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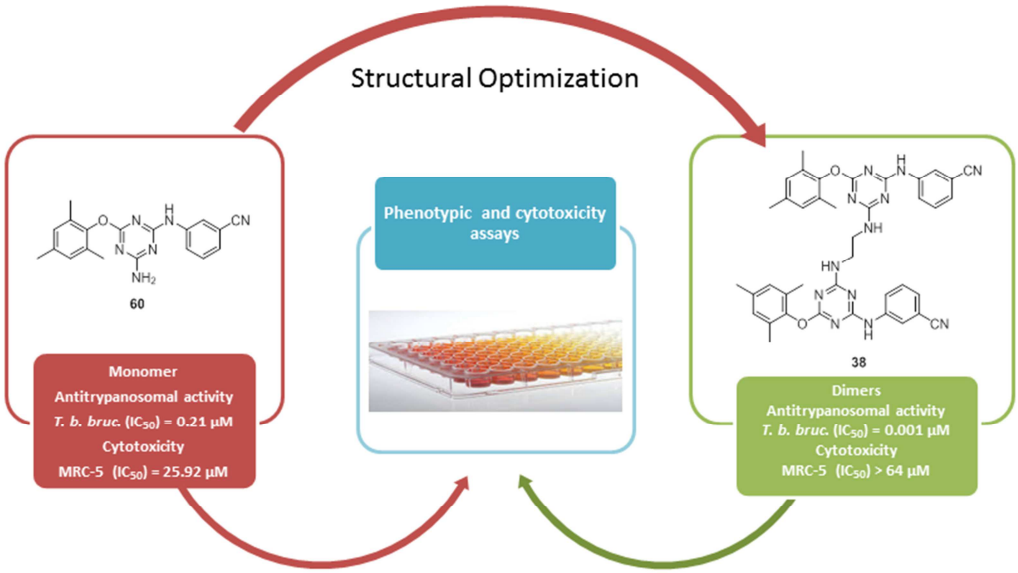

Novel Triazine Dimers with potent antitrypanosomal activity

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ABSTRACT

Human African trypanosomiasis (HAT), also known as *sleeping sickness* is a parasitic disease transmitted by the bite of the 'Glossina' insect, commonly known as the tsetse fly. This disease affects mostly poor populations living in remote rural areas of Africa. Untreated, it is usually fatal. Currently, safe and effective treatments against this disease are lacking. Phenotypic screening of triazine non-nucleoside HIV-1 reverse transcriptase inhibitors (monomers) resulted in potent and selective antitrypanosomal compounds. This serendipitous discovery and the presence of dimers in many compounds active against these neglected tropical diseases prompted us to investigate antitrypanosomal activity of triazine dimers. Optimization of the triazine dimers resulted in 3,3'-(((ethane-1,2-diylbis(azanediyl))bis(4-(mesityloxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (compound **38**), a compound with very potent *in vitro* and moderate *in vivo* antitrypanosomal activity.

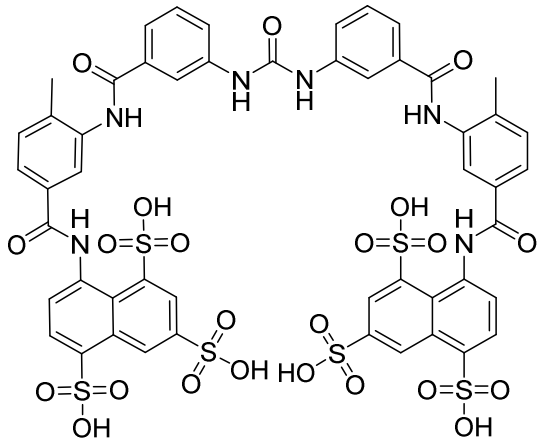
KEYWORDS

Trypanosoma brucei, dimer, microsomal stability, phenotypic screening.

INTRODUCTION

Human African trypanosomiasis (HAT) remains one of the most neglected life threatening diseases [1]. Today, the cases of sleeping sickness are being reduced year by year through control programs: this is how, in 2009, the number of new reported cases was reduced to less than 10 000 for the first time in half a century and in 2016 a historic low of 2184 cases was reported. In the last ten years, over 75% of reported cases occurred in the Democratic Republic of the Congo [2]. HAT is caused by two subspecies of the parasite *Trypanosoma brucei* (*T. b.*), known as *T. b. rhodesiense* and *T. b. gambiense*, while the animal form of the disease (Nagana) is caused by *T. b. brucei* [3]. The treatment of HAT has been unsatisfactory for many years, with four of the main drugs used for early-stage and late-stage disease being orally unavailable, often toxic, and sometimes ineffective [4].

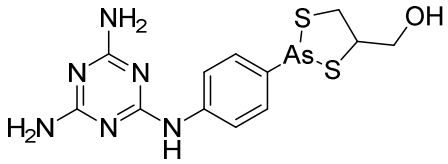
Early stage treatment:



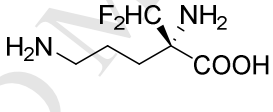
Suramin (IV)

Pentamidine (IM)

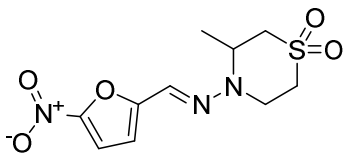
Late stage treatment:



Melarsoprol (IV)



Eflornithine (IV)



Nifurtimox (oral)

Figure 1. Current treatments against HAT. IV: intravenous; IM: intramuscular.

Five licensed compounds are used against HAT today (Figure 1), depending on the causative subspecies and the stage of the disease [5]. In stage 1 of the disease suramin is the first line treatment used for *T. b. rhodesiense* whereas pentamidine is applied against *T. b. gambiense*. This stage occurs 5 to 15 days after the bite of the tsetse fly in which the parasites proliferate in the bloodstream, lymph nodes, liver, and spleen and the symptoms are usually mild. In the late stage, also known as stage 2, where parasites have crossed the blood-brain barrier and have infected the central nervous system. The first line treatment against *T. b. rhodesiense* is melarsoprol and for *T. b. gambiense* a cocktail of eflornithine plus nifurtimox

is administered. This stage is reached after a few weeks in the case of rhodesiense disease, or months in the case of gambiense disease [6].

These drugs produce dangerous side effects such as renal failure in the case of suramin, or encephalopathy caused by melarsoprol. Furthermore, they become ineffective after some time due to an increase in resistance [3,7]. In Table 1, the mechanism of action of these drugs is shown [8]. Most of the mode of actions are still hypothetical.

Drug	Mechanism
Pentamidine	Accumulates in trypanosomes; disrupts mitochondrial processes
Suramin*	Binds to enzymes in the glycosome; disrupts glycolysis
Melarsoprol	Disrupts trypanosomal redox metabolism and glycolysis
Eflornithine	Irreversibly inhibits ornithine decarboxylase (ODC); disruption of proliferation and vulnerability to oxidative attack
NECT (nifurtimox–eflornithine combination treatment)	Eflornithine inhibits ODC; nifurtimox induces oxidative attack upon weakened trypanosomes

**Suramin is also effective against stage I T. b. gambiense, but its use remains confined to infections causes by stage I T. b. rhodesiense.*

Table 1. Treatments used against HAT and their mechanism of action.

RESULTS AND DISCUSSION

Discovery of new *T. b.* inhibitors. It has been demonstrated that phenotypic screening is a successful approach for the discovery of first-in-class drugs [9]. This methodology has the advantage of identifying compounds that are active against the whole cell being especially efficient in infectious disease drug development [10]. As part of our research program for new anti-HIV microbicides [11-14], we have developed a library of triazines [13] as non-nucleoside reverse transcriptase inhibitors (NNRTIs). The structural resemblance of those molecules with melarsoprol, and the results of the pharmacophore of the purine transporter of *T. brucei* [15] prompted us to evaluate the compounds on the parasite. Four triazines were identified as very potent antitrypanosomal compounds (IC_{50} < 0.25 µM) through phenotypic screening [16].

As a tool in drug development, the bivalent ligand approach has been particularly successful in the area of antiprotozoal compounds (**Figure 2**). For example, pentamidine and suramin are dimeric compounds that have been used for more than half a century against HAT [2-3]. Pentostam belongs to the class known as pentavalent antimonials, used for the first-line treatment of leishmaniasis [2, 17]. The dimers such as bis(4-aminoquinolines) [18-19], bis(8-aminoquinolines) [20], bis-artemisinins [21-23], bisacridines [24], bispyrroloquinoxalines [25], bisnaphthalamide [26], bistacrine [27] and bisbenzimidazoles [28-29] are known to display superior antiprotozoal activities.

