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From human immunodeficiency virus non-nucleoside reverse transcriptase inhibitors to potent and selective antitrypanosomal compounds

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ABSTRACT

The presence of a structural recognition motif for the nucleoside P2 transporter in a library of pyrimidine and triazine non-nucleoside HIV-1 reverse transcriptase inhibitors, prompted for the evaluation of antitrypanosomal activity. It was demonstrated that the structure–activity relationship for anti-HIV and antitrypanosomal activity was different. Optimization in the diaryl triazine series led to 6-(mesityloxy)-N_2-phenyl-1,3,5-triazine-2,4-diamine (**69**), a compound with potent in vitro and moderate in vivo antitrypanosomal activity.

1. Introduction

After malaria and lymphatic filariasis, human African trypanosomiasis (HAT, sleeping sickness) is estimated to have one of the highest disease burdens of the neglected parasitic diseases (NPD).[1] HAT is endemic in 36 sub-Saharan countries and approximately 70 million people are estimated to be at risk, with at least 10,000 mortalities annually.[2,3] However, gross underreporting due to difficulties in diagnosis and remoteness of some affected areas makes estimation of accurate rates of morbidity and mortality difficult. African trypanosomiasis is transmitted by the tsetse fly and caused by *Trypanosoma brucei gambiense*, causing endemic or chronic disease in Central and West Africa and *Trypanosoma brucei rhodesiense*, causing the more acute disease in East and Southern Africa. The animal form of African trypanosomiasis is called Nagana and caused by *Trypanosoma brucei brucei*.[4] Together with HAT, Nagana is a major cause of rural underdevelopment with significant economic damage to cattle farming. Chemotherapy currently remains the only treatment option. However the available drugs that were developed decades ago show limited efficacy, are not affordable, cause severe adverse effects and are threatened by increasing drug resistance.[4,5] Currently used drugs are pentamidine, suramin, melarsoprol, eflornithine and nifurtimox. HAT is classified as a neglected disease since investments are negligible compared to its impact on human and veterinary health. In the last decades, little progress has been made in addressing this lack of effective treatments and the initiative to tackle this pressing medical need has largely moved to academic research in collaboration with public–private partnerships, such as the Drugs for Neglected Diseases initiative (DNDi).

It has been well established that phenotypic screening is a successful approach for the discovery of first-in-class drugs, which is especially valid for drug discovery in infectious diseases, since the 'whole micro-organism' is the best possible target.[6,7] Hence, the screening/evaluation of existing libraries of drug-like molecules for novel anti-infectives is the most logical and straightforward approach. In the framework of a drug discovery programme for new anti-HIV microbicides,[8–11] we developed a library of novel

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0968-0896/

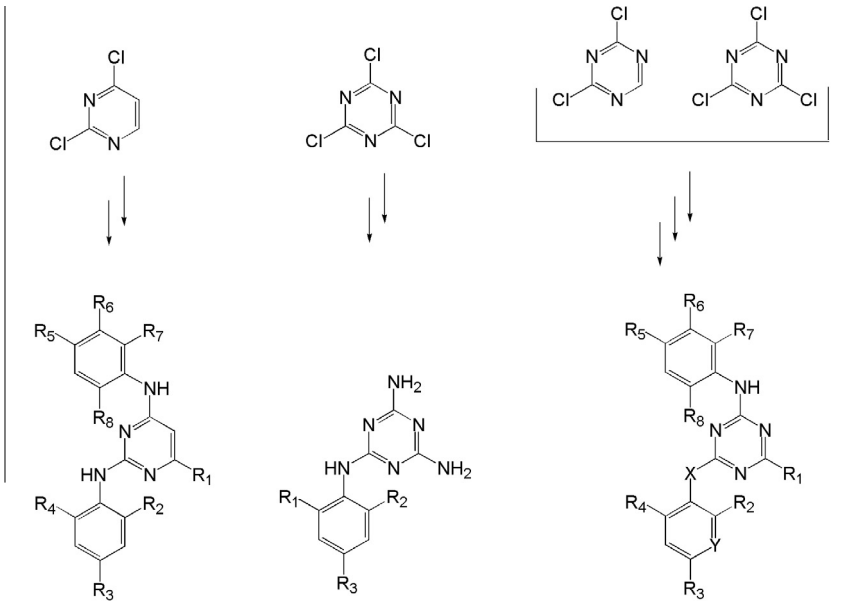
pyrimidines and triazines[11] related to TMC120[12] (**1**) as non-nucleoside reverse transcriptase inhibitors (NNRTIs). The antiprotozoal activity of NNRTIs has never been described, but the triazine core is a substrate for the unique nucleoside P2 transporter in *T. brucei* and is present in melarsoprol.[13] The triazine core has been used in drug delivery to trypanosomes for compounds such as polyamine analogues and nitroheterocycles.[14–19] However, since there are no reports of diarylsubstituted pyrimidines and triazines as antitrypanosomal agents, we broadly screened and optimized our compounds against *T. b. brucei* and *T. b. rhodesiense* (Fig. 1).

