furan-*C*), 166.1 (triazine-*C*); *m/z* (ES$^+$) 561.3 (M + H^+, 100%); 561.239 (M + H^+. $C_{27}H_{33}N_8O_4Si^+$ requires 561.2389).

5-Nitro-2-furaldehyde {[N^4-(3-hydroxypropyl-amino)-4,6-diamino-[1,3,5]-triazin]-2-yl}-hydrazone (5g)

Crude N^4-(3-hydroxy-propyl)-2,4-diamino-1,3,5-triazin-2-yl-hydrazine (1.466 g, 14.72 mmol) was dissolved in MeOH (10 mL) and 5-nitrofuraldehyde (2.096 g, 99%, 14.72 mmol), previously dissolved in MeOH (10 mL), was added to the solution. The solution turned into a suspension after 30 min. The mixture was left stirring at room temperature for 2 h. The solvent was then removed under vacuum and the solid purified by flash chromatography (2 × 200 mL DCM 100%, 2 × 200 mL MeOH 0.5% in DCM, 2 × 200 mL MeOH 1% in DCM) giving **5g** (417 mg, 17%) as an orange solid; (Found: C, 39.7; H, 4.5; N, 33.6; Cl, 1.6. $C_{11}H_{14}N_8O_4{\cdot}0.2$ MeOH·0.2 HCl requires C, 40.0; H, 4.5; N, 33.3; Cl, 2.1%); mp 143–145 °C; δ_H(500 MHz, DMSO-d_6) 1.64 (2 H, m, $CH_2CH_2CH_2$), 3.34 (2 H, m, CH_2-NH-), 3.44 (2 H, m, CH_2-OH), 4.46–4.65 (1 H, br m, -OH), 6.32–7.11 (3 H, br m, NH_2, -N*H*-), 7.05 (1 H, d, *J* 2.84, furan-C*H*), 7.77 (1 H, d, *J* 2.84, furan-C*H*), 8.02–8.04 (1 H, m, =C*H*-), 11.08–11.23 (1 H, br s, 1H, -N*H*=N-); δ_C(125 MHz, DMSO-d_6) 32.4 ($CH_2CH_2CH_2$), 32.7 (CH_2C*H_2CH_2), 36.9 (CH_2-NH-), 37.2 (C*H_2-NH-),

58.5 (CH_2-OH), 113.7 (furan-*C*H), 115.2 (furan-*C*H), 128.9 (=*C*H-), 129.2 (=*C**H-), 151.5 (furan-*C*), 152.9 (furan-*C*), 164.0 (triazine-*C*), 166.1 (triazine-*C*), 167.1 (triazine-*C*); δ_C(100 °C, 125 MHz, DMSO-d_6) 34.6 ($CH_2CH_2CH_2$), 60.7 (CH_2-OH), 114.4 (furan-*C*H), 116.6 (furan-*C*H), 131.6 (=*C*H-), 153.5 (furan-*C*), 155.3 (furan-*C*), 166.3 (triazine-*C*), 168.4 (triazine-*C*), 169.2 (triazine-*C*); *m*/*z* (ES^+) 323.12 ($(M + H)^+$, 100%); 323.1211 (M + H^+. $C_{11}H_{15}N_8O_4^+$ requires 323.1211).

3-(4-(2-((5-Nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)propanoic acid (5h)

5-Nitro-furaldehyde (721 mg, 5.1 mmol) added to starting material **4h** (700 mg, 3.27 mmol) dissolved in MeOH (20 mL) and resultant yellow suspension was stirred overnight at room temperature. Tlc analysis (RP C18 plates, 1:1 MeCN:H_2O) showed the triazine starting material (Rf = 0.7) still present. A further aliquot of 5-nitro-furaldehyde (400 mg, 2.8 mmol) was added and the reaction was stirred at 35 °C overnight whereupon tlc analysis showed complete loss of triazine starting material. The reaction was cooled to room temperature, filtered, washed with MeOH (10 ml) and air dried 10 min to yield the crude compound as a dark brown solid (1.284 g). The crude material was purified by ion exchange chromatography on DOWEX WX8/200 sulfonic acid resin eluting with a step wise gradient of water, 0.5 M HCl, 1.0 M HCl, 1.5 M HCl, (200 mL per step), 2.0 M HCl (the compound now begins to elute 1000 mL), 5% MeCN in 2.0 M HCl, (400 mL), 10% MeCN in 2.0 M HCl, (400 mL). UV absorbance of each fraction was measured at 240 nm and the fractions containing the compound were reduced in batches of 5 and checked by mass spec analysis before combining to yield the title compound **5h** (32%) as a pale yellow solid; ν_{max}/cm^{-1} (KBr) 3500–2700 {OH (water), CO_2H, NH_2}, ~2900 (CH_2), ~1700 (C=O acid), 1642 (C=N), 1526, 1352 (C-NO_2); δ_H(500 MHz, D_2O) 2.5 (2 H, m, CH_2), 3.5 (2 H, m, CH_2), 7.0 (1 H, m, furan-C*H*), 7.5 (1 H, s, N=C*H*), 8.1 (1 H, m, furan C*H*); δ_C(125 MHz, DMSO-d_6) 33.3 (CH_2), 36.8 (CH_2), 114.9 (2 furan-*C*H), 134.9 (N=*C*H), 151.7 (2 furan-*C*), 156.2 (triazine-*C*-NH-alkyl), 172.5 (*C*=O), 187.3 (triazine-C-NHN=); *m*/*z* 337 $(M + H)^+$; 337.1003 (M + H^+. $C_{11}H_{13}N_8O_5^+$ requires 337.1014).