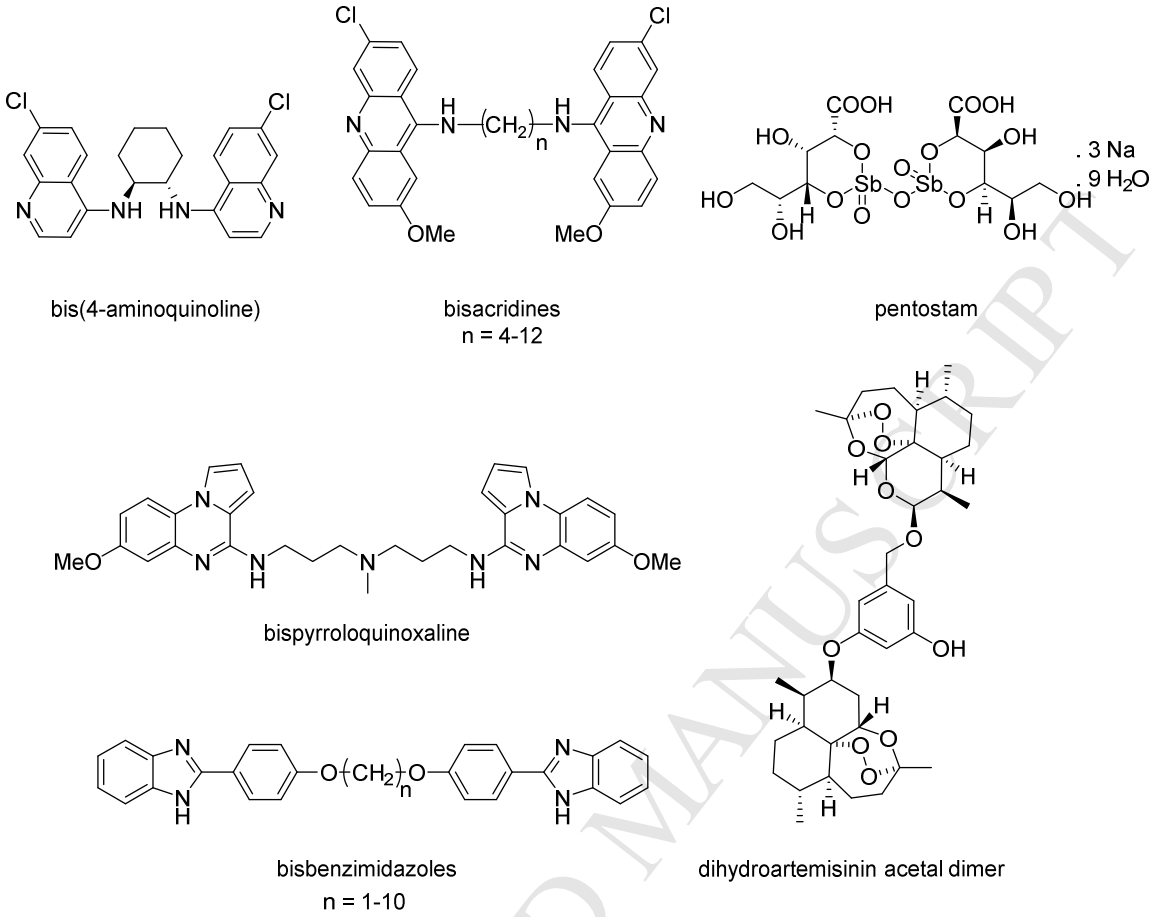


Figure 2. Structure of some antiprotozoal dimers.

The success of dimers in neglected tropical diseases and the feasible chemical modifications of our previously reported antitrypanosomal compounds motivated us to examine the unexplored potential of triazine dimers.

We report the synthesis and antitrypanosomal activity of triazine dimers linked through a set of aliphatic and aromatic linkers **1 – 46** in comparison with their corresponding monomers (**Figure 3**) [16].

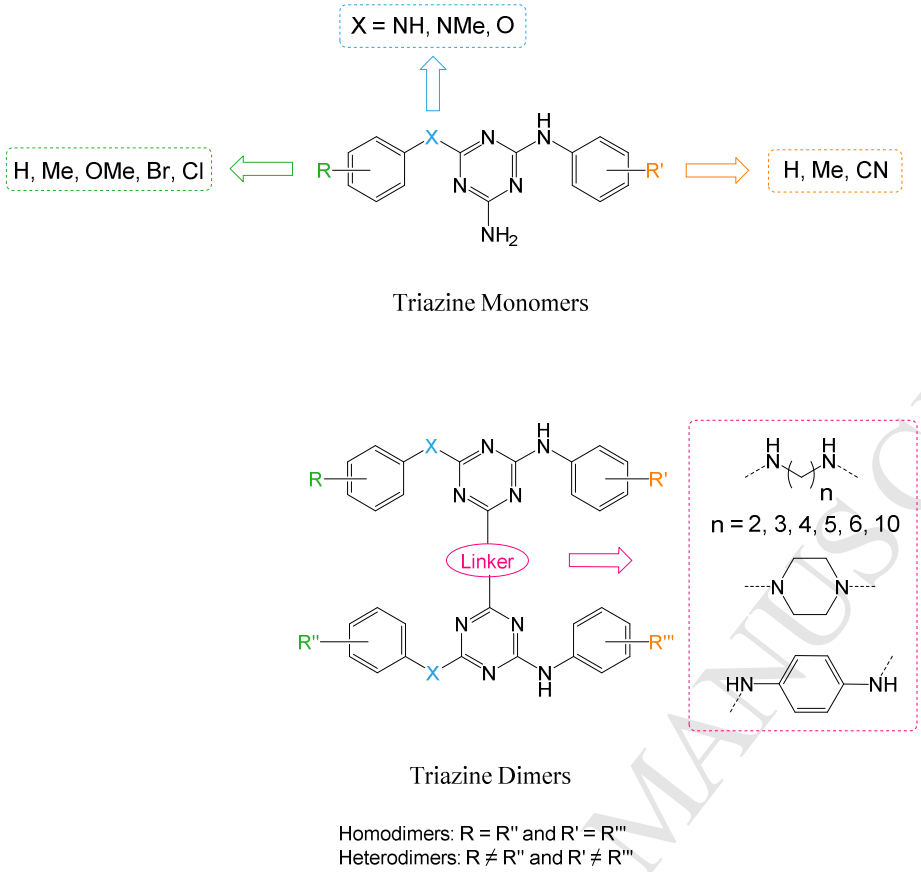
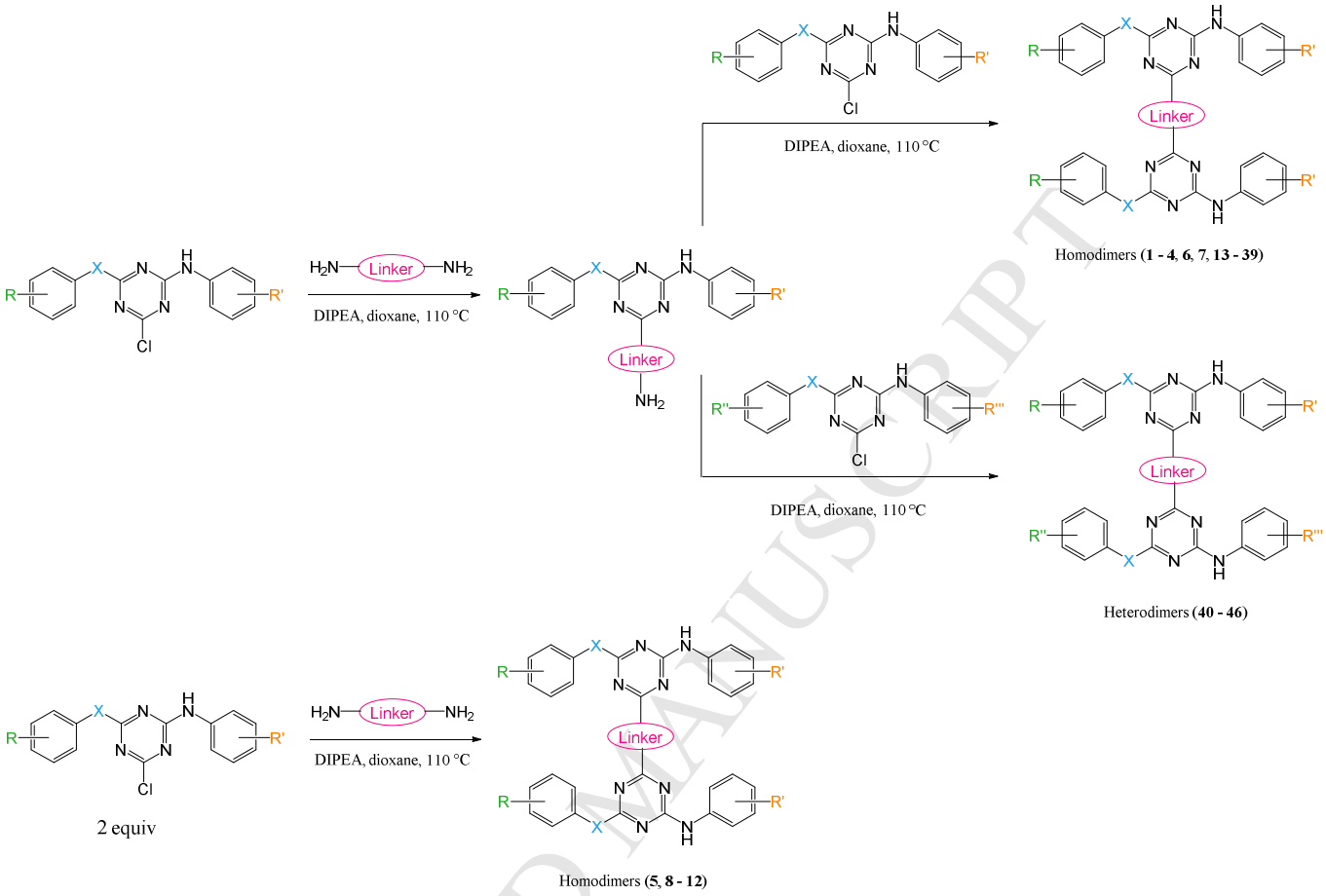


Figure 3. Triazine derivatives synthesized using the previously reported monomers as starting material.