2. Results and discussion

The target compounds were synthesized from 2,4-dichloropyrimidines, 2,4-dichlorotriazines and 2,4,6-trichlorotriazines by nucleophilic aromatic substitution (S_NAr) reactions (Schemes 1–5 in Supplementary content).

The activity of compounds **1–5**, **6–10** and **11–70** toward *T. b. brucei* and *T. b. rhodesiense* and their cytotoxicity on a human cell line (MRC5) is presented in comparison with anti-HIV-1 activity (Tables 1–5). The compounds were also tested against *Trypanosoma cruzi*, *Leishmania infantum* and *Plasmodium falciparum* (Tables 1–3 in Supplementary content). As a control, we used standard anti-parasitic compounds as well as known NNRTIs. The antiparasitic potential of all compounds was analyzed by applying the WHO/TDR screening activity criteria specified for each parasite.[20] All the disubstituted pyrimidines including TMC120 (**1**) as well as the monosubstituted triazines **6–10** were inactive (Table 1). In contrast, among the diaryltriazines several compounds exhibited submicromolar activity against both *T. b. brucei* and *T. b. rhodesiense* (Tables 2–5), with **69** being equipotent to suramin and melarsoprol.

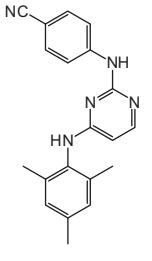
Based on the activities of the 60 diaryltriazines, we were able to deduce a structure–activity relationship (SAR) (Fig. 2). At R_1, the presence of NH_2 (e.g., **29**) is clearly favoured, whereas hydrogen (e.g., **14**), chloro- (e.g., **16**) and some alkylamino- (**37–44**) substituents are less active and/or cytotoxic. Other small substituents at this position such as cyano- (e.g., **24**) and methoxy- (e.g., **26**) are not tolerated. The presence of methyl groups at R_2, R_3 and R_4 originating from the SAR of the anti-HIV-1 NNRTIs (TMC120, **1**) resulted in potent antitrypanosomal compounds. Generally, these methyl groups can be replaced with Br or Cl, especially at R_2 and R_4, but removal of one of the methyls is less well tolerated. The *para*-cyano group on the other aryl group (R_5), which is known to be crucial for anti-HIV-1 activity can be removed and offers a way to dissociate antitrypanosomal and anti-HIV-1 activity. For instance, in the series **60** (*para*-cyano), **70** (*meta*-cyano) and **69** (no cyano group), the antiviral activity decreases whereas the antitrypanosomal activity increases. Replacement of one aryl group with a pyridyl group (**45–48**) was not tolerated. Changing the arylamino substituent (X = NH) to an aryloxy substituent (X = O, Table 5) was more favourable in almost all cases (e.g., **60** vs **28**). X = NMe did not seem to alter the activity much (**36** vs **28**). Concludingly, **16**, **29**, **63** and **69** are the four most potent antitrypanosomal compounds (IC_{50} = <0.25 μM) from the library of disubstituted triazines **11–70**.



