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Design, synthesis and biological evaluation of 6-aryl-1,6-dihydro-1,3,5-triazine-2,4-diamines as antiplasmodial antifolates†

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The design, synthesis and biological evaluation of a series of 6-aryl-1,6-dihydro-1,3,5-triazine-2,4-diamines is described. These compounds exhibited *in vitro* antiplasmodial activity in the low nanomolar range against both drug sensitive and drug resistant strains of *P. falciparum*, with 1-(3-(2,4-dichlorophenoxy) propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride identified as the most potent compound from this series against the drug resistant FCR-3 strain (IC_{50} 2.66 nM). The compounds were not toxic to mammalian cells at therapeutic concentrations and were shown to be inhibitors of parasitic DHFR in a biochemical enzyme assay.

Introduction

Malaria continues to pose a health risk to much of the world population despite a dramatic decrease in malaria morbidity over the past 15 years.[1] An estimated 214 million cases of malaria and 438 000 malaria fatalities were reported in 2014, with 88% of these infections originating in Africa and caused by the parasite *Plasmodium falciparum*.[1] The increasing occurrence of insecticide resistance and the emergence of multidrug resistant strains of the parasite threaten the progress made in the fight against malaria and highlight the need for the development of new antimalarial therapies.[2]

Until the emergence of drug resistance, folate metabolism was successfully targeted for both prophylaxis and the treatment of malaria.[3] Folates are essential cellular cofactors required by the parasite for a number of key processes, including the initiation of protein synthesis, and the biosynthesis of nucleotides and some amino acids.[4] The malaria parasite relies on these cofactors for growth, and is able to obtain the required folate derivatives by *de novo* synthesis or *via* a folate salvage pathway.

A key enzyme crucial to both pathways that has been targeted for malaria chemotherapy is dihydrofolate reductase-thymidylate synthase (DHFR-TS), a bifunctional enzyme. DHFR-TS is one of the few well-defined, validated targets in malaria chemotherapy.[5] Unfortunately, point mutations in the active site of the DHFR domains of DHFR-TS have resulted in resistance to the most widely used antifolates, cycloguanil **1** and pyrimethamine **2** (Fig. 1).[6] In many cases, the Ser108Asn

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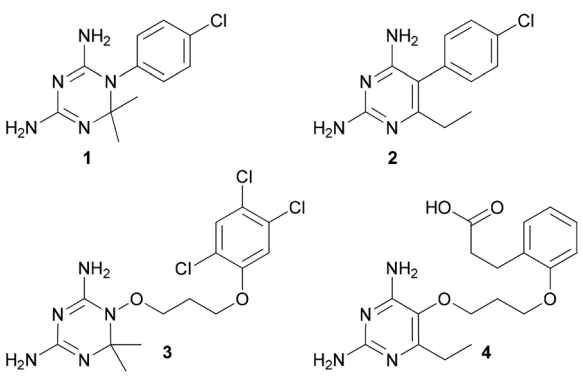


Fig. 1 Known antifolates cycloguanil **1**, pyrimethamine **2**, WR99210 **3** and P-218 **4**.

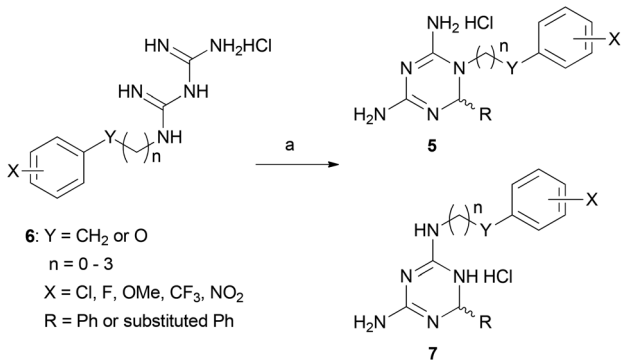
mutation, followed by Cys59Arg, results in steric clashes with inhibitors in the DHFR active site. This has also been observed for parasitic DHFR bearing Ser108Thr and Ala16Val mutations (in the FCR-3 clone). Subsequent mutations, Asn51Ile and Ile164Leu, cause a shift in the two chains around the active site, opening it up and reducing the binding affinity of small inhibitors. By comparison, compounds containing a flexible linker between the two rings, such as WR99210 **3** (Fig. 1), have been shown to maintain a high binding affinity to all mutant forms of *P. falciparum* DHFR.[7] The flexibility imparted by this linker enables the inhibitor to avoid unfavourable contacts with mutant amino acid residues. In a similar vein, flexible pyrimethamine derivatives, including P218 **4**, have proven to be active against DHFR-resistant parasites.[8]

As part of an ongoing malarial research programme, we utilised published crystal structures of wild type and mutant *P. falciparum* DHFR-TS [9] to design novel, flexible analogues of cycloguanil. We now report the results of this study, in which a series of compounds with *in vitro* antimalarial activity in the low nanomolar range were identified.[10]

Results and discussion

The crystal structures of both wild type *Pf*DHFR-TS and the quadruple mutant (bearing the following mutations: Asn51Ile, Cys59Arg, Ser108Asn and Ile164Leu) were selected for *in silico* screening.[11] In each case, the active site was defined at chain A of the DHFR region with the co-factor NADPH bound, in order to assess the comparative binding of the inhibitor molecules at the dihydrofolate binding site. The use of flexible docking protocols enabled us to identify that inhibitors bearing substituents larger than methyl at the 6-position of the dihydrotriazine ring could be accommodated in the dihydrofolate binding pocket. In particular, the side chain of Met55 is able to rotate away from the active site, enabling bulkier inhibitors to bind without disruption of the key H-bonding to Asp54, Ile14 and, in the mutant, Leu164. Examination of the literature revealed that Baker *et al.* had considered the use of larger substituents at the 6-position of the dihydrotriazine ring in their work on inhibitors of dihydrofolate reductase, but they did not explore this further.[12] In 2000, a series of rigid dihydrodiaminotriazines with one bulky substituent at the 6-position, designed with the aid of molecular modelling, displayed promising activity against A16V+S108T mutants.[5,13] In light of this, the initial series of novel analogues designed in this study contained flexible linkers ranging in length from 1 to 4 atoms between the dihydrotriazine and aromatic rings, and all contained a phenyl substituent at the 6-position of the dihydrotriazine ring (**5**).

Our initial approach to the synthesis of cycloguanil analogues **5** involved preparation of a biguanide precursor **6**, followed by reaction with a suitably substituted aldehyde (Scheme 1).[14] However, this approach resulted in the formation of a mixture of products in most cases, with the isomeric compound **7** predominating.[15] The isomeric

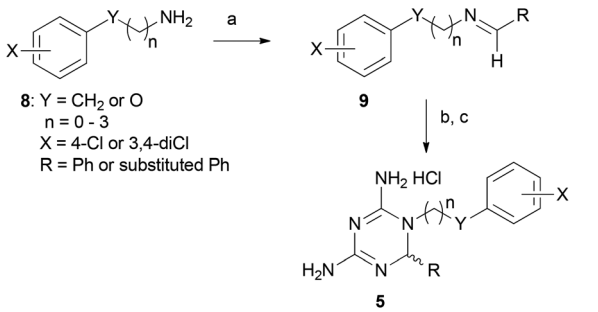


Scheme 1 Reagents and conditions: (a) RCHO, 1,4-dioxane, conc. HCl (cat), 100 W, 85 °C, 30 min.[15]

compounds were found to have only moderate activity against *P. falciparum in vitro* (0.991–49.8 μM).[15]

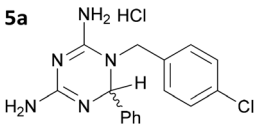
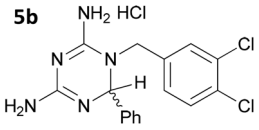
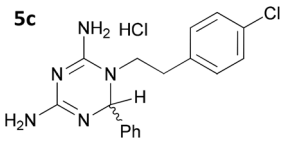
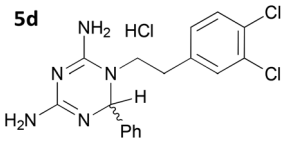
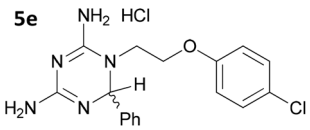
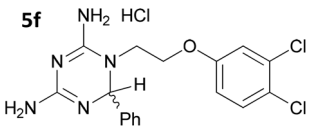
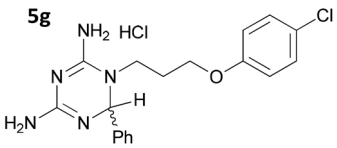
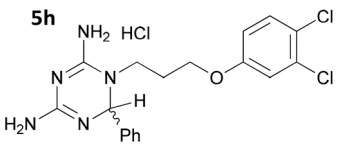
We therefore modified our approach to avoid the formation of isomeric mixtures of products by first forming a substituted imine and reacting this with dicyandiamide (Scheme 2).[16] To this end, suitably substituted amines **8** were treated with aromatic aldehydes in the presence of molecular sieves or the acidic clay Montmorillonite K-10 to afford substituted imines **9** (Scheme 2). Alternatively, imines **9** could be prepared neat at 100 °C, followed by treatment with 1,4-dioxane and molecular sieves. The imines prepared were then converted to hydrochloride salts by treatment with anhydrous HCl gas, followed by reaction with dicyandiamide to afford the desired cycloguanil analogues **5** in low yields. The reaction sequence was most commonly carried out in a one-pot multi-step fashion using microwave irradiation, however, conventional heating could also be employed both in the formation of the imine and in the final ring closing step. Amines **8** with $n = 0$ or 1 and Y = CH_2 were commercially available, while those with $n = 2$–3 were prepared from suitably substituted phenols (affording **8** with Y = O) as described previously.[15]

The initial series of compounds synthesised (Table 1) were assessed for antimalarial activity *in vitro* in a whole cell *P. falciparum* assay against a cycloguanil resistant strain



Scheme 2 Reagents and conditions: (a) RCHO, Montmorillonite K-10, 1,4-dioxane, 150 W, 100 °C, 30 min; or RCHO, 100 °C 2 h, then 1,4-dioxane, molecular sieves (b) HCl (g); (c) dicyandiamide, DMF, 150 W, 100 °C, 30 min (21–42% over three steps).

