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Paper

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Pyrimidine-2,4-diamines as antiplasmodial antifolates

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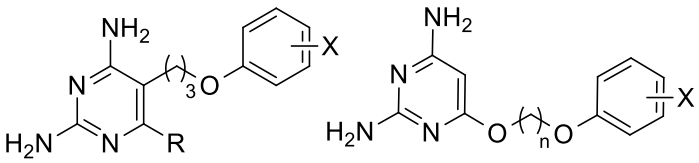
Abstract

Two series of substituted pyrimidine-2,4-diamines with a flexible side chain at either the 5- or 6-position of the pyrimdine ring were designed as potential inhibitors of *P. falciparum* dihydrofolate reductase (DHFR). The compounds were synthesised and evaluated for antiplasmodial activity *in vitro* against a cycloguanil-resistant strain of the *P. falciparum* parasite. 5-(3-(3,5-Dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine was identified as the most promising compound (IC_{50} 0.86 µM). In general, pyrimidine-2,4-diamines substituted at the 5-position of the pyrimidine ring showed better antiplasmodial activity (IC_{50} 0.86 - 26.55 µM) than those bearing a modified side chain at the 6-position of the pyrimidine ring (IC_{50} 4.46 – 83.45 µM).



R = Ph, X = Hal
IC_{50} 0.86 - 26.55 µM
(Gambian FCR-3)

X = Hal, n= 3 or 4
IC_{50} 4.46 - 83.45 µM
(Gambian FCR-3)

Keywords: Pyrimidine-2,4-diamines, antifolates, dihydrofolate reductase, antiplasmodial activity

Introduction

In 2018, approximately half of the world's population was at risk of contracting malaria, a disease caused by parasitic protozoa of the genus *Plasmodium*.[1] According to the most recent World Malaria Report, 93% of the estimated 228 million malaria cases and 94% of the 405,000 deaths worldwide in 2018 were caused by *Plasmodium falciparum*, one of the *Plasmodium* species responsible for causing malaria in humans which is prevalent in sub-Saharan Africa. While malaria-prevention tools such as indoor residual spraying (IRS) of insecticides, the use of insecticide-treated nets (ITNs), and intermittent preventative therapy in pregnancy (IPTp) have played a major role in decreasing malaria incidence and mortality over the last five years, the WHO estimates that 43% of people at risk of contracting malaria in sub-Saharan Africa do not have access to these preventative tools.[1] Furthermore, although there has been a significant decline in the number of malaria fatalities over the last 15 years, the rate of decline has decreased, with mortality rates remaining at similar levels since 2014.[1]

Folates are cellular cofactors that play an essential role in the life cycle of the malaria parasite.[2] Antimalarial antifolates target two key parasitic enzymes, dihydropteroate synthase (DHPS) and dihydrofolate reductase, which, in the parasite, is a bifunctional dimer with thymidylate synthase (DHFR-TS). These enzymes are responsible for maintaining adequate cellular levels of folate derivatives either via a folate salvage pathway or a *de novo* synthetic pathway.[3] The combination therapy of sulfadoxine-pyrimethamine, used in IPT both in pregnancy and in infants under the age of 5 years, targets these enzymes in the parasite, with pyrimethamine (**1**) targeting DHFR (Figure 1). Unfortunately, resistance to pyrimethamine and other anti-DHFR drugs, such as cycloguanil (**2**), is widespread and has limited their clinical usefulness.[4-5] Point mutations in the active site of the DHFR domains of DHFR-TS have been shown to be responsible for the development of resistance to these antifolates, which contain a rigid biaryl axis.[6] Related compounds with inherent flexibility, such as WR99210 (**3**) and P65 (**4**) (Figure 1), however, have been shown to maintain a high binding affinity to all mutant forms of *P. falciparum* DHFR.[7] These compounds contain a flexible linker between the two rings, which enables the compounds to avoid unfavourable contacts with mutant amino acid residues in the DHFR active site.

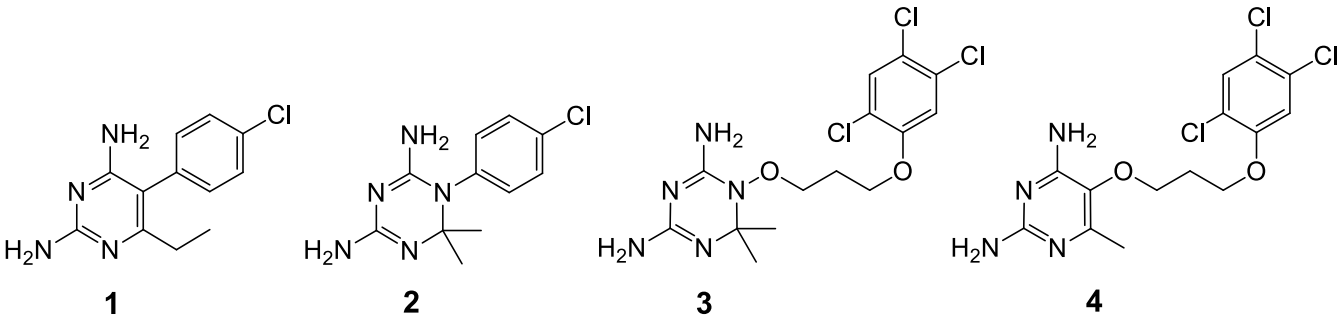
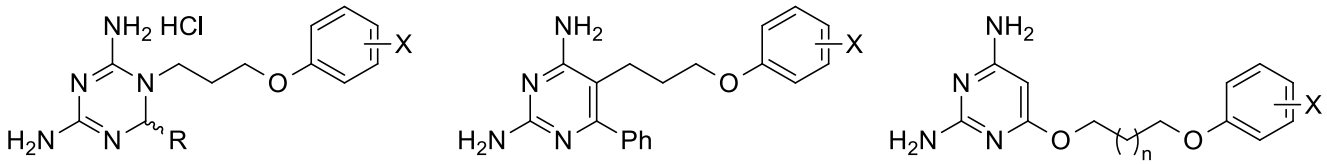


Figure 1. Known antifolates pyrimethamine (**1**), cycloguanil (**2**), WR99210 (**3**) and P65 (**4**).

Based on these findings, we reported the synthesis of a series of novel, flexible analogues of cycloguanil bearing a 4-atom linker between the two rings (see general structure (**5**), Figure 2), with *in vitro* antimalarial activity in the low nanomolar range against both drug-sensitive and drug-resistant

strains of *P. falciparum*.[8] The compounds were shown to inhibit parasitic DHFR, and were not cytotoxic. Despite the promising biological activity of these compounds; however, they were isolated as racemic mixtures in generally low yields. Furthermore, the synthesis proved challenging[9] and could not readily be adapted for asymmetric synthesis. Herein, we report the synthesis of pyrimidine analogues of general structure (**6**) of our dihydrotriazine series (**5**), and their biological activity in a whole cell *P. falciparum* assay. We also embarked on the synthesis of analogues of P65, of general structure (**7**), bearing a longer side chain moved to the 6-position of the pyrimidine ring in order to assess the effect of this on biological activity *in vitro.*



5 R = Ph or substituted Ph
X = Hal, OMe

6 X = Hal

7 X = Hal, n= 1 or 2

Figure 2. Dihydrotriazines (**5**) prepared previously, and pyrimidine analogues (**6**) and (**7**) prepared in this work.