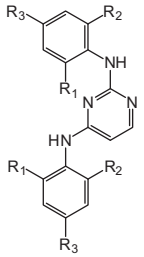
R_1 = H, Cl, OMe, CN, NH_2, NHR_9, NR_9R_{10}, Piperidinyl, N-Me piperazinyl
R_2 = H, Me, OMe, Br, Cl
R_3 = H, Me, Br, Cl, CN, CHO
R_4 = H, Me, OMe, Br
R_5 = R_6 = H, CN
R_7 = R_8 = H, Me
R_9 = Me, iPr, cyclopropyl, 3-Methoxypropyl, phenyl
R_{10} = Me
X = NH, NMe, O
Y = CH, N

Figure 1. Structures of target compounds.

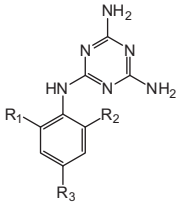
Table 1
Antitrypanosomal and anti-HIV-1 activity of disubstituted pyrimidines **1–5** and monosubstituted triazines **6–10**



1 (TMC120)



2-5

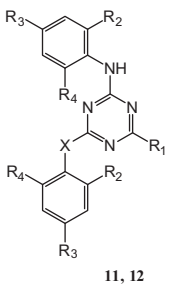


6-10

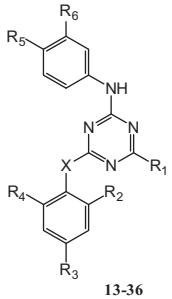
Compd	R_1	R_2	R_3	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
				T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
1				>16	19.5	>64	0.002	2.47
2	Me	Me	Me	7.19	3.84	>64	>10	nd[f]
3	H	H	H	7.27	2.53	13.2	>10	nd[f]
4	H	H	CN	>64	4	>64	>10	nd[f]
5	H	Me	CN	>64	39.77	>64	4.5	4.70
6	Me	Me	Me	>32	>32	>32	>10	nd[f]
7	Br	Br	Me	>32	>32	>32	>10	nd[f]
8	Br	Me	Me	>32	>32	>32	>10	nd[f]
9	Br	H	Me	>32	>32	>32	>10	nd[f]
10	H	H	CN	>32	>32	>32	>10	nd[f]
Suramin[g]				0.02	0.02	>64		
Melarsoprol[g]				0.03	0.02	7.4		
Lersivirine[g]							0.003	>100
MIV170[g]							0.001	>100

[a] Antitrypanosomal activity against the suramin-sensitive strain *T. b. brucei* Squib 427.
[b] Antitrypanosomal activity against *T. b. rhodesiense* strain STIB-900.
[c] Cytotoxicity measurement using human lung fibroblast MRC-5 SV_2 cells.
[d] 50% effective concentration from TZM-bl virus inhibition assay.
[e] 50% cytotoxic concentration from the Water Soluble Tetrazolium-1 (WST-1) Cell Proliferation Assay.
[f] Not determined.
[g] Reference compounds.

Table 2
Antitrypanosomal and anti-HIV-1 activity of disubstituted triazines **11–36**



11, 12



13-36

Compd	X	R_1	R_2	R_3	R_4	R_5	R_6	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
								T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
11	NH	Cl	Me	Me	Me	—	—	>64	>64	>64	4.35	>100
12	NH	NH_2	Me	Me	Me	—	—	2.33	0.58	>64	7.82	>100
13	NH	H	Me	Me	Me	CN	H	22.79	8.01	>64	0.002	29.65
14	NH	H	Br	Me	Br	CN	H	0.96	nd[f]	43.07	0.003	1
15	NH	Cl	Me	Me	Me	CN	H	45.74	6.66	>64	0.002	>10
16	NH	Cl	Br	Me	Br	CN	H	0.21	0.11	5.58	0.01	1.73
17	NH	Cl	Br	Me	Me	CN	H	2.86	2.96	>32	0.004	2.15
18	NH	Cl	Br	Me	H	CN	H	>32	>32	>32	0.49	10.24
19	NH	Cl	Me	H	Me	CN	H	32.46	5.53	>64	0.008	5
20	NH	Cl	Me	Br	Me	CN	H	1.99	2.4	5.84	0.008	9.14
21	NH	Cl	Br	Me	Br	H	H	>32	>32	>32	0.16	9.98
22	NH	Cl	Br	Me	Br	H	CN	8.06	4.01	>64	0.065	6.61
24	NH	CN	Br	Me	Br	CN	H	>32	>32	>32	0.008	0.15
25	NH	CN	Br	Me	Me	CN	H	11.61	13.27	>32	0.009	0.67
26	NH	OMe	Br	Me	Br	CN	H	4.16	2.84	0.52	0.003	0.19