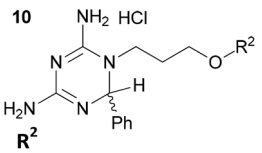
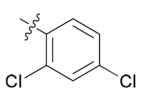
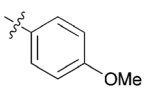
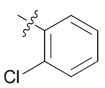
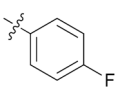
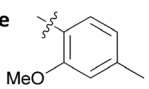
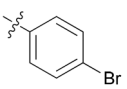
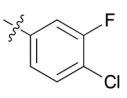
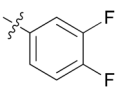
Table 1 Structure and antimalarial activity of first series of analogues **5** against cycloguanil resistant mutant

Entry	Inhibitor	*Pf* FCR-3 IC_{50} [a] (μM)
1	**5a** 	6.42 ± 2.24
2	**5b** 	4.32 ± 1.19
3	**5c** 	7.16 ± 0.63
4	**5d** 	4.77 ± 0.30
5	**5e** 	4.88 ± 0.69
6	**5f** 	2.12 ± 0.75
7	**5g** 	0.0550 ± 0.0131
8	**5h** 	0.0398 ± 0.0971
9	Cycloguanil	14.5 ± 2.5
10	Pyrimethamine	0.0962 ± 0.0148
11	Chloroquine	0.0722 ± 0.0118

[a] Values are expressed as the mean ± SD of triplicate determinations.

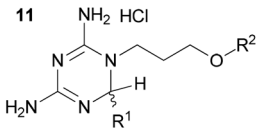
(Gambian FCR-3; ^{3}H hypoxanthine incorporation assay). A clear structure–activity relationship became apparent from this initial series of compounds synthesised, with the two compounds bearing a flexible linker of 4 atoms (**5g** and **5h**, entries 7 and 8 of Table 1) exhibiting activity in the low nanomolar range comparable with pyrimethamine (IC_{50} 55.0 nM and 39.8 nM respectively, *cf.* pyrimethamine IC_{50} 96.2 nM in this assay, entry 10 of Table 1). All other compounds of the series bearing flexible linkers of 1, 2 or 3 atoms only showed activity comparable with cycloguanil against the drug resistant *P. falciparum* strain (IC_{50} 2.12–7.16 μM; *cf.* cycloguanil IC_{50} 14.5 μM in this assay, entry 9 of Table 1). Significantly, both **5g** and **5h** were found to be non-toxic to mammalian cells at therapeutic concentrations, with a selectivity window >700 between cytotoxic and effective concentrations (HeLa cell line, sulforhodamine B (SRB) assay, IC_{50} 39.0 μM for **5g** and 33.5 μM for **5h**). We later synthesised compounds bearing 5 and 6 atom linkers (general structure **5**, n = 4 and n = 5), but these compounds once again only exhibited antimalarial activity in the low micromolar range (2.57–3.98 μM), indicating that the 4 atom linker was essential for potent antimalarial activity.

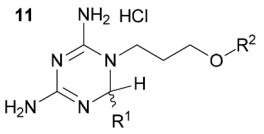
Table 2 Structure and antimalarial activity of second series of analogues **10** against cycloguanil resistant mutant

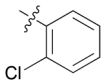
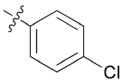
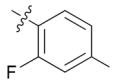
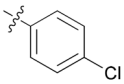
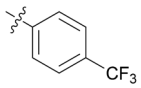
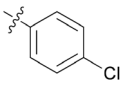
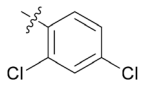
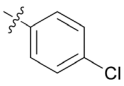
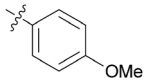
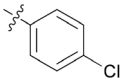
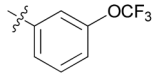
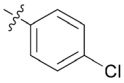
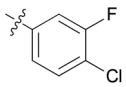
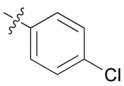
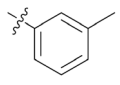
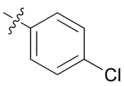
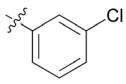
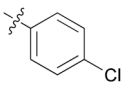
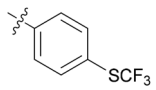
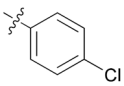
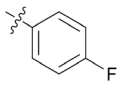
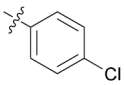
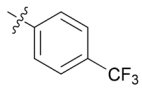
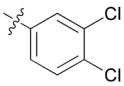
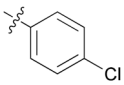
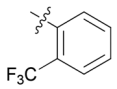
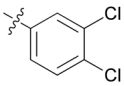
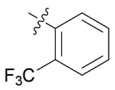
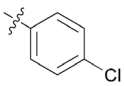
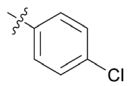
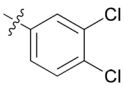
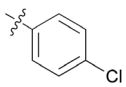
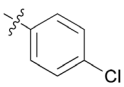
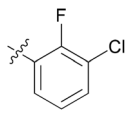
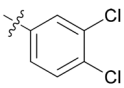
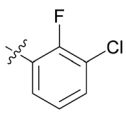
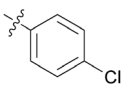
Entry	**10**  R^2	*Pf* FCR-3 IC_{50} [a] (μM)
1	**10a** 	0.00266 ± 0.00056
2	**10b** 	0.0603 ± 0.0087
3	**10c** 	0.171 ± 0.027
4	**10d** 	0.0387 ± 0.0163
5	**10e** 	0.0465 ± 0.0095
6	**10f** 	0.0241 ± 0.0168
7	**10g** 	0.00955 ± 0.0127
8	**10h** 	0.0908 ± 0.066
9	Cycloguanil	14.5 ± 2.5
10	Pyrimethamine	0.0962 ± 0.0148
11	Chloroquine	0.0722 ± 0.0118

[a] Values are expressed as the mean ± SD of triplicate determinations.

Table 3 Structure and antimalarial activity of third series of analogues **11** and **12** against cycloguanil resistant mutant





Entry		R^1	R^2	*Pf* FCR-3 IC_{50} [a] (μM)	Entry		R^1	R^2	*Pf* FCR-3 IC_{50} [a] (μM)
1	**11a**			0.859 ± 0.606	13	**11m**			0.163 ± 0.050
2	**11b**			3.37 ± 0.17	14	**11n**			2.51 ± 0.67
3	**11c**			0.0457 ± 0.0172	15	**11o**			>50[b]
4	**11d**			1.59 ± 0.30	16	**11p**			0.0449 ± 0.0405
5	**11e**			0.156 ± 0.012	17	**11q**			2.39 ± 0.03
6	**11f**			0.155 ± 0.048	18	**11r**			4.53 ± 0.63
7	**11g**			0.412 ± 0.080	19	**11s**			3.06 ± 0.04
8	**11h**			3.24 ± 0.45	20	**11t**			0.0607 ± 0.0361
9	**11i**			0.156 ± 0.129	21	**11u**			0.0977 ± 0.0049
10	**11j**			0.679 ± 0.221	22	**12**			0.463 ± 0.059
11	**11k**			0.926 ± 0.128					
12	**11l**			0.0772 ± 0.0319	23	Cycloguanil			14.5 ± 2.5
					24	Pyrimethamine			0.0962 ± 0.0148
					25	Chloroquine			0.0722 ± 0.0118

[a] Values are expressed as the mean ± SD of triplicate determinations. [b] IC_{50} not determined.

With these promising results in hand, we then embarked on the synthesis of a second series of analogues **10**, all of which contained an unsubstituted phenyl substituent at the 6-position of the dihydrotriazine ring and a flexible linker of 4 atoms (Table 2). This series of compounds contained variation in the nature of the aromatic substituent R^2 of the flexible linker, and required amines **8** with Y = O, n = 3 and bearing a variety of substituents X. Although commercially available, these amines were also prepared as described previously.[15] The final dihydrotriazine hydrochlorides **10a–h** were isolated in low yields in general (4%–48%), without optimisation of the reaction conditions at this stage. Gratifyingly, all of the compounds prepared in this series exhibited antimalarial activity in the low nanomolar range, indicating that both electron-withdrawing and electron-donating substituents are tolerated on the aromatic ring R^2. The most potent compound in this series (**10a**, entry 1 of Table 2, IC_{50} 2.66 nM) contained a 2,4-dichlorophenoxy substituent at the end of the flexible linker.

We then explored the effect of substitution on the phenyl ring at the 6-position of the dihydrotriazine, by preparing a third series of analogues **11** (Table 3). This series of analogues contained either a 4-chlorophenoxy or a 3,4-dichlorophenoxy substituent at the end of the flexible linker, with a range of substituted phenyl groups introduced at the 6-position of the dihydrotriazine ring. By comparison, this series of compounds did not perform as well in the *in vitro* assay, with only 5 of the compounds in this series displaying activity below 100 nM, and 6 of the compounds exhibiting IC_{50} values in the low micromolar range. In general, compounds bearing a 3,4-dichlorophenoxy substituent at the end of the flexible linker were more potent than the corresponding compound bearing a 4-chlorophenoxy substituent (see for *e.g.* **11t** *vs.* **11i** or **11u** *vs.* **11j**, Table 3). Compounds bearing bulkier $-CF_3$, $-SCF_3$ or $-OCF_3$ substituents on the 6-phenyl ring were the least active, most likely due to steric clashes in the DHFR active site (**11b**, **11h**, **11o**, **11q**, **11r**, **11s**, Table 3, IC_{50} 2.39– >50 μM). Finally, we prepared an analogue bearing an alkyl flexible chain for comparison with those bearing an oxygen atom (**12**, entry 22 of Table 3). This compound contained an unsubstituted phenyl ring at the end of the flexible linker, owing to the commercial availability of the precursor 1-bromo-4-phenylbutane.

