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Optimization of the pharmacokinetic properties of potent anti-trypanosomal triazine derivatives

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ABSTRACT

Human African trypanosomiasis is causing thousands of deaths every year in the rural areas of sub-saharan Africa. There is a high unmet medical need since the approved drugs are poorly efficacious, show considerable toxicity and are not easy to administer. This work describes the optimization of the pharmacokinetic properties of a previously published family of triazine lead compounds. One compound (**35** (UAMC-03011)) with potent anti-trypanosomal activity and no cytotoxicity was selected for further study because of its good microsomal stability and high selectivity for *Trypanosoma brucei* over a panel including *Trypanosoma cruzi*, *Leishmania infantum*, and *Plasmodium falciparum*. *In vivo* pharmacokinetic parameters were determined and the compound was studied in an acute *in vivo* mouse disease model. One of the important learnings of this study was that the rate of trypanocidal activity is an important parameter during the lead optimization process.

KEYWORDS

Trypanosoma brucei, triazine, phenotypic screening, microsomal stability, cidal efficacy

INTRODUCTION

Neglected tropical diseases have a significant impact on human health each year in countries with a less developed economic status and particularly suffer from a lack of research due to a limited return on investment. Among these disorders, African trypanosomiasis affects the rural areas of sub-Saharan Africa and affects either farm-animals named Nagana caused by *Trypanosoma brucei brucei* and human beings caused by *T. b. rhodesiense* and *T. b. gambiense* and share the same vector spreading the disease, *e.g.* the tse-tse fly. The current drugs are pentamidine and suramin for the first-stage, while melarsoprol, eflornithine and nifurtimox are used during the second stage when the parasite has spread to the central nervous system.[1] New and safer drugs that are easily administered are needed since the existing drugs show serious side effects and in some cases have fatal consequences.[2]

Triazine derivatives have been extensively investigated in our group. In the framework of a drug discovery programme for new-anti HIV microbicides, about 50 triazine and pyrimidine derivatives were synthesized and evaluated for anti-HIV-1 activity while one compound was further developed for prevention of sexual HIV transmission.[3-7] Although the antiprotozoal activity of anti-HIV-1 compounds had never been reported, the triazine core is known for its ability to act as substrate for the nucleoside P2 transporter[8, 9]. These compounds were therefore evaluated against *T. brucei* producing several interesting hits.[10, 11] During lead optimization, more than 100 monomers and dimers were evaluated for which it could be concluded that the triazine scaffold yields more potent compounds than the pyrimidine core. Compound **a** (6-(mesityloxy)-*N*²-phenyl-1,3,5-triazine-2,4-diamine) showed sub-micromolar

activity on *T. brucei*, no cytotoxicity on MRC5 cells, and was not active against *T. cruzi*, *Leishmania infantum* and *Plasmodium falciparum*. Furthermore, the anti-HIV activity was considerably reduced, indicating selective anti-trypanosomal activity. However, due to a low *in vitro* metabolic stability (only 20% of parent compound left after 15 min upon incubation with mouse microsomal S9 fraction), *in vivo* activity could not be demonstrated in a mouse model of acute *T. brucei* infection. We report here on the design, synthesis and characterization of compounds with improved *in vivo* pharmacokinetic properties while maintaining the selective anti-trypanosomal activity.

Figure 1. Previously reported compound **a** with antitrypanosomal activity.

RESULTS AND DISCUSSION

Synthesis of new *T. brucei* inhibitors

The design of the molecules was made based on the SAR obtained from the previously synthesized molecules.[10] Since the most likely metabolic hotspots are the benzylic methyl groups and the *para*-position of the phenyl ring, we introduced modifications such as halogen atoms to block aromatic hydroxylations.[12] Different linkers such as thioether or ether in both linker positions (X and Y) between the triazine ring and the aromatic moieties were also studied.

Figure 2. Different structural modifications based on compound **a**.

The synthesis was performed by subsequent nucleophilic aromatic substitutions. Different pathways were followed depending on the nature of the linker (Scheme 1).

Reaction conditions: i) When X = NH: DIPEA or K_2CO_3 , aniline derivative, dioxane, 25 – 101°C, 40 min – 48 h. When X = O: $(NBu_4)HSO_4$, NaOH, phenol derivative, toluen/water (10:1), 0 – 25°C, 40 min – 24 h. ii) When Y = NH: DIPEA or K_2CO_3 , aniline derivative, dioxane, 25 – 101°C, 45 min – 5 days. When Y = O: $(NBu_4)HSO_4$, NaOH, phenol derivative, toluen/water (10:1), 0 – 25°C, 5 min – 5 days. When Y = S: DIPEA, dioxane, 0 °C, 1 h. iii) When Z = NH_2 , NH_3 in methanol or dioxane, 25 – 101°C, 16 h – 8 days. When Z = NHMe, NH_2Me , DIPEA, dioxane, 101°C, 16 h. When Z = piperidiny, piperidine, DIPEA, dioxane, 101 °C, 16 h.

Scheme 1. Synthetic pathway to obtain different trisubstituted triazine derivatives.

In addition, two disubstituted triazines were synthesized to study the importance of the free amino group on the potency of the compound (Scheme 2).

88

89

90 Reaction conditions i) DIPEA or K₂CO₃, aniline derivative, dioxane, 25°C, 5 min. ii)
 91 tetrabutylammoniumhydrogensulfate, sodium hydroxide, dioxane, 25°C, 30 min.

92 **Scheme 2.** Synthetic pathway to obtain different disubstituted triazine derivatives.

93

94 **In vitro parasitology assays.** The compounds were evaluated on a panel including *T. brucei*,
 95 *T. cruzi*, *L. infantum* and *P. falciparum* (results presented in supporting information).
 96 Cytotoxicity on a human cell line (MRC-5) and on primary peritoneal mouse macrophages
 97 (PMM) was also tested. The activity of all the compounds against *T. brucei* and MRC-5 are
 98 shown in **Table 1**.

99

100 **Table 1.** In vitro antitrypanosomal activity and cytotoxicity of the final compounds and
 101 derivative b.

102

Compd.	X	Y	Z	R	R'	Antitrypanosomal activity and cytotoxicity IC ₅₀ (μM)	
						<i>T. b. bruc.</i> ^a	MRC5 ^b
a	NH	O	NH ₂	H	2,4,6-trimethyl	0.88	> 64
27	NH	O	NH ₂	4-F	2,4,6-trimethyl	0.31	> 64
28	NH	O	piperidinyl	4-F	2,4,6-trimethyl	8.17	> 64
29	NH	O	NH ₂	4-Cl	2,4,6-trimethyl	1.52	6.39
30	NH	O	NH ₂	4-CF ₃	2,4,6-trimethyl	6.94	6.5
31	NH	O	NH ₂	3,5-diF	2,4,6-trimethyl	1.29	4.44
32	NH	O	NHMe	4-F	2,4,6-trimethyl	10.86	> 64
33	NH	O	NH ₂	2,6-diF	2,4,6-trimethyl	> 64	> 64

34	O	O	NH ₂	4-F	2,4,6-trimethyl	4.27	> 64
35	NH	O	NH ₂	4-F	4-F	0.63	> 64
36	NH	O	NH ₂	4-F	4-Me	0.39	> 64
37	NH	O	NH ₂	H	4-Me	1.08	> 64
38	NH	O	NH ₂	4-F	2-Cl,4-Me	0.51	> 64
39	NH	O	NH ₂	4-CN	4-Me	0.96	> 64
40	NH	S	NH ₂	4-F	4-F	>64	>64
41	NH	NH	NH ₂	4-Me	2,6-diBr-4-Me	9.13	8.06
42	NH	NH	NH ₂	4-F	2,6-diBr-4-Me	7.1	6.7
43	NH	NH	NH ₂	4-F	4-F	2.77	> 64
44	NH	NH	NH ₂	4-CN	4-F	39.83	> 64
45	NH	NH	NH ₂	4-CN	4-Me	>64	>64
46	NH	NH	NH ₂	4-CN	2-Cl,4-Me	7.7	> 64
47	NH	NH	NH ₂	4-CN	2,6-diMe-4-F	7.87	23.74
50	NH	O	H	4-F	2,4,6-trimethyl	31.05	8.00
51	NH	O	H	H	2,4,6-trimethyl	32.46	17.76
Suramin^c						0.03	

103 ^a Each value is the mean of at least two independent determinations. ^b Cytotoxicity
104 measurement using human lung fibroblast MRC-5 cells. ^c reference compound
105

106 The structure-activity relationship follows the same pattern as the one previously found for
107 this type of compounds.[10] The nature of the linker atom Y considerably influences the
108 potency. If Y = O, the compounds are more potent than when Y = NH or Y = S (Compound
109 **35** vs **40** and vs **43** or compound **39** vs **45**). When X = NH, the compound is more potent than
110 when X = O (compound **27** vs **34**). The nature of Z also plays an important role in the
111 potency. If Z = NH₂ the compound is 25-100 fold more potent than a piperidinyI, NHMe or H
112 (compound **27** vs **28**, **32** and **50**, respectively).