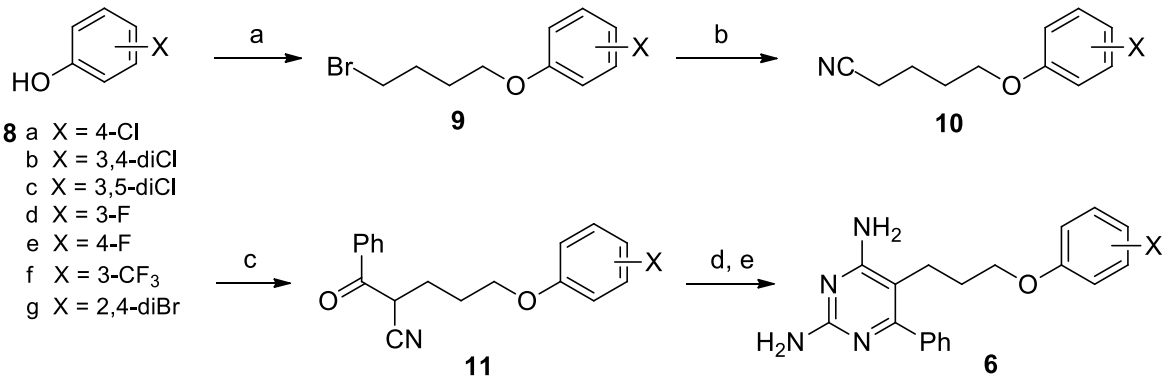
Results and Discussion

The dihydrotriazines of general structure (**5**) that we have prepared previously[8] contained an atypical phenyl substituent at the 6-position of the dihydrotriazine ring. The majority of known antifolates targeting DHFR (such as those shown in Figure 1) contain a simple alkyl substituent at this position. Owing to the difficulties associated with the synthesis of the dihydrotriazines (**5**) prepared previously,[8-9] and the fact that they were isolated as racemic mixtures, we planned to prepare the fully aromatic pyrimidine equivalents which lack the stereogenic centre.

Our approach to the pyrimidine analogues of general structure (**6**) began with simple alkylation of commercially available phenols (**8a-g**) with 1,4-dibromobutane to afford the ethers (**9a-g**) in good yields (Scheme 1). Functional-group interconversions by reacting ethers (**9a-g**) with potassium cyanide gave pentanenitriles (**10a-g**) in reasonable yields. Reaction of **10a-g** with ethyl benzoate in the presence of base afforded α-cyanoketones (**11a-g**), once again in good yields.

At this stage, we envisaged utilising methodology reported for the synthesis of 5-*aryl*pyrimidines[10] on our substrates (**11a-g**) to afford the desired 5-*alkyl*pyrimidines. This methodology involved formation of an intermediate enol ether, followed by reaction with guanidine hydrochloride to afford the desired pyrimidine products. Formation of the intermediate enol ethers was achieved by reaction with diazomethane generated *in situ* from diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) and potassium hydroxide. In general, the enol ethers were used as the crude products in the subsequent reactions with guanidine hydrochloride, after confirmation of the formation of the enol ether by ^{1}H NMR spectroscopy.

Unfortunately, the final reaction with guanidine hydrochloride either afforded the products in low yields or was unsuccessful, possibly due to the lack of an aromatic substituent at the α-position of our substrates **11a-g**. Of the seven enol ethers prepared, only four were successfully converted into pyrimidine products (**6a-d**), however, in disappointingly low yields.



Scheme 1. Reagents, conditions, and yields: (a) 1,4-dibromobutane, K_2CO_3, CH_3CN, reflux, 20 h, 63 – 98%; (b) KCN, EtOH/H_2O, reflux, 48 h, 62 – 89%; (c) ethyl benzoate, KO^{t}Bu, THF, rt, 18 h, 63 – 94%; (d) diazomethane, CH_2Cl_2, rt, 18 h; (e) guanidine HCl, DMSO, NaOMe, 90°C, 24 h, 9 – 11% for **6a-d**.

Nonetheless, we subjected compounds (**6a-d**) to an initial *in vitro* assessment of antiplasmodial activity in a whole cell *P. falciparum* assay, at a single concentration of 20 µM. The compounds were screened against a cycloguanil-resistant strain (Gambian FCR-3), for direct comparison with our original series of dihydrotriazine compounds (of general structure (**5**)[8]) which had been screened against this strain. At this single concentration, pyrimidines (**6a-d**) inhibited parasitaemia by more than 45% in each case (Table 1). IC_{50} values were then determined for the compounds from the log sigmoid dose response curves generated by GraphPad Prism® software. Disappointingly, only one of the compounds, 5-(3-(3,5-dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine (**6c**) (entry 3, Table 1, IC_{50} 0.86 µM) showed activity in the nanomolar range comparable with the dihydrotriazines[8] prepared previously. The remainder of the compounds displayed only moderate activity in the low micromolar range. This could be attributed to the inflexibility of the biaryl axis now present between the 6-phenyl substituent and the pyrimidine ring, which was not present in the original series of dihydrotriazines, where the phenyl substituent was bonded to an sp^3 hybridised carbon with tetrahedral geometry. Notably, the DHFR inhibitor methotrexate also displayed antiplasmodial activity in the low micromolar range against the cycloguanil-resistant strain (entry 7, Table 1, IC_{50} 1.71 µM).

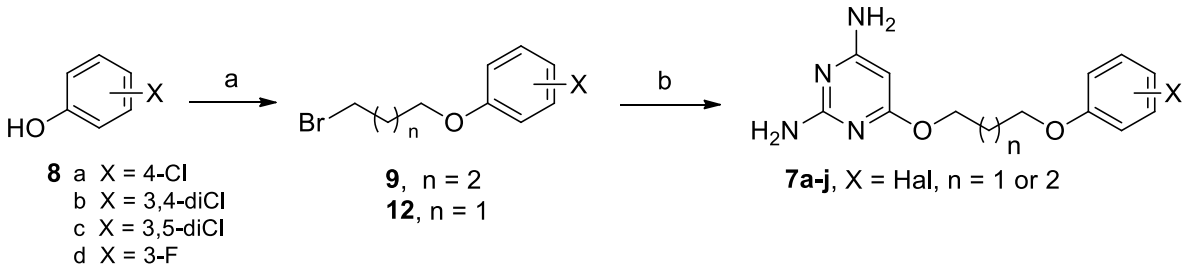
Table 1. Whole cell antiplasmodial activity of analogues (**6a-d**)[a]

Entry	Inhibitor	Substituent X	% Inhibition of parasitaemia at 20 µM	IC_{50} (µM)[b]
1	**6a**	4-Cl	59.84 ± 12.00	3.69 ± 0.35
2	**6b**	3,4-diCl	48.91 ± 16.49	14.80 ± 2.97
3	**6c**	3,5-diCl	59.94 ± 24.48	0.86 ± 0.01
4	**6d**	3-F	48.84 ± 17.33	26.55 ± 5.84
6	Control	DHA[c]	60.64 ± 0.17	$6.13\times10^{-3} \pm 1.11 \times10^{-3}$
7	Control	MTX[d]	59.69 ± 1.02	1.71 ± 0.51

[a]Data are presented as the mean ± SD of three experiments. [b]IC_{50} values determined by SYBR green assay against the Gambian FCR-3 strain. [c]DHA = Dihydroartemisinin. [d]MTX = Methotrexate

In another aspect of this work, we wanted to assess the effect of moving the flexible side chain from C-5 to C-6 on the pyrimidine ring. This would eliminate the biaryl axis created by the presence of a phenyl substituent at C-6 as was present in **6a-d**, and preliminary modelling studies suggested that the longer flexible chain could place the aromatic substituent at the correct binding position in the DHFR active site.[11]

The preparation of analogues of general structure (**7**) bearing a modified side chain could be achieved by simple substitution of a 4-hydroxypyrimidine. As shown in Scheme 2, commercially available phenols (**8a-d**) were alkylated with 1,3-dibromopropane, using the method described previously to afford bromoethers (**12a-d**). 2,4-Diaminopyrimidin-6-ol was then reacted with bromoethers (**12a-d**) to give pyrimidines (**7g-j**) bearing a 5-atom linker at the 6-position of the pyrimidine ring in moderate yields. 2,4-Diaminopyrimidin-6-ol was also treated with previously-prepared bromoethers (**9a-f**) to afford pyrimidines (**7a-f**) bearing a 6-atom linker at the 6-position of the pyrimidine ring. Pyrimidines (**7a-j**) prepared by this method were then assessed in the whole cell *P. falciparum* screen described previously (Table 2).