Table 4 Antimalarial activity (3D7) and cytotoxicity (HeLa cell line) of flexible cycloguanil analogues

Entry	Inhibitor	*Pf* 3D7 IC_{50} [a] (μM)	HeLa IC_{50} (μM)
1	**5g**	0.130 ± 0.027	39.0 ± 3.8[b]
2	**5h**	0.0183 ± 0.0121	33.6 ± 1.6
3	**10a**	0.0610 ± 0.0291	32.7 ± 2.1
4	**10b**	0.0590 ± 0.0014	>100
5	**10c**	0.627 ± 0.174	88.8 ± 20.8
6	**10d**	0.048	>100
7	**10e**	0.252 ± 0.140	ND[c]
8	**10f**	0.165 ± 0.104	ND
9	**10g**	0.0385 ± 0.0176	ND
10	**10h**	0.155 ± 0.033	ND
11	**11a**	0.290 ± 0.017	15.7 ± 2.1
12	**11b**	0.0663 ± 0.0085	11.0 ± 1.8
13	**11c**	0.0493 ± 0.0153	25.0 ± 3.3
14	**11d**	0.140 ± 0.020	13.7 ± 2.1
15	**11e**	0.0520 ± 0.0061	14.1 ± 2.2
16	**11f**	0.687 ± 0.019	ND
17	**11g**	0.0783 ± 0.0164	24.3 ± 3.0
18	**11h**	0.371 ± 0.081	18.9 ± 1.6
19	**11i**	0.0170 ± 0.0017	15.6 ± 2.5
20	**11j**	0.143 ± 0.042	23.0 ± 1.7
21	**11k**	0.340 ± 0.010	10.9 ± 0.4
22	**11l**	0.463 ± 0.086	33.3 ± 4.9
23	**11m**	0.150 ± 0.035	31.8 ± 1.7
24	**11n**	0.193 ± 0.038	12.2 ± 1.5
25	**11o**	1.49 ± 1.17	ND
26	**11p**	0.294 ± 0.142	ND
27	**11q**	1.04 ± 0.34	ND
28	**11r**	0.109 ± 0.036	13.1 ± 1.7
29	**11s**	0.530 ± 0.150	11.8 ± 0.2
30	**11t**	0.122 ± 0.034	13.4 ± 1.5
31	**11u**	0.0570 ± 0.0157	13.6 ± 1.6
32	**12**	0.195 ± 0.074	ND
33	Cycloguanil	0.00413 ± 0.00110	
34	Emetine		0.0202 ± 0.006

[a] Values are expressed as the mean ± SD of triplicate determinations. [b] Standard error calculated using Gnuplot. [c] ND = Not determined.

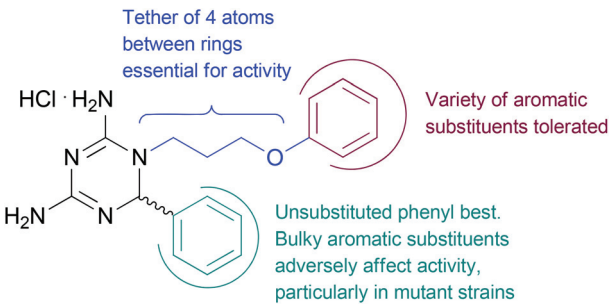


Fig. 2 Key structure–activity relationships identified for the 6-aryl-1,6-dihydro-1,3,5-triazine-2,4-diamines prepared.

Table 5 Enzyme inhibition studies of active compounds against wild type and double mutant *Pf*DHFR

Entry	Inhibitor	K_i Wild type *Pf*DHFR[a] (nM)	K_i A16VS108T *Pf*DHFR[a] (nM)
1	**5g**	4.9 ± 0.5	8.2 ± 1.1
2	**5h**	2.3 ± 0.2	4.9 ± 0.7
3	**10a**	2.5 ± 0.0	6.5 ± 1.1
4	**10b**	10.3 ± 2.0	15.5 ± 2.4
5	**10d**	29.5 ± 5.8	25.9 ± 3.8
6	**10f**	7.7 ± 1.3	14.1 ± 1.4
7	**10g**	5.0 ± 0.6	11.2 ± 1.6
8	**10h**	17.6 ± 1.3	29.1 ± 2.5
9	**11c**	7.6 ± 1.6	10.1 ± 1.3
10	**11l**	28.6 ± 1.3	37.6 ± 4.7
11	**11p**	12.2 ± 0.2	12.3 ± 1.1
12	**11t**	8.3 ± 2.0	12.6 ± 1.0
13	**11u**	8.0 ± 0.5	9.5 ± 1.6
14	Cycloguanil	1.60 ± 0.30 [17]	1518 ± 503 [17]
15	WR99210	0.4 ± 0.2 [17]	0.7 ± 0.1 [17]

[a] Values are expressed as the mean ± SD of triplicate determinations.

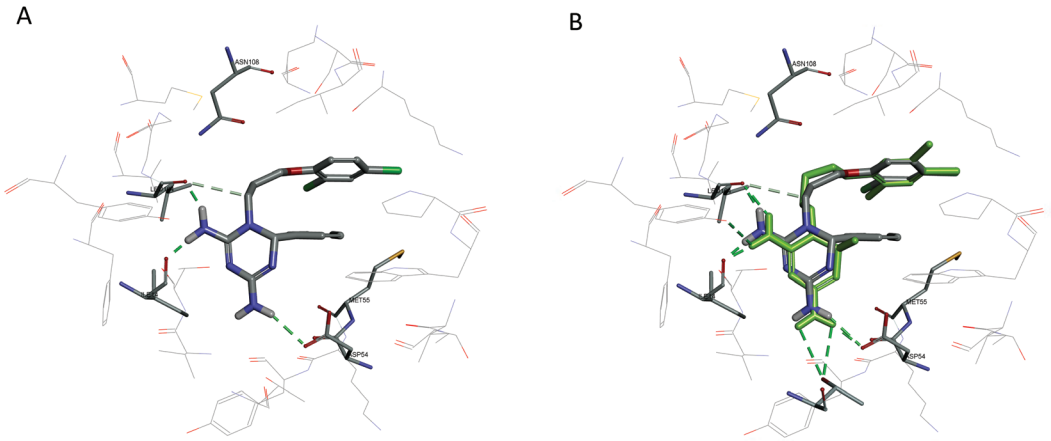


Fig. 3 (A) **5g** docked in the active site of *Pf*DHFR (quadruple mutant) with key H-bonds to Asp54, Ile14 and Leu164; (B) image of **5g** superimposed with WR99210 from the crystal structure (in green); additional H-bond to Thr185 shown.

Compound **12** displayed activity in the nanomolar range, although somewhat less potent than the halogenated analogues **10**.

These compounds were also assessed for *in vitro* antimalarial activity against the drug sensitive 3D7 strain using the pLDH assay (Table 4). Similar trends were observed against this strain, with the majority of compounds tested displaying activity in the nanomolar range. The exceptions contained bulky $-OCF_3$ (**11o**) or $-SCF_3$ (**11q**) groups on the 6-phenyl ring (Table 4, IC_{50} 1.49 μM and 1.04 μM respectively), as had been observed against the drug resistant FCR-3 strain. Surprisingly, however, the compounds substituted with a $-CF_3$ group on the 6-phenyl ring (**11b**, **11h**, **11r** and **11s**) that had performed poorly against the FCR-3 strain, showed promising activity against the 3D7 strain bearing wildtype DHFR (Table 4, IC_{50} 66.3 nM–530 nM). Key structure–activity relationships are summarised in Fig. 2. Cytotoxicity of the majority of compounds was tested by SRB assay on the HeLa cell line. Significantly, none of the compounds tested were cytotoxic at nanomolar concentrations, with IC_{50} values in the micromolar range (Table 4, IC_{50} 10.9– >100 μM).

All compounds with an IC_{50} value below 100 nM in the whole cell *in vitro* assay against the drug resistant strain (FCR-3) were assessed in a *Pf*DHFR biochemical enzyme assay against both the *Pf*DHFR wild type and the Ala16Val+Ser108Thr double mutant (Table 5). The data obtained from these assays confirm that the compounds act as inhibitors of parasitic DHFR, with all compounds exhibiting K_i values in the low nanomolar range against both the wild type and mutant forms of the enzyme. From our molecular modelling studies, the dihydrotriazines prepared in this study bind in the *Pf*DHFR active site in a similar manner to WR99210 (Fig. 3).

Conclusion

In summary, we have designed and synthesised a series of flexible analogues of cycloguanil bearing an aromatic substituent at the 6-position of the dihydrotriazine ring. The compounds displayed potent activity against both drug resistant and drug sensitive forms of *P. falciparum* in a whole cell *in vitro* assay, and were shown to act as inhibitors of parasitic DHFR in a biochemical enzyme assay. Good activities against the mutant enzyme (Ala16Val+Ser108Thr DHFR) can be explained by the flexibility of the 5-side chain which can avert steric hindrance introduced by the Ser108Thr mutation, while the 6-aryl substituent, in contrast to the 6,6-dimethyl groups of cycloguanil, can avert steric hindrance caused by the Ala16Val mutation. This is indicative of the potential application of these compounds as antimalarial leads for further development.