113 **Microsomal stability.** The more potent compounds **27**, **35**, **36** and **38** were studied for *in*
114 *vitro* metabolic stability with human and mouse microsomal fraction (S9) (**Figure 3**).

Figure 3. *In vitro* mouse microsomal stability of compounds **27**, **35**, **36** and **38**. Diclofenac was used as a reference compound.

As could be expected, the substituents on the two phenyl rings play an important role in the metabolic stability. Compound **35** (UAMC-03011) with a *para*-F in both phenyl rings is the most stable with 76% and 93% of remaining parent compound after 15 min upon incubation with mouse and human microsomes, respectively. All compounds had a (slightly) better metabolic stability compared to the starting lead compound **a**, which showed only 20% remaining parent compound after 15 min incubation with mouse microsomes. However, compounds **27**, **36** and **38** showed insufficient stability in mouse microsomes (remaining parent compound at 15 min of 22%, 57% and 25% respectively) to allow further study in mouse disease models. The plasma stability was also evaluated and compounds **27**, **35**, **36** and **38** remained fully stable after incubation in human plasma for 24 h at 37°C (Supporting information). Mouse plasma protein binding of **35** was 96% and plasma protein binding in the *T. brucei* culture medium was 50%, demonstrating a sufficiently high unbound fraction to exert anti-trypanosomal effect.

Pharmacokinetics

in view of its potent and selective anti-trypanosomal activity, and excellent microsomal and plasma stability, compound **35** was selected first for *in vivo* pharmacokinetic properties in mice and subsequent study in the acute *T. brucei* disease model. Compound **35** was administered orally to groups of mice using a single dose of either 50 mg/kg or 25 mg/kg. **Figure 4** shows the plasma concentrations of compound **35** vs time for the 50 mg/kg dose. Peak concentrations rise to about 10-fold the *in vitro* anti-trypanosomal IC₅₀, and then fall to about the IC₅₀ of 0.63 µM at 24 h with a T_{1/2} of about 6 h (**Table 2**).

Figure 4. Concentration in blood of compound **35** after a single oral dose of 50 mg/kg in mice.

Table 2. Pharmacokinetic parameters of derivative **35**.

	T _{max} (h)	C _{max} (µM)	T _{1/2} (h)	AUC ₀₋₂₄ (ng.h/mL)	V _z (l)	Cl (mL/min)

50 mg/kg PO	0.5	5.66	6.51	28609	15.6	27.8
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Acute mouse model of *T. b. brucei*

The encouraging pharmacokinetic data and the high potency and selectivity of **35** motivated to study this compound in an acute mouse disease model of *T. b. brucei*. The compound was administered orally (PO) or injected intraperitoneally (IP) at doses differing from 10 to 50 mg/kg once (s.i.d.) or twice daily (b.i.d.) for 5 days after infection. Disappointingly, no *in vivo* activity was demonstrated since the treated animals showed the same mean survival time (MST < 7 days) as the vehicle-treated infected controls (**Table 3**).

Table 3. *In vivo* activities of compound **35** on the acute *T. b. brucei* model.

Compd.	dose (mg/kg)	freq.	MST	survivors on 14 dpi
Vehicle			7	0/3
Suramin	10	SID × 5 (IP)	> 7	3/3
	25	BID × 5 (PO)	6	0/3
35	50	BID × 5 (PO)	5.7	0/3
	50	SID × 5 (PO)	7	0/3
	10	SID × 5 (IP)	7	0/3

Rate of trypanocidal activity of compound **35** compared to suramin

To explain the lack of potency in the *in vivo* model for a compound of high potency and with good pharmacokinetic properties, we decided to study the rate at which the compound is able

to kill the parasites.[13, 14] Based on the *in vivo* $C_{\max}$ that was around 10-fold the anti-trypanosomal IC_{50} , we investigated the parasite reduction over time using concentrations that were seven (**Figure 5A**) and fourteen (**Figure 5B**) times higher than the IC_{50} of **35** and compared this with the reference compound suramin at concentrations which exceeded its IC_{50} in a similar way. Whereas suramin gave a very significant reduction after 18 h and a complete eradication after 48 h at both 7 and 14 times the IC_{50} , compound **35** only gave a very minor reduction of parasites after 48 h. It is clear that **35** is much slower acting than suramin.

Figure 5. Graph A: % parasite growth vs time of compound **35** at 5 µM and suramin at 0.125 µM. Graph B: % parasite growth vs time of compound **35** at 10 µM and suramin at 0.25 µM.

CONCLUSIONS

A novel compound series was synthesized presenting a potent and selective anti-trypanosomal activity. By careful design considering the previously determined SAR and metabolic hotspots in the starting compound, we developed compound **35** (UAMC-03011) with a high *in vitro* microsomal stability and good pharmacokinetic properties while maintaining the excellent potency and selectivity. After oral administration at 50 mg/kg, **35** reached blood concentrations exceeding the IC₅₀ by 10-fold and keeping the concentration in blood above or at the IC₅₀ for 24 h until the next dose was administered. Despite these properties, compound **35** did not result in any activity in the acute mouse model of *T. b. brucei*, whereas the reference drug suramin at 100 mg/kg resulted in complete cure. Published pharmacokinetic data of suramin in rats treated with a single oral dose of 100 mg/kg showed a plasma concentration after 24 h of 2.3 µM, which is 76-fold its IC₅₀. [15]. Although rats and mice are not comparable, this indicates that the slightly higher plasma

concentrations of suramin compared to **35**, combined with its superior potency against *T. b. brucei* demonstrated by the 20-fold lower IC₅₀ is probably responsible for the failure to demonstrate of *in vivo* activity of **35**. Furthermore, the rate of trypanocidal activity of **35** is much slower compared to suramin. In conclusion, the present study demonstrated that next to potency, selectivity, lack of toxicity and excellent pharmacokinetics, the rate of trypanocidal activity should be considered as an important parameter in lead selection and lead optimization.

EXPERIMENTAL PART

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). ¹H 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: spectral width 16 ppm, pulse width 9 μs (57 °), data size 32 K. ¹³C 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: spectral width 16 ppm, pulse width 9 μs (57 °), data size 32 K. Chemical shifts are reported in values (ppm) relative to internal Me₄Si, and *J* values are reported in Hz. The UPLC (Ultra Performance liquid chromatography), used to quantify the purity of the products was an ACQUITY UPLC H-Class System with an TUV detector Waters coupled to a MS detector Waters QDa. An Acquity UPLC BEH C18 1.7μm (2.1 x 50 mm) column was used and as eluent a mixture of 0.1% FA in water, 0.1% FA in acetonitrile, water, acetonitrile. Final compounds were analyzed by high resolution mass. 10 μL of 10⁻⁵ M solution of each sample was injected using the CapLC system (Waters,

Manchester, UK) and electrosprayed using a standard electrospray source. Samples were injected with an interval of 5 min. Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, UK). The MS was calibrated prior to use with a 0.2% H₃PO₄ solution. The spectra were lock mass corrected using the known mass of the nearest H₃PO₄ cluster. All the compounds were obtained as amorphous solids. All the final compounds presented a purity at least 95% determined by UPLC and HNMR.