4-(4-(2-((5-Nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)butanoic acid (5i)

5-Nitrofuraldehyde (300 mg, 2.1 mmol) was added to starting material **4i** (263 mg, 1.2 mmol) dissolved in MeOH (10 mL) and the resulting yellow suspension stirred overnight at room temperature. The orange mixture was filtered, washed with MeOH (2 × 20 mL) and air dried 20 min to afford 551 mg of crude. The crude material was purified by ion exchange chromatography on DOWEX WX8/200 sulfonic acid resin eluting with a step wise gradient of water, 200 mL HCl (0.5M), 200 mL HCl (1.0M), 200 mL HCl (1.5M), 1600 mL HCl (2.0M) (the compound now begins to elute), 400 mL 5% MeCN in HCl (2.0M), 400 mL 10% MeCN in HCl (2.0M), 400 mL 15% MeCN in HCl (2.0M), 400 mL 20% MeCN in HCl (2.0M). UV absorbance of each fraction was measured at 240 nm and the fractions containing the compound reduced under vacuum to yield a pale yellow solid **5i** (321 mg, 79%). mp 185–188 °C; ν_{max}/cm^{-1} (KBr) 3500–2700 {OH (water), NH_2}, ~2900 (CH_2), ~1700 (C=O acid), 1642 (C=N), 1526, 1352 (C-NO_2); δ_H(500 MHz, DMSO-d_6) 1.72 (2 H, m, CH_2), 2.24 (2 H, m, CH_2), 3.28 (2 H, m, CH_2), 7.81 (1 H, m, furan-C*H*), 7.83 (s, 1 H, N=C*H*), 8.15 (1H, d, *J* 16.4, furan-C*H*), 8.20–9.20 (4H, 3 br s, NH_2 + 2 N*H*), 12.90 (1H, m, CO_2H); δ_C(125 MHz, DMSO-d_6) 24.0 (CH_2), 30.9 (CH_2), 39.6 (CH_2), 115.0 and 115.1 (2 furan-*C*H), 134.9 (N=*C*H), 151.7 (furan-*C*), 151.8 (furan-*C*), 156.2, 156.6 and 158.2 (triazine-*C*), 174.0 (*C*=O); *m*/*z* (ES^+) 351$(M + H)^+$; 351.1163 (M + H^+. $C_{12}H_{15}N_8O_5^+$ requires 351.1160).

6-(4-(2-((5-Nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)hexanoic acid (5j)

5-Nitrofuraldehyde (77 mg, 0.5 mmol) was added to starting material **4j** (76 mg, 0.3 mmol) dissolved in MeOH (2.8 mL) with molecular sieves (4 Å) and the resulting yellow suspension stirred for 24 h at room temperature. Mass spec analysis of the solution showed the presence of starting material **4j**, therefore, more 5-nitrofuraldehyde (25 mg, 0.2 mmol) was added and the resulting solution stirred a further 4 h at 40 °C. Mass spec of the solution showed the only presence of compound **5j**. The solution was left to cool down to room temperature (1 h), filtered and washed with MeOH (10 mL) to afford a pure solid as **5j** (63 mg, 56%). mp 174–176 °C; ν_{max}/cm^{-1} (KBr) 3500–2700 {OH (water), CO_2H, NH_2}, ~2900 (CH_2), ~1700 (C=O acid), 1641 (C=N), 1522, 1354 (C-NO_2); δ_H(500 MHz, DMSO-d_6) 1.29 (2 H, m, CH_2), 1.50 (4 H, m, 2 x CH_2), 2.21 (2 H, t, *J* 7.0, CH_2), 3.21 (2 H, m, CH_2), 6.2–7.3 (3 H, 3 br s, NH_2 and N*H*), 7.06 (1 H, d, *J* 3.7, furan-C*H*), 7.78 (1H, d, *J* 3.7, furan-C*H*), 8.02 (1H, d, J 17.9, N=C*H*), 11.12 (1 H, m, N*H*); δ_C(125 MHz, DMSO-d_6) 24.3 and 29.0 (2 CH_2), 26.0 (CH_2), 33.6 (CH_2), 39.7 (CH_2), 113.8 and 115.3 (2 furan-*C*H), 128.8 (N=*C*H), 151.5 (furan-*C*), 153.0 (furan-*C*), 164.1, 166.1 and 167.2 (triazine-*C*), 174.5 (*C*=O); *m*/*z* (ES^+) 379 $(M + H)^+$; 379.1473 (M + H^+. $(C_{14}H_{19}N_8O_5)^+$ requires 379.1478).

5-Nitro-2-furaldehyde [N^4-(3-acetyl-oxy)-propyl]-4,6-diamino-[1,3,5]-triazin-2-yl-hydrazone (6a)