Experimental section

Chemistry

General. All reagents were of reagent grade purchased from Sigma-Aldrich (Steinheim, Germany) and were used without any further purification unless specified. Solvents used for reactions, chromatography or extractions, such as ethyl acetate (EtOAc) and hexane, were distilled prior to use. *N*,*N*-Dimethyl formamide (DMF) was distilled and stored over 4 Å molecular sieves. All microwave reactions were carried out in a CEM Discover Focused Microwave Synthesis System. Reactions were monitored by thin-layer chromatography (TLC) using precoated aluminium-backed plates (Merck silica gel 60 F254) visualised under UV light (λ = 254 nm). Intermediates and final compounds were purified by column chromatography on Fluka silica gel 60 (70–230 mesh). NMR spectra were run on either a Varian 200 MHz Gemini 2000 instrument, or on a 400 MHz Varian INOVA instrument. High resolution mass spectra were generated on a Waters SYNAPT G1 HDMS mass spectrometer operated in electrospray mode. Melting points were determined on a Stuart SMP20 melting point apparatus and are uncorrected. Purity of all final compounds was determined by UPLC, using a Waters Acquity UPLC coupled in tandem to a UPDA detector (200–500 nm).

General method for the preparation of 4,6-diamino-1,2-dihydro-1,3,5-triazines 5. Amine **8** (1.50 mmol) and the appropriate aldehyde (1.2 eq., 1.75 mmol) were dissolved in dry diethyl ether in a nitrogen-purged oven-dried flask. Activated 4 Å molecular sieves and Montmorillonite K-10 (spatula-full) were added and the resulting heterogeneous mixture heated under reflux for 3 h. The mixture was allowed to cool to room temperature and was then filtered into an oven-dried nitrogen-purged flask. The solids were washed with additional anhydrous ether. HCl gas was gently bubbled through this solution until saturation was reached. A white precipitate formed. The resulting suspension was subjected to vacuum and the diethyl ether and excess HCl evaporated *in vacuo*. The flask was re-purged with nitrogen. Dicyandiamide and anhydrous *N*,*N*-dimethylformamide (DMF) were added and the resulting mixture stirred under nitrogen overnight (20 h). The reaction mixture was poured into diethyl ether : acetone (9 : 1) (50 ml) and stirred until a solid had formed at the bottom of the flask. The desired product was filtered and washed with diethyl ether.

Alternatively, Montmorillonite K-10 (150 mg) was added to a microwave tube containing a solution of amine **8** (1.50 mmol) and the appropriate aldehyde (3 eq., 4.50 mmol) in 1,4-dioxane (2 ml). The contents were irradiated with microwave energy (power = 150 W, temperature = 100 °C, time = 30 min). The mixture was allowed to cool to ambient temperature and dried with $MgSO_4$. Hydrogen chloride gas was bubbled through the mixture until saturation. Dicyandiamide (1.2 eq., 1.75 mmol) dissolved in a minimum volume of anhydrous DMF was added to the above mixture. The tube was inserted into the microwave synthesiser and was irradiated with microwave energy (power = 150 W, temperature = 100 °C, time = 30 min). After cooling to ambient temperature, the contents were poured into diethyl ether. The resulting suspension was then filtered through Celite. The solids were washed copiously with diethyl ether. Thereafter methanol was used to elute the desired product.

The following compounds were prepared using these methods:

1-(4-Chlorobenzyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride[15] (5a). Prepared from 4-chlorobenzylamine and benzaldehyde, isolated as a colourless amorphous solid (880 mg, 31%). M.p. 188–192 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.45–7.41 (m, 3H), 7.40–7.34 (m, 4H), 7.27–7.23 (m, 2H), 5.68 (s, 1H), 4.88 (d, J = 16.9, 1H), 4.28 (d, J = 16.9, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.5, 159.8, 140.4, 135.9, 135.2, 131.8, 131.3, 131.0, 130.8, 128.3, 70.2, 51.6; compound purity (UPLC) 96.3%.

1-(3,4-Dichlorobenzyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (5b). Prepared from 3,4-dichlorobenzylamine and benzaldehyde, isolated as a pale yellow amorphous solid (452 mg, 21%). M.p. 204–208 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (d, J = 8.3, 1H), 7.46–7.39 (m, 3H), 7.39–7.33 (m, 3H), 7.18 (dd, J = 8.3, 2.1, 1H), 5.73 (s, 1H), 4.80 (d, J = 17.2, 1H), 4.38 (d, J = 17.2, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.5, 159.8, 140.5, 137.6, 134.6, 133.8, 132.9, 131.9, 131.3, 131.1, 128.8, 128.4, 70.7, 51.3; HRMS (ESI) found $(M - Cl^-)^+$ 348.0792, $C_{16}H_{16}{}^{35}Cl_2N_5$ requires 348.0783; compound purity (UPLC) 99.8%.

1-(4-Chlorophenethyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (5c). Prepared from 4-chlorophenethylamine and benzaldehyde, isolated as a white solid (1.13 g, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 7.74 (s, 2H), 7.52–7.20 (m, 9H), 5.81 (s, 1H), 3.89 (ddd, J = 14.9, 9.1, 6.1, 1H), 3.19 (ddd, J = 15.0, 9.4, 5.8, 1H), 3.02–2.84 (m, 1H), 2.82–2.68 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.1, 156.9, 139.5, 136.7, 131.1, 130.9, 130.6, 129.2, 129.0, 128.4, 128.1, 125.9, 66.7, 47.9, 31.8; HRMS (ESI) found $(M + H)^+$ 328.1330, $C_{17}H_{19}{}^{35}ClN_5$ requires 328.1329; compound purity (UPLC) 99.6%.

1-(3,4-Dichlorophenethyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (5d). Prepared from 3,4-dichlorophenethylamine and benzaldehyde, isolated as a white solid (171 mg, 53%). M.p. 194–198 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.50–7.44 (m, 4H), 7.40–7.30 (m, 3H), 7.21–7.09 (m, 1H), 5.52 (s, 1H), 3.79 (ddd, J = 14.6, 7.8, 5.9 Hz, 1H), 3.38–3.32 (m, 1H), 2.98 (dt, J = 14.5, 7.4 Hz, 1H), 2.82 (dt, J = 13.7, 6.8 Hz, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.1, 158.8, 140.0, 139.8, 132.3, 132.0, 131.8, 131.0, 130.5, 130.1, 129.9, 127.3, 69.9, 49.4, 33.1; HRMS (ESI) found $(M + H)^+$ 362.0943, $C_{17}H_{18}{}^{35}Cl_2N_5$ requires 362.0939; compound purity (UPLC) 99.2%.

1-(2-(4-Chlorophenoxy)ethyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (5e). Prepared from 2-(4-chlorophenoxy)ethanamine and benzaldehyde, isolated as a white solid (621 mg, 26%). M.p. 164–168 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.43–7.32 (s, 5H), 7.30–7.14 (m, 2H), 6.93–6.71 (m, 2H), 5.91 (s, 1H), 4.19–3.96 (m, 2H), 3.92 (ddd, J = 16.0, 5.0, 3.4 Hz, 1H), 3.71 (ddd, J = 15.9, 8.0, 3.8 Hz, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.8, 158.7, 158.3, 140.3, 130.8, 130.4, 130.4, 129.8, 127.3, 127.3, 127.0, 117.1, 70.5, 66.9; HRMS (ESI) found $(M + H)^+$ 344.1287, $C_{17}H_{19}{}^{35}ClN_5O$ requires 344.1278; compound purity (UPLC) 86.4%.

1-(2-(3,4-Dichlorophenoxy)ethyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (5f). Prepared from 2-(3,4-dichlorophenoxy)ethanamine and benzaldehyde, isolated as white solid (350 mg, 27%). M.p. 195–197 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (s, 5H), 7.35 (d, J = 9.3 Hz, 1H, overlapped with previous signal), 6.99 (d, J = 2.9 Hz, 1H), 6.78 (dd, J = 8.9, 2.9 Hz, 1H), 5.85 (s, 1H), 4.13–3.95 (m, 2H), 3.87 (ddd, J = 16.0, 5.1, 3.4 Hz, 1H), 3.68 (ddd, J = 16.0, 7.8, 3.8 Hz, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 158.3, 157.4, 157.2, 138.9, 132.3, 130.5, 129.4, 128.9, 125.8, 123.9, 116.1, 114.3, 114.3, 68.9, 65.6; HRMS (ESI) found $(M + H)^+$ 378.0883, $C_{17}H_{18}{}^{35}Cl_2N_5O$ requires 378.0888; compound purity (UPLC) 99.4%.

1-(3-(4-Chlorophenoxy)propyl)-6-phenyl-1,2-dihydro-1,3,5-triazine-4,6-diamine hydrochloride (5g). Prepared from 3-(4-chlorophenoxy)propan-1-amine and benzaldehyde, isolated as a white solid (223 mg, 13%). M.p. 200–202 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 (s, 5H), 7.24–7.15 (m, 2H), 6.86 (d, J = 7.1 Hz, 2H), 5.71 (s, 1H), 3.99 (t, J = 6.1 Hz, 2H), 3.62–3.23 (m, 2H), 2.06–1.92 (m, 2H); ^{13}C NMR (101 MHz,

CD_3OD) δ 159.7, 159.1, 158.6, 140.7, 130.8, 130.3, 130.1, 127.5, 126.6, 117.1, 67.0, 64.9, 39.1, 30.4; HRMS (ESI) found $(M + H)^+$ 358.1405, $C_{18}H_{21}{}^{35}ClN_5O$ requires 358.1435; compound purity (UPLC) 95.8%.