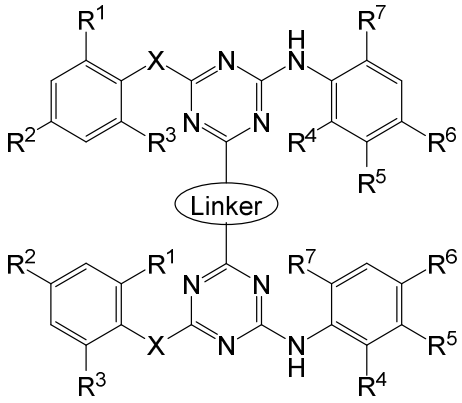
Target compounds were prepared by a linear synthesis (**Scheme 1**) using the corresponding chloro-derivative monomer as starting material. The *N*-alkylation of the monomer with the linker was performed by a conventional way in the presence of a base such as *N*,*N*-diisopropylethylamine (DIPEA). Using the same conditions, the second monomer was coupled to the previous intermediate. In some cases the homodimers were synthesized in a one-pot reaction (see Experimental Part).



Scheme 1. General synthetic procedure for the newly designed *Trypanosome* inhibitors.

Phenotypic assay and Structure-Activity relationship. The phenotypic assay was carried out against a parasitic panel including *T. b. brucei, T. b. rhodesiense, T. cruzi, L. infantum* and *P. falciparum* (Full panel in Supporting information, **Table 1S**); furthermore the cytotoxicity on a human cell line (MRC-5) was also tested. The activity results of all the compounds against *T. b. brucei* and the human pathogenic subspecies *T. b. rhodesiense* are shown in **Tables 2** and **3**.

Most of the triazine dimers were highly active against *T. b. brucei* and/or *T. b. rhodesiense*. We determined a structure-activity relationship including the substituents on the phenyl rings and the nature and length of the linker.



1 - 39

Compd	X	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Linker	Antitrypanosomal activity and cytotoxicity IC_{50} (µM)		
										T. b. bruc.[a,b]	*T. b. rhod.*[b,c]	MRC-5[b,d]
1	NH	Me	Me	Me	Me	H	Me	Me	$HN(CH_2)_2NH$	33.19	0.21	> 64
2	NH	Me	Me	Me	Me	H	Me	Me	$HN(CH_2)_3NH$	31.51	0.65	> 64
3	NH	Me	Me	Me	Me	H	Me	Me	$HN(CH_2)_4NH$	> 64	39.01	> 64
4	NH	Me	Me	Me	Me	H	Me	Me	$HN(CH_2)_5NH$	> 64	41.90	> 64
5	NH	Me	Me	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.07	0.05	> 64
6	NH	Me	Me	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.08	0.16	> 64
7	NH	Me	Me	Me	H	H	CN	H	$HN(CH_2)_4NH$	1.21	0.25	> 64
8	NH	Me	Me	Me	H	H	CN	H	$HN(CH_2)_5NH$	13.99	0.17	> 64
9	NH	Me	Me	Me	H	H	CN	H	$HN(CH_2)_6NH$	20.48	0.46	> 64
10	NH	Me	Me	Me	H	H	CN	H	$HN(CH_2)_{10}NH$	32.46	7.00	> 64
11	NH	Me	Me	Me	H	H	CN	H		0.32	0.07	> 64
12	NH	Me	Me	Me	H	H	CN	H		0.53	0.02	> 64
13	NH	Br	Me	Br	H	H	CN	H	$HN(CH_2)_3NH$	0.03	0.007	> 64

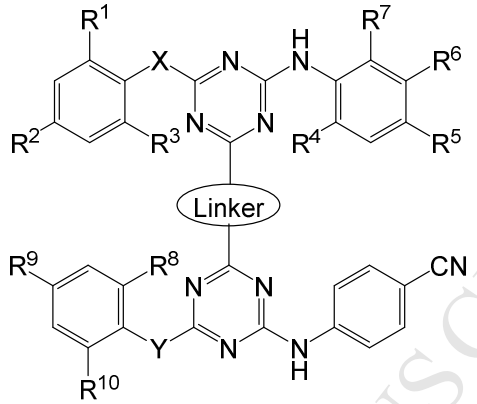
14	NH	Br	Me	Br	H	H	CN	H	$HN(CH_2)_4NH$	0.56	0.73	> 64
15	NH	Br	Me	Br	H	H	CN	H	$HN(CH_2)_5NH$	2.14	1.51	> 64
16	NH	Br	Me	H	H	H	CN	H	$HN(CH_2)_2NH$	0.087	0.21	> 64
17	NH	Br	Me	H	H	H	CN	H	$HN(CH_2)_3NH$	0.14	0.21	> 64
18	NH	Br	Me	H	H	H	CN	H	$HN(CH_2)_5NH$	0.51	0.43	> 64
19	NH	Me	H	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.04	0.25	> 64
20	NH	Me	H	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.10	0.09	> 64
21	NH	Me	H	Me	H	H	CN	H	$HN(CH_2)_5NH$	0.3	0.47	> 64
22	NH	Me	Br	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.13	0.17	> 64
23	NH	Me	Br	Me	H	H	CN	H	$HN(CH_2)_4NH$	0.13	0.16	> 64
24	NH	Me	Br	Me	H	H	CN	H	$HN(CH_2)_5NH$	30.22	8.29	> 64
25	NMe	Me	Me	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.13	0.04	> 64
26	NMe	Me	Me	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.03	0.05	> 64
27	O	Me	Me	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.015	0.01	> 64
28	O	Me	Me	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.01	0.01	> 64
29	O	Me	Me	Me	H	H	CN	H	$HN(CH_2)_5NH$	0.01	0.01	> 64
30	O	Me	H	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.03	0.01	> 64
31	O	Me	H	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.03	0.04	> 64

32	O	Me	Br	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.01	0.03	> 64
33	O	Me	Br	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.02	0.02	> 64
34	O	Me	Cl	Me	H	H	CN	H	$HN(CH_2)_2NH$	0.02	0.01	> 64
35	O	Me	Cl	Me	H	H	CN	H	$HN(CH_2)_3NH$	0.13	0.13	> 64
36	O	OMe	Me	OMe	H	H	CN	H	$HN(CH_2)_2NH$	0.13	0.10	> 64
37	O	OMe	Me	OMe	H	H	CN	H	$HN(CH_2)_3NH$	0.13	0.09	> 64
38	O	Me	Me	Me	H	CN	H	H	$HN(CH_2)_2NH$	0.002	0.01	> 64
39	O	Me	Me	Me	H	H	H	H	$HN(CH_2)_2NH$	0.05	0.16	> 64
Suramin[d]										0.02	0.02	> 64
Melarsoprol[d]										0.03	0.02	7.4

[a]Antitrypanosomal activity against the suramin-resistant strain T. b. brucei Squib 427. [b]Each value is the mean of at least two independent determinations. [c]Antitrypanosomal activity against T.b. rhodesiense strain STIB-900. [d]Cytotoxicity measurement using human lung fibroblast MRC-5 SV_2cells. [d]reference compounds

Table 2. Antitrypanosomal activity of triazine homodimers **1** – **39**.

The data in **Table 2** indicate that X is preferably an oxygen atom instead of a nitrogen for *T. rhod* (e.g. compounds **5**, **6**, **19** and **22** *vs*. compounds **27**, **28**, **31** and **32** respectively). These results are in agreement with the structure – activity relationship (SAR) data reported for the monomers [12]. Regarding the linker between the two triazine rings, different natures and lengths were explored. The best inhibitory activities are obtained with an aliphatic chain of 2 or 3 methylenes. (e.g. compare compound **13** *vs*. **14** and **15**, or compound **22** *vs.* **23** and **24**). The potency seriously decreased with 5 methylenes or above, and was also much less if piperazine or benzene is used as linker. Concerning the substituents in the phenyl ring, when $R^4 = R^5 = R^7 = H$ and $R^6 = CN$ the compounds show better activities (e.g. compounds **1** and **2** *vs.* compounds **5** and **6**, respectively). Triazine dimers **5**, **13**, **20** and **26 - 34** showed a high potency against both *T. b. brucei* and *T. b. rhodesiense*. Derivative **38** is more active than the controls suramin and melarsoprol.



40 - 46

Compd	X	Y	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	Linker	Antitrypanosomal activity and cytotoxicity IC_{50} (µM)		
														T. b. bruc.[a]	*T. b. rhod.*[b]	MRC-5[c]
40	NH	NH	Me	Me	Me	H	CN	H	H	Br	Me	Br	$HN(CH_2)_2NH$	0.13	0.03	> 64
41	NH	NH	Me	Me	Me	H	CN	H	H	Br	Me	Br		7.69	0.48	> 64
42	NH	NH	Me	Me	Me	Me	Me	H	Me	Me	Me	Me	$HN(CH_2)_2NH$	0.04	0.03	> 64
43	NH	NH	Me	Me	Me	Me	Me	H	Me	Me	Me	Me	$HN(CH_2)_3NH$	0.13	0.12	> 64
44	O	O	Me	Me	Me	H	H	CN	H	Me	Me	Me	$HN(CH_2)_2NH$	0.02	0.02	> 64
45	O	O	Me	Me	Me	H	H	H	H	Me	Me	Me	$HN(CH_2)_2NH$	0.01	0.05	> 64
46	NH	O	Me	Me	Me	H	CN	H	H	Me	Me	Me	$HN(CH_2)_2NH$	0.03	0.02	> 64
Suramin[d]														0.02	0.02	> 64
Melarsoprol[d]														0.03	0.02	7.4

[a]Antitrypanosomal activity against the suramin-sensitive strain T. b. brucei Squib 427. [b]Each value is the mean of at least two independent determinations. [c]Antitrypanosomal activity against T.b. rhodesiense strain STIB-900. [d]Cytotoxicity measurement using human lung fibroblast MRC-5 SV_2cells. [d]reference compounds

Table 3. Antitrypanosomal activity of triazine heterodimers **40** – **46**.

Triazine heterodimers **42** and **44 - 46** had comparable potency values comparing to the activity of standards suramin and melarsoprol (**Table 3**).