5-Nitro-2-furaldehyde [N^4-(3-hydroxypropyl)-4,6-diamino-[1,3,5]-triazin]-2-yl-hydrazone (117 mg, 0.36 mmol) was suspended in dry pyridine (5 mL) and acetic anhydride (39.8 mg, d = 1.08, 0.04 mL, 0.39 mmol) was added to the suspension at 0 °C. The mixture was left stirring for 30 min and DMAP (catalytic, 10 mg) was then added. The temperature was left stirring at room temperature for 20 min. The reaction was quenched with MeOH and the solvent was removed under vacuum. The crude was purified by flash chromatography (200 mL DCM, 2 × 100 mL 2% MeOH in DCM, 2 × 100 mL 5% MeOH in DCM) giving **6a** (85 mg, 65%) as an orange product; (Found: C, 42.8; H, 4.5; N, 29.2; Cl, 0.4. $C_{13}H_{16}N_8O_5$·0.5 MeOH ·0.05 HCl requires C, 42.4; H, 4.7; N, 29.3; Cl, 0.5%); mp 142–144 °C; δ_H(500 MHz, DMSO-d_6) 1.81 (2 H, t, *J* 6.33, $CH_2CH_2CH_2$), 2.01 (3 H, s, CH_3-C=O), 3.25 (2 H, m, CH_2-NH, 4.04 (2 H, t, *J* 6.33, CH_2-O-), 6.40–7.58 (3 H, br m, N*H*-, NH_2-), 7.05 (1 H, s, furan-C*H*), 7.77 (1 H, d, *J* 3.93, furan-C*H*), 8.01, 8.04 (1 H, m, N=C*H*), 11.08, 11.16 (1 H, br m, N=C*H*); δ_C(125 MHz, DMSO-d_6) 20.6 (CH_3-C=O), 20.7 (*C**H_3-C=O), 28.2 (-$CH_2CH_2CH_2$-), 28.4 (-CH_2*C**H_2CH_2), 36.9 (-CH_2-NH-), 37.1 (-*C**H_2-NH-), 61.7 (-CH_2-O-), 61.8 (-*C**H_2-O-), 113.7 (furan-*C*H), 114.4 (furan-*C**H), 115.2 (furan-*C*H), 116.0

(furan-*C**H), 129.0 (N=*C*H), 151.5 (furan-*C*), 152.9 (furan-*C*), 164.0 (triazine-*C*-NH-alkyl), 166.1 (triazine-*C*-NHN=), 167.1 (triazine-*C*-NH_2), 170.4 (*C*=O); *m/z* (ES^+) 365 (M + H^+, 100%); 365.1331 (M + H^+. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 365.1316).

Ethyl 3-(4-(2-((5-nitrofuran-2-yl)methylene)-hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)propanoate (6b)

HCl (g) generated by the addition of H_2SO_4 to NaCl was bubbled for 30 min through a suspension of the starting material **5h** (40 mg, 1.1mmol) in EtOH (20 mL) at room temperature. The flask was sealed and the reaction mixture was stirred overnight at room temperature. The resultant solution was reduced in vacuum to yield the title compound (36 mg, 84%) as a pale yellow solid. ν_{max}(KBr)/cm^{-1} 3500–2700 OH (water), NH_2, ~2900 CH_2, CH_3, 1760 C=O ester, 1639 C=N, 1529, 1354 C-NO_2; δ_H(500 MHz, acetone-d_6) 1.3 (3 H, m, CH_3), 2.7 (2 H, bm, CH_2), 3.7 (2 H, bm, CH_2), 4.2 (2 H, m, -OCH_2-), 7.5 (1 H, m, furan-C*H*), 8.0 (1 H, bs, N=C*H*), 8.4 (1 H, m, furan-C*H*); δ_C(125 MHz, DMSO-d_6) 14.2 (*C*H_3), 33.2 (*C*H_2), 36.6 (*C*H_2), 60.2 (-O*C*H_2), 115.2 (2 furan-*C*H), 135 (N=*C*H), 151.7 (furan-*C*), 151.8, 170.9 (*C*=O). *m/z* (ES^+) 365 (M + H^+, 100%); 365.1310 (M + H^+. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 365.1316).

Benzyl 3-(4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)propanoate (6c)

Thionyl chloride (10 µl, 0.13 mmol) was added to a solution of the starting material **5h** (45 mg, 0.13 mmol) in BnOH (0.5 mL) cooled to 0 °C. The resultant solution was stirred at 0 °C for 10 min and then at room temperature for 48 h. Mass spec analysis showed the starting material triazine was still present therefore a further aliquot of thionyl chloride (10 µL, 0.13 mmol) was added and the mixture was stirred at 65 °C for 90 min whereupon mass spec analysis showed complete loss of the triazine starting material. The viscous reaction mixture was washed with hexane (3 × 5 ml), diethyl ether (2 × 5 mL) and dried under high vacuum for 4 h to yield the title compound (36 mg, 57%) as a pale yellow solid. ν_{max}(KBr)/cm^{-1} 3500–2700 OH (water), NH_2, ~2900 CH_2, ~1710 C=O ester, 1636 C=N, 1530, 1402 C-NO_2, 740, 698 5 adjacent Ph; δ_H(500 MHz, DMSO-d_6) 2.7 (2 H, bm, CH_2), 3.6 (2 H, bm, CH_2), 5.1 (2 H, s, Bn-CH_2), 7.4–7.3 (5 H, m, Ar-C*H*), 7.6–7.4 (5 H, m, furan-C*H*, -NH_2, 2 N*H*), 7.9 (1 H, bs, N=C*H*), 8.4 (1 H, m, furan C*H*); δ_C(125 MHz, DMSO-d_6) 33.1 (*C*H_2), 38.9 (*C*H_2), 65.7 (Bn-*C*H_2), 115 (2 furan-*C*H), 126.4 (Ar-*C*H), 126.6 (Ar-*C*H), 127.9 (Ar-*C*H), 128.0 (Ar-*C*H), 128.4 (Ar-*C*H), 135.9 (N=C*H*), 142.4 (Ar-*C*), 151.6 (furan-*C*), 151.8 (furan-*C*), 170.8 (triazine-*C*), 187.3 (*C*=O). *m/z* (ES^+) 427.1 (M + H^+, 100%); 427.1473 (M + H^+. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 427.1476).