1-(3-(3,4-Dichlorophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (5h). Prepared from 3-(3,4-dichlorophenoxy)propan-1-amine and benzaldehyde, isolated as a white solid (152 mg, 26%). M.p. 189–192 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.48–7.43 (m, 3H), 7.42–7.37 (m, 3H), 7.09 (d, J = 2.9 Hz, 1H), 6.87 (dd, J = 8.9, 2.9 Hz, 1H), 5.74 (s, 1H), 4.07–3.99 (m, 2H), 3.70 (ddd, J = 15.4, 7.8, 5.9 Hz, 1H), 3.39–3.26 (m, 1H), 2.19–1.93 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.1 (2 C), 159.6, 141.1, 134.6, 132.9, 131.8, 131.3, 128.2, 125.8, 118.3, 116.7, 70.8, 67.3, 46.4, 28.3; HRMS (ESI) found $(M + H)^+$ 392.1065, $C_{18}H_{20}{}^{35}Cl_2N_5O$ requires 392.1045; compound purity (UPLC) 99.9%.

1-(3-(2,4-Dichlorophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10a). Prepared from 3-(2,4-dichlorophenoxy)propan-1-amine and benzaldehyde, isolated as an off-white solid (134 mg, 21%). M.p. 192–195 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.45–7.31 (m, 6H), 7.25–7.20 (m, 1H), 7.00 (d, J = 8.9 Hz, 1H), 5.69 (s, 1H), 4.10–4.03 (m, 2H), 3.62 (ddd, J = 15.4, 8.0, 5.2 Hz, 1H), 3.42–3.33 (m, 1H), 2.11 (s, 1H), 2.02–1.91 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 154.2, 140.3, 131.1, 130.8, 130.5, 129.1, 127.5, 127.1, 124.5, 115.5, 70.4, 66.9, 45.3, 27.5; HRMS (ESI) found $(M + H)^+$ 392.1059, $C_{18}H_{20}{}^{35}Cl_2N_5O$ requires 392.1045; compound purity (UPLC) 98.8%.

1-(3-(4-Methoxyphenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10b). Prepared from 3-(4-methoxyphenoxy)propan-1-amine and benzaldehyde, isolated as an off-white solid (30 mg, 11%). M.p. 184–188 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (2 H, dd, J 1.9, 5.3), 7.41 (2 H, dd, J 2.4, 7.2), 6.96–6.78 (5 H, m), 5.75 (1 H, s), 4.04–3.97 (2 H, m), 3.76 (3H, s), 3.74–3.65 (1 H, m), 3.41–3.37 (1 H, m), 2.15–2.06 (1 H, m), 2.06–1.93 (1 H, m); ^{13}C NMR (101 MHz, CD_3OD) δ 156.5, 156.2, 155.4, 154.8, 141.2, 131.9, 131.4, 128.2, 117.4, 116.5, 70.9, 67.1, 56.9, 46.4, 28.7; HRMS (ESI) found $(M + H)^+$ 354.1911, $C_{19}H_{24}N_5O_2$ requires 354.1930; compound purity (UPLC) 97.8%.

T1-(3-(2-chlorophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10c). Prepared from 3-(2-chlorophenoxy)propan-1-amine and benzaldehyde, isolated as an off-white solid (273 mg, 48%). M.p. 180–183 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.44–7.30 (m, 6H), 7.25–7.19 (m, 1H), 7.04–7.00 (m, 1H), 6.93–6.87 (m, 1H), 5.71 (d, J = 0.7 Hz, 1H), 4.12–4.03 (m, 2H), 3.71–3.56 (m, 1H), 3.39 (dt, J = 15.3, 7.6 Hz, 1H), 2.20–2.07 (m, 1H), 2.04–1.90 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 155.2, 140.3, 131.2, 131.0, 130.5, 129.3, 127.4, 123.6, 122.9, 114.6, 70.3, 66.4, 45.3, 27.6; HRMS (ESI) found $(M + H)^+$ 358.1391, $C_{18}H_{21}{}^{35}ClN_5O$ requires 358.1435; compound purity (UPLC) 98.3%.

1-(3-(4-Fluorophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10d). Prepared from 3-(4-fluorophenoxy)propan-1-amine and benzaldehyde, isolated as a white solid (67 mg, 10%). M.p. 214–217 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.47–7.30 (m, 5H), 7.03–6.91 (m, 2H), 6.91–6.82 (m, 2H), 5.71 (s, 1H), 4.01–3.90 (m, 2H), 3.67 (ddd, J = 15.4, 7.7, 5.8 Hz, 1H), 3.35–3.21 (m, 1H), 2.13–1.90 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.8 (d, J_{C-F} = 237.0 Hz), 158.7, 153.1 (d, J_{C-F} = 2.2 Hz), 140.3, 131.0, 130.5, 127.4, 116.8 (d, J_{C-F} = 21.5 Hz), 116.6 (d, J_{C-F} = 6.1 Hz), 70.0, 66.2, 45.6, 27.7; HRMS (ESI) found $(M + H)^+$ 342.1734, $C_{18}H_{21}FN_5O$ requires 342.1730; compound purity (UPLC) 96.2%.

1-(3-(2-Methoxy-4-methylphenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10e). Prepared from 3-(2-methoxy-4-methylphenoxy)propan-1-amine and benzaldehyde, isolated as a white solid (18 mg, 4%). M.p. 186 °C (decomp); ^{1}H NMR (400 MHz, CD_3OD) δ 7.49–7.37 (m, 5H), 6.83 (d, J = 8.2 Hz, 2H), 6.69 (dd, J = 8.1, 1.9 Hz, 1H), 5.80 (s, 1H), 4.04–3.93 (m, 2H), 3.82 (s, 3H), 3.68–3.59 (m, 1H), 3.42 (dt, J = 15.2, 7.4 Hz, 1H), 2.28 (s, 3H), 2.06–1.90 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 150.5, 146.7, 140.6, 132.7, 131.0, 130.5, 127.4, 122.2, 114.7, 113.9, 70.2, 66.5, 56.2, 45.4, 28.0, 21.1; HRMS (ESI) found $(M + H)^+$ 368.2051, $C_{20}H_{26}N_5O_2$ requires 368.2087; compound purity 96.3%.

1-(3-(4-Bromophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10f). Prepared from 3-(4-bromophenoxy)propan-1-amine and benzaldehyde, isolated as a white solid (227 mg, 39%). M.p. 215 °C (decomp); ^{1}H NMR (400 MHz, CD_3OD) δ 7.47–7.35 (m, 7H), 6.91–6.80 (m, 2H), 5.73 (s, 1H), 4.07–3.97 (m, 2H), 3.70 (ddd, J = 15.3, 7.7, 5.8 Hz, 1H), 3.34 (s, 1H), 2.18–2.07 (m, 1H), 2.06–1.95 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 157.8, 157.7, 157.3, 138.8, 132.0, 129.5, 129.0, 125.9, 116.0, 112.6, 68.5, 64.5, 44.1, 26.1; HRMS (ESI) found $(M + H)^+$ 402.0891, $C_{18}H_{21}{}^{79}BrN_5O$ requires 402.0929; compound purity (UPLC) 98.2%.

1-(3-(4-Chloro-3-fluorophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10g). Prepared from 3-(4-chloro-3-fluorophenoxy)propan-1-amine and benzaldehyde, isolated as an off-white solid (64 mg, 10%). M.p. 224–227 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.50–7.42 (m, 3H), 7.41–7.31 (m, 3H), 6.93–6.81 (m, 1H), 6.78–6.64 (m, 1H), 5.73 (s, 1H), 4.09–3.97 (m, 2H), 3.75–3.64 (m, 1H), 2.19–2.07 (m, 1H), 2.06–1.94 (m, 1H), 3.39–3.23 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.0 (d, J_{C-F} = 9.9 Hz), 159.7 (d, J_{C-F} = 246.6 Hz), 159.2, 158.8, 140.2, 131.9 (d, J_{C-F} = 1.3 Hz), 131.0, 130.5, 127.4, 113.2 (d, J_{C-F} = 18.2 Hz), 112.6 (d, J_{C-F} = 3.3 Hz), 104.4 (d, J_{C-F} = 24.8 Hz) 69.9, 66.6, 49.4, 45.6, 27.5; HRMS (ESI) found $(M + H)^+$ 376.1312, $C_{18}H_{20}{}^{35}ClFN_5O$ requires 376.1340; compound purity (UPLC) 98.4%.

1-(3-(3,4-Difluorophenoxy)propyl)-6-phenyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (10h). Prepared from 3-(3,4-difluorophenoxy)propan-1-amine and benzaldehyde, isolated as an off-white solid (60 mg, 12%). M.p. 215–217 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.50–7.34 (m, 5H), 7.24–6.97 (m, 1H), 6.94–6.80 (m, 1H), 6.75–6.65 (m, 1H), 5.76 (s, 1H), 4.09–3.94 (m, 2H), 3.78–3.66 (m, 1H), 3.40–3.26 (m, 1H), 2.16–2.07 (m, 1H), 2.07–1.94 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.8, 156.5 (dd, J_{C-F} = 8.8, 2.2 Hz), 151.7 (dd, J_{C-F} = 245.9, 13.8 Hz), 146.3 (dd, J_{C-F} = 238.7, 12.9 Hz), 140.2,

131.0, 130.5, 127.4, 118.4 (dd, J_{C-F} = 18.7, 1.4 Hz), 111.2 (dd, J_{C-F} = 5.7, 3.5 Hz), 105.1 (d, J_{C-F} = 20.5 Hz) 69.9, 66.7, 45.6, 27.6; HRMS (ESI) found $(M + H)^+$ 360.1607, $C_{18}H_{20}F_2N_5O$ requires 360.1636; compound purity (UPLC) 93.2%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2-chlorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11a). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 2-chlorobenzaldehyde, isolated as an off-white solid (107 mg, 17%). M.p. 224–226 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.57–7.49 (m, 1H), 7.49–7.38 (m, 2H), 7.38–7.33 (m, 1H), 7.29–7.22 (m, 2H), 6.97–6.85 (m, 2H), 6.17 (s, 1H), 4.22–3.94 (m, 2H), 3.85–3.71 (m, 1H), 3.32–3.25 (m, 1H), 2.21–2.11 (m, 1H), 2.06–1.96 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.3, 159.5, 159.4, 137.7, 134.6, 133.4, 132.7, 131.3, 130.1, 129.6, 127.8, 117.8, 68.3, 66.7, 46.5, 28.4; HRMS (ESI) found $(M + H)^+$ 392.1016, $C_{18}H_{20}{}^{35}Cl_2N_5O$ requires 392.1045; compound purity (UPLC) 96.8%.