The antiprotozoal activity of triazine dimers was compared against their monomeric counterparts (**Table 3S**, in Supporting Information). Triazine dimers demonstrated generally a substantial increase in antitrypanosomal activity (4 - 400 fold) and a decrease in cytotoxicity in comparison with their monomers. No cytotoxicity was observed for all the dimers against MRC-5 cells (IC_{50} > 64 µM).

Microsomal and plasma stability. Since low nanomolar potencies on the whole parasites are very exceptional, we selected two compounds for further profilation. Compound **13** (IC_{50} *T. b. brucei* = 30 nM, *T. b. rhodesiense* = 7 nM) was selected as the most potent compound from the arylamino series, and compound **38** (IC_{50} *T.b.brucei* = 2 nM, *T. b. rhodesiense* = 10 nM) as the best compound from the aryloxy series. Both compounds were exposed to mouse and human liver microsomes (S9) to investigate the *in vitro* metabolic stability.

Microsomal stability							
		% remaining parent compound					
Microsomes	Phase I/II	Time (min)	**13**	**std**	**38**	**std**	diclofenac
Mouse	CYP450-NADPH	0	100	---	100	---	100
		15	104	7,2	96	10	110
		30	109	8,9	105	6,6	96
		60	105	3,7	101	4,6	58
	UGT enzymes	0	100	---	100	---	100
		15	79	13,9	97	7,8	39
		30	81	11,8	101	7,9	33
		60	86	16,1	97	14,9	27
Human	CYP450 - NADPH	0	100	---	100	---	100
		15	77	11,6	87	11,2	46
		30	81	8,1	90	4,7	18
		60	81	5,8	75	5,2	5
	UGT enzymes	0	100	---	100	---	100
		15	99	10,8	96	1,7	20
		30	100	2,8	97	4	17
		60	99	6,9	97	3,2	0

[a]*For the CYP450 both compounds were incubated at 5 μM together with 0.5 mg/mL liver microsomes in potassium phosphate buffer in a reaction started by the addition of 1 mM NADPH and stopped at the above listed sampling times. For the UGT enzymes, both compounds were incubated at 5 μM together*

with 0.5 mg/mL liver microsomes in a reaction started by the addition of 2 mM UDPGA cofactor (Detailed protocol in the Suplementary Information).

Table 4. Microsomal stability of compounds **13** and **38**.[a]

Compounds **13** and **38** did not demonstrate extensive phase 1 nor phase 2 in both mouse and human microsomes with half-lives well above 1 hour, indicating satisfactory metabolic stability (**Table 4**). Furthermore, **13** and **38** remained fully stable after incubation in human plasma for 24 h at 37 °C (Supplementary Information, Table 5).

***In vivo T. b. brucei.* acute mouse model.** Due to the good results in the microsomal stability assay, these compounds were studied in a preliminary acute mouse model of *T. b. brucei* infection. The compounds were injected intraperitoneally (IP) at different doses once (SID) or twice per day (BID) for 5 days after infection. Both **13** and **38** showed a moderate reduction in parasitemia 4 days post infection (**Table 5**). Compound **38** showed a reduction in parasitemia of 46% at intraperitoneal dosing of 25 mg/kg once a day for 5 days, whereas the reduction was complete for the control compound suramin. This incomplete suppression of the parasitic infection led to the survival of only one animal out of 6 at 14 days post infection.

Compd	dose (mg/kg)	freq.	MST[a]	reduction (%) in parasitemia on 4 dpi[b]	survivors on 14 dpi
control[c]			7.2		0/6
13	50	SID × 5	7.7	28	0/6
	50	BID × 5	7.2	nd[d]	0/6
38	25	SID × 5	12.8	46	1/6
Suramin	10	SID × 5	> 21	100	6/6

[a]Mean Survival time. [b]day post-infection. [c]Vehicle-treated infected control. [d]not determined.

Table 5. *In vivo* activities of selected triazine dimers against *T. b. brucei* model

CONCLUSIONS

Based on the serendipitous discovery of the antitrypanosomal activity of triazines derived from non-nucleoside HIV reverse transcriptase inhibitors and on the presence of dimer structures in many antiparasitic compounds, we synthesized a series of 46 homo- and heterodimers of triazines. We observed that several triazine dimers showed an increase in antitrypanosomal activity (4 - 400 fold) and a decrease in cytotoxicity in comparison to their monomers. A linker with two or three methylenes proved to be optimal. Two of the most potent compounds (**13** and **38**) showed excellent metabolic stability upon incubation with mouse and human microsomes. However in a model of acute infection with *T.b.brucei* both compounds showed only moderate reduction of parasitemia, resulting in a low survival rate. The reason for this remains unclear and will deserve further investigation of antiparasitic and pharmacokinetic properties.

EXPERIMENTAL SECTION

Chemistry. Reagents were purchased from commercial sources and without further purification. The products were purified with flash chromatography on a Flashmaster II (Jones chromatography) or on or

IsoleraOne flash purification system from Biotage. Compounds were detected with UV light (254 nm). ^{1}H NMR spectra were obtained on a 400 MHz Bruker Avance DRX-400 and 400 MHz Bruker Avance III nanobay spectrometer with ultrashield. Typical spectral parameters: special width 16 ppm, pulse width 9 μs (57 °), data size 32 K. For target compounds such as derivatives **13** and **38**, ^{13}C NMR experiments were carried out on the Bruker 400 MHz Bruker Avance DRX-400 and 400 MHz Bruker Avance III nanobay spectrometer with ultrashield operating at 100 MHz. The acquisition parameters: special width 16 ppm, pulse width 9 μs (57 °), data size 32 K. Chemical shifts are reported in values (ppm) relative to internal Me_4Si, and *J* values are reported in Hz. Purity was verified using two diverse HPLC systems, an HPLC from Agilent (HPLC system A) and a Waters SQD ESI mass spectrometer (HPLC system B). Water (A) and CH_3CN (B) were used as eluents. The first LC-MS system is an Agilent 1100 Series HPLC system equipped with a C18 column (2.1 x 50 mm, 5 μm, Supelco, Sigma-Aldrich) coupled with and Esquire 3000 plus iontrap mass spectrometer from Bruker Daltonics as MS detector. A typical method was used 95-5% A (5-95% B), 20 min gradient with a flow rate from 0.2 mL/min. The wavelength for the UV detection was 254 nm and 214 nm. The second LC-MS, Waters SQD ESI mass spectrometer was used in combination with a Waters TUV detector. Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm column was used. Solvent A consisted of water with 0.1% formic acid. Solvent B consisted of acetonitrile with 0.1% formic acid. Method I involved the following: 0.15 min 95% A, 5% B, then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25 min (0.350 mL/min), 95% B, 5% A. The wavelength for UV detection was 254 nm. Method II involved the following: flow 0.4 mL/min, 0.25 min 95% A, 5% B, then in 4.75 min to 95% B, 5% A, then 0.25 min 95% B, 5% A, followed by 0.75 min 95% A, 5% B. The wavelength for UV detection was 214 nm. All the compounds were obtained as amorphous solids.

General Procedure for the Synthesis of triazine dimers 1- 4, **6**, **7**, **13 - 46**. To a solution of the corresponding monomer (1 equiv) in dioxane (10 mL) was added DIPEA (1 - 2 equiv) and the appropriate

diamine derivative (1 equiv) and allowed to reflux for 48 h. Removal of solvent afforded the intermediate, which was used in the next step without further purification.

A mixture of the intermediate previously synthesized (1 equiv), the appropriate monomer (1 equiv), *N*,*N*-diisopropylethylamine (DIPEA) (1 - 4 equiv) and dioxane, was heated (24 – 48 h, 101 °C). The crude was purified by IsoleraOne using ethyl acetate and hexane as eluents, to afford the final compound.

N*^{2},*N*$^{2'}$-(Ethane-1,2-diyl)bis(*N*^{4},*N*^{6}-dimesityl-1,3,5-triazine-2,4,6-triamine)** (1***). Reagents: *N*^{2}-(2-aminoethyl)-*N*^{4},*N*^{6}-dimesityl-1,3,5-triazine-2,4,6-triamine (1 mmol), ethane-6-chloro-*N*^{2},*N*^{4}-dimesityl-1,3,5-triazine-2,4-diamine (1 mmol), DIPEA (1 mmol) and dioxane (15 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (290 mg, 39%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 6.92 - 6.81 (m, 8H), 3.51 - 3.35 (m, 4H), 2.26 - 2.00 (m, 36H). HPLC: purity = 87 %. m/z (ESI) 751 [M + 1] (HPLC system B).

N*^{2},*N*$^{2'}$-(Propane-1,3-diyl)bis(*N*^{4},*N*^{6}-dimesityl-1,3,5-triazine-2,4,6-triamine)** (2***). Reagents: *N*^{2}-(3-aminopropyl)-*N*^{4},*N*^{6}-dimesityl-1,3,5-triazine-2,4,6-triamine (1 mmol), 6-chloro-*N*^{2},*N*^{4}-dimesityl-1,3,5-triazine-2,4-diamine (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (270 mg, 35%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 6.92 - 6.70 (m, 8H), 3.35 (bs, 4H), 2.28 - 2.07 (m, 36H), 1.65 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 766 [M + 1] (HPLC system B).