Scheme 2. Reagents, conditions, and yields: (a) For **12** (n = 1); 1,3-dibromopropane, K_2CO_3, CH_3CN, reflux, 20 h, 87 – 95%; for **9** (n = 2); 1,4-dibromobutane, reflux, 20 h, 63 – 98%; (b) 2,4-diaminopyrimidin-6-ol, K_2CO_3, CH_3CN, reflux, 20 h, 33 – 55%.

Table 2. Whole cell antiplasmodial activity of analogues (**7a-j**)[a]

Entry	Inhibitor	Substituent X	n	% Inhibition of parasitaemia at 20 μM	IC_{50} (μM)[b]
1	**7a**	4-Cl	2	66.04 ± 9.4	83.45 ± 8.36
2	**7b**	3,4-diCl	2	55.15 ± 5.1	4.46 ± 0.29
3	**7c**	3,5-diCl	2	40.30 ± 14.1	22.80 ± 2.96
4	**7d**	3-F	2	53.95 ± 9.4	11.18 ± 1.06
5	**7e**	4-F	2	48.18 ± 5.6	14.96 ± 1.78
6	**7f**	3-CF_3	2	66.90 ± 3.1	18.02 ± 2.80
7	**7g**	4-Cl	1	57.36 ± 4.4	44.88 ± 7.76
8	**7h**	3,4-diCl	1	52.68 ± 2.8	11.10 ± 1.71
9	**7i**	3,5-diCl	1	33.95 ± 16.8	7.66 ± 1.03
10	**7j**	4-F	1	57.50 ± 8.5	54.74 ± 10.91
11	Control	DHA[c]		60.64 ± 0.17	$6.13\times10^{-3} \pm 1.11\times10^{-3}$
12	Control	MTX[d]		59.69 ± 1.02	1.71 ± 0.51

[a]Data are presented as the mean ± SD of three experiments. [b]IC_{50} values determined by SYBR green assay against the Gambian FCR-3 strain. [c]DHA = Dihydroartemisinin. [d]MTX = Methotrexate

The results presented in Table 2 show that moving the side chain, albeit a modified side chain, to the 6-position of the pyrimidine ring, has a detrimental effect on antiplasmodial activity *in vitro*, with the majority of pyrimidines (**7a-j**) being less active than pyrimidines (**6a-d**) against the drug-resistant strain of the *P. falciparum* parasite. Although from our initial docking studies it appeared that analogues bearing a longer side chain at the 6-position of the pyrimidine ring could adopt a suitable conformation for binding in the DHFR active site, the biological data suggest that this could be energetically unfavourable.

Conclusions

In summary, we have prepared two series of substituted pyrimidine-2,4-diamines with a flexible side chain at either the 5- or 6-position of the pyrimdine ring as potential inhibitors of *P. falciparum* dihydrofolate reductase (DHFR). The synthesis of the first series of compounds involved construction of the pyrimidine ring by reaction of the enol ether of a suitably substituted α-cyanoketone with guanidine hydrochloride while the second series was prepared by substitution of the commercially available 2,4-diaminopyrimidin-6-ol. The compounds prepared were evaluated for antiplasmodial activity *in vitro* against a cycloguanil-resistant strain of the *P. falciparum* parasite. Compounds bearing a substituent at the 5-position of the pyrimidine ring showed better activity, in general, than those substituted at the 6-position, with 5-(3-(3,5-dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine identified as the most active compound in the series (IC_{50} 0.86 μM). The remaining pyrimidine-2,4-diamines showed antiplasmodial activity in the micromolar range.

Experimental Section

Chemistry

General. Reagents purchased from Sigma-Aldrich (Steinheim, Germany) were of reagent grade and used without any further purification unless specified. Ethyl acetate (EtOAc) and hexane used for chromatography or extractions were distilled prior to use. Dimethyl sulfoxide (DMSO) was distilled and stored over 4 Å molecular sieves. Acetonitrile (CH_3CN) was distilled from calcium hydride and tetrahydrofuran (THF) was distilled from sodium prior to use. Reactions were monitored by thin-layer chromatography (TLC) using precoated aluminium-backed plates (Merck silica gel 60 F254) and 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 acquired on a Bruker 300 or 500 MHz spectrometer at room temperature, using the specified deuterated solvent. For those compounds soluble in deuterated chloroform ($CDCl_3$), the solvent contained tetramethylsilane (TMS, 0.05% v/v) as internal standard. For others, the residual solvent signal was used for referencing (MeOD: 3.310 ppm; DMSO-d_6: 2.500 ppm). Data processing was done using MestreNova Software under license from Mestrelab Research, CA, USA. Infra-red spectra were recorded on a Bruker Tensor-27 Fourier Transform spectrometer. Mass Spectra (High Resolution) were recorded on a SYNAPT G2 HDMS mass spectrometer (ESI) at Stellenbosch University. Melting points were determined on a Stuart SMP10 melting point apparatus and are uncorrected.

General procedure for the synthesis of (4-bromobutoxy)benzenes (9). To a solution of substituted phenol **8** in dry acetonitrile was added 1,4-dibromobutane (3 eq) and potassium carbonate (1.5 eq). The resultant mixture

was heated at reflux overnight under a nitrogen atmosphere. After this time, TLC analysis showed consumption of the starting material and the reaction mixture was allowed to cool to room temperature and filtered through celite. The filtrate was concentrated on a rotary evaporator and excess 1,4-dibromobutane was removed by distillation under high vacuum. Compounds were purified by silica gel column chromatography (EtOAc/hexane 20:80).

1-(4-Bromobutoxy)-4-chlorobenzene (9a).[9] Prepared from 4-chlorophenol (**8a**) (5.00 g, 38.9 mmol), 1,4-dibromobutane (13.9 ml, 117 mmol) and potassium carbonate (8.06 g, 58.3 mmol) in dry acetonitrile (250 ml), isolated as a white crystalline solid (10.0 g, 98%). mp 29-30 °C. IR (ν_{max}/cm^{-1}): 2958, 1593, 1578, 1104, 823. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.21 (2H, d, *J* 8.9 Hz), 6.74 (2H, d, *J* 8.9 Hz), 3.93 (2H, t, *J* 6.0 Hz), 3.46 (2H, t, *J* 6.5 Hz), 2.14-1.97 (2H, m), 1.97-1.84 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.5, 129.3, 125.4, 115.7, 67.1, 33.5, 29.4, 27.8.

1-(4-Bromobutoxy)-3,4-dichlorobenzene (9b).[9] Prepared from 3,4-dichlorophenol (**8b**) (5.05 g, 31.0 mmol), 1,4-dibromobutane (11.1 ml, 92.9 mmol) and potassium carbonate (6.42 g, 46.5 mmol) in dry acetonitrile (250 ml), isolated as a white solid (8.50 g, 92%). mp 25-27 °C. IR (ν_{max}/cm^{-1}): 3041, 1578, 1145. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.31 (1H, d, *J* 8.9 Hz), 6.98 (1H, d, *J* 2.8 Hz), 6.74 (1H, dd, *J* 8.9, 2.8 Hz), 3.96 (2H, t, *J* 5.9 Hz), 3.48 (2H, t, *J* 6.4 Hz), 2.13-1.86 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.9, 132.9, 130.7, 124.0, 116.3, 114.5, 67.5, 33.2, 29.3, 27.7.

1-(4-Bromobutoxy)-3,5-dichlorobenzene (9c).[9] Prepared from 3,5-dichlorophenol (**8c**) (5.00 g, 30.7 mmol), 1,4-dibromobutane (10.9 ml, 92.0 mmol) and potassium carbonate (6.36 g, 46.0 mmol) in dry acetonitrile (250 ml), isolated as a white crystalline solid (8.02 g, 88%). mp 26-28 °C. IR (ν_{max}/cm^{-1}): 2953, 1581, 1576, 1141. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 6.91 (1H, t, *J* 1.8 Hz), 6.75 (2H, d, *J* 1.8 Hz), 3.92 (2H, t, *J* 5.9 Hz), 3.45 (2H, t, *J* 6.4 Hz), 2.08-1.96 (2H, m), 1.96-1.84 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 160.2, 135.6, 121.2, 113.8, 67.8, 33.5, 29.6, 27.9.