1-(3-(4-Chlorophenoxy)propyl)-6-(4-(trifluoromethyl)phenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11b). Prepared from 3-(4-chlorophenoxy)propan-1-amine and α,α,α-trifluoro-*p*-tolualdehyde, isolated as a white solid (275 mg, 47%). M.p. 227–230 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 7.32–7.26 (m, 2H), 6.97–6.91 (m, 2H), 5.91 (s, 1H), 4.10–3.99 (m, 2H), 3.90–3.78 (m, 1H), 3.29–3.21 (m, 1H), 2.14–1.82 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.3, 158.8, 158.6, 144.5, 132.8 (q, J_{C-F} = 32.5 Hz), 130.4, 128.1, 127.4 (q, J_{C-F} = 3.8 Hz), 126.9, 125.3 (q, J_{C-F} = 271.5 Hz), 117.0, 69.1, 66.1, 46.1, 27.2; HRMS (ESI) found $(M + H)^+$ 426.1302, $C_{19}H_{20}{}^{35}ClF_3N_5O$ requires 426.1308; compound purity (UPLC) 99.7%.

1-(3-(4-Chlorophenoxy)propyl)-6-(4-methoxyphenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11c). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 4-methoxybenzaldehyde, isolated as an off-white solid (25 mg, 4%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.34–7.30 (m, 2H), 7.27–7.23 (m, 2H), 6.99–6.95 (m, 2H), 6.90–6.86 (m, 2H), 5.68 (s, 1H), 4.04–3.96 (m, 2H), 3.80 (d, J = 0.8 Hz, 3H), 3.70–3.59 (m, 1H), 3.38–3.25 (m, 1H), 2.13–2.03 (m, 1H), 2.02–1.91 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 162.4, 159.2, 158.7, 158.6, 132.0, 130.4, 129.0, 126.9, 117.0, 115.7, 69.8, 66.1, 55.9, 45.3, 27.6; HRMS (ESI) found $(M + H)^+$ 388.1541, $C_{19}H_{23}{}^{35}ClN_5O_2$ requires 388.1540; compound purity (UPLC) 95.7%.

6-(4-Chloro-3-fluorophenyl)-1-(3-(4-chlorophenoxy)propyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11d). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 4-chloro-3-fluorobenzaldehyde, isolated as an off-white solid (118 mg, 18%). M.p. 215–218 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.91–7.81 (m, 2H), 7.76–7.67 (m, 2H), 7.51–7.41 (m, 1H), 7.14–7.09 (m, 1H), 6.97–6.85 (m, 1H), 6.19 (s, 1H), 4.17–4.02 (m, 2H), 3.80–3.66 (m, 1H), 3.31–3.17 (m, 1H), 2.27–2.14 (m, 1H), 2.12–1.96 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.4 (d, J_{C-F} = 250.0 Hz), 160.1, 159.6, 159.5, 142.4 (d, J_{C-F} = 5.5 Hz), 133.7, 131.2, 127.7, 125.0 (d, J_{C-F} = 3.8 Hz), 124.1 (d, J_{C-F} = 17.8 Hz), 117.9, 116.6 (d, J_{C-F} = 22.4 Hz), 69.4, 67.0, 47.0, 28.6; HRMS (ESI) found $(M + H)^+$ 410.0947, $C_{18}H_{19}{}^{35}Cl_2FN_5O$ requires 410.0951; compound purity (UPLC) 94.3%.

1-(3-(4-Chlorophenoxy)propyl)-6-(3-chlorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11e). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 3-chlorobenzaldehyde, isolated as an off-white solid (33 mg, 6%). M.p. 216–218 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.48–7.43 (m, 2H), 7.41–7.38 (m, 1H), 7.34–7.29 (m, 1H), 7.29–7.23 (m, 2H), 6.94–6.87 (m, 2H), 5.76 (s, 1H), 4.07–4.00 (m, 2H), 3.76 (ddd, J = 15.4, 7.6, 5.9 Hz, 1H), 3.39–3.32 (m, 1H), 2.20–2.09 (m, 1H), 2.09–1.96 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 158.6, 142.6, 136.3, 132.2, 131.0, 130.4, 127.4, 126.9, 125.7, 117.0, 69.2, 66.0, 45.8, 27.7; HRMS (ESI) found $(M + H)^+$ 392.1041, $C_{18}H_{20}{}^{35}Cl_2N_5O$ requires 392.1045; compound purity (UPLC) 99.7%.

1-(3-(4-Chlorophenoxy)propyl)-6-(4-fluorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11f). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 4-fluorobenzaldehyde, isolated as a white crystalline solid (22 mg, 4%). M.p. 194–196 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.53–7.45 (2 H, m), 7.36–7.29 (2 H, m), 7.29–7.21 (2 H, m), 6.99–6.93 (2 H, m), 5.82 (1 H, s), 4.13–4.04 (2 H, m), 3.82–3.71 (1 H, m), 3.45–3.40 (1 H, m), 2.26–2.12 (1 H, m), 2.12–1.99 (1 H, m); ^{13}C NMR (101 MHz, CD_3OD) δ 165.7 (d, J_{C-F} = 247.9 Hz), 160.0, 159.6, 159.5, 137.3 (d, J_{C-F} = 3.2 Hz), 131.3, 130.5 (d, J_{C-F} = 8.7 Hz), 127.7, 118.1 (d, J_{C-F} = 22.2 Hz), 117.8, 70.2, 66.9, 46.4, 28.4; HRMS (ESI) found $(M + H)^+$ 376.1334, $C_{18}H_{20}{}^{35}ClFN_5O$ requires 376.1340; compound purity (UPLC) 93.1%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2-fluorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11g). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 2-fluorobenzaldehyde, isolated as an off-white solid (107 mg, 20%). M.p. 221–224 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.54–7.43 (m, 1H), 7.35 (tt, J = 7.6, 2.0 Hz, 1H), 7.30–7.19 (m, 4H), 6.96–6.86 (m, 2H), 6.08 (d, J = 1.8 Hz, 1H), 4.09–4.00 (m, 2H), 3.81–3.69 (m, 1H), 3.41–3.30 (m, 1H), 2.23–2.08 (m, 1H), 2.08–1.95 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 162.6 (d, J_{C-F} = 247.6 Hz), 160.1, 159.6, 159.5, 133.9 (d, J_{C-F} = 8.6 Hz), 131.3, 129.6 (d, J_{C-F} = 3.1 Hz), 127.8 (d, J_{C-F} = 12.7 Hz), 127.8, 127.1 (d, J_{C-F} = 3.6 Hz), 118.3 (d, J_{C-F} = 21.4 Hz), 117.9, 66.9, 66.0 (d, J_{C-F} = 3.9 Hz), 46.5, 28.5; HRMS (ESI) found $(M + H)^+$ 376.1341, $C_{18}H_{20}{}^{35}ClFN_5O$ requires 376.1340; compound purity (UPLC) 96.7%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2-(trifluoromethyl)phenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11h). Prepared from 3-(4-chlorophenoxy)propan-1-amine and α,α,α-trifluoro-*o*-tolualdehyde, isolated as a white crystalline solid (95 mg, 11%). M.p. 230–234 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (dd, J = 15.7, 7.6 Hz, 2H), 7.68 (t, J = 7.4 Hz, 2H), 7.28 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 6.16 (s, 1H), 4.12–3.97 (m, 2H), 3.70 (ddd, J = 15.5, 7.7, 5.5 Hz, 1H), 3.27–3.16 (m, 1H), 2.25–2.11 (m, 1H), 2.08–1.94 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.3, 159.4, 159.1, 140.2, 136.0, 132.5, 131.2, 129.6, 129.4 (q, J_{C-F} = 30.7 Hz), 128.4 (q, J_{C-F} = 5.7 Hz), 127.7, 126.3 (q, J_{C-F} = 273.6 Hz), 117.7, 67.0 (q, J_{C-F} = 2.3 Hz), 66.8, 46.7, 28.1; HRMS (ESI) found $(M + H)^+$ 426.1239, $C_{19}H_{20}{}^{35}ClF_3N_5O$ requires 426.1308; compound purity (UPLC) 97.4%.

1-(3-(4-Chlorophenoxy)propyl)-6-(4-chlorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11i). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 4-chlorobenzaldehyde, isolated as a white crystalline solid (58 mg, 11%). M.p. 226–228 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.51–7.42 (m, 2H), 7.41–7.33 (m, 2H), 7.31–7.21 (m, 2H), 6.94–6.86 (m, 2H), 5.77 (s, 1H), 4.06–3.99 (m, 2H), 3.73 (ddd, J = 15.3, 7.7, 5.8 Hz, 1H), 3.38–3.32 (m, 1H), 2.19–2.07 (m, 1H), 2.06–1.94 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 158.6, 139.1, 136.8, 130.6, 130.4, 129.1, 126.9, 117.0, 69.3, 66.1, 45.7, 27.6; HRMS (ESI) found $(M + H)^+$ 392.1017, $C_{18}H_{20}{}^{35}Cl_2N_5O$ requires 392.1045; compound purity (UPLC) 98.5%.