Ethyl 4-(4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-yl amino)butanoate (6d)

Thionyl chloride (30 µl, 0.39 mmol) was added to a solution of the starting material **5i** (25 mg, 0.07 mmol) in EtOH (5 mL) at room temperature. The resultant solution was stirred at room temperature for 15 h whereupon a faint precipitate formed. The reaction mixture was reduced in vacuum to give a pale orange solid which was dissolved in MeOH (0.5 mL), triturated with hexane (5 mL), washed with diethyl ether (5 mL), reduced in vacuum and then dried under high vacuum for 4 h to yield the title compound (24 mg, 88%) as a pale yellow solid. mp 172–174 °C; ν_{max}(KBr)/cm^{-1} 3500–2700 OH (water), NH_2, ~2900 CH_2, CH_3, ~1750 C=O ester, 1641 C=N, 1530, 1354 C-NO_2; δ_H(500 MHz, DMSO-d_6) δ 1.12 (3 H, m, CH_3) 1.73 (2 H, t, *J* 6.5, CH_2), 2.29 (2 H, t, *J* 7.2, CH_2), 3.27 (2 H, m, CH_2), 3.99 (2 H, q, *J* 7.0, OCH_2), 7.80 (1 H, m, furan-C*H*), 8.13 (1 H, d, *J* 9.1, furan-C*H*), 8.30 (1 H, m, N=C*H*), 8.5–9.2 (3H, m, NH_2 and N-N*H*), 12.80 (1H, bs, N*H*); δ_C(125 MHz, DMSO-d_6) 14.1 (*C*H_3), 24.1 (*C*H_2), 30.7 (*C*H_2), 39.8 (*C*H_2), 59.9 (O*C*H_2), 115.0 (2 furan-*C*H), 135.0 (N=*C*H), 151.4 (furan-*C*), 151.8 (furan-*C*), 151.9 (furan-*C**), 172.5 (*C*=O). *m/z* (ES^+) 379.1 (M + H^+, 100%); 379.1473 (M + H^+. $C_{14}H_{19}N_8O_5^{+\bullet}$ requires 379.1473).

Benzyl 4-(4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)butanoate (6e)

Thionyl chloride (30 µl, 0.39 mmol) was added to a solution of the starting material **5i** (40 mg) in BnOH (0.5 mL) at room temperature. The resultant viscous solution was heated at 85 °C for 30 min. The viscous reaction mixture was allowed to cool to room temperature, washed with hexane (2 × 5 mL), diethyl ether (2 × 5 mL) and dried under high vacuum for 2 × 8 h to yield the title compound (27 mg, 54%) as a pale yellow solid. ν_{max}(KBr)/cm^{-1} 3500–2700 OH (water), NH_2, ~2900 CH_2, ~1710 C=O ester, 1636 C=N, 1522, 1397 C-NO_2, 737, 698 5 adjacent Ph; δ_H(500 MHz, DMSO-d_6) δ 1.8 (2 H, bm, CH_2), 2.4 (2 H, bm, CH_2), 3.4 (2 H, bm, CH_2), 5.1 (2 H, s, Bn-CH_2), 7.4-7.3 (6 H, m, furan-C*H*, 5 Ar-C*H*), 7.9 (1 H, bs, -N=C*H*), 8.3 (1 H, m, furan-C*H*), 8.6 (1 H, bm, N*H*); δ_C(125 MHz, DMSO-d_6) 24.0 (*C*H_2) 30.7 (*C*H_2), 65.4 (Bn-*C*H_2), 114.9 (furan-*C*H), 115 (furan-*C*H), 126.4 (Ar-*C*H), 126.6 (Ar-*C*H), 127.9 (Ar-*C*H), 128.0 (Ar-*C*H), 128.4 (Ar-*C*H), 134.5 (N=C*H*), 136.1 (Ar-*C*), 151.5 (furan-*C*), 151.7 (furan-*C*), 151.8 (furan-*C**), 172.4 (triazine-*C*), 187.4 (*C*=O). *m/z* (ES^+) 441 (M + H^+, 100%); 441.1629 (M + H^+. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 441.1630).

Ethyl 6-(4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)hexanoate (6f)

Thionyl chloride (30 µl, 0.39 mmol) was added to a solution of the starting material **5j** (45 mg, 0.11 mmol) in EtOH (5 mL) at room temperature. The resultant solution was stirred at room temperature for 15 h whereupon a faint precipitate formed. The reaction mixture was reduced in vacuum to give a pale orange solid which was dissolved in MeOH (0.5 mL), triturated with hexane (5 mL), washed with diethyl ether (5 mL), reduced in vacuum and then dried under high vacuum for 4 h to yield the title compound (32 mg, 67%) as a pale yellow solid. ν_{max}(KBr)/cm^{-1} 3500–2700 OH (water), NH_2, ~2900 CH_2, CH_3, ~1750 C=O ester, 1641 C=N, 1530, 1354 C-NO_2; δ_H(500 MHz, DMSO-d_6) 1.2 (3 H, m, CH_3) 1.3 (2 H, m, CH_2), 1.6 (4 H, m, 2 CH_2), 2.3 (2 H, m, CH_2), 3.4 (2 H, m, CH_2), 4.1 (2 H, quartet, OCH_2), 7.7 (1 H, m, furan-C*H*), 7.9 (1 H, bs, -N=C*H*), 8.3 (1 H, m, furan-C*H*); δ_C(125 MHz, DMSO-d_6) 18.4 (*C*H_3) 21.9, 24.0 (*C*H_2), 25.7 (*C*H_2), 28.3 (*C*H_2), 33.5 (*C*H_2), 55.8 (O*C*H_2), 114.8 (furan-*C*H), 114.9 (furan-*C*H), 134.9 (N=C*H*), 151.6 (furan-*C*), 151.7 (furan-*C*), 151.8 (furan-*C**), 172.8 (*C*=O), 174.3 (triazine-*C*). *m/z* (ES^+) 407 (M + H^+, 100%); 407.1785 (M + H^+. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 407.1787).