1-(4-Bromobutoxy)-3-fluorobenzene (9d).[12] Prepared from 3-fluorophenol (**8d**) (5.00 g, 44.6 mmol), 1,4-dibromobutane (15.9 ml, 134 mmol) and potassium carbonate (9.25 g, 66.9 mmol) in dry acetonitrile (250 ml), isolated as an oil (9.51 g, 86%). IR (ν_{max}/cm^{-1}): 2956, 1593, 1577, 1084. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.19 (1H, td, *J* 8.2, 6.8 Hz), 6.71-6.54 (3H, m), 3.94 (2H, t, *J* 6.0 Hz), 3.46 (2H, t, *J* 6.5 Hz), 2.11-1.98 (2H, m), 1.98-1.86 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 163.7 (d, $J_{C\text{-}F}$ 245.3 Hz), 160.3 (d, $J_{C\text{-}F}$ 10.8 Hz), 130.3 (d, $J_{C\text{-}F}$ 10.0 Hz), 110.3 (d, $J_{C\text{-}F}$ 2.9 Hz), 107.6 (d, $J_{C\text{-}F}$ 21.3 Hz), 102.2 (d, $J_{C\text{-}F}$ 24.7 Hz), 67.2, 33.4, 29.5, 27.9.

1-(4-Bromobutoxy)-4-fluorobenzene (9e).[13] Prepared from 4-fluorophenol (**8e**) (5.02 g, 44.8 mmol), 1,4-dibromobutane (16.0 ml, 134 mmol) and potassium carbonate (9.28 g, 67.2 mmol) in dry acetonitrile (250 ml), isolated as a white solid (6.92 g, 63%). mp 32-33 °C. IR (ν_{max}/cm^{-1}): 2930, 1599, 1578, 1133. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.01-6.93 (2H, m), 6.86-6.75 (2H, m), 3.95 (2H, t, *J* 6.0 Hz), 3.48 (2H, t, *J* 6.6 Hz), 2.11-2.01 (2H, m), 2.00-1.86 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.3 (d, $J_{C\text{-}F}$ 238.3 Hz), 154.9 (d, $J_{C\text{-}F}$ 2.0 Hz), 115.8 (d, $J_{C\text{-}F}$ 23.1 Hz), 115.4 (d, $J_{C\text{-}F}$ = 8.0 Hz), 67.4, 33.4, 29.4, 27.9. HRMS (ESI) found $[M + Na]^+$ 268.9953, $C_{10}H_{12}{}^{79}BrFONa$ requires 268.9956.

1-(4-Bromobutoxy)-3-(trifluoromethyl)benzene (9f).[14] Prepared from 3-(trifluoromethyl)phenol (**8f**) (7.00 g, 43.2 mmol), 1,4-dibromobutane (15.5 ml, 126 mmol) and potassium carbonate (8.95 g, 64.8 mmol) in dry acetonitrile (250 ml), isolated as a pale-yellow oil (11.5 g, 89%). IR (ν_{max}/cm^{-1}): 2963, 1594, 1066, 1224. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.39-7.34 (1H, m), 7.21-7.17 (1H, m), 7.13-7.09 (1H, m), 7.04 (1H, dd, *J* 8.4, 2.7 Hz), 4.01 (2H, t, *J* 6.0 Hz), 3.48 (2H, t, *J* 6.6 Hz), 2.10-2.03 (2H, m), 2.00-1.92 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 159.1, 131. 9 (q, $J_{C\text{-}F}$ 32.2 Hz), 130.0, 124.1 (q, $J_{C\text{-}F}$ 272.4 Hz), 118.0, 117.4 (q, $J_{C\text{-}F}$ 3.8 Hz), 111.3 (q, $J_{C\text{-}F}$ 3.7 Hz), 67.2, 33.3, 29.4, 27.8.

2,4-Dibromo-1-(4-bromobutoxy)benzene (9g). Prepared from 2,4-dibromophenol (**8g**) (5.00 g, 19.8 mmol), 1,4-dibromobutane (7.11 ml, 59.5 mmol) and potassium carbonate (4.12 g, 30.0 mmol) in dry acetonitrile (260 ml), isolated as a light-brown solid (6.13 g, 80%). mp 30-33 °C. IR (ν_{max}/cm^{-1}): 3042, 1583, 1095. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.65 (1H, d, *J* 2.4 Hz), 7.34 (1H, dd, *J* 8.7, 2.4 Hz), 6.73 (1H, d, *J* 8.7 Hz), 4.01 (2H, t, *J* 5.8 Hz), 3.52 (2H, t, *J* 6.4 Hz), 2.16-2.04 (2H, m), 2.04-1.93 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 154.6, 135.4, 131.3, 114.3, 113.2, 113.1, 68.5, 33.6, 29.4, 27.7. HRMS (ESI): found $[M + Na]^+$ 406.8266, $C_{10}H_{11}{}^{79}Br_3ONa$ requires 406.8258.

General procedure for the synthesis of 5-phenoxypentanenitriles (10). To a solution of the substituted (4-bromobutoxy)benzene **9** in ethanol/water (3:1) was added potassium cyanide (1.1 eq). The resulting heterogeneous mixture was stirred and heated at reflux under a nitrogen atmosphere for two days. The reaction progress was monitored by TLC (EtOAc/hexane 40:60). When complete, the reaction mixture was cooled to room temperature, quenched with aq. NaOH (0.1 M, 100 ml) and concentrated on a rotary evaporator. The residue was extracted with CH_2Cl_2 (3 × 100 ml), the combined organic layers were dried with $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. Products were purified by silica gel column chromatography (EtOAc/hexane 10:90 -20:80).

5-(4-Chlorophenoxy)pentanenitrile (10a). Prepared from 1-(4-bromobutoxy)-4-chlorobenzene (**9a**) (4.00 g, 15.2 mmol) and potassium cyanide (1.10 g, 16.7 mmol) in ethanol/water (160 ml); isolated as a pale yellow oil (2.85 g, 89%). IR (ν_{max}/cm^{-1}): 3084, 2965, 2252, 1585, 1570. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.21 (2H, d, *J* 9.0 Hz), 6.79 (2H, d, *J* 9.0 Hz), 3.94 (2H, t, *J* 5.6 Hz), 2.41 (2H, t, *J* 6.8 Hz), 1.97-1.77 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.3, 129.3, 125.6, 119.5, 115.7, 67.0, 28.1, 22.4, 16.9. HRMS (ESI): found $[M + Na]^+$ 232.0500, $C_{11}H_{12}{}^{35}ClNONa$ requires 232.0507.

5-(3,4-Dichlorophenoxy)pentanenitrile (10b). Prepared from 1-(4-bromobutoxy)-3,4-dichlorobenzene (**9b**) (5.05 g, 16.9 mmol) and potassium cyanide (1.21 g, 18.6 mmol) in ethanol/water (160 ml), isolated as pale yellow oil (3.55 g, 86%). IR (ν_{max}/cm^{-1}): 3083, 2964, 2250, 1599, 1574. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.31 (1H, d, *J* 8.9 Hz), 6.97 (1H, d, *J* 2.8 Hz), 6.74 (1H, dd, *J* 8.9, 2.8 Hz), 3.97 (2H, t, *J* 5.8 Hz), 2.44 (2H, t, *J* 7.0 Hz), 2.00-1.81 (4H, m). ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 157.7, 132.9, 130.7, 124.1, 119.3, 116.3, 114.5, 67.3, 28.0, 22.3, 17.0. HRMS (ESI): found $[M + Na]^+$ 266.011, $C_{11}H_{11}{}^{35}Cl_2NONa$ requires 266.0118.