N*^{2},*N*$^{2'}$-(Butane-1,4-diyl)bis(*N*^{4},*N*^{6}-dimesityl-1,3,5-triazine-2,4,6-triamine)** (3***). Reagents : *N*^{2}-(4-aminobutyl)-*N*^{4},*N*^{6}-dimesityl-1,3,5-triazine-2,4,6-triamine (1 mmol), 6-chloro-*N*^{2},*N*^{4}-dimesityl-1,3,5-triazine-2,4-diamine (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (280 mg, 36%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 - 7.82 (m, 4H), 6.88 - 6.82 (m, 8H), 6.64 (bs, 2H), 3.31 - 3.21 (m, 4H), 2.23 - 2.03 (m, 36H), 1.44 (bs, 2H). HPLC: purity = 95%. m/z (ESI) 779 [M + 1] (HPLC system B)

N^2,$N^{2'}$-(Pentane-1,5-diyl)bis(N^4,N^6-dimesityl-1,3,5-triazine-2,4,6-triamine) (***4***). Reagents: N^2-(5-aminopentyl)-N^4,N^6-dimesityl-1,3,5-triazine-2,4,6-triamine (1 mmol), 6-chloro-N^2,N^4-dimesityl-1,3,5-triazine-2,4-diamine (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (240 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98 - 7.82 (m, 4H), 6.88 - 6.83 (m, 8H), 6.57 (bs, 2H), 3.31 - 3.18 (m, 4H), 2.24 - 2.07 (m, 36H) 1.44 (bs, 6H). HPLC: purity ≥ 99%. m/z (ESI) 794 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***6***). Reagents: 4-(4-(3-aminopropylamino)-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (1.1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (100 mg, 55%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.97 (bs, 2H), 7.61 (bs, 4H), 7.36 (bs, 2H), 6.96 (bs, 4H), 3.56 - 3.49 (m, 4H), 2.33 (bs, 6H), 2.19 (bs, 12H), 1.88 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 731 [M + 1] (HPLC system A).

4,4'-(((Butane-1,4-diylbis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***7***). Reagents: 4-(4-(4-aminobutylamino)-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (1.1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (210 mg, 51%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.96 (bs, 2H), 7.61 (bs, 4H), 7.36 (bs, 2H), 6.90 (bs, 4H), 3.56 - 3.49 (m, 4H), 2.33 (bs, 6H), 2.19 (bs, 12H), 1.76 - 1.66 (m, 4H). HPLC: purity ≥ 99%. m/z (ESI) 745 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-((2,6-dibromo-4-methylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***13***). Reagents: 4-(4-(3-aminopropylamino)-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.2 mmol), 4-(4-chloro-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.2 mmol), DIPEA (0.21 mmol) and dioxane

(5 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (80 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 – 7.24 (m, 12H), 3.40 (bs, 4H), 2.30 (bs, 6H), 1.80 (bs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 164.4, 164.0, 143.3, 142.9, 140.4, 132.8, 124.5, 119.4, 119.3, 105.0, 37.7, 29.8, 20.7. HPLC: purity ≥ 99%. m/z (ESI) 985 [M - 5], 987 [M - 3], 989 [M - 1], 991 [M + 1], 993 [M + 3].

4,4'-(((Butane-1,4-diylbis(azanediyl))bis(4-((2,6-dibromo-4-methylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***14***). Reagents: 4-(4-(4-aminobutylamino)-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.14 mmol); 4-(4-chloro-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.14 mmol), DIPEA (0.15 mmol) and dioxane (5 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (60 mg, 43%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.9 - 7.5 (bs, 12H), 3.44 (bs, 4H), 2.34 (bs, 6H), 1.67 (bs, 4H). HPLC: purity ≥ 99%. m/z (ESI) 999 [M - 5], 1001 [M - 3], 1003 [M - 1], 1005 [M + 1], 1007 [M + 3] (HPLC system B).

4,4'-(((Pentane-1,5-diylbis(azanediyl))bis(4-((2,6-dibromo-4-methylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***15***). Reagents: 4-(4-(5-aminopentylamino)-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.17 mmol), 4-(4-chloro-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.17 mmol), DIPEA (0.2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (90 mg, 52%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.7 - 7.1 (bs, 12H), 3.11 (bs, 4H), 2.1(bs, 6H), 1.7 (bs, 4H), 1.41 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 1013 [M - 5], 1015 [M - 3], 1017 [M - 1], 1019 [M + 1], 1021 [M + 3] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-((2-bromo-4-methylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***16***). Reagents: 4-(4-(2-aminoethylamino)-6-(2-bromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.49 mmol), 4-(4-(2-bromo-4-methylphenylamino)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (0.49 mmol), DIPEA (0.98 mmol) and

dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (150 mg, 37%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.77 (bs, 6H), 7.40 (bs, 6H), 7.04 (bs, 2H), 3.61 (bs, 4H), 2.27 (bs, 6H). HPLC: purity ≥ 99%. m/z (ESI) 817 [M - 1], 819 [M + 1], 821 [M + 3] (HPLC system A).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-((2-bromo-4-methylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***17***). Reagents: 4-(4-(3-aminopropylamino)-6-(2-bromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), 4-(4-(2-bromo-4-methylphenylamino)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), DIPEA (1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (80 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 2H), 8.31 (bs, 2H), 7.94 (bs, 4H), 7.40 (bs, 6H), 7.27 (bs, 2H), 7.16 (bs, 4H), 3.39 (bs, 4H), 2.27 (bs, 6H), 1.90 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 831 [M - 1], 833 [M + 1], 835 [M + 3] (HPLC system B).

4,4'-(((Pentane-1,5-diylbis(azanediyl))bis(4-((2-bromo-4-methylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***18***). Reagents: 4-(4-(5-aminopentylamino)-6-(2-bromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), 4-(4-(2-bromo-4-methylphenylamino)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), DIPEA (1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (110 mg, 26%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.81 (bs, 6H), 7.33 (bs, 6H), 7.04 (bs, 2H), 3.35 (bs, 4H), 2.30 (bs, 6H), 1.7 (bs, 4H), 1.44 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 859 [M - 1], 861 [M + 1], 863 [M + 3] (HPLC system A).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-((2,6-dimethylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***19***). Reagents: 4-(4-(2-aminoethylamino)-6-(2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.9 mmol), 4-(4-chloro-6-(2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.9 mmol), DIPEA (1.8 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to

afford an amorphous solid (140 mg, 23%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.90 (bs, 1H), 7.55 (bs, 4H), 7.28 (bs, 3H), 7.10 (bs, 6H), 3.69-3.54 (m, 4H), 2.17 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 689 [M + 1] (HPLC system A).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-((2,6-dimethylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***20***). Reagents: 4-(4-(3-aminopropylamino)-6-(2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (260 mg, 37%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.90 (bs, 1H), 7.60 (bs, 4H), 7.25 (bs, 3H), 7.12 (bs, 6H), 3.69 - 3.56(m, 4H), 2.22 (bs, 12H), 1.90 - 1.75 (m, 2H). HPLC: purity ≥ 99%. m/z (ESI) 703 [M + 1] (HPLC system B).

4,4'-(((Pentane-1,5-diylbis(azanediyl))bis(4-((2,6-dimethylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***21***). Reagents: 4-(4-(5-aminopentylamino)-6-(2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (240 mg, 33%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.90 (bs, 1H), 7.60 (bs, 4H), 7.35 (bs, 3H), 7.11 (bs, 6H), 3.6 - 3.5 (m, 4H), 2.26 (bs, 12H), 1.75 - 1.60 (m, 4H), 1.5 - 1.4 (m, 2H). HPLC: purity ≥ 99%. m/z (ESI) 731 [M + 1] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-((4-bromo-2,6-dimethylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***22***). Reagents: 4-(4-(2-aminoethylamino)-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (3.5 mmol), 4-(4-(4-bromo-2,6-dimethylphenylamino)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (3.5 mmol), DIPEA (7 mmol) and dioxane (25 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (1.1 g,

41%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.96 (bs, 2H), 7.63 (bs, 4H), 7.33 (bs, 6H), 2.10 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 847 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-((4-bromo-2,6-dimethylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***23***). Reagents: 4-(4-(5-aminopentylamino)-6-(4-bromo-2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-(4-bromo-2,6-dimethylphenylamino)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (370 mg, 37%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 8.00 (bs, 2H), 7.64 (bs, 4H), 7.40 (bs, 6H), 3.69 (bs, 4H), 2.10 (bs, 12H), 1.88 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 860 [M + 1] (HPLC system B).

4,4'-(((Pentane-1,5-diylbis(azanediyl))bis(4-((4-bromo-2,6-dimethylphenyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***24***). Reagents: 4-(4-(5-aminopentylamino)-6-(4-bromo-2,6-dimethylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-(4-bromo-2,6-dimethylphenylamino)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (370 mg, 42%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.98 (bs, 2H), 7.62 (bs, 4H), 7.31 (bs, 6H), 3.34 (bs, 4H), 2.10 (bs, 12H), 1.81 (bs, 4H), 1.56 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 889 [M + 1] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(mesityl(methyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***25***). Reagents: 4-(4-(2-aminoethylamino)-6-(mesityl(methyl)amino)-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), 4-(4-chloro-6-(mesityl(methyl)amino)-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), DIPEA (1 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (70 mg, 19%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.43 (bs, 4H), 7.22 (bs, 4H), 6.96 - 6.85 (m, 4H), 3.66 - 3.48 (m, 4H), 2.38 (s, 6H), 2.00 (bs, 18H). HPLC: purity = 95%. m/z (ESI) 745 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(mesityl(methyl)amino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***26***). *Reagents:* 4-(4-(3-aminopropylamino)-6-(mesityl(methyl)amino)-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), 4-(4-chloro-6-(mesityl(methyl)amino)-1,3,5-triazin-2-ylamino)benzonitrile (0.5 mmol), DIPEA (1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (110 mg, 29%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.50 (bs, 4H), 7.25 (bs, 4H), 6.97 (bs, 4H), 3.52 - 3.48 (m, 4H), 2.35 (s, 6H), 2.04 (bs, 18H), 1.80 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 759 [M + 1] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(mesityloxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***27***). Reagents: 4-(4-(2-aminoethylamino)-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.75 mmol), 4-(4-chloro-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.75 mmol), DIPEA (1.3 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (280 mg, 52%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.85 (bs, 4H), 7.56 (bs, 4H), 7.08 (bs, 4H), 3.74 - 3.81 (m, 4H), 2.47 (bs, 6H), 2.26 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 719 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(mesityloxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***28***). Reagents: 4-(4-(3-aminopropylamino)-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (15 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (240 mg, 33%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.7 (bs, 4H), 7.45 (bs, 4H), 6.88 (bs, 4H), 3.35 - 3.29 (m, 4H), 2.28 (bs, 6H), 2.00 (bs, 12H), 1.88 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 733 [M + 1] (HPLC system B).