Benzyl 6-(4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)hexanoate (6g)

Thionyl chloride (30 µl, 0.39 mmol) was added to a solution of the starting material **5j** (35 mg, 0.1 mmol) in BnOH (0.5 mL) at room temperature overnight. The resultant viscous solution was stirred at room temperature. The viscous reaction mixture was washed with hexane (2 × 5 ml), diethyl ether (2 × 5 mL) and dried under high vacuum for 2 × 8 h to yield the title compound (42 mg, 97%) as a pale yellow solid. ν_{max}(KBr)cm^{-1} 3500–2700 OH (water), NH_2, ~2900 CH_2, ~1710 C=O ester, 1636 C=N, 1522, 1397 C-NO_2, 737, 698 5 adjacent Ph; δ_H(500 MHz, DMSO-d_6) 1.3 (2 H, m, CH_2), 1.5 (4 H, bm, 2 CH_2), 2.3 (2 H, t, *J* 6.9, CH_2), 3.4 (2 H, t, *J* 7.0, CH_2), 5.1 (2 H, s, Bn-CH_2), 7.4–7.3 (6 H, m, furan-C*H*, 5 Ar-C*H*), 7.9 (1 H, s, N=C*H*), 8.3 (1 H, m, furan-C*H*); δ_C(125 MHz, DMSO-d_6) 22.4 (*C*H_2) 24.1 (*C*H_2), 25.7 (*C*H_2), 28.3 (*C*H_2), 33.4 (*C*H_2), 65.4 (Bn-*C*H_2), 114.9 (furan-*C*H), 115 (furan-*C*H), 126.4 (Ar-*C*H), 126.6 (Ar-*C*H), 127.9 (Ar-*C*H), 128.0 (Ar-*C*H), 128.4 (Ar-*C*H), 134.0 (N=*C*H), 136.2 (Ar-*C*), 151.7 (furan-*C*), 152.2 (furan-*C*), 172.7 (*C*=O). *m*/*z* (ES$^+$) 469.2 (M + H$^+$, 100%); 469.1942 (M + H$^+$. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 469.1942).

Methyl 6-(4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-6-amino-1,3,5-triazin-2-ylamino)hexanoate (6h)

Thionyl chloride (30 µl, 0.39 mmol) was added to a solution of the starting material **5j** (45 mg, 0.11 mmol) in BnOH (0.5 mL) at room temperature. The resultant viscous solution was stirred at room temperature overnight. The viscous reaction mixture was washed with hexane (2 × 5 ml), diethyl ether (2 × 5 mL) and dried under high vacuum for 2 × 8 h to yield the title compound (30 mg, 65%) as a pale yellow solid. δ_H (500 MHz, DMSO-d_6) 1.3 (2 H, bm, CH_2), 1.5 (4 H, m, 2 CH_2), 2.3 (2 H, bm, CH_2), 3.3 (2 H, bm, CH_2), 7.3 (1 H, m, furan C*H*), 7.9 (1 H, bs, N=C*H*), 8.3 (1 H, m, furan-C*H*); δ_C (125 MHz, DMSO-d_6) 24.0 (*C*H_2), 25.6 (*C*H_2), 28.3 (*C*H_2), 33.1 (*C*H_2), 33.5 (*C*H_2), 51.2 (*C*H_3-O), 114.3 (furan-*C*H), 114.7 (furan-*C*H), 134.5 (N=*C*H), 151.5 (furan-*C*), 151.7 (furan-*C*), 151.8 (furan-*C**), 173.4 (*C*=O), 174.3 (triazine C). *m*/*z* (ES$^+$) 393.2 (M + H$^+$, 100%); 393.1629 (M + H$^+$. $C_{13}H_{17}N_8O_5^{+\bullet}$ requires 393.1633).

2-Acetyl-5-nitro-furan (8b)

In a first flask, 2-acetylfuran **7b** (252 mg, 2.27 mmol) was dissolved in acetic anhydride (0.4 mL, 4.20 mmol) and stirred at 0 °C. In a second flask, nitric acid 70% (0.2 mL, 3.10 mmol) was slowly added to acetic anhydride (0.6 mL, 6.30 mmol) at 0 °C. The nitric acid solution was then added dropwise to the starting material at 0 °C. The resulting mixture was left stirring 3 h at rt. TEA was then added and the solution stirred a further 1 h at rt, extracted with $CHCl_3$ (2 × 25 mL), dried, concentrated and purified by column chromatography ($CHCl_3$/MeOH: 100–96%/0–4%) to afford the pure product (90 mg, 26%) **8b**; δ_H(500 MHz, MeOD) 2.45 (3 H, s, CH_3), 7.35 (1 H, d, *J* 3.9, furan-C*H*), 7.44 (1 H, d, *J* 3.9, furan-C*H*); δ_C(125 MHz, MeOD) 26.2 (*C*H_3), 113.0 (furan-*C*H), 119.0 (furan-*C*H), 152.7 (2 furan-*C*), 188.3 (*C*=O); *m*/*z* (ES$^+$) 155 (M$^+$, 100%); 155.0211 (M$^+$. $C_6H_5NO_4^{+\bullet}$ requires 155.0213).