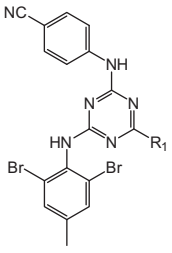
(*continued on next page*)

Table 2 (*continued*)

Compd	X	R_1	R_2	R_3	R_4	R_5	R_6	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
								T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
27	NH	OMe	Br	Me	Me	CN	H	11.60	11.97	>32	0.002	0.19
28	NH	NH_2	Me	Me	Me	CN	H	4.84	2.49	20.51	0.004	44
29	NH	NH_2	Br	Me	Br	CN	H	0.12	0.08	16.61	0.002	5.9
30	NH	NH_2	Br	Me	Me	CN	H	6.15	3.21	>32	0.001	2.63
31	NH	NH_2	Br	Me	H	CN	H	4.47	30.82	>64	0.004	>100
32	NH	NH_2	Me	H	Me	CN	H	16.28	8.68	>64	0.009	22.23
33	NH	NH_2	Me	Br	Me	CN	H	7.01	7.5	7.51	0.003	15.16
34	NH	NH_2	Br	Me	Br	H	H	6.44	3.89	33.94	0.13	26.46
35	NH	NH_2	Br	Me	Br	H	CN	6.84	1.12	6.74	0.033	18.51
36	NMe	NH_2	Me	Me	Me	CN	H	4.47	1.45	23.57	0.007	24.63
Suramin[g]								0.02	0.02	>64		
Melarsoprol[g]								0.03	0.02	7.4		
Lersivirine[g]											0.003	>100
MIV170[g]											0.001	>100

[a] Antitrypanosomal activity against the suramin-sensitive strain *T. b. brucei* Squib 427.
[b] Antitrypanosomal activity against *T. b. rhodesiense* strain STIB-900.
[c] Cytotoxicity measurement using human lung fibroblast MRC-5 SV_2 cells.
[d] 50% effective concentration from TZM-bl virus inhibition assay.
[e] 50% cytotoxic concentration from the Water Soluble Tetrazolium-1 (WST-1) Cell Proliferation Assay.
[f] Not determined.
[g] Reference compounds.

Table 3
Antitrypanosomal and anti-HIV-1 activity of disubstituted triazines **37–44**



37-44

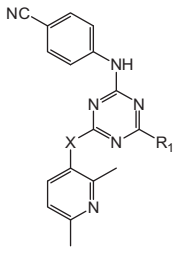
Compd	R_1	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
		T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
37	NHMe	0.59	0.45	1.03	0.002	1.07
38	NMe_2	1.54	1.01	53.50	0.005	12.55
39	$NHCHMe_2$	1.54	2.05	>64	0.036	17.59
40	NHcyclopropyl	2.05	0.40	2.12	0.011	2.90
41	$NH(CH_2)_3OMe$	12.16	11.18	22.92	0.011	35.53
42	NHPh	0.42	0.37	2.11	0.15	2.72
43	Piperidinyl	1.68	1.22	>64	0.12	71.85
44	N-Me piperazinyl	1.88	0.82	5.64	0.011	8.66
Suramin[f]		0.02	0.02	>64		
Melarsoprol[f]		0.03	0.02	7.4		
Lersivirine[f]					0.003	>100
MIV170[f]					0.001	>100

[a] Antitrypanosomal activity against the suramin-sensitive strain *T. b. brucei* Squib 427.
[b] Antitrypanosomal activity against *T. b. rhodesiense* strain STIB-900.
[c] Cytotoxicity measurement using human lung fibroblast MRC-5 SV_2 cells.
[d] 50% effective concentration from TZM-bl virus inhibition assay.
[e] 50% cytotoxic concentration from the Water Soluble Tetrazolium-1 (WST-1) Cell Proliferation Assay.
[f] Reference compounds.