4,4'-(((Pentane-1,5-diylbis(azanediyl))bis(4-(mesityloxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***29***). Reagents: 4-(4-(5-aminopentylamino)-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1

mmol), DIPEA (2 mmol) and dioxane (15 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (230 mg, 30%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.73 (bs, 4H), 7.45 (bs, 4H), 6.88 (bs, 4H), 3.35 - 3.25 (m, 4H), 2.28 (bs, 6H), 2.06 (bs, 12H), 1.63 (bs, 6H). HPLC: purity ≥ 99%. m/z (ESI) 761 [M + 1] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(2,6-dimethylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***30***). Reagents: 4-(4-(2-aminoethylamino)-6-(2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (270 mg, 39%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.64(bs, 4H), 7.37 (bs, 4H), 7.14 - 7.0 (m, 6H), 3.47 - 3.3 (m, 4H), 2.16 - 2.03 (m, 12H). HPLC: purity ≥ 99%. m/z (ESI) 691 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(2,6-dimethylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***31***). Reagents: 4-(4-(3-aminopropylamino)-6-(2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (220 mg, 31%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.67(bs, 4H), 7.37 (bs, 4H), 7.14 - 7.05 (m, 6H), 3.54 - 3.35 (m, 4H), 2.12 - 2.04 (m, 12H), 1.77 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 705 [M + 1] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(4-bromo-2,6-dimethylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***32***). Reagents: 4-(4-(2-aminoethylamino)-6-(4-bromo-2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.7 mmol), 4-(4-(4-bromo-2,6-dimethylphenoxy)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (0.7 mmol), DIPEA (1.3 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (260 mg, 47%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.64 (bs, 4H), 7.44 -

7.37 (m, 4H), 7.25 - 7.15 (m, 4H), 3.69 - 3.53 (m, 4H), 2.15 - 2.04 (m, 12H). HPLC: purity ≥ 99%. m/z (ESI) 847 [M - 1], 849 [M + 1], 851 [M + 3] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(4-bromo-2,6-dimethylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***33***). Reagents: 4-(4-(3-aminopropylamino)-6-(4-bromo-2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.95 mmol), 4-(4-(4-bromo-2,6-dimethylphenoxy)-6-chloro-1,3,5-triazin-2-ylamino)benzonitrile (0.95 mmol), DIPEA (1.9 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (230 mg, 28%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.69 (bs, 4H), 7.46 (bs, 4H), 7.24 - 7.2 (m, 4H), 3.52 - 3.35 (m, 4H), 2.3 - 2.06 (m, 12H), 1.87 (bs, 2H). HPLC: purity = 93%. m/z (ESI) 861 [M - 1], 863 [M + 1], 865 [M + 3] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(4-chloro-2,6-dimethylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***34***). Reagents: 4-(4-(2-aminoethylamino)-6-(4-chloro-2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(4-chloro-2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (260 mg, 34%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.69 (bs, 4H), 7.43 (bs, 4H), 7.15 (bs, 4H), 3.73 - 3.57 (m, 4H), 2.19 - 2.04 (m, 12H). HPLC: purity ≥ 99%. m/z (ESI) 759 [M], 761 [M + 2], 763 [M + 4] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(4-chloro-2,6-dimethylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***35***). Reagents: 4-(4-(3-aminopropylamino)-6-(4-chloro-2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 4-(4-chloro-6-(4-chloro-2,6-dimethylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (240 mg, 31%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.76 - 7.41(m, 8H), 7.13 - 7.04 (m,

4H), 3.51 - 3.32 (m, 4H), 2.16 - 2.03 (m, 12H), 1.88 (bs, 2H). HPLC: purity = 91%. m/z (ESI) 773 [M], 775 [M + 2], 773 [M + 4] (HPLC system B).

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(2,6-dimethoxy-4-methylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***36***). Reagents: 4-(4-(2-aminoethylamino)-6-(2,6-dimethoxy-4-methylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.75 mmol), 4-(4-chloro-6-(2,6-dimethoxy-4-methylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.75 mmol), DIPEA (1.5 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (240 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (bs, 4H), 7.37 (bs, 4H), 6.57 (bs, 4H), 3.66 (bs, 12H), 3.52 - 3.48 (m, 4H), 2.38 (s, 6H). HPLC: purity ≥ 99%. m/z (ESI) 783 [M + 1] (HPLC system B).

4,4'-(((Propane-1,3-diylbis(azanediyl))bis(4-(2,6-dimethoxy-4-methylphenoxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***37***). Reagents: 4-(4-(3-aminopropylamino)-6-(2,6-dimethoxy-4-methylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.4 mmol), 4-(4-chloro-6-(2,6-dimethoxy-4-methylphenoxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.4 mmol), DIPEA (0.8 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (70 mg, 22%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.66 (bs, 4H), 7.43 (bs, 4H), 6.57 (bs, 4H), 3.72 (bs, 12H), 3.52 - 3.35 (m, 4H), 2.40 (bs, 6H), 1.86 (bs, 2H). HPLC: purity = 95%. m/z (ESI) 797 [M + 1] (HPLC system B).

3,3'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(mesityloxy)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***38***). Reagents: 3-(4-(2-aminoethylamino)-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), 3-(4-chloro-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (280 mg, 39%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.90 (bs, 2H), 7.55 (bs, 2H), 7.16 (bs, 4H), 6.83 (s, 4H), 3.69-3.54 (m, 4H), 2.24 (bs, 6H), 2.06 (bs, 12H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.5, 166.8, 165.3, 147.0, 140.6, 129.6, 129, 125.4,

124, 121.8, 118.8, 111.3, 31.3, 20.4, 16.1. HPLC: purity ≥ 99%. m/z (ESI) 719 [M + 1] (HPLC system B).

N^2,$N^{2'}$-(Ethane-1,2-diyl)bis(6-(mesityloxy)-N^4-phenyl-1,3,5-triazine-2,4-diamine) (***39***). Reagents: N^2-(2-aminoethyl)-6-(mesityloxy)-N^4-phenyl-1,3,5-triazine-2,4-diamine (0.5 mmol), 4-chloro-6-(mesityloxy)-*N*-phenyl-1,3,5-triazin-2-amine (0.5 mmol), DIPEA (1 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 1:1) to afford an amorphous solid (120 mg, 36%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.46 (bs, 4H), 7.11 (bs, 4H), 6.86 (bs, 6H), 6.90 (bs, 4H), 3.66 - 3.50 (m, 4H), 2.26 (bs, 6H), 2.06 (bs, 12H). HPLC: purity = 97%. m/z (ESI) 669 [M + 1] (HPLC system B).

4-((4-((2-((4-((4-Cyanophenyl)amino)-6-((2,6-dibromo-4-methylphenyl)amino)-1,3,5-triazin-2-yl)amino)ethyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (***40***). Reagents: 4-(4-(2-aminoethylamino)-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.6 mmol), 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.6 mmol), DIPEA (1.1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (50 mg, 11%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.91 (bs, 2H), 7.57 (bs, 4H), 7.31 (bs, 2H), 6.93 (bs, 4H), 3.67 - 3.58 (m, 4H), 2.33 (bs, 6H), 2.17 (bs, 6H). HPLC: purity ≥ 99%. m/z (ESI) 845 [M - 1], 847 [M + 1], 849 [M + 3] (HPLC system B).

4-((4-(4-(4-((4-Cyanophenyl)amino)-6-((2,6-dibromo-4-methylphenyl)amino)-1,3,5-triazin-2-yl)piperazin-1-yl)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (***41***). Reagents: 4-(4-(mesitylamino)-6-(piperazin-1-yl)-1,3,5-triazin-2-ylamino)benzonitrile 2,2,2-trifluoroacetate (0.8 mmol), 4-(4-chloro-6-(2,6-dibromo-4-methylphenylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.8 mmol), DIPEA (3.2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (70 mg, 10%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.64 (bs, 1H), 9.56 (bs, 1H), 9.13 (bs, 1H), 8.57 (bs, 1H), 8.0 (bs, 1H), 7.72 (bs, 4H), 7.49 (bs, 3H),

6.94 (bs, 4H), 3.9 (bs, 8H), 2.23 (bs, 6H), 2.13 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 871 [M - 1], 873 [M + 1], 875 [M + 3] (HPLC system A).

4-((4-((2-((4,6-bis(Mesitylamino)-1,3,5-triazin-2-yl)amino)ethyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (***42***). Reagents: N_2-(2-aminoethyl)-N^4,N^6-dimesityl-1,3,5-triazine-2,4,6-triamine (0.85 mmol), 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.85 mmol), DIPEA (1.7 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 4:1) to afford an amorphous solid (210 mg, 34%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 8.48 - 7.47 (m, 4H), 6.94 - 6.83 (m, 6H), 3.51 - 3.46 (m, 4H), 2.26 - 2.03 (m, 27H). HPLC: purity ≥ 99%. m/z (ESI) 734 [M + 1] (HPLC system B).