5-(3,5-Dichlorophenoxy)pentanenitrile (10c). Prepared from 1-(4-bromobutoxy)-3,5-dichlorobenzene (**9c**) (4.20 g, 14.1 mmol) and potassium cyanide (1.01 g, 15.5 mmol) in ethanol/water (160 ml), isolated as a white solid (2.45 g, 71%). mp 52-53 °C. IR (ν_{max}/cm^{-1}): 3083, 2966, 2253, 1585, 1570. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 6.96 (1H, t, *J* 1.8 Hz), 6.78 (2H, d, *J* 1.8 Hz), 3.98 (2H, t, *J* 5.6 Hz), 2.45 (2H, t, *J* 6.8 Hz), 2.0-1.80 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 159.7, 135.5, 121.3, 119.3, 113.6, 67.3, 28.0, 22.3, 17.0. HRMS (ESI): found $[M + Na]^+$ 266.0115, $C_{11}H_{11}{}^{35}Cl_2NONa$ requires 266.0118.

5-(3-Fluorophenoxy)pentanenitrile (10d). Prepared from 1-(4-bromobutoxy)-3-fluorobenzene (**9d**) (5.00 g, 20.2 mmol) and potassium cyanide (1.58 g, 24.3 mmol) in ethanol/water (160 ml), isolated as a brown oil (3.22 g, 72%). IR (ν_{max}/cm^{-1}): 2961, 2932, 2239, 1596, 1503. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.26 -7.14 (1H, m), 6.70-6.55 (3H, m), 3.95 (2H, t, *J* 5.6 Hz), 2.41 (2H, t, *J* 6.8 Hz), 1.99-1.75 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 163.7 (d, $J_{C\text{-}F}$ = 245.7 Hz), 160.1 (d, $J_{C\text{-}F}$ = 10.8 Hz), 130.4 (d, $J_{C\text{-}F}$ = 10.1 Hz), 119.5, 110.3 (d, $J_{C\text{-}F}$ = 3.0 Hz), 107.8 (d, $J_{C\text{-}F}$ = 21.4 Hz), 102.2 (d, $J_{C\text{-}F}$ = 24.8 Hz), 67.0, 28.2, 22.5, 17.1. HRMS (ESI): found $[M + Na]^+$ 216.0807, $C_{11}H_{12}FNONa$ requires 216.0803.

5-(4-Fluorophenoxy)pentanenitrile (10e). Prepared from 1-(4-bromobutoxy)-4-fluorobenzene (**9e**) (4.00 g, 16.2 mmol) and potassium cyanide (1.20 g, 17.8 mmol) in ethanol/water (160 ml), isolated as a white solid (1.93 g, 62%). mp 26-27 °C. IR (ν_{max}/cm^{-1}): 2960, 2933, 2240, 1596, 1503. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.09-6.88 (2H, m), 6.88-6.71 (2H, m), 3.96 (2H, t, *J* 5.5 Hz), 2.44 (2H, t, *J* 6.6 Hz), 2.04-1.76 (4H, m). ^{13}C NMR (75

MHz, $CDCl_3$): δ_C 157.3 (d, J_{C-F} 238.3 Hz), 154.8 (d, J_{C-F} 2.1 Hz), 119.5, 115.8 (d, J_{C-F} 23.1 Hz), 115.4 (d, J_{C-F} 8.0 Hz), 67.3, 28.2, 22.5, 17.0. HRMS (ESI): found $[M + Na]^+$ 216.0809, $C_{11}H_{12}FNONa$ requires 216.0803.

5-(3-(Trifluoromethyl)phenoxy)pentanenitrile (10f). Prepared from 1-(4-bromobutoxy)-3-(trifluoromethyl)benzene (**9f**) (6.00 g, 20.2 mmol) and potassium cyanide (1.45 g, 22.2 mmol) in ethanol/water (160 ml), isolated as a yellow oil (3.65 g, 74%). IR (ν_{max}/cm^{-1}): 3039, 2930, 2239, 1599. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.37 (1H, t, *J* 8.1 Hz), 7.23-7.16 (1H, m), 7.12 (1H, br s), 7.05 (1H, dd, *J* 8.3, 2.6 Hz), 4.01 (2H, t, *J* 5.7 Hz), 2.42 (2H, t, *J* 6.8 Hz), 2.04-1.73 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 158.9, 132.0 (q, J_{C-F} 32.3 Hz), 130.1, 124.0 (q, J_{C-F} 272.3 Hz), 119.5, 118.0, 117.7 (q, J_{C-F} 3.9 Hz), 111.3 (q, J_{C-F} 3.9 Hz), 67.0, 28.2, 22.5, 17.1. HRMS (ESI): found $[M + Na]^+$ 266.0773, $C_{12}H_{12}F_3NONa$ requires 266.0771.

5-(2,4-Dibromophenoxy)pentanenitrile (10g). Prepared from 2,4-dibromo-1-(4-bromobutoxy)benzene (**9g**) (5.00 g, 12.9 mmol) and potassium cyanide (1.01 g, 15.5 mmol, 1.2 eq) in ethanol/water (160 ml), isolated as white solid (3.12 g, 73%). mp 49-50 °C. IR (ν_{max}/cm^{-1}): 3091, 2948, 2253, 1580, 1480. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.66 (1H, d, *J* 2.4 Hz), 7.36 (1H, dd, *J* 8.8, 2.4 Hz), 6.74 (1H, d, *J* 8.8 Hz), 4.04 (2H, t, *J* 5.4 Hz), 2.50 (2H, t, *J* 6.7 Hz), 2.03-1.89 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 154.4, 135.5, 131.2, 119.5, 114.2, 113.2, 113.1, 68.3, 27.9, 22.5, 17.1. HRMS (ESI): found $[M + Na]^+$ 353.9108, $C_{11}H_{11}{}^{79}Br_2NONa$ requires 353.9107.

General procedure for the synthesis of 2-benzoyl-5-phenoxypentanenitriles (11). To a solution of the substituted 5-phenoxypentanenitrile **10** (1 eq) in dry THF was added potassium *tert*-butoxide (3 eq) and ethyl benzoate (4 eq). The reaction mixture was stirred at room temperature under a nitrogen atmosphere overnight. After consumption of the starting material, the reaction was quenched with sat. aq. NH_4Cl and THF removed under reduced pressure. The remaining aqueous residue was washed with EtOAc (3 × 100 ml) and the organic layers were combined, dried over $MgSO_4$ and filtered through celite. Crude products were purified by column chromatography (EtOAc/hexane 20:80).

2-Benzoyl-5-(4-chlorophenoxy)pentanenitrile (11a). Prepared from 5-(4-chlorophenoxy)pentanenitrile (**10a**) (2.32 g, 11.1 mmol), potassium *tert*-butoxide (3.72 g, 33.2 mmol) and ethyl benzoate (6.33 ml, 44.3 mmol) in dry THF (50 ml), isolated as yellow crystals (2.82 g, 81%). mp 124-126 °C. IR (ν_{max}/cm^{-1}): 2878, 2253, 1694, 1596, 1580. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.97 (2H, d, *J* 7.2 Hz), 7.65 (1H, t, *J* 7.4 Hz), 7.52 (2H, dd, *J* 8.3, 6.9 Hz), 7.21 (2H, d, *J* 9.0 Hz), 6.77 (2H, d, *J* 9.0 Hz), 4.48 (1H, dd, *J* 8.4, 5.7 Hz), 4.04-3.99 (2H, m), 2.37-1.99 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 190.4, 157.1, 134.6, 133.9, 129.4, 129.2, 128.8, 125.9, 117.1, 115.7, 67.0, 39.4, 26.7, 26.5. HRMS (ESI): found $[M + Na]^+$ 336.0757, $C_{18}H_{16}{}^{35}ClNO_2Na$ requires 336.0770.