As could be expected, several diaryltriazines also demonstrated low nanomolar activity against HIV-1. However, the structure–activity relationship for the anti-HIV-1 and antitrypanosomal compounds is different (e.g., compare **60** and **69**). Since this is the first report of antitrypanosomal activity of non-nucleoside reverse transcriptase inhibitors (NNRTIs), we decided to test several other NNRTI drugs and drug candidates (Table 4 in Supplementary content). None of the other NNRTIs demonstrated antitrypanosomal activity, indicating that the antitrypanosomal activity is uniquely linked to the diaryl triazine structural motif.

After the extensive SAR study to optimize the antitrypanosomal activity, we selected the most potent diaryltriazine from the arylamino series (**29**) and from the aryloxy series (**69**) for evaluation of their in vitro ADME properties in comparison with melarsoprol (Table 6). Compounds **29** and **69** are highly lipophilic ($\log D$ = >4) with a rather low solubility. Both compounds show excellent stability in a buffer of pH 7.4 and in plasma. Compared to melarsoprol **29** has a similar (human) to slightly improved (mouse) microsomal stability, whereas the metabolism of **69** is clearly faster.

Table 4
Antitrypanosomal and anti-HIV-1 activity of disubstituted triazines **45–48**

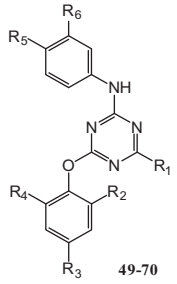


45-48

Compd	X	R_1	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
			T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
45	NH	Cl	>64	5.82	4.38	1.42	11.87
47	NH	NH_2	50.55	12.57	>64	0.41	>100
48	NMe	NH_2	>64	29.32	>64	0.06	>100
Suramin[f]			0.02	0.02	>64		
Melarsoprol[f]			0.03	0.02	7.4		
Lersivirine[f]						0.003	>100
MIV170[f]						0.001	>100

[a] Antitrypanosomal activity against the suramin-sensitive strain *T. b. brucei* Squib 427.
[b] Antitrypanosomal activity against *T. b. rhodesiense* strain STIB-900.
[c] Cytotoxicity measurement using human lung fibroblast MRC-5 SV_2 cells.
[d] 50% effective concentration from TZM-bl virus inhibition assay.
[e] 50% cytotoxic concentration from the Water Soluble Tetrazolium-1 (WST-1) Cell Proliferation Assay.
[f] Reference compounds.

Table 5
Antitrypanosomal and anti-HIV-1 activity of disubstituted triazines **49–70**



49-70

Compd	R_1	R_2	R_3	R_4	R_5	R_6	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
							T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
49	Cl	Me	Me	Me	CN	H	2.12	1.05	7.32	1.04	17.38
50	Cl	Br	Me	Br	CN	H	1.45	0.78	>64	2.02	15.89
51	Cl	Br	Me	H	CN	H	5.18	8.50	8.17	3.82	20.84
52	Cl	Cl	Me	H	CN	H	5.51	4.53	8.92	2.14	21.18
53	Cl	Me	H	Me	CN	H	4.10	2.15	>64	5.36	16.38
54	Cl	Me	Br	Me	CN	H	5.32	2.11	6.36	2.57	18.55
55	Cl	Me	Cl	Me	CN	H	2.61	1.65	10	2.69	16.56
56	Cl	Me	CHO	Me	CN	H	5.20	1.46	17.41	5.21	19.30
57	Cl	OMe	Me	OMe	CN	H	9.12	7.09	>64	1.92	18.81
58	Cl	Me	Me	Me	H	H	15.36	19.75	25.5	nd[f]	nd[f]
59	Cl	Me	Me	Me	H	CN	5.2	21.4	8.01	nd[f]	nd[f]
60	NH_2	Me	Me	Me	CN	H	1.17	0.59	7.48	0.002	17.27
61	NH_2	Br	Me	Br	CN	H	1.37	0.43	2.82	0.002	2.31
62	NH_2	Br	Me	H	CN	H	3.52	2.37	>64	0.002	>100
63	NH_2	Cl	Me	H	CN	H	0.05	0.1	>64	0.002	94.76
64	NH_2	Me	H	Me	CN	H	1.13	1.15	>64	0.008	20.56
65	NH_2	Me	Br	Me	CN	H	0.52	0.35	7.63	0.002	6.48
66	NH_2	Me	Cl	Me	CN	H	0.78	0.46	5.25	0.003	4.49
67	NH_2	Me	CHO	Me	CN	H	2.03	1.93	>64	0.012	26.70
68	NH_2	OMe	Me	OMe	CN	H	0.79	0.71	34.05	0.003	19.68
69	NH_2	Me	Me	Me	H	H	0.015	0.24	>64	0.158	20.63
70	NH_2	Me	Me	Me	H	CN	0.21	0.84	25.92	0.098	23.08