Synthesis of intermediates:

General procedure for Compounds 1 and 2, 14 - 19:

A solution of NaOH (1 equiv) in water (12 mL for 36.7 mmol) was added to a solution of phenol derivative (1 equiv), monoaryl-bischloro-triazine derivative (1 equiv) and tetrabutylammoniumhydrogensulfate (0.1 equiv) in toluene (120 mL for 36.7 mmol) at 0 °C. The reaction was stirred for a certain time at a fixed temperature specified in each case. The solvent was evaporated, dichloromethane (50 mL) and brine (50 mL) were added. The organic solvent was evaporated and the crude was used in the next step without further purification.

2,4-Dichloro-6-(mesityloxy)-1,3,5-triazine (1). Reagents: NaOH (1.45 g, 36.7 mmol), mesitol (5 g, 36.7 mmol), 2,4,6-trichloro-1,3,5-triazine (6.77 g, 36.7 mmol) and tetrabutylammoniumhydrogensulfate (1.25 g, 3.67 mmol). Reaction conditions: 2 h at 0 °C and 24 h at room temperature. Yield: 7.70 g, 65%. UPLC: purity 88%. m/z (ES) 284 [M], 286 [M + 2], 288 [M + 4].

2,4-Dichloro-6-(4-fluorophenoxy)-1,3,5-triazine (2). Reagents: NaOH (76 mg, 1.89 mmol), 4-fluorophenol (213 mg, 1.89 mmol), 2,4,6-trichloro-1,3,5-triazine (350 mg, 1.89 mmol) and tetrabutylammoniumhydrogensulfate (64.4 mg, 0.19 mmol). Reaction conditions: 2 h at 0 °C and 40 min at room temperature. Yield: 321.1 mg, 65%. UPLC: purity 70%. m/z (ES) 260 [M], 262 [M + 2], 264 [M + 4].

243 **2-Chloro-4-(4-fluorophenoxy)-6-(mesityloxy)-1,3,5-triazine (14).** Reagents: NaOH (23.07
244 mg, 0.57 mmol), mesitol (79 mg, 0.57 mmol), intermediate **2** (150 mg, 0.57 mmol) and
245 tetrabutylammoniumhydrogensulfate (19.58 mg, 0.06 mmol). Reaction conditions: 5 min at 0
246 °C. Yield: 125 mg, 60%. UPLC: purity 50%. m/z (ES) 360 [M + 1], 362 [M + 3].

247 **4-Chloro-6-(4-fluorophenoxy)-N-phenyl-1,3,5-triazin-2-amine (15).** Reagents: NaOH
248 (19.91 mg, 0.49 mmol), 4-fluorophenol (55.8 mg, 0.49 mmol), intermediate **3** (120 mg, 0.49
249 mmol) and tetrabutylammoniumhydrogensulfate (16.9 mg, 0.05 mmol). Reaction conditions:
250 5 min at 0 °C and after that, 1 h at room temperature. Yield: 102.7 mg, 65%. UPLC: purity
251 65%. m/z (ES) 317 [M + 1], 319 [M + 3].

252 **4-Chloro-N-(4-fluorophenyl)-6-(p-tolyloxy)-1,3,5-triazin-2-amine (16).** Reagents: NaOH
253 (18.53 mg, 0.46 mmol), *p*-cresol (50.1 mg, 0.46 mmol), intermediate **3** (120 mg, 0.46 mmol)
254 and tetrabutylammoniumhydrogensulfate (15.73 mg, 0.05 mmol). Reaction conditions: 5 min
255 at 0 °C and after that 1 h at room temperature. Yield: 96.3 mg, 63%. UPLC: purity 63%. m/z
256 (ES) 331 [M + 1], 333 [M + 3].

257 **4-Chloro-N-phenyl-6-(p-tolyloxy)-1,3,5-triazin-2-amine (17).** Reagents: NaOH (33.2 mg,
258 0.83 mmol), *p*-cresol (90 mg, 0.83 mmol), intermediate **4** (200 mg, 0.83 mmol) and
259 tetrabutylammoniumhydrogensulfate (28.2 mg, 0.08 mmol). Reaction conditions: 5 min at 0
260 °C and after that 40 min at room temperature. Yield: 176 mg, 68%. UPLC: purity 68%. m/z
261 (ES) 313 [M + 1], 315 [M + 3].

262 **4-Chloro-6-(2-chloro-4-methylphenoxy)-N-(4-fluorophenyl)-1,3,5-triazin-2-amine (18).**
263 Reagents: NaOH (18.5 mg, 0.46 mmol), 2-chloro-4-methylphenol (66 mg, 0.46 mmol),
264 intermediate **3** (120 mg, 0.46 mmol) and tetrabutylammoniumhydrogensulfate (15.7 mg, 0.04
265 mmol). Reaction conditions: 5 min at 0 °C and after that 1 h at room temperature. Yield:
266 109.8 mg, 68%.

267 **4-((4-Chloro-6-(*p*-tolylloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (19).** Reagents: NaOH
268 (29.6 mg, 0.74 mmol), *p*-cresol (0,08 ml, 0,74 mmol), intermediate **5** (250 mg, 0,74 mmol),
269 and tetrabutylammonium hydrogensulfate (252 mg, 0,74 mmol). Reaction conditions: 5 min
270 at 0 °C and after that 5 days at room temperature. Yield: 0,13 g, 52%. UPLC: purity 50%.
271 *m/z* 338 [M], 340 [M + 2].

272 General procedures for compounds **3** – **13**, **21** - **26**:

273 A solution of the specified aniline (1 equiv), 2,4,6-trichloro-1,3,5-triazine (1 equiv) and a
274 determined base (1 – 1.4 equiv) in dioxane (12 mL for 5.42 mmol) was stirred for a specified
275 time at fixed temperature. The solvent was evaporated and DCM (50 mL) was added, the
276 crude was extracted with a saturated solution of NaHCO₃ (50 mL) and a saturated solution of
277 NaCl (50 mL). The organic phase was dried and the solvent was evaporated under vacuum.
278 The product was purified by IsoleraOne using the eluents specified for each reaction.

279 **4,6-Dichloro-*N*-(4-fluorophenyl)-1,3,5-triazin-2-amine (3).** Reagents: 4-fluorobenzenamine
280 (603 mg, 5.42 mmol), 2,4,6-trichloro-1,3,5-triazine (1 g, 5.42 mmol) and potassium carbonate
281 (1.05 g, 7.59 mmol). Reaction conditions: 40 min at 25 °C. Purification: heptane/ ethyl
282 acetate (0 – 100 ethyl acetate). Yield: 270 mg, 20%. UPLC: purity > 99%. *m/z* (ES) 259 [M],
283 261 [M + 2], 263 [M + 4].

284 **4,6-Dichloro-*N*-phenyl-1,3,5-triazin-2-amine (4).** Reagents: aniline (177 mg, 1.89 mmol),
285 2,4,6-trichloro-1,3,5-triazine (350 mg, 1.89 mmol) and potassium carbonate (367 mg, 2.66
286 mmol. Reaction conditions: 5 min at 25 °C. Purification: heptane/ ethyl acetate (0 – 100 ethyl
287 acetate). Yield: 371.9 mg, 57%. UPLC: purity > 99%. *m/z* (ES) 241 [M], 243 [M + 2], 245
288 [M + 4].

289 **4-((4,6-Dichloro-1,3,5-triazin-2-yl)amino)benzonitrile (5).** Reagents: 2,4,6-trichloro-1,3,5-
290 triazine (5 g, 27,1 mmol), 4-aminobenzonitrile (3,36 g, 28,45 mmol) and potassium carbonate
291 (3,93 g, 28,5 mmol). Reaction conditions: 48 h at 25 °C and 2 h extra at 101 °C. Purification:

292 heptane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 1,7 g, 23%. UPLC: purity 79%. m/z (ES)
293 266 [M], 268 [M + 2], 270 [M + 4].

294 **4,6-Dichloro-N-(4-fluorophenyl)-1,3,5-triazin-2-amine (6).** Reagents: *N*-ethyl-*N*-
295 isopropylpropan-2-amine (2,26 ml, 13,01 mmol), 4-fluorobenzenamine (1,03 ml, 10,85
296 mmol) and 2,4,6-trichloro-1,3,5-triazine (2g, 10,85 mmol). Reaction conditions: 25 °C for 2
297 h. Purification: heptane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 1,67 g, 60%. UPLC:
298 purity > 99%. m/z (ES) 259 [M], 261 [M + 2], [M + 4].