6-(3-Chloro-2-fluorophenyl)-1-(3-(4-chlorophenoxy)propyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11j). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 3-chloro-2-fluorobenzaldehyde, isolated as a white solid (25 mg, 10%). M.p. 184–186 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.62–7.51 (m, 1H), 7.32–7.21 (m, 4H), 6.96–6.85 (m, 2H), 6.11 (d, J = 1.8 Hz, 1H), 4.05 (t, J = 5.5 Hz, 2H), 3.78 (ddd, J = 15.5, 7.6, 5.8 Hz, 1H), 3.41–3.32 (m, 1H), 2.23–2.10 (m, 1H), 2.09–1.97 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 158.6, 157.1 (d, J_{C-F} = 250.2 Hz), 133.3, 130.4, 128.8 (d, J_{C-F} = 12.8 Hz), 127.2 (d, J_{C-F} = 2.9 Hz), 126.9 (d, J_{C-F} = 4.9 Hz), 123.0 (d, J_{C-F} = 17.5 Hz), 119.9, 117.0, 66.0, 65.2 (d, J_{C-F} = 3.8 Hz), 45.8, 27.6; HRMS (ESI) found $(M + H)^+$ 410.0937, $C_{18}H_{19}{}^{35}Cl_2FN_5O$ requires 410.0951; compound purity (UPLC) 99.4%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2,5-dichlorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11k). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 2,5-dichlorobenzaldehyde, isolated as a white solid (84 mg, 16%). M.p. 197–201 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.56–7.42 (m, 2H), 7.35–7.20 (m, 3H), 6.94–6.87 (m, 2H), 6.14 (d, J = 3.0 Hz, 1H), 4.09–4.02 (m, 2H), 3.81–3.70 (m, 1H), 3.28 (s, 1H), 2.22–2.11 (m, 1H), 2.08–1.95 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.4, 158.6, 158.6, 138.6, 135.0, 133.4, 132.5, 132.3, 130.4, 128.7, 127.0, 117.0, 67.3, 65.9, 45.8, 27.5; HRMS (ESI) found $(M + H)^+$ 426.0631, $C_{18}H_{19}{}^{35}Cl_3FN_5O$ requires 426.0655; compound purity (UPLC) 97.2%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2-fluoro-3-methoxyphenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11l). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 2-fluoro-3-methoxybenzaldehyde, isolated as a white solid (246 mg, 39%). M.p. 204–206 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.31–7.21 (m, 2H), 7.20–7.15 (m, 2H), 6.95–6.82 (m, 3H), 6.07 (d, J = 1.4 Hz, 1H), 4.03 (t, J = 5.7 Hz, 2H), 3.79–3.68 (m, 1H), 3.40–3.29 (m, 1H), 2.22–2.09 (m, 1H), 2.07–1.95 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.8, 158.6, 151.3 (d, J_{C-F} = 248.3 Hz), 149.7 (d, J_{C-F} = 10.2 Hz), 130.4, 127.8 (d, J_{C-F} = 9.2 Hz), 126.9, 126.2 (d, J_{C-F} = 4.9 Hz), 119.1, 117.0, 116.1, 66.0, 64.9 (d, J = 5.2 Hz), 57.0, 45.6, 27.6; HRMS (ESI) found $(M + H)^+$ 406.1435, $C_{19}H_{22}{}^{35}ClFN_5O_2$ requires 406.1446; compound purity (UPLC) 95.3%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2,4-difluorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11m). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 2,4-difluorobenzaldehyde, isolated as a white solid (166 mg, 33%). M.p. 225–228 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (td, J = 8.6, 6.1 Hz, 1H), 7.13–7.06 (m, 1H), 7.34–7.24 (m, 2H), 7.19–7.06 (m, 2H), 6.98–6.91 (m, 2H), 6.07 (s, 1H), 4.08 (t, J = 5.7 Hz, 2H), 3.76 (ddd, J = 15.4, 7.6, 5.7 Hz, 1H), 3.42–3.36 (m, 1H), 2.26–2.11 (m, 1H), 2.11–1.97 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 166.1 (dd, J_{C-F} = 250.8, 12.3 Hz), 163.0 (dd, J_{C-F} = 250.5, 12.4 Hz), 160.0, 159.6, 159.5, 131.3, 131.1 (dd, J_{C-F} = 10.2, 4.9 Hz), 127.8, 124.4 (dd, J_{C-F} = 13.1, 3.9 Hz), 117.8, 114.2 (dd, J_{C-F} = 21.9, 3.6 Hz), 106.7 (dd, J_{C-F} = 25.9, 25.9 Hz), 66.8, 65.7, 46.4, 28.4; HRMS (ESI) found $(M + H)^+$ 394.1209, $C_{18}H_{19}{}^{35}ClF_2N_5O$ requires 394.1246; compound purity (UPLC) 92.7%.

1-(3-(4-Chlorophenoxy)propyl)-6-(2,4-dichlorophenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11n). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 2,4-dichlorobenzaldehyde, isolated as a white solid (120 mg, 23%). M.p. 209–211 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.57–7.51 (m, 1H), 7.45–7.35 (m, 1H), 7.30 (dd, J = 8.4, 4.7 Hz, 1H), 7.26–7.15 (m, 2H), 6.93–6.79 (m, 2H), 6.11 (d, J = 4.5 Hz, 1H), 4.06–3.96 (m, 2H), 3.75–3.64 (m, 1H), 3.29–3.18 (m, 1H), 2.17–2.04 (m, 1H), 2.02–1.89 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.4, 158.6, 158.5, 137.6, 135.7, 134.7, 131.5, 130.4, 130.1, 129.5, 126.9, 117.0, 67.1, 65.9, 45.7, 27.5; HRMS (ESI) found $(M + H)^+$ 426.0643, $C_{18}H_{19}{}^{35}Cl_3FN_5O$ requires 426.0655; compound purity (UPLC) 97.8%.

1-(3-(4-Chlorophenoxy)propyl)-6-(3-(trifluoromethoxy)phenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11o). Prepared from 3-(4-chlorophenoxy)propan-1-amine and 3-(trifluoromethoxy)benzaldehyde, isolated as an off-white solid (30 mg, 6%). M.p. 203–206 °C (decomp); ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (t, J = 8.0 Hz, 1H), 7.44–7.34 (m, 2H), 7.31 (s, 1H), 7.28–7.20 (m, 2H), 6.95–6.86 (m, 2H), 5.86 (s, 1H), 4.08–3.99 (m, 2H), 3.81 (ddd, J = 15.3, 7.6, 5.9 Hz, 1H), 3.42–3.33 (m, 1H), 2.22–2.09 (m, 1H), 2.09–1.97 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 158.6, 151.0, 143.0, 132.4, 130.4, 126.8, 126.1, 123.2, 121.8 (q, J_{C-F} = 256.3 Hz), 120.0, 117.0, 68.9, 66.1, 46.0, 27.7; HRMS (ESI) found $(M + H)^+$ 442.1222, $C_{19}H_{20}{}^{35}ClF_3N_5O_2$ requires 442.1258; compound purity (UPLC) 96.4%.

1-(3-(4-Chlorophenoxy)propyl)-6-*m*-tolyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11p). Prepared from 3-(3,4-dichlorophenoxy)propan-1-amine and *m*-tolualdehyde, isolated as a white solid (243 mg, 48%). M.p. 228–231 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.33 (t, J = 7.6 Hz, 1H), 7.30–7.22 (m, 3H), 7.23–7.12 (m, 2H), 6.89 (d, J = 8.9 Hz, 2H), 5.68 (s, 1H), 4.06–3.97 (m, 2H), 3.73–3.63 (m, 1H), 3.39–3.26 (m, 1H), 2.17–2.07 (m, 1H), 2.05–1.94 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.8, 158.7 (2C), 140.6, 140.2, 131.7, 130.4, 130.4, 127.9, 126.9, 124.5, 117.0, 70.1, 66.0, 45.5, 27.6, 21; HRMS (ESI) found $(M + H)^+$ 372.1570, $C_{19}H_{23}{}^{35}ClN_5O$ requires 372.1591; compound purity (UPLC) 98.8%.

1-(3-(4-Chlorophenoxy)propyl)-6-(4-(trifluoromethylthio)phenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11q). Prepared from 3-(4-chlorophenoxy)propan-1-amine and

4-((trifluoromethyl)thio)benzaldehyde, isolated as an off-white solid (194 mg, 33%). M.p. 189–193 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.32–7.20 (m, 2H), 6.96–6.86 (m, 2H), 5.86 (s, 1H), 4.08–3.99 (m, 2H), 3.81 (ddd, J = 15.2, 7.4, 5.8 Hz, 1H), 3.41–3.32 (m, 1H), 2.22–2.09 (m, 1H), 2.08–1.99 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.2, 158.7, 158.6, 143.5, 138.3, 131.03 (d, J_{C-F} = 306.8 Hz), 130.4, 128.6, 126.9 (q, J_{C-F} = 2.1 Hz), 126.8, 117.0, 69.1, 66.0, 46.0, 27.7; HRMS (ESI) found $(M + H)^+$ 458.1006, $C_{19}H_{20}{}^{35}ClF_3N_5OS$ requires 458.1029; compound purity (UPLC) 97.5%.

1-(3-(3,4-Dichlorophenoxy)propyl)-6-(4-(trifluoromethyl)phenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11r). Prepared from 3-(3,4-dichlorophenoxy)propan-1-amine and α,α,α-trifluoro-*p*-tolualdehyde, isolated as a white solid (272 mg, 42%). M.p. 231–234 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.9 Hz, 1H), 7.12 (d, J = 2.9 Hz, 1H), 6.89 (dd, J = 9.0, 2.9 Hz, 1H), 5.88 (s, 1H), 4.10–4.00 (m, 2H), 3.85–3.76 (m, 1H), 3.41–3.32 (m, 1H), 2.23–2.10 (m, 1H), 2.10–1.98 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.4, 159.0, 158.2, 139.3, 135.2, 133.8, 132.0, 131.6, 130.4, 128.8, 128.5 (q, J_{C-F} = 30.3 Hz), 127.5 (q, J_{C-F} = 5.5 Hz), 125.5 (q, J_{C-F} = 273.5 Hz), 125.0, 117.4, 115.8, 66.3, 66.1, 45.7, 27.1; HRMS (ESI) found $(M + H)^+$ 460.0908, $C_{19}H_{19}{}^{35}Cl_2F_3N_5O$ requires 460.0919; compound purity (UPLC) 94.8%.