2-Butyryl-5-nitro-furan (8c)

In a first flask, 2-butyrylfuran (97 mg, 0.7 mmol) was dissolved in acetic anhydride (0.24 mL, 2.5 mmol) and stirred at 0 °C. In a second flask, nitric acid 70% (0.14 mL, 2.2 mmol) was slowly added to acetic anhydride (0.34 mL, 3.6 mmol) at 0 °C. The nitric acid solution was then dropwise added to the starting material at 0 °C. The resulting mixture was left stirring 3 h at room temperature. TEA (0.13 mL, 0.9 mmol) was then added and the solution stirred a further 1 h at room temperature, extracted with $CHCl_3$ (2 x 15 mL), dried, concentrated and purified by column chromatography ($CHCl_3$/MeOH: 100–96%/0–4%) to afford product **8c** (69 mg, 53%); δ_H(500 MHz, MeOD) 1.04 (3 H, t, *J* 7.4, CH_3), 1.80 (2 H, qt, *J* 7.4, CH_2-CH_3), 2.95 (2 H, t, *J* 7.3, -CH_2-CO-), 7.28 (1 H, d, *J* 3.8, furan-C*H*), 7.39 (1 H, d, *J* 3.8, furan-C*H*,); δ_C(125 MHz, MeOD): 13.7 (*C*H_3-), 17.0 (-*C*H_2-CH_3,), 40.6 (-*C*H_2-CO-), 111.9 (furan-*C*H), 116.5 (furan-*C*H), 151.6 (furan-*C*), 189.6 (*C*=O).

5-Nitro-2-imidazolcarboxaldehyde (8d)

Nitric acid 70% (3.0 mL, 46.5 mmol) was added to 2-imidazolecarboxaldehyde **7c** (127 mg, 1.3 mmol) at room temperature and stirred 30 min at room temperature. Then the solution was concentrated under vacuum to afford compound **8d** (185 mg, 100%); mp = 148–149 °C; (Found: C, 29.6; H, 3.1; N, 25.8 $C_4H_3N_3O_3{\cdot}1.2H_2O$ Requires C, 29.5 H, 3.3; N, 25.8%) δ_H(500 MHz, DMSO) 6.06 (1H, s, C*H**(OH)$_2$), 7.61 (2 H, s, imidaz-C*H** and N*H**), 7.81 (2 H, s, imidaz-C*H* and N*H*), 9.69 (1 H, s, -C*H*O), 14.30 (2 H, br s, 2-O*H**); δ_C(125 MHz, DMSO) 83.2 (-*C*H*(OH)$_2$), 119.2 (imidaz-*C*H* and -*C**-NO_2), 125.0 (imidaz-*C*H and -*C*-NO_2), 142.6 (-*C*-CHO), 148.2 (-*C**-CH(OH)$_2$), 178.6 (*C*=O).

2-Nitro-5-imidazolcarboxaldehyde (8e)

Nitric acid 70% (3.0 mL, 46.5 mmol) was added to 4(5)-imidazolecarboxaldehyde **7e** (143 mg, 1.5 mmol) at room temperature and stirred 30 min at room temperature. Then the solution was concentrated under vacuum to afford compound **8e** (197 mg, 94%); * indicates the hemiacetal form. mp = 160–161 °C; δ_H(500 MHz, DMSO-d_6) 5.90 (1 H, s, C*H**(OH)$_2$), 7.44 (1 H, s, imidaz-C*H**), 8.57 (1 H, s, imidaz-C*H*), 8.80 (1 H, s, imidaz-N*H**) 9.28 (1 H, s, imidaz-N*H*), 9.86 (1 H, s, -C*H*O); δ_C(125 MHz, DMSO-d_6) 83.0 (C*H**(OH)$_2$), 115.5 (imidaz-*C*H*), 128.4 (imidaz-*C*H), 132.9 (-*C*-CHO), 133.5 (-*C**-NO_2), 135.9 (-*C**-C(OH)$_2$), 138.2 (-*C*-NO_2), 181.1 (*C*=O).

5-Carboxylic acid-2-furaldehyde-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (9a)

A mixture of 2-hydrazino-4,6-diamino-1,3,5-triazine (51 mg, 0.36 mmol) and 5-formyl-2-furancarboxylic acid (50 mg, 0.36 mmol) were stirred in methanol (3 mL) at room temperature for 6 h. The mixture was filtered and the solid washed with methanol (20 mL) to afford a pure product (50 mg, 53%) (C, 37.3 H, 3.8; N, 32.9. $C_9H_9N_7O_7{\cdot}0.5HCl{\cdot}0.5H_2O{\cdot}0.2MeOH$ requires C, 37.2; H, 3.8; N, 33.0); δ_H(500 MHz, DMSO-d_6) 6.40 (4H, br s, 2 N*H*$_2$), 6.83 (1 H, d, *J* 3.6, furan-C*H*), 7.26 (1 H, d, *J* 3.6, furan-C*H*), 7.97 (1 H, s, N=C*H*), 10.85 (1 H, bs, N=N*H*); δ_C(125 MHz, DMSO-d_6) 112.9 (furan-*C*H), 119.3 (furan-*C*H), 130.4 (N=*C*H), 144.8 (furan-*C*), 153.1 (furan-*C*), 159.3 (*C*OOH), 164.6 (2 triazine-*C*-NH_2), 167.2 (triazine-*C*-NH-N=); *m*/*z* (ES$^+$)

m/z 264.1 (M + H$^+$, 100%), 264.0836 (M + H$^+$. $C_9H_{10}N_7O_3^{+\bullet}$ requires 264.0840).

Methyl-5-nitro-furyl ketone-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (9b and 9c)

2-Hydrazino-4,6-diamino-1,3,5-triazine (**4a**, 245 mg, 1.74 mmol.) and 5-nitro-2-acetylfuran (8b, 235 mg, 1.52 mmol) were dissolved in methanol (30 mL) and stirred 24 h at room temperature with molecular sieves. Starting material was filtered off and washed with methanol (50 mL). The organic phase was concentrated and purified by column chromatography ($CHCl_3$/MeOH: 100–70/0–30) to afford a mixture of diastereoisomers. Preparative chromatography ($CHCl_3$/MeOH: 90/10) allowed the purification of compounds **9c** (80 mg, 32%) and **9b** (46 mg, 18%).