2-Benzoyl-5-(3,4-dichlorophenoxy)pentanenitrile (11b). Prepared from 5-(3,4-dichlorophenoxy)pentanenitrile (**10b**) (2.50 g, 10.2 mmol), potassium *tert*-butoxide (3.45 g, 30.7 mmol) and ethyl benzoate (5.89 ml, 40.9 mmol) in dry THF (60 ml), isolated as a white solid (3.34 g, 94%). mp 43-44 °C. IR (ν_{max}/cm^{-1}): 3041, 2954, 2252, 1695, 1586, 1574. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.96 (2H, d, *J* 6.9 Hz), 7.69-7.61 (1H, m), 7.51 (2H, t, *J* 8.0 Hz), 7.28 (1H, dd, *J* 8.7, 2.9 Hz), 6.91 (1H, s), 6.73-6.67 (1H, m), 4.64-4.40 (1H, m), 4.07-3.92 (2H, m), 2.34-2.12 (2H, m), 2.12-1.95 (2H, m). ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 190.6, 157.6, 134.6, 133.9, 132.8, 130.7, 129.2, 128.8, 124.1, 117.3, 116.4, 114.4, 67.3, 39.5, 26.6, 26.4. HRMS (ESI): found $[M + Na]^+$ 370.0377, $C_{18}H_{15}{}^{35}Cl_2NO_2Na$ requires 370.0380.

2-Benzoyl-5-(3,5-dichlorophenoxy)pentanenitrile (11c). Prepared from 5-(3,5-dichlorophenoxy)pentanenitrile (**10c**) (1.98 g, 8.11 mmol), potassium *tert*-butoxide (2.73 g, 24.3 mmol) and ethyl benzoate (4.64 ml, 32.4 mmol) in dry THF (40 ml), isolated as a light yellow solid (2.80 g, 91%). mp 45-46 °C. IR (ν_{max}/cm^{-1}): 2889, 2255, 1697, 1582, 1570. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.97 (2H, d, *J* 7.1 Hz), 7.74-7.61 (1H, m), 7.53 (2H, dd, *J* 8.3, 7.0 Hz), 6.95 (1H, t, *J* 1.8 Hz), 6.72 (2H, d, *J* 1.8 Hz), 4.46 (1H, dd, *J* 8.1, 5.9 Hz), 4.06-3.88 (2H, m), 2.36-1.82 (4H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 190.2, 159.5, 135.5 (2C), 134.7, 133.9, 129.2, 128.8, 121.3, 117.0, 113.6, 113.5, 67.3, 39.3, 26.5, 26.4. HRMS (ESI): found $[M + Na]^+$ 370.0381, $C_{18}H_{15}{}^{35}Cl_2NO_2Na$ requires 370.0380.

2-Benzoyl-5-(3-fluorophenoxy)pentanenitrile (11d). Prepared from 5-(3-fluorophenoxy)pentanenitrile (**10d**) (4.88 g, 25.3 mmol), potassium *tert*-butoxide (8.50 g, 75.8 mmol) and ethyl benzoate (14.4 ml, 101 mmol) in dry THF (70 ml), isolated as a yellow solid (6.53 g, 87%). mp 32 °C. IR (ν_{max}/cm^{-1}) 2876, 2254, 1689, 1596, 1576. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.96 (2H, d, *J* 7.1 Hz), 7.70-7.59 (1H, m), 7.51 (2H, dd, *J* 8.4, 7.1 Hz), 7.28-7.12 (1H, m), 6.73-6.46 (3H, m), 4.50 (1H, dd, *J* 8.4, 5.7 Hz), 4.04-3.92 (2H, m), 2.36-1.99 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 190.5, 163.6 (d, J_{C-F} 245.3 Hz), 159.9 (d, J_{C-F} 10.8 Hz), 134.6, 133.9, 130.3 (d, J_{C-F} 10.0 Hz), 129.1, 128.8, 117.2, 110.2 (d, J_{C-F} 2.9 Hz), 107.8 (d, J_{C-F} 21.3 Hz), 102.2 (d, J_{C-F} 24.8 Hz), 66.9, 39.4, 26.7, 26.4. HRMS (ESI): found $[M + Na]^+$ 320.1063, $C_{18}H_{16}FNO_2Na$ requires 320.1065.

2-Benzoyl-5-(4-fluorophenoxy)pentanenitrile (11e). Prepared from 5-(4-fluorophenoxy)pentanenitrile (**10e**) (1.55 g, 8.02 mmol), potassium *tert*-butoxide (2.70 g, 24.1 mmol) and ethyl benzoate (4.60 ml, 32.1 mmol) in dry THF (45 ml), isolated as a yellow solid (1.99 g, 83%). mp 86-87 °C. IR (ν_{max}/cm^{-1}): 2956, 2930, 2252, 1703, 1586, 1577. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.04-7.90 (2H, m), 7.72-7.57 (1H, m), 7.51 (2H, dd, *J* 8.4, 7.1 Hz), 7.03-6.90 (2H, m), 6.87-6.70 (2H, m), 4.50 (1H, dd, *J* 8.5, 5.7 Hz), 4.08-3.93 (2H, m), 2.34-1.78 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 190.4, 157.4 (d, J_{C-F} 238.7 Hz), 154.6 (d, J_{C-F} 2.1 Hz), 134.6, 133.9, 129.1, 128.8, 117.2, 115.8 (d, J_{C-F} 23.1 Hz), 115.4 (d, J_{C-F} 8.0 Hz), 67.3, 39.4, 26.8, 26.6. HRMS (ESI): found $[M + Na]^+$ 320.1100, $C_{18}H_{16}FNO_2Na$ requires 320.1065.

2-Benzoyl-5-(3-(trifluoromethyl)phenoxy)pentanenitrile (11f). Prepared from 5-(3-(trifluoromethyl)phenoxy)pentanenitrile (**10f**) (2.50 g, 10.3 mmol), potassium *tert*-butoxide (3.46 g, 30.8 mmol) and ethyl benzoate (5.88 ml, 41.1 mmol) in dry THF (60 ml), isolated as a yellow viscous oil (2.24 g, 63%). IR (ν_{max}/cm^{-1}): 2941, 2249, 1696, 1592, 1571. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.97 (2H, d, *J* 7.3 Hz), 7.66 (1H, t, *J* 7.4 Hz), 7.52 (2H, t, *J* 7.7 Hz), 7.38 (1H, t, *J* 7.9 Hz), 7.21 (1H, d, *J* 7.7 Hz), 7.08-6.96 (2H, m), 4.49 (1H, dd, *J* 8.2, 5.8 Hz), 4.16-4.00 (2H, m), 2.40-2.00 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 190.3, 158.6, 134.6, 134.0, 131.9 (q, J_{C-F} 32.3 Hz), 130.1, 129.2, 128.8, 123.9 (q, J_{C-F} 272.4 Hz), 117.8, 117.7 (q, J_{C-F} 3.9 Hz), 117.1, 111.3 (q, J_{C-F} 3.8 Hz), 67.0, 39.3, 26.7, 26.5. HRMS (ESI): found $[M + H]^+$ 348.1227, $C_{19}H_{17}F_3NO_2$ requires 348.1213.

2-Benzoyl-5-(2,4-dibromophenoxy)pentanenitrile (11g). Prepared from 5-(2,4-dibromophenoxy)pentanenitrile (**10g**) (2.94 g, 8.83 mmol), potassium *tert*-butoxide (2.97 g, 26.5 mmol) and ethyl benzoate (5.05 ml, 35.3 mmol) in dry THF (50 ml), isolated as a yellow solid (3.03 g, 79%). mp 35-36 °C. IR (ν_{max}/cm^{-1}): 3010, 2255, 1699, 1578, 1569. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.00 (2H, d, *J* 7.0 Hz), 7.70-7.61 (2H, m), 7.56-7.47 (2H, m), 7.37 (1H, d, *J* 7.9 Hz), 6.76 (1H, dd, *J* 8.9, 2.9 Hz), 4.66 (1H, p, *J* 3.8 Hz), 4.19-4.00 (2H, m), 2.43-2.18 (2H, m), 2.19-2.06 (2H, m). ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 190.7, 154.2, 135.5, 134.6, 133.9, 131.3, 129.1, 128.9, 117.3, 114.2, 113.3, 113.0, 68.4, 39.8, 26.9, 26.3. HRMS (ESI): found $[M + Na]^+$ 457.9370, $C_{18}H_{15}{}^{79}Br_2NO_2Na$ requires 457.9270.