(*continued on next page*)

Table 5 (*continued*)

Compd	R_1	R_2	R_3	R_4	R_5	R_6	Antitrypanosomal activity and cytotoxicity IC_{50} (μM)			Anti-HIV-1 activity and cytotoxicity (μM)	
							T. b. brucei[a]	*T. b. rhodesiense*[b]	MRC-5[c]	EC_{50}[d]	CC_{50}[e]
Suramin[g]							0.02	0.02	>64		
Melarsoprol[g]							0.03	0.02	7.4		
Lersivirine[g]										0.003	>100
MIV170[g]										0.001	>100

[a] Antitrypanosomal activity against the suramin-sensitive strain *T. b. brucei* Squib 427.
[b] Antitrypanosomal activity against *T. b. rhodesiense* strain STIB-900.
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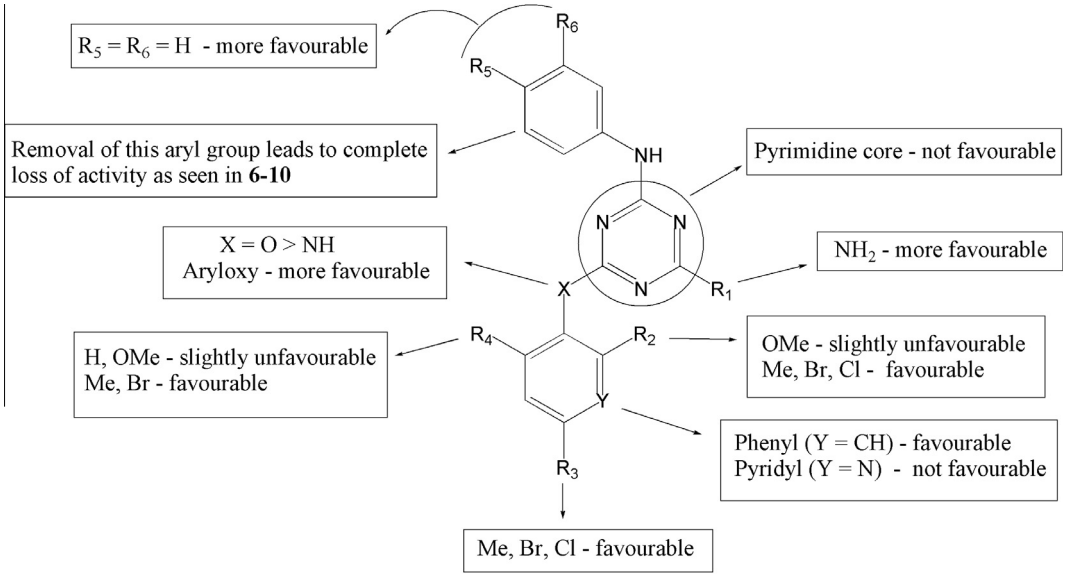
[g] Reference compounds.



R_1 = H, Cl, OMe, CN, NH_2, NHR_7, NR_7R_8, piperidinyl, N-Me piperazinyl
R_2 = Me, OMe, Br, Cl
R_3 = H, Me, Br, Cl, CHO and R_4 = H, Me, OMe, Br
R_5 = R_6 = H, CN
R_7 = Me, iPr, cyclopropyl, 3-methoxypropyl, phenyl
R_8 = Me
X = NH, NMe, O and Y = CH, N

Figure 2. SAR derived from screening of target compounds against *Trypanosoma brucei*.