4-*((4-((3-((4,6-bis(Mesitylamino)-1,3,5-triazin-2-yl)amino)propyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile* (***43***). Reagents: N^2-(3-aminopropyl)-N^4,N^6-dimesityl-1,3,5-triazine-2,4,6-triamine (1 mmol), 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), DIPEA (2 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (260 mg, 35%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.58 (bs, 2H), 7.30 (bs, 2H), 6.94 - 6.74 (m, 6H), 3.35 - 3.31 (bs, 4H), 2.32 - 2.0 (m, 27H), 1.75 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 748 [M + 1] (HPLC system B).

3-((4-((2-((4-((4-Cyanophenyl)amino)-6-(mesityloxy)-1,3,5-triazin-2-yl)amino)ethyl) amino)-6-(mesityloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (***44***). Reagents: 3-(4-(2-aminoethylamino)-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.95 mmol), 4-(4-chloro-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.95 mmol), DIPEA (1.9 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (240 mg, 35%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.64 (bs, 4H), 7.36 (bs, 4H), 6.87 (bs, 4H), 3.68 - 3.60 (m, 4H), 2.27 (bs, 6H), 2.07 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 719 [M + 1] (HPLC system A).

3-((4-(Mesityloxy)-6-((2-((4-(mesityloxy)-6-(phenylamino)-1,3,5-triazin-2-yl)amino) ethyl)amino) -1,3,5-triazin-2-yl)amino)benzonitrile (***45***). N^2-(2-aminoethyl)-6-(mesityloxy)-N^4-phenyl-1,3,5-triazine-

2,4-diamine (1.2 mmol), 4-(4-chloro-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (1.2 mmol), DIPEA (2.4 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude product was purified (AcOEt/hexane 3:2) to afford an amorphous solid (210 mg, 25%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.70 (bs, 2H), 7.39 (bs, 4H), 7.11 (bs, 2H), 6.88 (bs, 5H), 3.67 - 3.50 (m, 4H), 2.28 (bs, 6H), 2.07 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 694 [M + 1] (HPLC system B).

4-((4-((2-((4-((4-Cyanophenyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)ethyl)amino)-6-(mesityloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (***46***). Reagents: 4-(4-(2-aminoethylamino)-6-(mesityloxy)-1,3,5-triazin-2-ylamino)benzonitrile (0.65 mmol), 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.65 mmol), DIPEA (0.65 mmol) and dioxane (15 mL). Reaction conditions: 48 h at 101 °C. The crude product was purified (AcOEt/hexane 7:3) to afford an amorphous solid (250 mg, 51%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.91 (bs, 1H), 7.59 (bs, 4H), 7.39 (bs, 3H), 6.90 (bs, 4H), 3.65 (bs, 4H), 2.3-2.0 (m, 18H). HPLC: purity ≥ 99%. m/z (ESI) 718 [M + 1] (HPLC system B).

General Procedure for the Synthesis of triazine homodimers 5, **8 - 12**. To a solution of the corresponding monomer (2 equiv) in dioxane (10 mL) was added DIPEA (2 equiv) and the appropriate diamine derivative (1 equiv) and allowed to reflux for 24 - 48 h. The crude was purified by IsoleraOne using ethyl acetate and hexane as eluents, to afford the final compound.

4,4'-(((Ethane-1,2-diylbis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***5***). Reagents: 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.7 mmol), ethane-1,2-diamine (0.35 mmol), DIPEA (0.8 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude was purified (AcOEt/hexane 7:3) to afford an amorphous solid (30 mg, 12%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 8.09 (bs, 2H), 7.75 (bs, 4H), 7.48 (bs, 2H), 7.12 (bs, 4H), 3.86 - 3.51 (m, 4H), 2.50 (bs, 6H), 2.32 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 717 [M + 1] (HPLC system B).

4,4'-(((Pentane-1,5-diylbis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***8***). Reagents: 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-

ylamino)benzonitrile (0.6 mmol), pentane-1,5-diamine (0.3 mmol), DIPEA (0.7 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude was purified (AcOEt/hexane 7:3) to afford an amorphous solid (30 mg, 13%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.98 (bs, 2H), 7.61 (bs, 4H), 7.37 (bs, 2H), 6.93 (bs, 4H), 3.42-3.37 (m, 4H), 2.33 (bs, 6H), 2.19 (bs, 12H), 1.72-1.66 (m, 4H) 1.41 (bs, 2H). HPLC: purity ≥ 99%. m/z (ESI) 759 [M + 1] (HPLC system B).

4,4'-(((Hexane-1,6-diylbis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***9***). Reagents: 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), hexane-1,6-diamine (0.5 mmol), DIPEA (1.1 mmol) and dioxane (10 mL). Reaction conditions: 24 h at 101 °C. The crude was purified (AcOEt/hexane 7:3) to afford an amorphous solid (100 mg, 26%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.98 (bs, 2H), 7.61 (bs, 4H), 7.37 (d, J = 7.6 Hz, 2H), 6.92 (bs, 4H), 3.42 (bs, 4H), 2.33 (bs, 6H), 2.19 (bs, 12H), 1.66 (bs, 4H) 1.47 (bs, 4H). HPLC: purity ≥ 99%. m/z (ESI) 773 [M + 1] (HPLC system A).

4,4'-(((Decane-1,10-diylbis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***10***). Reagents: 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (2 mmol), decane-1,10-diamine (1 mmol), DIPEA (2.2 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude was purified (AcOEt/hexane 7:3) to afford an amorphous solid (60 mg, 15%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.97 (bs, 2H), 7.66 - 7.58 (m, 4H), 7.37 (d, J = 8.0 Hz, 2H), 6.91 (bs, 4H), 3.40 (bs, 4H), 2.31 (bs, 6H), 2.19 (bs, 12H), 1.6 (bs, 4H), 1.35 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 829 [M + 1] (HPLC system A).

4,4'-(((Piperazine-1,4-diyl)bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl)) dibenzonitrile (***11***). Reagents: 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (1 mmol), piperazine (0.5 mmol), DIPEA (1.1 mmol) and dioxane (10 mL). Reaction conditions: 48 h at 101 °C. The crude was purified (AcOEt/hexane 7:3) to afford an amorphous solid (50 mg, 14%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.56 (bs, 2H), 8.52 (bs, 2H), 8.0 (bs, 1H), 7.72 (bs, 4H), 7.49 (d, J = 7.0 Hz, 3H), 6.92 (bs, 4H), 3.9 (bs, 8H), 2.23 (bs, 6H), 2.13 (bs, 12H). HPLC: purity ≥ 99%. m/z (ESI) 743 [M + 1] (HPLC system A).

4,4'-(((1,4-Phenylenebis(azanediyl))bis(4-(mesitylamino)-1,3,5-triazine-6,2-diyl))bis(azanediyl))dibenzonitrile (***12***). Reagents: 4-(4-chloro-6-(mesitylamino)-1,3,5-triazin-2-ylamino)benzonitrile (0.75 mmol), benzene-1,4-diamine (0.4 mmol), DIPEA (0.8 mmol) and dioxane (10 mL); Reaction conditions: 24 h at 101 °C. The crude was purified (AcOEt/hexane 7:3) to afford an amorphous solid (25 mg, 9%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (bs, 1H), 9.51 (bs, 1H), 9.14 (bs, 2H), 8.55 (bs, 2H), 8.07 (bs, 2H), 7.84 (bs, 2H), 7.68 (bs, 4H), 7.49 (bs, 4H), 6.92 (bs, 4H), 2.26 (bs, 6H), 2.13 (bs, 12H). HPLC: purity = 96%. m/z (ESI) 765 [M + 1] (HPLC system A).

Antiprotozoal assays

***In vitro* assays**

The antiprotozoal assays were performed at the Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Antwerp University, adopting the set of standard protocols as described by Cos *et al.* (1) and IC_{50} values were determined from five 4-fold dilutions, starting from a maximum concentration of 64 μg/mL.

Cytotoxicity assay

Human lung fibroblast MRC-5 SV_2 cells (purchased from Sigma Aldrich) were cultured in Earl's MEM, supplemented with 5% heat-inactivated FBS, 20 mM L-glutamine and 16.5 mM sodium bicarbonate. Assays were performed in 96-well microtiter plates, each well containing 1×10^4 cells. IC_{50} values were determined from five 4-fold dilutions starting at 64 μg/mL. After incubation for 72 h in a humidified atmosphere (37 °C, 5% CO_2) and addition of resazurin, the cell viability was assessed fluorimetrically (λ_{ex} 550 nm, λ_{em} 590 nm). The results were expressed as % reduction in cell growth/ viability compared to untreated control wells and IC_{50} values were determined. Tamoxifen was included as reference drug. The experiments were performed with low passage number cryostabilate and the cell line was checked and free of mycoplasma.

anti-*Trypanosoma brucei brucei* activity

The suramin-sensitive strain *Trypanosoma b. brucei* Squib 427 was maintained in HMI-9- medium, supplemented with 10% heat-inactivated FBS. Assays were performed in 96-well microtiter plates, each well containing 10 μL of the dilution of compound together with 190 μL of the parasite suspension (7×10^4 parasites/mL). After incubation in a humidified atmosphere (37 °C, 5% CO_2) for 72 h, resazurin was added for another 24 h and parasite growth was assessed fluorimetrically (λ_{ex} = 550 nm, λ_{em} = 590 nm). The results were expressed as % reduction in parasite growth/viability compared to control wells and IC_{50} values were calculated from five 4-fold dilutions starting with 64 μg/mL. Suramin and melarsoprol were included as reference drugs.