Compound 9c. δ_H(500 MHz, MeOD) 1.30 (3 H, s, CH_3-), 3.61 (4 H, bs, 2-NH_2), 7.14 (1 H, bs, N=N*H*), 7.34 (1 H, d, *J* 8.6, furan-C*H*), 8.05 (1 H, d, *J* 8.7, furan C-*H*). δ_C(125 MHz, MeOD) 23.8 (*C*H$_3$), 119.9 (furan-*C*H) and 130.1 (furan-*C*H), 136.6 and 156.5 (N=*C*H) and (furan-*C*), 159.3 (furan-*C*-NO$_2$), 165.7 (triazine-*C*-NH-N=), 168.6 (triazine-*C*-NH$_2$). *m/z* (ES$^+$): *m/z* 278.9 (M + H$^+$, 100%); 279.0957 (M + H$^+$. $C_9H_{11}N_8O_3^{+\bullet}$ requires 279.0949).

Compound 9b. δ_H(500 MHz, MeOD) 1.34 (3 H, s, C*H*$_3$), 3.52 (4 H, bs, 2-NH_2), 7.17 (1 H, bs, N=N*H*), 7.53 (1 H, d, *J* 8.5, furan-C-*H*), 7.65 (1 H, d, *J* 8.5, furan-C-*H*); δ_C(125 MHz, MeOD) 23.6 (*C*H$_3$), 119.2 (furan-*C*H) and 128.8 (furan-*C*H), 136.4 and 153.0 (N=*C* and furan-*C*), 159.1 (furan-*C*-NO$_2$), 165.9 (triazine-*C*-NH-N=), 168.3 (triazine-*C*-NH$_2$).

Propyl-5-nitro-furyl ketone-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (9d)

2-Hydrazino-4,6-diamino-1,3,5-triazine (**4a**, 138 mg, 1.0 mmol) and 5-nitro-2-butyrylfuran (163 mg, 0.9 mmol) were dissolved in methanol (21 mL) and stirred 48 h at room temperature with molecular sieves. Molecular sieves were filtered, washed with methanol (20 mL) and the solute concentrated. The organic phase was purified by column chromatography ($CHCl_3$/MeOH:100–0/0–10) to afford a non separable mixture of diastereoisomers **9d** (49 mg, 18%). mp: 249–251 °C; δ_H(500 MHz, MeOD) 0.91 (3 H, t, *J* 7.4, C*H*$_3$), 0.96 (3 H, t, *J* 7.4, C*H*′$_3$), 1.58 (4 H, m, CH$_3$-C*H*$_2$- and CH$_3$-C′*H*$_2$-), 2.60 (2 H, m, -CH$_2$-C*H*$_2$-), 2.69 (2 H,m, -CH$_2$-C′*H*$_2$-), 7.14 (1 H, d, *J* 4.0, furan-C-*H*), 7.21 (1 H, d, *J* 3.9, furan-C′-*H*), 7.44 (1 H, d, *J* 3.9, furan-C-*H*,), 7.50 (1 H, d, *J* 4.0, furan-C′-*H*,); δ_C(125 MHz, MeOD) 14.1 (*C*H$_3$), 14.2 (*C*′H$_3$), 20.5 (CH$_3$-*C*′H$_2$-), 22.8 (CH$_3$-*C*H$_2$-), 28.3 (-CH$_2$-*C*′H$_2$-), 30.8 (-CH$_2$-*C*H$_2$-), 112.3 (furan-*C*′H), 113.4 (furan-*C*H), 114.9 (furan-*C*′H), 116.9 (furan-*C*H), 143.5 (N=*C*H) + 150.3 (furan-*C*) + 153.0 (N=*C*′H) + 153.0 (furan-*C*′), 156.2 (furan-*C*-NO$_2$ and furan-*C*′-NO$_2$), 165.5 (triazine-*C*-NH-N= and triazine-*C*′-NH-N=), 168.3 (2 triazine-*C*-NH$_2$ and 2 triazine-*C*′-NH$_2$). *m/z* (ES$^+$): *m/z* 307.1 (M + H$^+$, 100%); 307.1257 (M + H$^+$. $C_{11}H_{15}N_8O_3^{+\bullet}$ requires 307.1262).

5-Nitro-2-imidazolcarboxaldehyde-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (9e)

5-Nitro-2-imidazolecarboxaldehyde **8d** (33 mg, 0.23 mmol) and compound **4a** (39 mg, 0.28 mmol) were dissolved in methanol (3 mL) in the presence of molecular sieves, and stirred overnight at room temperature. The precipitate was filtered off and washed with methanol (10 mL). The organic phase was concentrated and the product purified by reverse phase column chromatography (H_2O/CH_3CN: 100–85%/0–15%) to afford product **9e** (14 mg, 19%); mp 247–250 °C; (C, 26.7; H, 3.6; N, 43.8. $C_7H_8N_{10}O_2 \cdot 1.4H_2O \cdot 0.8HCl$ requires C, 26.4 H, 3.7; N, 44.0%), δ_H(500 MHz, DMSO-d$_6$) 6.37 (4 H, bs, 2 NH_2), 7.01 (1 H, s, NH-N=C*H*), 7.17 (1 H, s, imidaz-C*H*), 8.00 (1 H, s, triazine-N*H*-N), 12.50 (1 H, s, imidaz-N*H*); δ_C(125 MHz, DMSO-d$_6$) 118.3 (imidaz-*C*H), 129.3 (N=*C*H) and 133.4 (imidaz-*C*-NO$_2$), 143.3 (imidaz-*C*-C=), 164.6 (triazine-*C*), 167.2 (2 triazine-*C*-NH$_2$); *m/z* 220.1 (M + H − NO$_2^+$, 100%).