Table 6
In vitro ADME of selected compounds

Compd	Solubility[a] (μM)	log$D_{7.4}$[b]	Stability at pH 7.4% recovery after 24 h[c]	Microsomal stability % recovery after 15 min[d]		Plasma stability % recovery after 1 h[e]	
				Human	Mouse	Human	Mouse
29	25–50	4.51	100	84	72	100	100
69	25–50	4.14	100	26	18	100	100
Melarsoprol	>200	2.11	100	95	45	100	100

[a] Final test compound concentration range of between 3.125 μM and 200 μM [4 μl DMSO solution in 196 μl buffer solution (10 mM PBS pH 7.4)].
[b] The partition coefficient between octanol and aqueous phase (10 mM PBS pH 7.4).
[c] Stability measurement using a 100 μl aliquot of the 10 μM solution in DMSO+1900 μl of PBS (10 mM PBS, pH 7.4).
[d] The metabolism by microsomes (CYP_{450} and other NADPH-dependent enzymes) was monitored and expressed as percentage of remaining parent compound.
[e] Percentage of remaining parent compound.

The two selected compounds **29** and **69** were also evaluated against *T. b. brucei* (suramin-sensitive Squib 427 strain) in Swiss mice, with suramin as positive control compound. The mean survival time (MST), the reduction in parasitemia at day 4 post infection and the number of survivors at day 14 post-infection were measured as end points (Table 7). With **29**, we noted a dose-dependent increase of survival, with a doubling of the MST compared to vehicle at a dose of 50 mg/kg twice daily. However, a complete

Table 7
Activities of compounds in the *T. b. brucei* mouse model

Compd	Dose (mg/kg)	Freq.	Formulation	MST[a]	Reduction (%) in parasitemia on 4 dpi[b]	Survivors on 14 dpi
Control[c]				7.2		0/4
29	25	SID × 5	100% DMSO	7	nd[d]	0/4
	50	SID × 5	100% DMSO	8	41	0/4
	80	SID × 5	100% DMSO	9	nd[d]	0/4
	25	BID × 5	100% DMSO	10.5	nd[d]	0/4
	50	BID × 5	100% DMSO	15.5	nd[d]	2/4
69	25	SID × 5	100% DMSO/PEG200 (1:50)	8.7	66	0/6
Suramin	10	SID × 5	100% PBS	>21	100	6/6

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

cure as with suramin was not observed. A similar result was observed for **69**. Since **29** and **69** are equipotent with suramin in the in vitro assay against *T. b. brucei*, the most likely reasons for the weaker in vivo profile must be linked to the pharmacokinetic properties. As indicated in Table 6, the solubility of **29** and **69** and the metabolic stability of **69** are suboptimal.

3. Conclusion

The antiprotozoal activity of NNRTIs has never been described, but the triazine core is a substrate for the unique nucleoside P2 transporter in *T. b. brucei* and is present in melarsoprol. This prompted us to evaluate our drug-like library of pyrimidine and triazine NNRTIs. For the first time, it was demonstrated that diarylsubstituted triazines show potent antitrypanosomal activity, a property that is not present in other NNRTI drug classes. A structure–antitrypanosomal activity relationship could be established that was different from the SAR of the anti-HIV-1 activity. Several compounds displayed nanomolar antitrypanosomal activity with **69** being equipotent to suramin and melarsoprol. Two selected compounds showed a dose-dependent anti-trypanosomal activity in vivo, but complete cure as for suramin was not observed. The most likely reason for the suboptimal in vivo properties was found in their pharmacokinetic properties. Hence, optimization of the diarylsubstituted triazines with enhanced solubility and metabolic stability is now in progress.