299 **4,6-Dichloro-N-(2,6-dibromo-4-methylphenyl)-1,3,5-triazin-2-amine (7).** Reagents: 2,4,6-
300 trichloro-1,3,5-triazine (1 g, 5,42 mmol), 2,6-dibromo-4-methylaniline (1,51 g, 5,69 mmol)
301 and potassium carbonate (0,78 g, 5,70 mmol). Reaction conditions: 25 °C for 48 h.
302 Purification: heptane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 0,79 g, 34%. UPLC: purity:
303 84%. m/z (ES) [M - 2], [M + 2], [M + 4].

304 **6-Chloro-*N*²,*N*⁴-bis(4-fluorophenyl)-1,3,5-triazine-2,4-diamine (8).** Reagents: *N*-ethyl-*N*-
305 isopropylpropan-2-amine (0,34 ml, 1,95 mmol), 4-fluorobenzenamine (0,31 ml, 3,25 mmol)
306 and 2,4,6-trichloro-1,3,5-triazine (300 mg, 1,63 mmol). Reaction conditions: 25 °C for 24 h.
307 Purification: heptane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 224,5 mg, 41%. UPLC:
308 purity: >99%. m/z (ES) 334 [M + 1], 336 [M + 3].

309 **4-Chloro-N-(4-fluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (9).** Reagents:
310 intermediate **1**, (2 g, 7.04 mmol), 4-fluoroaniline (0.78 g, 7.04 mmol), *N*-ethyl-*N*-
311 isopropylpropan-2-amine (0.9 g, 7.04 mmol). Reaction conditions: 4 days at room
312 temperature. Purification: hexane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 1.3 g, 52%.
313 UPLC: purity 88%. m/z (ES) 359 [M + 1], 361 [M + 3].

314 **4-Chloro-N-(4-chlorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (10).** Reagents:
315 intermediate **1** (1 g, 3.5 mmol), 4-chloroaniline (0.45 g, 3.5 mmol), *N*-ethyl-*N*-
316 isopropylpropan-2-amine (0.45 g, 3.5 mmol). Reaction conditions: 4 days at room

317 temperature. Purification: hexane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 638.1 mg,
318 49%. UPLC: purity 99%. m/z (ES) 375 [M], 377 [M + 2], 379 [M + 4].

319 **4-Chloro-6-(mesityloxy)-N-(4-(trifluoromethyl)phenyl)-1,3,5-triazin-2-amine (11).**

320 Reagents: intermediate **1** (500 mg, 1.76 mmol), 4-(trifluoromethyl)aniline (284 mg, 1.76
321 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (273 mg, 2.1 mmol). Reaction conditions: 45
322 min at 101 °C. Purification: hexane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 409 mg,
323 57%. UPLC: purity > 99%. m/z (ES) 407 [M - 1], 309 [M + 1].

324 **4-Chloro-N-(3,5-difluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (12).** Reagents:

325 intermediate **1** (500 mg, 1.76 mmol), 3,5-difluoroaniline (227 mg, 1.76 mmol), *N*-ethyl-*N*-
326 isopropylpropan-2-amine (273 mg, 2.1 mmol). Reaction conditions: 45 min at 101 °C.
327 Purification: hexane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 500.8 mg, 76%. UPLC:
328 purity 85%. m/z (ES) 377 [M + 1], 379 [M + 3].

329 **4-Chloro-N-(2,6-difluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (13).** Reagents:

330 intermediate **1** (616 mg, 2.17 mmol), 2,6-difluoroaniline (280.2 mg, 2.17 mmol), *N*-ethyl-*N*-
331 isopropylpropan-2-amine (280.2 mg, 2.17 mmol). Reaction conditions: 6 h at 101 °C.
332 Purification: hexane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 570 mg, 70%. UPLC: purity
333 >99%. m/z (ES) 377 [M + 1], 379 [M + 3].

334 **6-Chloro-*N*²-(2,6-dibromo-4-methylphenyl)-*N*⁴-(*p*-tolyl)-1,3,5-triazine-2,4-diamine (21).**

335 Reagents: intermediate **7** (300 mg, 0,61 mmol), *p*-toluidine (65,4 mg, 0,61 mmol), *N*-ethyl-*N*-
336 isopropylpropan-2-amine (0,12 ml, 0,67 mmol). Reaction conditions: 19 h at 101 °C.
337 Purification: heptane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 216 mg, 73%. UPLC:
338 purity > 99%. m/z (ES) [M - 2], [M + 2], [M + 4].

339 **6-Chloro-*N*²-(2,6-dibromo-4-methylphenyl)-*N*⁴-(4-fluorophenyl)-1,3,5-triazine-2,4-diamine**

340 **(22).** Reagents: intermediate **6** (300 mg, 0,62 mmol), 4-fluoroaniline (0,06 ml, 0,62 mmol),

341 *N*-ethyl-*N*-isopropylpropan-2-amine (0,11 ml, 0,62 mmol). Reaction conditions: 19 h at 101

°C. Purification: heptane/ ethyl acetate (0 – 100 ethyl acetate). Yield: 0,21 g, 69%. UPLC: purity > 99%. m/z (ES) 488 [M - 2], 487 [M], 489 [M + 2], 491 [M + 4].

4-((4-Chloro-6-((4-fluorophenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (23).

Reagents: intermediate **5** (300 mg, 1,13 mmol), 4-fluoroaniline (0,11 ml, 1,13 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0,21 ml, 1,24 mmol). Reaction conditions: 1 h at 101 °C. The organic crude was used in the next step without further purification (270.6 mg, 71%). UPLC: purity: 97%. m/z (ES) 341 [M + 1], 343 [M + 3].

4-((4-Chloro-6-(*p*-tolylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (24). Reagents:

intermediate **5** (300 mg, 1,13 mmol), *p*-toluidine (121 mg, 1,127 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0,21 ml, 1,24 mmol) and. Reaction conditions: 1 h at 101 °C. The organic crude was used in the next step without further purification (342 mg, 90%). UPLC: purity: 90%. m/z (ES) 337[M + 1], 339 [M + 3].

4-((4-Chloro-6-((2-chloro-4-methylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile

(25). Reagents: intermediate **5** (250 mg, 0,74 mmol), 2-chloro-4-methylaniline (0,09 ml, 0,74 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0,13 ml, 0,74 mmol). Reaction conditions: 22 h at 101 °C. The organic crude was used in the next step without further purification (0,3 g, 99% yield). UPLC: purity: 92%. m/z (ES) 371 [M], 373 [M + 2], 375 [M + 4].

4-((4-Chloro-6-((4-fluoro-2,6-dimethylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile

(26). Reagents: intermediate **5** (250 mg, 0,74 mmol), 4-fluoro-2,6-dimethylaniline (103 mg, 0,74 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0,12 mL, 0,74 mmol). Reaction conditions: 4 h at 101 °C. The crude was used in the next reaction without further purification (0,29 g, 99%). UPLC: purity 91%. m/z (ES) 369 [M], 371 [M + 2].

4-Chloro-*N*-(4-fluorophenyl)-6-((4-fluorophenyl)thio)-1,3,5-triazin-2-amine (20). A

solution of 4,6-dichloro-*N*-(4-fluorophenyl)-1,3,5-triazin-2-amine (300 mg, 1,16 mmol) in THF (4,2 mL) was cooled down to 0 °C. A second solution containing 4-fluorobenzenethiol

(135 mg, 1,05 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (0,18 mL, 1,05 mmol) in THF (9 mL) was added dropwise to this first solution. The final solution was stirred for 1 h at 0 °C. The solution was concentrated in vacuo and the residue was redissolved in ethyl acetate (25 mL) and washed with water (25 mL), a saturated solution of NaHCO₃ (25 mL) and brine (25 mL). The crude was purified by IsoleraOne using heptane and ethyl acetate (0 – 100% ethyl acetate) as eluents (0,31 g, 56%). UPLC: purity: 42%. *m/z* (ES) 351 [M + 2].

4-Chloro-*N*-(4-fluorophenyl)-1,3,5-triazin-2-amine (48). A solution of 2,4-dichloro-1,3,5-triazine (300 mg, 2.0 mmol), 4-fluorobenzenamine (222 mg, 2.0 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (284 mg, 2.2 mmol) in dioxane (4 mL) was stirred at room temperature during 5 min. Dichloromethane (50 mL) and water (50 mL) were added and the organic phase was dried over magnesium sulphate and the organic solvent was evaporated under vacuum. The crude was used in the next step without further purification (449 mg, 99%). UPLC: purity 99%. *m/z* (ES) 225 [M + 1], 227 [M + 3].