2-Nitro-5-imidazolcarboxaldehyde-(4,6-diamino)-[1,3,5]-triazin-2-yl-hydrazone (9f)

2-Nitro-5-imidazolecarboxaldehyde **8e** (115 mg, 0.82 mmol) and compound **4a** (140 mg, 0.99 mmol) were dissolved in methanol (7 mL) in the presence of molecular sieves, and stirred overnight at room temperature. The precipitate was filtered off and washed with methanol (40 mL). The organic phase was concentrated and the product purified by reverse phase column chromatography (H_2O/CH_3CN: 100–75%/0–25%) to afford product **9f** (32 mg, 12%); mp 270–272 °C; δ_H (500 MHz, DMSO-d$_6$) 6.40 (4 H bs, 2 NH_2), 7.16 and 7.33 (1 H, 2 s, imidaz-C*H*), 7.59 and 7.70 (1 H, 2 s, N=C-*H*), 7.99 and 8.01 (1 H, 2 s, N=N*H*), 12.57 and 12.72 (1 H, 2bs, imidaz-N*H*); δ_C(125 MHz, DMSO-d$_6$) 119.7 (imidaz-*C*-C=N), 130.1 (imidaz-*C*-H), 135.8 (N=*C*-H) and 136.1 (imidaz-*C*-NO$_2$), 164.3 (triazine-*C*) 167.1 (2 triazine-*C*).

Acknowledgements

We would like to thank DNDi and Cardiff University for funding. The EPSRC National Mass Spectrometry Service Centre at Swansea is acknowledged for accurate mass spectroscopy.

References

1 M. J. Dinah and H. M. Gilles, *Human antiparasitic drugs: pharmacology and usage*, Wiley, Chichester, 1985.
2 M. P. Barrett and A. H. Fairlamb, *Parasitology Today*, 1999, **15**, 136.
3 H. P. de Koning, C. J. Watson and S. M. Jarvis, *J. Biol. Chem.*, 1998, **273**, 9486.
4 H. P. DeKoning and S. M. Jarvis, *Eur. J. Biochem.*, 1997, **247**, 1102.
5 N. S. Carter, B. J. Berger and A. H. Fairlamb, *J. Biol. Chem.*, 1995, **270**, 28153.
6 N. S. Carter and A. H. Fairlamb, *Nature*, 1993, **361**, 173.
7 M. Griffiths, N. Beaumont, S. Y. M. Yao, M. Sundaram, C. E. Boumah, A. Davies, F. Y. P. Kwong, I. Coe, C. E. Cass, J. D. Young and S. A. Baldwin, *Nature Medicine*, 1997, **3**, 89.
8 M. Griffiths, S. Y. M. Yao, F. Abidi, S. E. V. Phillips, C. E. Cass, J. D. Young and S. A. Baldwin, *Biochem. J.*, 1997, **328**, 739.
9 H. P. De Koning and S. M. Jarvis, *Mol. Pharmacol.*, 1999, **56**, 1162.
10 P. Maser, C. Sutterlin, A. Kralli and R. Kaminsky, *Science*, 1999, **285**, 242.
11 H. P. de Koning, *Mol. Pharmacol.*, 2001, **59**, 586.
12 P. G. Bray, M. P. Barrett, S. A. Ward and H. P. de Koning, *Trends Parasitol.*, 2003, **19**, 232.
13 D. J. Bridges, M. K. Gould, B. Nerima, P. Mäser, R. J. Burchmore and H. P. de Koning, *Mol. Pharmacol.*, 2007, **71**, 1098.
14 C. A. Lanteri, M. Stewart, J. M. Brock, V. P. Alibu, S. R. Meshnick, R. R. Tidwell and M. P. Barrett, *Mol. Pharmacol.*, 2006, **70**, 1585.
15 M. P. Barrett and I. H. Gilbert, *Advan. Parasitol.*, 2006, **63**, 126.
16 R. Millet, L. Maes, V. Landry, C. Sergheraert and E. Davioud-Charvet, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3601.

17 F. W. Jennings, G. M. Urquhart, P. K. Murray and B. M. Miller, *Trans. R. Soc. Trop. Med. Hyg.*, 1983, **77**, 693.
18 J. Williamson, in *Review of chemotherapeutic and chemoprophylatic agents in the trypanosomiases* ed. H. W. Mulligan, George Allen and Unwin, London, 1970, pp. 126-221.
19 D. Legros, G. Ollivier, M. Gastellu-Etchegorry, C. Paquet, C. Burri, J. Jannin and P. Buscher, *Lancet Infect. Dis.*, 2002, **2**, 437.
20 J. Pepin, F. Milford, F. Meurice, L. Ethier, L. Loko and B. Mpia, *Trans. R. Soc. Trop. Med. Hyg.*, 1992, **86**, 254.
21 A. Baliani, G. J. Bueno, M. L. Stewart, V. Yardley, R. Brun, M. P. Barrett and I. H. Gilbert, *J. Med. Chem.*, 2005, **48**, 5570.
22 V. M. Kolb, S. D. Darling, D. F. Koster and C. Y. Meyers, *J. Org. Chem.*, 1984, **49**, 1636.
23 G. Balina, P. Kesler, J. Petre, D. Pham and A. Vollmar, *J. Org. Chem.*, 1986, **51**, 3811.
24 M. L. Stewart, G. J. Bueno, A. Baliani, B. Klenke, R. Brun, J. M. Brock, I. H. Gilbert and M. P. Barrett, *Antimicrob. Agents Chemother.*, 2004, **48**, 1733.