4. Experimental section

4.1. Chemistry

Reagents were obtained from Sigma–Aldrich or Acros. Characterization of all the compounds was done with NMR and mass spectrometry. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance DRX-400 and 400 MHz Bruker Avance III nanobay spectrometer with ultrashield. Chemical shifts were in ppm and coupling constants were in Hertz (Hz). ES mass spectra were obtained from an Esquire 3000plus iontrap mass spectrometer from Bruker Daltonics. LC–MS spectra were recorded on an Agilent 1100 Series HPLC system using a Alltech Prevail C18 column (2.1 × 50 mm, 5 mm) coupled with an Esquire 3000plus as MS detector; solvent A: H_2O+0.1% formic acid, solvent B: CH_3CN+0.1% formic acid; gradient: 5% B → 100% B over 30 min at 0.2 mL min^{-1}. A wavelength of 214 nm was used. HPLC was run on a Gilson instrument equipped with an Ultrasphere ODS column (4.6 × 250 mm, 5 mm); solvent A: H_2O+0.1% trifluoroacetic acid (TFA), solvent B: CH_3CN+0.1% TFA; gradient: 10% B → 100% B over 36 min at 1 mL min^{-1}. A wavelength of 214 nm was used. Waters acquity UPLC system coupled to a waters TQD ESI mass spectrometer and waters TUV detector was used with UPLC BEH C18 1.7 μm 2.1 × 50 mm column. Water (A) and MeCN (B) were used as eluents. 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. Formic acid 0.1% was added to solvents A and B. The wavelength for UV detection was 214 nm. The products were purified with flash chromatography on a Flashmaster II (Jones chromatography) or on a Biotage® ISOLERA One flash system equipped with a internal variable dual-wavelength diode array detector (200–400 nm), with a 30 min gradient of 0–80% EtOAc in hexanes or 0–10% DCM in MeOH in necessary cases. The purity of all the target compounds were ⩾95%, except for compound **14** (88%) which could not be purified further. As a representative example the experimental and analytical data for **29** and **69** were given. Detailed experimental and analytical data for all intermediates and remaining target compounds can be found in Supplementary content of this publication.

4.2. General procedure for synthesis of 29 and 69

Compounds **16** or **58** (1 mmol) was taken in a pressure tube and dissolved in 2.0 M ammonia in isopropanol (10 mL) and allowed to stir at 100 °C overnight. Solvents were evaporated and the crude product was purified by flash chromatography using 70% EtOAc in hexanes or 10% DCM in methanol as eluent. After evaporation, the product was obtained as white powder.

4.2.1. 4-((4-Chloro-6-((2,6-dibromo-4-methylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (29)

Yield: 0.24 g, 51%. ^{1}H NMR (MeOD, 400 MHz) δ 7.96 (d, 1H, J = 8.7 Hz), 7.62 (d, 2H, J = 8.5 Hz), 7.55 (s, 2H), 7.40 (br s, 1H), 2.38 (br s, 3H). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167, 165.3, 164.4, 145.1, 139.9, 134.1, 132.7, 132.4, 125.2, 119.5, 118.9, 102.2, 19.8. MS (ESI) m/z 476 [M+H]$^{+}$. HPLC (214 nm) t_r 19.6 min, 100%. LC–MS (214 nm) t_r 16.9 min, 100%.

4.2.2. 6-(Mesityloxy)-N_2-phenyl-1,3,5-triazine-2,4-diamine (69)

Yield: 0.19 g, 59%. ^{1}H NMR (MeOD, 400 MHz) δ 7.47 (br s, 2H), 7.15 (br s, 2H), 6.90 (br s, 3H), 2.33 (br s, 3H), 2.09 (br s, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170, 168.4, 165.6, 147.2, 139.8, 133.9, 129.6, 128.9, 128.2, 121.9, 119.7, 20.3, 16.1. MS (ESI) m/z 322 [M+H]$^{+}$. UPLC–MS (214 nm) t_r 4.8 min, 100%. LC–MS (214 nm) t_r 18.1 min, 100%.

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Supplementary data

Supplementary data (chemistry and synthetic schemes, antiprotozoal and antiviral activity, spectroscopic details of the intermediate and target compounds, in vitro antiprotozoal assays, in vivo drug screening model against *Trypanosoma brucei brucei* Squib 427, in vitro antiviral assays, in vitro ADME assays and additional references) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.08.005.

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