4-Chloro-*N*-phenyl-1,3,5-triazin-2-amine (49). A solution of 2,4-dichloro-1,3,5-triazine (300 mg, 2.0 mmol), aniline (186 mg, 2.0 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (284 mg, 2.2 mmol) in dioxane (4 mL) was stirred at room temperature during 5 min. Dichloromethane (50 mL) and water (50 mL) were added and the organic phase was dried over magnesium sulphate and the organic solvent was evaporated under vacuum. The crude was used in the next step without further purification (413 mg, 99%). UPLC: purity 99%. *m/z* (ES) 207 [M + 1], 209 [M + 3].

Synthesis of final compounds:

General procedure for compounds 27 - 47:

The triazine derivative (1 equiv) was dissolved in a solution of ammonia in a specified solvent with a determined normality. The solution was heated at a specified temperature for a

different time, given for each reaction. The solvent was evaporated and the crude was purified by IsoleraOne using different eluents for each reaction.

***N*²-(4-Fluorophenyl)-6-(mesityloxy)-1,3,5-triazine-2,4-diamine (27)**: Reagents: 4-chloro-*N*-(4-fluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (300 mg, 0.8 mmol) and ammonia 7 N in methanol (40 mL). Reaction conditions: 65 °C for 4 h. Purification: IsoleraOne using DCM/MeOH (0 – 10% MeOH). Yield: 171 mg, 60%. ¹H-NMR (400 MHz, DMSO-*d*₆) δ: 9.51 (s, 1H), 7.65 (s, 2H), 7.22 - 6.84 (m, 6H), 2.24 (s, 3H), 2.04 (s, 6H). ¹³C-NMR (100 MHz, DMSO-*d*₆) δ: 169.9, 168.4, 165.5, 157.41 (d, *J* = 239.1 Hz), 147.2, 136.2 (d, *J* = 2.3 Hz), 134.0, 129.7, 129.0, 121.4, 114.77 (d, *J* = 21.8 Hz), 20.3, 16.1. UPLC: purity > 99%. *m/z* (ES) 340.1 [M + 1]. HRMS: Calc: 339.15 Found: 340.1584 [M + 1].

***N*²-(4-Chlorophenyl)-6-(mesityloxy)-1,3,5-triazine-2,4-diamine (29)**: Reagents: 4-chloro-*N*-(4-chlorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (300 mg, 0.8 mmol) and ammonia 7 N in methanol (40 mL). Reaction conditions: 65 °C for 16 h. Purification: IsoleraOne using DCM/MeOH (0 – 10% MeOH). Yield: 184.1 mg, 65%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.63 (s, 1H), 7.68 (s, 2H), 7.36 - 6.99 (m, 4H), 6.90 (s, 2H), 2.24 (s, 3H), 2.03 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 170.0, 168.4, 165.5, 147.2, 138.9, 134.0, 129.7, 129.0, 128.1, 125.5, 121.1, 20.3, 16.1. UPLC: purity > 99%. *m/z* (ES) 356.1 [M + 1], 358.1 [M + 3]. HRMS: Calc: 355.12 Found: 356.1278 [M + 1].

6-(Mesityloxy)-*N*²-(4-(trifluoromethyl)phenyl)-1,3,5-triazine-2,4-diamine (30): Reagents: 4-chloro-6-(mesityloxy)-*N*-(4-(trifluoromethyl)phenyl)-1,3,5-triazin-2-amine (409 mg, 1.0 mmol) and ammonia 7 N in methanol (40 mL). Reaction conditions: 65 °C for 2 h. Purification: IsoleraOne using hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 209.7 mg, 54%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.89 (s, 1H), 7.85 (s, 2H), 7.50 (d, *J* = 7.9 Hz, 2H), 7.25 (d, *J* = 34.8 Hz, 2H), 6.92 (s, 2H), 2.25 (s, 3H), 2.04 (s, 6H). ¹³C NMR (100 MHz,

416 DMSO-*d*₆) δ : 170.1, 168.5, 165.6, 147.2, 143.62 (c, $J = 1.0$ Hz), 134.1, 129.7, 129.0, 125.9
417 (c, $J = 271.1$ Hz), 125.5 (c, $J = 3.6$ Hz), 121.9 (c, $J = 32.0$ Hz), 119.3, 20.3, 16.1. UPLC:
418 purity > 99%. m/z (ES) 390.2 [M + 1]. HRMS: Calc: 389.15 Found: 390.1542 [M + 1].

419 ***N*²-(3,5-Difluorophenyl)-6-(mesityloxy)-1,3,5-triazine-2,4-diamine (31)**: Reagents: 4-chloro-
420 *N*-(3,5-difluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (409 mg, 1.0 mmol) and
421 ammonia 7 N in methanol (40 mL). Reaction conditions: 65 °C for 2 h. Purification:
422 IsoleraOne using hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 173.2 mg, 36%. ¹H
423 NMR (400 MHz, DMSO-*d*₆) δ : 9.85 (s, 1H), 7.77 - 7.01 (m, 4H), 6.90 (s, 2H), 6.70 (t, $J = 8.9$
424 Hz, 1H), 2.23 (s, 3H), 2.03 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 170.0, 168.4, 165.5,
425 162.31 (dd, $J = 241.6, 15.7$ Hz), 147.0, 142.6 (t, $J = 14.2$ Hz), 134.2, 129.5, 129.0, 102.0 (d, J
426 = 29.3 Hz), 96.7 (t, $J = 26.3$ Hz), 20.3, 16.1. UPLC: purity > 99%. m/z (ES) 358.1 [M + 1].
427 HRMS: Calc: 357.14 Found: 358.1479 [M + 1].

428 ***N*²-(2,6-Difluorophenyl)-6-(mesityloxy)-1,3,5-triazine-2,4-diamine (33)**: Reagents: 4-chloro-
429 *N*-(2,6-difluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (500 mg, 1.3 mmol) and
430 ammonia 7 N in methanol (40 mL). Reaction conditions: 65 °C for 16 h. Purification:
431 IsoleraOne using hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 248 mg, 52%. ¹H
432 NMR (400 MHz, DMSO-*d*₆) δ : 9.00 (s, 1H), 7.32 (tt, $J = 7.9, 6.3$ Hz, 1H), 7.13 (t, $J = 8$ Hz,
433 2H), 7.01 (d, $J = 13.2$ Hz, 2H), 6.87 (s, 2H), 2.22 (s, 3H), 2.03 (s, 6H). ¹³C NMR (100 MHz,
434 DMSO-*d*₆) δ : 170.2, 168.6, 167.1, 158.6 (d, $J = 248.4$ Hz), 147.1, 133.9, 129.6, 128.9, 127.7
435 (t, $J = 5.5$ Hz), 115.5 (t, $J = 16.4$ Hz), 111.8 (dd, $J = 18.1, 5.4$ Hz), 20.3, 16.1. UPLC: purity
436 > 99%. m/z (ES) 358.2 [M + 1]. HRMS: Calc: 357.14 Found: 358.1485 [M + 1].

437 ***4*-(4-Fluorophenoxy)-6-(mesityloxy)-1,3,5-triazin-2-amine (34)**: Reagents: 2-chloro-4-(4-
438 fluorophenoxy)-6-(mesityloxy)-1,3,5-triazine (205 mg, 0.57 mmol) and ammonia 7 N in
439 methanol (20 mL). Reaction conditions: 65 °C for 16 h. Purification: IsoleraOne using
440 hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 38.1 mg, 20%. ¹H NMR (400 MHz,

441 CDCl_3) δ : 7.15 - 7.02 (m, 4H), 6.88 (d, $J = 0.5$ Hz, 2H), 2.29 (s, 3H), 2.11 (s, 6H). ^{13}C NMR
442 (100 MHz, CDCl_3) δ : 172.8, 172.1, 169.5, 160.3 (d, $J = 244.2$ Hz), 147.8, 147.1, 135.5,
443 130.0, 129.3, 123.2 (d, $J = 8.5$ Hz), 116.1 (d, $J = 23.5$ Hz), 20.9, 16.5. UPLC: purity > 99%.
444 m/z (ES) 341.1 $[\text{M} + 1]$. HRMS: Calc: 340.13 Found: 341.1412 $[\text{M} + 1]$.