General procedure for the synthesis of 6-phenylpyrimidine-2,4-diamines (6). Carbitol, diethyl ether and aqueous potassium hydroxide (10.7 M, 20.0 eq) were placed in a diazomethane apparatus fitted with a condenser and a dropping funnel containing diazald (3.0 eq) dissolved in diethyl ether. The diazomethane apparatus was lowered into a water bath at 70-80 °C. Diazomethane gas, generated from the reaction of diazald and the above reaction mixture was passed into a flask containing the starting material **11a-g** (1.0 eq), in dry dichloromethane (50 ml). Diethyl ether (10 ml) was used to rinse the dropping funnel. After distillation was complete, the reaction mixture was left to stir at room temperature overnight. Any excess diazomethane was quenched with glacial acetic acid. The reaction was evaporated to dryness *in vacuo* to give the crude product, which was taken to the next step without further purification. Partial characterisation of the

intermediate enol ethers was done by ^{1}H and ^{13}C NMR spectroscopy (exemplified in the synthesis of **6a** below). In the subsequent step, guanidine hydrochloride was treated with sodium methoxide (2.0 eq) in methanol (20 ml) to furnish free guanidine after filtration and removal of methanol *in vacuo*. The enol ether prepared in the first step (1.0 eq) was dissolved in dry DMSO and then added to the flask containing free guanidine. The reaction mixture was then heated at 80-100 °C overnight. After formation of a new product spot visible by TLC, the reaction mixture was heated to 120 °C to remove DMSO under vacuum. The resulting solid residue was dissolved in a methanol-ethyl acetate mixture and purified by column chromatography (EtOAc/hexane 20:80) to give the desired pyrimidine product **6**.

5-(3-(4-Chlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine (6a). Diazomethane gas, produced from the reaction of diazald (2.05 g, 9.56 mmol) with potassium hydroxide solution (10.7 M, 6 ml), in a mixture of carbitol (4 ml) and diethyl ether (20 ml) was passed through a solution of 2-benzoyl-5-(4-chlorophenoxy)-pentanenitrile (**11a**) (2.10 g, 3.19 mmol), in dry DCM. The resulting enol ether was isolated as a mixture of *E* and *Z* isomers in a ratio of 1.4:1. Major isomer: ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.56-7.33 (4H, m), 7.23-7.18 (1H, m), 7.23 (2H, d, *J* 9.1 Hz), 6.84 (2H, d, *J* 9.0 Hz), 4.00 (2H, t, *J* 6.1 Hz), 3.43 (3H, s), 2.57 (2H, dd, *J* 8.2, 6.7 Hz), 2.11-2.01 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 168.9, 157.6, 131.5, 130.5, 129.3, 128.9, 128.8, 125.5, 120.1, 115.8, 94.2, 67.1, 58.3, 28.1, 25.0. Minor isomer: ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.56-7.33 (4H, m), 7.23-7.18 (1H, m), 7.18 (2H, d, *J* 9.1 Hz), 6.66 (2H, d, *J* 8.9 Hz), 3.85 (2H, t, *J* 5.9 Hz), 3.47 (3H, s), 2.23 (2H, dd, *J* 8.1, 6.5 Hz), 1.99-1.87 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 168.7, 157.2, 130.7, 130.3, 129.2, 128.8, 128.7, 125.5, 118.2, 115.6, 91.9, 66.1, 58.1, 27.7, 23.8. The enol ether (0.55 g, 1.68 mmol) was treated with free guanidine (0.32 g, 3.36 mmol) in dry DMSO (5.0 ml) as described to afford **6a** as a yellow solid (0.065 g, 11%). mp 130-132 °C. IR (ν_{max}/cm^{-1}): 3328, 3140, 2929, 1637, 1583, 1555. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.43-7.33 (5H, m), 7.21 (2H, d, *J* 8.9 Hz), 6.74 (2H, d, *J* 8.9 Hz), 5.29 (2H, s), 5.15 (2H, s), 3.84 (2H, t, *J* 5.6 Hz), 2.53 (2H, t, *J* 8.6 Hz), 1.93-1.84 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 165.6, 163.3, 160.4, 157.1, 129.7, 129.4, 128.4, 128.3, 128.0, 125.9, 115.7, 104.8, 67.1, 28.6, 22.2. HRMS (ESI): found $[M + H]^+$ 355.1326, $C_{19}H_{20}{}^{35}ClN_4O$ requires 355.1327.

5-(3-(3,4-Dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine (6b). 2-Benzoyl-5-(3,4-dichlorophenoxy)pentanenitrile (**11b**) (499 mg, 1.38 mmol) was treated with diazomethane as described. The resulting enol ether was treated with free guanidine (0.26 g, 2.76 mmol) in dry DMSO (5.0 ml) to afford a light-yellow solid (0.057 g, 11%). mp 102-104 °C. IR (ν_{max}/cm^{-1}): 3430, 3164, 2866, 1636, 1582, 1554. ^{1}H NMR (500 MHz, MeOD): δ_H 7.52 – 7.35 (5H, m), 6.97 (1H, t, *J* 1.8 Hz), 6.65 (2H, d, *J* 1.8 Hz), 3.81 (2H, t, *J* 5.7 Hz), 2.60 (2H, t, *J* 7.4 Hz), 1.91-1.83 (2H, m). ^{13}C NMR (126 MHz, MeOD): δ_C 171.6, 165.1, 160.0 (2C), 135.0 (2C), 129.7, 128.5, 128.1, 120.2 (2C), 116.7, 113.2, 105.9, 66.7, 27.0, 20.9. HRMS (ESI): found $[M + H]^+$ 389.0936, $C_{19}H_{19}{}^{35}Cl_2N_4O$ requires 389.0938.

5-(3-(3,5-Dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine (6c). 2-Benzoyl-5-(3,5-dichlorophenoxy)pentanenitrile (**11c**) (607 mg, 1.68 mmol) was treated with diazomethane as described. The resulting enol ether was treated with free guanidine (0.32 g, 3.36 mmol) in dry DMSO (5.0 ml) to afford a brown solid (0.048 g, 9%). mp 105-107 °C. IR (ν_{max}/cm^{-1}): 3430, 3164, 2956, 1637, 1599, 1575. ^{1}H NMR (300 MHz, MeOD): δ_H 7.98 (2H, d, *J* 7.5 Hz), 7.56-7.30 (5H, m), 6.98 (1H, t, *J* 1.8 Hz), 6.68 (2H, d, *J* 1.8 Hz), 3.83 (2H, t, *J* 5.8 Hz), 2.60 (2H, t, *J* 7.4 Hz), 1.98-1.72 (2H, m). ^{13}C NMR (126 MHz, MeOD): δ_C 164.7, 159.9, 157.7, 135.0, 130.8, 129.1, 128.2, 128.1, 127.5, 120.2, 113.2, 105.3, 66.9, 27.2, 21.1. HRMS (ESI): found $[M + H]^+$ 389.0936, $C_{19}H_{19}{}^{35}Cl_2N_4O$ requires 389.0938.