1-(3-(3,4-Dichlorophenoxy)propyl)-6-(2-(trifluoromethyl)phenyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11s). Prepared from 3-(3,4-dichlorophenoxy)propan-1-amine and α,α,α-trifluoro-*o*-tolualdehyde, isolated as a white solid (164 mg, 28%). M.p. 209–212 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.85–7.72 (m, 2H), 7.69–7.62 (m, 2H), 7.39 (d, J = 8.9 Hz, 1H), 7.04 (d, J = 2.9 Hz, 1H), 6.84 (dd, J = 8.9, 2.9 Hz, 1H), 6.10 (s, 1H), 4.07–3.96 (m, 2H), 3.71–3.59 (m, 1H), 3.24–3.09 (m, 1H), 2.20–2.07 (m, 1H), 2.03–1.88 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 160.2, 159.9, 159.0, 140.1, 136.0, 134.6, 132.9, 132.5, 129.6, 129.6 (q, J_{C-F} = 5.2 Hz), 128.4 (q, J_{C-F} = 5.8 Hz), 126.3 (q, J_{C-F} = 273.6 Hz), 125.9, 118.2, 116.6, 67.2, 67.0, 46.6, 27.9; HRMS (ESI) found $(M + H)^+$ 460.0883, $C_{19}H_{19}{}^{35}Cl_2F_3N_5O$ requires 460.0919; compound purity (UPLC) 94.3%.

6-(4-Chlorophenyl)-1-(3-(3,4-dichlorophenoxy)propyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11t). Prepared from 3-(3,4-dichlorophenoxy)propan-1-amine and 4-chlorobenzaldehyde, isolated as a white solid (41 mg, 15%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.52–7.45 (m, 2H), 7.45–7.38 (m, 3H), 7.12 (d, J = 2.8 Hz, 1H), 6.90 (dd, J = 8.9, 2.8 Hz, 1H), 5.82 (s, 1H), 4.11–4.03 (m, 1H), 3.84–3.71 (m, 1H), 3.38 (t, J = 7.4 Hz, 2H), 2.25–2.09 (m, 1H), 2.10–1.95 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 161.6, 161.5, 161.1, 141.3, 139.1, 136.1, 134.3, 132.8, 131.4, 127.4, 119.9, 118.2, 71.5, 68.9, 48.1, 29.8; HRMS (ESI) found $(M + H)^+$ 426.0611, $C_{18}H_{19}{}^{35}Cl_3FN_5O$ requires 426.0655; compound purity (UPLC) 91.0%.

6-(3-Chloro-2-fluorophenyl)-1-(3-(3,4-dichlorophenoxy)propyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (11u). Prepared from 3-(3,4-dichlorophenoxy)propan-1-amine and 3-chloro-2-fluorobenzaldehyde, isolated as a white solid (88 mg, 20%). M.p. 205–209 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (td, J = 7.2, 2.5 Hz, 1H), 7.38 (d, J = 8.9 Hz, 1H), 7.30–7.20 (m, 2H), 7.09 (d, J = 2.9 Hz, 1H), 6.87 (dd, J = 8.9, 2.8 Hz, 1H), 6.10 (d, J = 1.8 Hz, 1H), 4.08–4.03 (m, 2H), 3.77 (ddd, J = 15.5, 7.6, 5.9 Hz, 1H), 3.39–3.31 (m, 1H), 2.22–2.09 (m, 1H), 2.07–1.97 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 159.3, 159.2, 158.7, 157.1 (d, J_{C-F} = 249.9 Hz), 133.8, 133.3, 132.0, 130.4, 128.9 (d, J_{C-F} = 12.7 Hz), 127.1 (d, J_{C-F} = 2.8 Hz), 126.9 (d, J_{C-F} = 4.6 Hz), 125.1, 123.03 (d, J_{C-F} = 17.4 Hz), 117.5, 115.8, 66.4, 65.1 (d, J_{C-F} = 2.5 Hz), 45.7; HRMS (ESI) found $(M + H)^+$ 444.0539, $C_{18}H_{18}Cl_3FN_5O$ requires 444.0561; compound purity (UPLC) 94.4%.

6-Phenyl-1-(4-phenylbutyl)-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (12).[12] Prepared from 4-phenylbutylamine and benzaldehyde, isolated as an off-white solid (148 mg, 11%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.44–7.26 (m, 5H), 7.20 (dd, J = 8.7, 6.8 Hz, 2H), 7.14–7.03 (m, 3H), 5.66 (s, 1H), 3.47 (ddd, J = 14.7, 8.8, 5.5 Hz, 1H), 3.06 (ddd, J = 15.0, 9.1, 5.7 Hz, 1H), 2.54 (t, J = 7.2 Hz, 2H), 1.72–1.41 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 164.8, 159.1, 158.7, 143.1, 140.2, 130.9, 130.4, 129.4, 129.4, 127.3, 126.9, 119.9, 69.6, 36.4, 29.3, 27.6; HRMS (ESI) found $(M + H)^+$ 322.1993, $C_{19}H_{24}N_5$ requires 322.2032; compound purity (UPLC) 93.5%.

Biology

General

^{3}H Hypoxanthine incorporation assay. The chloroquine-resistant Gambian FCR-3 strain was cultured *in vitro* according to the method described by Jensen and Trager[18] and van Zyl *et al.*[19] For experimental purposes the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring stage.[20] The antimalarial activity of the various compounds was determined using the tritiated hypoxanthine incorporation assay.[21] The parasite suspension, consisting of predominately the ring stage, was adjusted to a 0.5% parasitaemia and 1% haematocrit in hypoxanthine-free RPMI-1640 culture medium with 10% human plasma and exposed to the 6 to 7 concentrations of each compound for a single cycle of parasite growth. All assays were carried out using untreated parasites and uninfected red blood cells as controls. Labeled ^{3}H-hypoxanthine was added after 24 h and the parasitic ^{3}H-DNA harvested after a further 24 h incubation period at 37 °C in a humidified environment. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined from the sigmoidal log dose response curve generated by the Enzfitter® and GraphPad Prism® software.

pLDH assay. Three-fold serial dilutions of the test compounds were incubated with 3D7 strain *P. falciparum* parasites (2% parasitaemia, 1% haematocrit) in 96-well plates containing RPMI 1640 medium supplemented with 25 mM HEPES, 0.5% (w/v) Albumax II, 22 mM glucose, 0.65 mM hypoxanthine, 0.05 mg mL^{-1} gentamicin and 1% (v/v) human erythrocytes. Incubations were initiated when parasites were predominantly in the trophozoite stage and continued for 48 h at 37 °C in sealed containers filled with an atmosphere of 5% CO_2, 5% O_2, 90% N_2. Subsequently, a colourimetric assay for parasite lactate

dehydrogenase (pLDH) activity in individual wells was carried out.[22] Twenty μL of culture was removed from the individual wells and transferred to a second 96-well plate containing 125 μL per well pLDH assay reagent (44 mM Tris buffer, pH 9, containing 0.18 M L-lactic acid, 0.13 mM acetylpyridine adenine dinucleotide, 0.39 mM nitrotetrazolium blue chloride, 0.048 mM phenazine ethosulphate and 0.16% (v/v) Triton X-100) and incubated at ambient temperature for 10–30 minutes, after which Abs_{620} was measured in a Tecan Infinite F500 multiwell plate reader. Absorbance values were used to calculate percentage parasite viability relative to control wells containing untreated parasite cultures. IC_{50} values for individual compounds were calculated from plots of % viability *vs.* log(concentration) by non-linear regression using GraphPad Prism®.

Cytotoxicity assay. HeLa cells were plated in 96 well plates at a density of 7×10^3 cells per well in EMEM medium containing 5% fetal bovine serum, 2 mM L-glutamine and 50 μg ml^{-1} gentamicin and incubated overnight at 37 °C in a 5% CO_2 incubator. Three-fold serial dilutions of test compounds were added and incubation was continued for a further 48 h. Remaining cell biomass in the wells was determined using a sulforhodamine B (SRB) assay.[23] Cells were fixed by removing the medium and briefly adding cold 50% trichloroacetic acid, followed by washing in water and staining with 0.4% (w/v) SRB in 1% (v/v) acetic acid. Unbound stain was removed by washing in 1% acetic acid, after which 10 mM Tris base was added to dissolve bound SRB. The SRB solutions were transferred to a duplicate 96 well plate and Abs_{540} measured in a Tecan Infinite F500 plate reader. Absorbance readings were converted to % cell viability relative to untreated control wells and used to generate sigmoidal log dose response curves and calculate IC_{50} values using GraphPad Prism® software.

Enzyme assays and inhibition by analogues. Wild-type and A16V+S108T mutant *Pf*DHFRs were prepared as described earlier.[17] The activity of *Pf*DHFR was determined spectrophotometrically. Briefly, the reaction (200 μL) contained 1× DHFR buffer (50 mM TES, pH 7.0, 75 mM β-mercaptoethanol, 1 mg mL^{-1} bovine serum albumin), 100 μM dihydrofolate, 100 μM NADPH, and *Pf*DHFR enzyme (0.001–0.005 units) of the affinity-purified enzymes. Inhibition of the enzymes by cycloguanil and its analogues was carried out in the presence of varying concentrations of the inhibitor. The K_i values of the inhibitors against the enzymes was determined by fitting to the equation $IC_{50} = K_i(1 + ([S]/K_m))$, where IC_{50} is the concentration of inhibitor which inhibits 50% of the enzyme activity under the standard assay condition and K_m is the Michaelis constant for dihydrofolate.[13,24]

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