445 **6-(4-Fluorophenoxy)- N^2 -(4-fluorophenyl)-1,3,5-triazine-2,4-diamine (35)**: Reagents: 4-
446 chloro-6-(4-fluorophenoxy)- N -(4-fluorophenyl)-1,3,5-triazin-2-amine (167 mg, 0.5 mmol)
447 and ammonia 7 N in methanol (20 mL). Reaction conditions: 65 °C for 16 h. Purification:
448 IsoleraOne using hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 46.7 mg, 30%. ^1H
449 NMR (400 MHz, $\text{MeOD}-d_4$) δ : 7.51 (s, 2H), 7.24 - 7.08 (m, 4H), 6.92 (s, 2H). ^{13}C NMR (100
450 MHz, $\text{MeOD}-d_4$) δ : 172.5, 169.9, 166.9, 161.5 (d, $J = 242.3$ Hz), 160.1 (d, $J = 240.6$ Hz),
451 149.8 (d, $J = 2.8$ Hz), 136.5 (d, $J = 2.3$ Hz), 124.8 (d, $J = 8.5$ Hz), 123.5 (d, $J = 6.2$ Hz),
452 116.9 (d, $J = 23.7$ Hz), 115.8 (d, $J = 22.5$ Hz). UPLC: purity > 99%. m/z (ES) 316.1 $[\text{M} + 1]$.
453 HRMS: Calc: 315.09 Found: 316.1016 $[\text{M} + 1]$.

454 **N^2 -(4-Fluorophenyl)-6-(p -tolylloxy)-1,3,5-triazine-2,4-diamine (36)**: Reagents: 4-chloro- N -
455 (4-fluorophenyl)-6-(p -tolylloxy)-1,3,5-triazin-2-amine (165 mg, 0.5 mmol) and ammonia 7 N
456 in methanol (20 mL). Reaction conditions: 65 °C for 16 h. Purification: IsoleraOne using
457 hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 49.6 mg, 32%. ^1H NMR (400 MHz,
458 $\text{DMSO}-d_4$) δ : 9.49 (s, 1H), 7.65 (s, 2H), 7.21 (d, $J = 8.3$ Hz, 2H), 7.12 (s, 1H), 7.08 – 6.96
459 (m, 4H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 170.95, 168.29, 165.36, 157.45 (d, $J =$
460 242.1 Hz), 150.10, 136.07 (d, $J = 2.3$ Hz), 134.18, 129.78, 121.79, 121.59 (d, $J = 7.5$ Hz),
461 114.77 (d, $J = 21.8$ Hz), 20.40. UPLC: purity > 99%. m/z (ES) 312.1 $[\text{M} + 1]$. HRMS: Calc:
462 311.12 Found: 312.1257 $[\text{M} + 1]$.

463 **N^2 -Phenyl-6-(p -tolylloxy)-1,3,5-triazine-2,4-diamine (37)**: Reagents: 4-Chloro- N -phenyl-6-
464 (p -tolylloxy)-1,3,5-triazin-2-amine (0.3 g, 0.8 mmol) and ammonia 7 N in methanol (20 mL).
465 Reaction conditions: 65 °C for 16 h. Purification: IsoleraOne using hexane/ethyl acetate (0 –

100% ethyl acetate). Yield: 79.9 mg, 33%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.45 (s, 1H), 7.64 (s, 2H), 7.29 – 6.98 (m, 8H), 6.93 (t, *J* = 7.3 Hz, 1H), 2.32 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 170.9, 168.3, 165.5, 150.1, 139.7, 134.2, 129.8, 128.3, 122.0, 121.8, 119.9, 20.4. UPLC: purity > 99%. *m/z* (ES) 294.1 [M + 1]. HRMS: Calc: 293.13 Found: 394.1345 [M + 1].

6-(2-Chloro-4-methylphenoxy)-*N*²-(4-fluorophenyl)-1,3,5-triazine-2,4-diamine (38):

Reagents: 4-chloro-6-(2-chloro-4-methylphenoxy)-*N*-(4-fluorophenyl)-1,3,5-triazin-2-amine (183 mg, 0.5 mmol) and ammonia 7 N in methanol (20 mL). Reaction conditions: 65 °C for 16 h. Purification: IsoleraOne using hexane/ethyl acetate (0 – 100% ethyl acetate). Yield: 56.8 mg, 33%. ¹H NMR (400 MHz, MeOD) δ 7.48 (s, 2H), 7.32 (s, 1H), 7.17 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 1H) 6.90 (s, 2H), 2.37 (s, 3H). ¹³C NMR (100 MHz, MeOD-*d*₄) δ: 172.0, 169.9, 166.9, 160.1 (d, *J* = 240.6 Hz), 147.6, 138.3, 136.5 (d, *J* = 2.7 Hz), 131.5, 129.6, 128.0, 124.9, 123.4, 115.8 (d, *J* = 22.6 Hz), 20.7. UPLC: purity > 99%. *m/z* (ES) 346.1 [M + 1]. HRMS: Calc: 345.08 Found: 346.0874 [M + 1].

4-((4-Amino-6-(*p*-tolylloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (39): Reagents: 4-(4-chloro-6-(*p*-tolylloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (130 mg, 0.4 mmol) and ammonia 0.5 N in dioxane (100 ml). Reaction conditions: 101 °C for 24 h. Purification: IsoleraOne using heptane/ethyl acetate (0 – 100% ethyl acetate). Yield: 9 mg, 7%. ¹H NMR (400 MHz, Acetone-*d*₆) δ: 9.02 (s, 1H), 7.95 (d, *J* = 7.3 Hz, 2H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.65 (s, 2H), 2.39 (s, 3H). UPLC: purity: 97%. *m/z* (ES) 319.1 [M + 1].

***N*²-(4-Fluorophenyl)-6-((4-fluorophenyl)thio)-1,3,5-triazine-2,4-diamine (40):** Reagents: 4-chloro-*N*-(4-fluorophenyl)-6-((4-fluorophenyl)thio)-1,3,5-triazin-2-amine (310.8 mg, 0.6 mmol) and ammonia 0.5 N in dioxane (50 ml). Reaction conditions: 101 °C for 17 h. Purification: IsoleraOne using heptane/ethyl acetate (0 – 100% ethyl acetate). Yield: 177.4

491 mg, 90%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.45 (s, 1H), 7.71 – 7.58 (m, 2H), 7.42 – 6.72
492 (m, 8H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 165.4, 162.9 (d, *J* = 244.9 Hz), 162.7, 157.3 (d,
493 *J* = 233.7 Hz), 138.4, 135.9 (d, *J* = 2.4 Hz), 124.1 (d, *J* = 3.0 Hz), 121.2, 116.3 (d, *J* = 21.9
494 Hz), 114.6 (d, *J* = 23.8 Hz). UPLC: purity > 99%. *m/z* (ES) 332.1 [M + 1]. HRMS: Calc:
495 331.07 Found: 332.0779 [M + 1].

496 ***N*²-(2,6-Dibromo-4-methylphenyl)-*N*⁴-(*p*-tolyl)-1,3,5-triazine-2,4,6-triamine (41):** Reagents:
497 6-chloro-*N*²-(2,6-dibromo-4-methylphenyl)-*N*⁴-(*p*-tolyl)-1,3,5-triazine-2,4-diamine (144.5
498 mg, 0.3 mmol) and ammonia 7 N in methanol (100 ml). Reaction conditions: 65 °C for 8
499 days. Purification: IsoleraOne using heptane/ethyl acetate (0 – 100% ethyl acetate). Yield:
500 71.7 mg, 52%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.95 – 8.75 (m, 1H), 8.75 – 8.50 (m, 1H),
501 7.78 – 7.21 (m, 4H), 7.18 – 6.69 (m, 2H), 6.43 (s, 2H), 2.39 – 2.28 (m, 3H), 2.28 – 2.12 (m,
502 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 167.1, 165.4, 139.7, 138.0, 134.4, 132.4, 129.8,
503 128.6, 125.4, 119.5, 20.4, 19.8. UPLC: purity > 99%. *m/z* (ES) 463.1 [M - 1], 465.1 [M + 1],
504 467.1 [M + 3]. HRMS: Calc: 463.98 Found: 462.9895 [M - 1].