anti-*Trypanosoma brucei rhodesiense* activity

T. rhodesiense (strain STIB-900) was maintained in HMI-9- medium, supplemented with 10% heat-inactivated FBS. Assays were performed in 96-well microtiter plates, each well containing 10 μL of the dilution of compound together with 190 μL of the parasite suspension (2×10^4 parasites/mL). After incubation in a humidified atmosphere (37 °C, 5% CO_2) for 72 h, resazurin was added for another 6 h and parasite growth was assessed fluorimetrically (λ_{ex} = 550 nm, λ_{em} = 590 nm). The results were expressed as % reduction in parasite growth/viability compared to control wells and IC_{50} values were calculated from five 4-fold dilutions starting with 64 μg/mL. Suramin and melarsoprol were included as reference drugs.

anti-*Trypanosoma cruzi* activity

The nifurtimox-sensitive *Trypanosoma cruzi*, Tulahuen CL2, βgalactosidase strain was maintained in MRC-5 SV2 cells in MEM medium, supplemented with 200 mM L-glutamine, 16.5 mM sodium bicarbonate and 5% heat-inactivated *FBS*. All cultures and assays were conducted under a humidified atmosphere (37 °C, 5% CO_2). Assays were performed in 96-well microtiter plates, each well containing 10 μL of the dilution of compound together with 190 μL of MRC-5 SV2 cell/parasite inoculum (2×10^4 cells/mL and 2×10^5 parasites/mL). After incubation for 168 h, parasite growth was compared to untreated-infected controls. Parasite burdens were assessed after adding the substrate: 50 μL/well of a

stock solution containing 15.2 mg CPRG (chlorophenolredβ-D-galactopyranoside) and 250 µL Nonidet in 100 ml PBS. The change in color was measured spectrophotometrically at 540 nm after 4 h at 37 °C. The results were expressed as % reduction in parasite burdens compared to control wells and IC_{50} values were calculated from five fourfold dilutions starting with 64 µg/mL. Benznidazole and nifurtimox were included as reference drugs.

anti-*Leishmania infantum* activity

Leishmania infantum MHOM/MA (BE)/67 was maintained in the golden hamster and spleen amastigotes were collected for preparing infection inocula. Primary peritoneal mouse macrophages were used as host cells and collected 48 h after peritoneal stimulation with a 2% potato starch suspension. Assays were performed in 96-well microtiter plates, each well containing 10 µL of the dilution of compound together with 190 µL of macrophage/parasite inoculum (3×10^5 cells and 3×10^6 parasites/well in RPMI-1640 + 5% heat-inactivated FBS). After incubation for 120 h in a humidified atmosphere (37 °C, 5% CO_2), total parasite burdens were microscopically assessed after Giemsa staining. The results were expressed as % reduction in parasite burden compared to untreated control wells and IC_{50} values were calculated. The compounds were tested using five fourfold dilutions starting with 64 µg/mL. Miltefosine was included as reference drug.

anti-*Plasmodium falciparum* activity

The chloroquine-resistant strain of *P. falciparum* (Pf-K1) was maintained in RPMI-1640 supplemented with 0.37 mM hypoxanthine, 25 mM HEPES buffer, 25 mM sodium bicarbonate and 10% human 0+ serum together with 2–4% washed human 0+ erythrocytes (2). All cultures and assays were conducted under a humidified atmosphere (37 °C, 4% CO_2, 3% O_2 and 93% N_2) with the assay being an adaptation of the procedure described by Desjardins *et al.* (3). Assays were performed in 96-well microtiter plates, each well containing 10 µL of the dilutions of compound together with 190 µL of the malaria parasite inoculum (1% parasitaemia, 2% hematocrit). After incubation for 72 h at 37 °C, the plates were frozen and stored at −20 °C. Upon thawing, 20 µL of each well was transferred into another plate together with

100 µL Malstat® reagent and 20 µL of a 1/1 mixture of PES (phenazine methosulfate, 2 mg/mL) and NBT (Nitro Blue Tetrazolium Grade III, 0.1 mg/ml). The plates were kept in the dark for 2 h and change in color was measured spectrophotometrically at 655 nm. The results were expressed as % reduction in parasitaemia compared to control wells. The compounds were tested using five 4-fold dilutions starting with 64 µg/mL. Chloroquine and artemether were included as reference drugs.

Microsomal and plasma stability

Components of the assay

Male mouse and human liver microsomes were purchased from commercial sources (Corning) and stored at -80°C. NADPH generating system solutions A and B and UGT reaction mix solutions A and B were purchased from a commercial source (Corning) and kept at -20°C. Human plasma was collected in the lab from volunteers.

Microsomal stability assay

The microsomal stability assay was carried out based on the BD Biosciences Guidelines for Use (TF000017 Rev1.0) (Addendum 2) with minor adaptations. The metabolic stability of the compounds was studied through the CYP450 superfamily (Phase-I metabolism) by fortification with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and through uridine glucuronosyl-transferase (UGT) enzymes (Phase-II metabolism) by fortification with uridine diphosphate glucuronic acid (UDPGA). For the CYP450 and other NADPH dependent enzymes, both compounds were incubated at 5 µM together with 0.5 mg/mL liver microsomes in potassium phosphate buffer in a reaction started by the addition of 1 mM NADPH and stopped at the above listed sampling times. At these time points, 20 µl was withdrawn from the reaction mixture and 80 µl cold acetonitrile (ACN), containing the internal standard tolbutamide, was added to inactivate the enzymes and precipitate the protein. The mixture was vortexed for 30 sec and centrifuged at 4 °C for 5 min at 15,000 rpm. The supernatant was stored at -80 °C until analysis. For the

UGT enzymes, both compounds were incubated at 5 µM together with 0.5 mg/mL liver microsomes in a reaction started by the addition of 2 mM UDPGA cofactor.

Plasma stability assay

The plasma stability assay was carried out by incubating 40 µl of the test compounds (1 mM -100% DMSO) in 360 µL plasma at 37°C. At the above listed sampling points, 20 µl was withdrawn and 80 µl cold acetonitrile (ACN), containing the internal standard tolbutamide was added to precipitate the protein. The mixture was vortexed for 30 sec and centrifuged at 4°C for 5 min at 15,000 rpm. The supernatant was stored at -80°C until analysis.

Bioanalytical method

The corresponding loss of parent compound was determined using liquid chromatography (UPLC) (Waters AquityTM) coupled with tandem quadrupole mass spectrometry (MS^2) (Waters XevoTM), equipped with an electrospray ionization (ESI) interface and operated in multiple reaction monitoring (MRM) mode. The optimal MS parameters and control of the chromatographic separation conditions were tuned in a preceding experiment

***In vivo* drug screening model against *Trypanosoma brucei* (suramin-sensitive Squib 427 strain) in Swiss mice**

This study using laboratory rodents was carried out in strict accordance with all mandatory guidelines and was approved by the ethical committee of the University of Antwerp, Belgium.

Artificial infection

Trypanosoma brucei (suramin-sensitive Squib 427 strain) is maintained in the laboratory by weekly mechanical sub-passage in Swiss mice. The infection inoculum was prepared by taking heparinized blood

collected from a clinically ill donor mouse and diluted in PBS to obtain an infection inoculum of about 1×10^4 haemoflagellates in 0.25 ml. The infection inoculum was administered intraperitoneally (day 0 of the experiment).

Compound solutions and reference drugs

Compound formulations are prepared in 100% DMSO (compound **13**) or 100% DMSO/PEG200 (1/50) (compound **38**) administered intraperitoneally. Suramin was used as the standard reference drug and is formulated at 5 mg/ml in PBS.

Primary evaluation

Female Swiss mice (6/group) are intraperitoneally infected with 1×10^4 haemoflagellates at day 0. The first dosing was given orally 1/2 hour before artificial infection and then next four/twenty daily administrations were given intraperitoneally (dose 25 and 50 mg/kg). The reference compound suramin (10 mg/kg IP) is included in the same treatment regimen. Untreated infected controls generally die before day 7 of infection. On days 4, 10 and 14, a drop of blood is obtained from the tail vein for determination of the levels of parasitaemia (microscopic reading of Giemsa-stained blood smears). Compounds are considered active if the parasitaemia is reduced by >80% on day 4 (i.e. during dosing) or if the mean survival time in the treated exceeds that of the untreated controls by at least 50%.

Parameters

Clinical symptoms: the animals are observed for the occurrence/presence of clinical and adverse effects during the course of the experiment. The occurrence of mortality is monitored daily. Deaths before day 5 are likely related to drug toxicity. Obviously ill animals are euthanized and survival time is set at the next day. The mean survival time (MST) of treated versus control animals is indicative for efficacy.

Parasitaemia: on day 4, 10 and 14 (or longer in survivors) – reduction as compared to infected control animals is a measure for drug activity. Parasitaemia is determined microscopically. The difference between the mean value of the control group (taken as 100 %) and those of the experimental groups is expressed as percent reduction using the equation:

$$\text{Activity} = 100 - \frac{mean\ parasitemia\ treated}{mean\ parasitemia\ control} * 100$$

Body weight: on days 0, 4, 10, 14 (or longer in survivors).

ASSOCIATED CONTENT

Supporting Information

Full parasitic panel of dimers and monomers, Solubility and plasma stability assays, Additional references.

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HIGHLIGHTS

-Design and synthesis of dimeric compounds against T.brucei obtaining extremely potent compounds in vitro

-The two selected compouynds were able to improve metabolic stability compared to the monomeric derivatives.

-In vivo data are not in line with the highly promising in vitro potency and in vitro ADME data.