505 ***N*²-(2,6-Dibromo-4-methylphenyl)-*N*⁴-(4-fluorophenyl)-1,3,5-triazine-2,4,6-triamine (42):**
506 Reagents: 6-chloro-*N*²-(2,6-dibromo-4-methylphenyl)-*N*⁴-(4-fluorophenyl)-1,3,5-triazine-2,4-
507 diamine (207.7 mg, 0.4 mmol) and ammonia 0.5 N in dioxane (75 ml). Reaction conditions:
508 101 °C for 5 days. Purification: IsoleraOne using DCM/methanol (0 – 10% methanol). Yield:
509 45 mg, 23%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.28 – 8.60 (m, 2H), 7.94 – 7.36 (m, 4H),
510 7.26 – 6.34 (m, 4H), 2.34 (s, 3H). ¹³C NMR (100 MHz, Acetone) δ: 167.5, 165.8, 164.8,
511 157.9 (d, *J* = 238.8 Hz), 140.1, 136.7, 134.3, 132.6, 125.3, 121.1, 114.5 (d, *J* = 27.4 Hz),
512 19.5. UPLC: purity > 99%. *m/z* (ES) 467.0 [M - 1], 469.0 [M + 1], 471.0 [M + 3]. HRMS:
513 Calc: 467.95 Found: 466.9628 [M - 1].

514 ***N*²,*N*⁴-bis(4-Fluorophenyl)-1,3,5-triazine-2,4,6-triamine (43):** Reagents: 6-chloro-*N*²,*N*⁴-
515 bis(4-fluorophenyl)-1,3,5-triazine-2,4-diamine (224 mg, 0.7 mmol) and ammonia 7 N in

methanol (200 mL). Reaction conditions: 65 °C for 8 days. Purification: IsoleraOne using heptane/ethyl acetate (0 – 100% ethyl acetate). Yield: 52.3 mg, 25%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.10 (s, 2H), 7.76 (s, 4H), 7.12 – 7.03 (m, 4H), 6.61 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 166.7, 164.3, 157.3 (d, *J* = 238.1 Hz), 136.9, 121.5 (d, *J* = 5.5 Hz), 114.8 (d, *J* = 21.9 Hz). UPLC: purity > 99%. *m/z* (ES) 315.1 [M + 1]. HRMS: Calc: 314.11 Found: 315.1169 [M + 1].

4-((4-Amino-6-((4-fluorophenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (44):

Reagents: 4-((4-chloro-6-((4-fluorophenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (338.3 mg, 0.8 mmol) and ammonia 7 N in methanol (200 ml). Reaction conditions: 65 °C for 24 h. Purification: IsoleraOne using water/methanol (0 – 100% methanol). Yield: 26.1 mg, 10% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.59 (s, 1H), 9.24 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.82 – 7.64 (m, 4H), 7.19 – 7.05 (m, 2H), 6.79 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 166.7, 164.3, 164.2, 157.4 (d, *J* = 237.6 Hz), 144.9, 136.3, 132.8, 132.7, 121.8 (d, *J* = 6.9 Hz), 119.6, 119.2, 114.8 (d, *J* = 22.0 Hz), 102.5. UPLC: purity > 99%. *m/z* (ES) 322.1 [M + 1]. HRMS: Calc: 321.11 Found: 322.1234 [M + 1].

4-((4-Amino-6-(*p*-tolylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (45): Reagents: 4-((4-chloro-6-(*p*-tolylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (269.9 mg, 0.8 mmol) and ammonia 7 N in methanol (200 ml). Reaction conditions: 65 °C for 48 h. Purification: IsoleraOne using water/methanol (0 – 100% methanol). Yield: 14 mg, 6%. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.59 (s, 1H), 9.13 (s, 1H), 8.03 (d, *J* = 8.6 Hz, 2H), 7.76 – 7.56 (m, 4H), 7.09 (d, *J* = 8.3 Hz, 2H), 6.77 (s, 2H), 2.26 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 166.7, 164.30, 164.2, 145.0, 137.4, 132.8, 128.8, 120.4, 119.6, 119.2, 102.5, 20.5. UPLC: purity > 99%. *m/z* (ES) 318.2 [M + 1]. HRMS: Calc: 317.14 Found: 318.1465 [M + 1].

4-((4-Amino-6-((2-chloro-4-methylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile

(46): Reagents: 4-((4-chloro-6-((2-chloro-4-methylphenyl)amino)-1,3,5-triazin-2-

yl)amino)benzonitrile (300.3 mg, 0.7 mmol) and ammonia 0.5 N in dioxane (100 ml).
Reaction conditions: 101 °C for 48 h. Purification: IsoleraOne using heptane/ethyl acetate (0 – 100% ethyl acetate). Yield: 164 mg, 63%. ¹H NMR (400 MHz, Acetone-*d*₆) δ: 8.82 (s, 1H), 8.09 (s, 1H), 8.05 (d, *J* = 8.7 Hz, 2H), 7.63 – 7.60 (m, 3H), 7.29 (d, *J* = 1.2 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.36 (s, 2H), 2.33 (s, 3H). ¹³C NMR (100 MHz, Acetone-*d*₆) δ: 168.6, 166.2, 165.8, 145.6, 135.5, 134.2, 133.5, 130.2, 128.7, 125.6, 120.3, 120.2, 119.9, 104.9, 20.5. UPLC: purity > 99 %. *m/z* (ES) 352.1 [M], 354.1 [M + 2]. HRMS: Calc: 351.10 Found: 352.1096 [M + 1].

4-((4-Amino-6-((4-fluoro-2,6-dimethylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile

(**47**): Reagents: 4-((4-chloro-6-((4-fluoro-2,6-dimethylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (0.2972 g, 0.733 mmol) and ammonia 0.5 N in dioxane (100 ml).
Reaction conditions: 101 °C for 6 days. Purification: IsoleraOne using heptane/ethyl acetate (0 – 100% ethyl acetate). Yield: 24.7 mg, 10%. ¹H NMR (400 MHz, MeOD-*d*₄) δ: 7.96 (t, *J* = 8.1 Hz, 1H), 7.66 – 7.57 (m, 2H), 7.37 (d, *J* = 8.6 Hz, 1H), 6.86 (dd, *J* = 15.2, 9.3 Hz, 2H), 2.22 (s, 5H). UPLC: purity: 95%. *m/z* (ES) 350.1 [M + 1]. HRMS: Calc: 345.15 Found: 350.1548 [M + 1].

N-(4-Fluorophenyl)-4-(mesityloxy)-6-(piperidin-1-yl)-1,3,5-triazin-2-amine (**28**): 4-Chloro-*N*-(4-fluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (300 mg, 0.8 mmol) was stirred with piperidine (85 mg, 1 mmol) and DIPEA (216 mg, 1.6 mmol) in dioxane (10 mL) at 101 °C overnight. After that, the solvent was evaporated under vacuum. The crude was purified by IsoleraOne using DCM and MeOH (0 – 10% methanol) as eluents obtaining the final compound (267.9 mg, 79%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.55 (s, 1H), 7.91 - 6.70 (m, 6H), 3.87 - 3.49 (m, 4H), 2.25 (s, 3H), 2.03 (s, 6H), 1.69 - 1.36 (m, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 169.9, 165.5, 165.1, 157.4 (d, *J* = 231.7 Hz), 147.2, 136.1, 133.9, 129.6, 128.9,

121.4, 114.8 (d, $J = 20.8$ Hz), 43.8, 25.3, 24.2, 20.4, 16.2. UPLC: purity >99%. m/z (ES) 408.2 $[M + 1]$. HRMS: Calc: 407.21 Found: 408.2200 $[M + 1]$.

***N*²-(4-Fluorophenyl)-6-(mesityloxy)-*N*⁴-methyl-1,3,5-triazine-2,4-diamine (32)**: 4-Chloro-*N*-(4-fluorophenyl)-6-(mesityloxy)-1,3,5-triazin-2-amine (300 mg, 0.8 mmol) was stirred with a solution 2 M of methanamine in THF (31.2 mg, 1 mmol) and DIPEA (216 mg, 1.6 mmol) in dioxane (10 mL) at reflux temperature for 16 h. After that, the solvent was evaporated under vacuum. The crude was purified by IsoleraOne using hexane and ethyl acetate (0 – 100 ethyl acetate) as eluents obtaining the final compound (162.1 mg, 22%). ¹H NMR (400 MHz, DMSO-*d*₆) δ : 9.64 – 9.41 (m, 1H), 7.94 – 7.39 (m, 17H), 7.26 – 6.80 (m, 4H), 2.76 (d, $J = 4.6$ Hz, 3H), 2.25 (d, $J = 4.5$ Hz, 3H), 2.04 (d, $J = 4.6$ Hz, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 169.4, 167.4, 164.7, 157.4 (d, $J = 237.9$ Hz), 147.2, 136.2, 134.0, 129.7, 128.9, 121.3 (d, $J = 6.9$ Hz), 114.8 (d, $J = 17.7$ Hz), 27.5, 20.3, 16.1. UPLC: purity > 99%. m/z (ES) 354.2 $[M + 1]$. HRMS: Calc: 357.17 Found: 358.1505 $[M + 1]$.

***N*-(4-Fluorophenyl)-4-(mesityloxy)-1,3,5-triazin-2-amine (50)**: To a solution of 2,4,6-trimethylphenol (272 mg, 2 mmol), 4-chloro-*N*-(4-fluorophenyl)-1,3,5-triazin-2-amine (449 mg, 2 mmol) and tetrabutylammoniumhydrogensulfate (67.9 mg, 0.2 mmol) in Toluene (6.5 mL); a solution of NaOH (80 mg, 2 mmol) in water (0.6 mL) was added at 0 °C. The solution was stirred at 0 °C for 30 min. The solvent was evaporated, and the crude was purified by IsoleraOne using hexane and ethyl acetate (0 – 100 ethyl acetate) as eluents obtaining the final product (132.6 mg, 20%). ¹H NMR (400 MHz, DMSO-*d*₆) δ : 10.58 – 10.11 (m, 1H), 8.52 (s, 1H), 7.77 – 7.38 (m, 2H), 7.24 – 6.86 (m, 4H), 2.28 (s, 3H), 2.02 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 169.8, 168.3, 164.8, 158.1 (d, $J = 243.2$ Hz), 146.9, 134.8, 134.6, 129.5, 129.1, 121.6, 115.0 (d, $J = 24.2$ Hz), 20.3, 16.0. UPLC: purity > 99%. m/z (ES) 325.2 $[M + 1]$. HRMS: Calc: 324.14 Found: 325.1459 $[M + 1]$.

4-(Mesityloxy)-N-phenyl-1,3,5-triazin-2-amine (51): To a solution of 2,4,6-trimethylphenol (272 mg, 2 mmol), 4-chloro-N-phenyl-1,3,5-triazin-2-amine (413 mg, 2 mmol) and tetrabutylammoniumhydrogensulfate (67.9 mg, 0.2 mmol) in Toluene (6.5 mL); a solution of NaOH (80 mg, 2 mmol) in water (0.6 mL) was added at 0 °C. The solution was stirred at 0 °C for 30 min. The solvent was evaporated, and the crude was purified by IsoleraOne using hexane and ethyl acetate (0 – 100 ethyl acetate) as eluents obtaining the final product (69.7 mg, 11%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 10.57 – 10.08 (m, 1H), 8.53 (s, 1H), 7.81 – 6.88 (m, 7H), 2.28 (s, 3H), 2.03 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 169.6, 168.5, 164.9, 146.9, 138.4, 134.6, 129.5, 129.1, 128.5, 123.2, 119.9, 39.5, 20.4, 16.0. UPLC: purity > 99%. m/z (ES) 307.2 [M + 1]. HRMS: Calc: 306.15 Found: 307.1546 [M + 1].

ASSOCIATED CONTENT

Full panel of parasitology screens for the final compounds, antiprotozoal *in vitro* and *in vivo* assays such as cytotoxicity, *T. cruzi*, *L. infantum*, *P. falciparum*, microsomal and plasma stability assays and results. Protocols to evaluate the pharmacokinetics of compound **35** and the pharmacokinetic parameters. Protocol to evaluate the rate of action is also included.

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Table 1. In vitro antitrypanosomal activity and cytotoxicity of the final compounds and derivative b.

Compd.	X	Y	Z	R	R'	Antitrypanosomal activity and cytotoxicity IC ₅₀ (μM)	
						<i>T. b. bruc.</i> ^a	MRC5 ^b
a	NH	O	NH ₂	H	2,4,6-trimethyl	0.88	> 64
27	NH	O	NH ₂	4-F	2,4,6-trimethyl	0.31	> 64
28	NH	O	piperidiny	4-F	2,4,6-trimethyl	8.17	> 64
29	NH	O	NH ₂	4-Cl	2,4,6-trimethyl	1.52	6.39
30	NH	O	NH ₂	4-CF ₃	2,4,6-trimethyl	6.94	6.5
31	NH	O	NH ₂	3,5-diF	2,4,6-trimethyl	1.29	4.44
32	NH	O	NHMe	4-F	2,4,6-trimethyl	10.86	> 64
33	NH	O	NH ₂	2,6-diF	2,4,6-trimethyl	> 64	> 64
34	O	O	NH ₂	4-F	2,4,6-trimethyl	4.27	> 64
35	NH	O	NH ₂	4-F	4-F	0.63	> 64
36	NH	O	NH ₂	4-F	4-Me	0.39	> 64
37	NH	O	NH ₂	H	4-Me	1.08	> 64
38	NH	O	NH ₂	4-F	2-Cl,4-Me	0.51	> 64
39	NH	O	NH ₂	4-CN	4-Me	0.96	> 64
40	NH	S	NH ₂	4-F	4-F	>64	>64
41	NH	NH	NH ₂	4-Me	2,6-diBr-4-Me	9.13	8.06
42	NH	NH	NH ₂	4-F	2,6-diBr-4-Me	7.1	6.7
43	NH	NH	NH ₂	4-F	4-F	2.77	> 64
44	NH	NH	NH ₂	4-CN	4-F	39.83	> 64
45	NH	NH	NH ₂	4-CN	4-Me	>64	>64
46	NH	NH	NH ₂	4-CN	2-Cl,4-Me	7.7	> 64
47	NH	NH	NH ₂	4-CN	2,6-diMe-4-F	7.87	23.74
50	NH	O	H	4-F	2,4,6-trimethyl	31.05	8.00
51	NH	O	H	H	2,4,6-trimethyl	32.46	17.76
Suramin^c						0.03	

Table 2. Pharmacokinetic parameters of derivative **35**.

	T _{max} (h)	C _{max} (μM)	T _{1/2} (h)	AUC ₀₋₄ (ng.h/mL)	V _Z (l)	Cl (mL/min)
50 mg/kg PO	0.5	5.66	6.51	28609	15.6	27.8

Table 3. *In vivo* activities of compound **35** on the acute *T. b. brucei* model.

Compd.	dose (mg/kg)	freq.	MST	survivors on 14 dpi
Vehicle			7	0/3
Suramin	10	SID × 5 (IP)	> 7	3/3
35	25	BID × 5 (PO)	6	0/3
	50	BID × 5 (PO)	5.7	0/3
	50	SID × 5 (PO)	7	0/3
	10	SID × 5 (IP)	7	0/3

Figure 1. Previously reported compound **a** with antitrypanosomal activity.

Figure 2. Different structural modifications based on compound **a**.

Figure 3. *In vitro* mouse microsomal stability of compounds **27**, **35**, **36** and **38**. Diclofenac was used as a reference compound.

Figure 4. Concentration in blood of compound **35** after a single oral dose of 50 mg/kg in mice.

Figure 5. Graph A: % parasite growth vs time of compound **35** at 5 μ M and suramin at 0.125 μ M. Graph B: % parasite growth vs time of compound **35** at 10 μ M and suramin at 0.25 μ M.

Scheme 1. Synthetic pathway to obtain different trisubstituted triazine derivatives.

Scheme 2. Synthetic pathway to obtain different disubstituted triazine derivatives.

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