5-(3-(3-Fluorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine (6d). 2-Benzoyl-5-(3-fluorophenoxy)pentanenitrile (**11d**) (450 mg, 1.45 mmol) was treated with diazomethane as described. The resulting enol ether was treated with free guanidine (0.28 g, 2.89 mmol) in dry DMSO (5.0 ml) to afford a creamy-white solid (0.043 g, 9%). mp 104-105 °C. IR (ν_{max}/cm^{-1}): 3479, 3112, 2875, 1612, 1576, 1505. ^{1}H NMR

(300 MHz, MeOD): δ_H 7.57-7.33 (4H, m), 7.29-7.09 (1H, m), 6.73-6.29 (3H, m), 3.82 (2H, t, *J* 5.9 Hz), 2.56 (2H, dd, *J* 8.6, 6.6 Hz), 1.99-1.83 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 175.04, 163.7 (d, $J_{C\text{-}F}$ 245.0 Hz), 163.4, 160.6, 160.3 (d, $J_{C\text{-}F}$ 10.9 Hz), 130.24, 130.1, 128.3, 128.29, 128.0, 110.3 (d, $J_{C\text{-}F}$ 3.0 Hz), 107.4 (d, $J_{C\text{-}F}$ 21.3 Hz), 104.7, 102.2 (d, $J_{C\text{-}F}$ 24.7 Hz), 67.8, 28.6, 22.1. HRMS (ESI) found $[M + H]^+$ 339.1621, $C_{19}H_{20}FN_4O$ requires 339.1703.

General procedure for the synthesis of (4-bromopropoxy)benzenes (12). (4-Bromopropoxy)benzenes (**12**) were prepared using the same method described for the synthesis of (4-bromobutoxy)benzenes **9** described above.

1-(3-Bromopropoxy)-4-chlorobenzene (12a).[9] Prepared from 4-chlorophenol (**8a**) (6.50 g, 50.6 mmol), 1,3-dibromopropane (15.4 ml, 152 mmol) and potassium carbonate (10.5 g, 75.8 mmol) in dry acetonitrile (300 ml), isolated as a white crystalline solid (12.5 g, 99%). mp 28-29 °C. IR (ν_{max}/cm^{-1}): 2959, 1596, 1578, 1102. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.23 (2H, d, *J* 8.9 Hz), 6.81 (2H, d, *J* 8.9 Hz), 4.02 (2H, t, *J* 5.7 Hz), 3.46 (2H, t, *J* 6.5 Hz), 2.19-1.95 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.3, 129.3, 125.6, 115.7, 67.2, 33.3, 27.8.

4-(3-Bromopropoxy)-1,2-dichlorobenzene (12b).[9] Prepared from 3,4-dichlorophenol (**8b**) (5.40 g, 33.1 mmol), 1,3-dibromopropane (10.1 ml, 99.4 mmol) and potassium carbonate (6.87 g, 49.7 mmol) in dry acetonitrile (250 ml), isolated as a white solid (8.96 g, 95%). mp 29-30 °C. IR (ν_{max}/cm^{-1}): 3041, 1578, 1145. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.31 (1H, d, *J* 8.9 Hz), 6.97 (1H, d, *J* 2.9 Hz), 6.73 (1H, dd, *J* 8.9, 2.9 Hz), 4.00 (2H, t, *J* 5.8 Hz), 3.45 (2H, t, *J* 6.4 Hz), 2.18-1.99 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.4, 132.7, 130.7, 124.2, 116.4, 114.5, 66.0, 25.2, 14.1.

1-(3-Bromopropoxy)-3,5-dichlorobenzene (12c).[9] Prepared from 3,5-dichlorophenol (**8c**) (5.00 g, 30.7 mmol), 1,3-dibromopropane (9.34 ml, 92.0 mmol) and potassium carbonate (6.36 g, 46.0 mmol) in dry acetonitrile (200 ml), isolated as a white solid (8.46 g, 97%). mp 27-28 °C. IR (ν_{max}/cm^{-1}): 2953, 1581, 1576, 1141. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 6.91 (1H, t, *J* 1.8 Hz), 6.75 (2H, d, *J* 1.9 Hz), 3.92 (2H, t, *J* 5.9 Hz), 3.45 (2H, t, *J* 6.4 Hz), 1.90-1.77 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 159.7, 135.5 (2C), 121.3, 114.0 (2C), 67.3, 28.0, 22.3.

1-(3-Bromopropoxy)-4-fluorobenzene (12d).[15] Prepared from 4-fluorophenol (**8f**) (4.00 g, 35.7 mmol), 1,3-dibromopropane (10.9 ml, 107 mmol) and potassium carbonate (7.40 g, 53.5 mmol) in dry acetonitrile (200 ml), isolated as a pale solid (7.26 g, 87%). mp 30-31 °C. IR (ν_{max}/cm^{-1}): 2956, 1593, 1577, 1084. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.08-6.94 (2H, m), 6.85-6.81 (2H, m), 4.02 (2H, t, *J* 5.6 Hz), 3.45 (2H, t, *J* 6.4 Hz), 2.15-2.07 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 157.3 (d, $J_{C\text{-}F}$ 238.3 Hz), 154.9 (d, $J_{C\text{-}F}$ 2.0 Hz), 115.8 (d, $J_{C\text{-}F}$ 23.1 Hz), 115.4 (d, $J_{C\text{-}F}$ 8.0 Hz), 66.0, 30.8, 14.2.

General procedure for the synthesis of 6-(4-(4-chlorophenoxy)butoxy)pyrimidine-2,4-diamine (7a) and analogues. To a stirred solution of 2,4-diamino-6-hydroxy pyrimidine in dry acetonitrile (20 ml) was added potassium carbonate (1.5 eq), followed by a suitably substituted bromoether **9** or **12** (1.0 eq) in dry acetonitrile (30 ml). The reaction was heated to reflux under an atmosphere of nitrogen overnight. The reaction mixture was allowed to cool to room temperature, filtered through celite and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/hexane 60:40) to furnish the desired product.

6-(4-(4-Chlorophenoxy)butoxy)pyrimidine-2,4-diamine (7a). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.48 g, 3.79 mmol), potassium carbonate (0.79 g, 5.69 mmol) and **9a** (1.00 g, 3.79 mmol) in dry acetonitrile, isolated as a white solid (0.58 g, 50%). mp 97-98 °C. IR (ν_{max}/cm^{-1}): 3520, 3359, 3011, 1576, 1124, 791. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.31 (2H, d, *J* 8.9 Hz), 6.95 (2H, d, *J* 9.0 Hz), 5.98 (2H, s), 5.82 (2H, s), 5.04 (1H, s), 4.15-4.11 (2H, m), 3.98 (2H, t, *J* 3.5 Hz), 1.81-1.68 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.1,

165.9, 162.9, 157.4, 129.2, 124.1, 116.2, 76.1, 67.5, 64.1, 25.3 (2C). HRMS (ESI): found $[M + H]^+$ 309.1117, $C_{14}H_{18}ClN_4O_2$ requires 309.1120.

6-(4-(3,4-Dichlorophenoxy)butoxy)pyrimidine-2,4-diamine (7b). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.43 g, 3.42 mmol), potassium carbonate (0.71 g, 5.13 mmol) and **9b** (1.02 g, 3.42 mmol) in dry acetonitrile, isolated as a white solid (0.53 g, 45%). mp 136-137 °C. IR (ν_{max}/cm^{-1}): 3520, 3359, 3011, 1576, 1124, 791. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.50 (1H, d, *J* 8.9 Hz), 7.22 (1H, d, *J* 2.9 Hz), 6.95 (1H, dd, *J* 8.9, 2.9 Hz), 5.99 (2H, s), 5.82 (2H, s), 5.04 (1H, s), 4.15-4.11 (2H, m), 4.05-4.01 (2H, m), 1.78-1.74 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.6, 166.5, 163.4, 158.6, 132.1, 131.4, 122.7, 116.8, 115.9, 76.6, 68.5, 64.6, 25.7 (2C). HRMS (ESI): found $[M + H]^+$ 343.0729, $C_{14}H_{17}Cl_2N_4O_2$ requires 343.0730.

6-(4-(3,5-Dichlorophenoxy)butoxy)pyrimidine-2,4-diamine (7c). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.40 g, 3.15 mmol), potassium carbonate (0.65 g, 4.73 mmol) and **9c** (0.94 g, 3.15 mmol) in dry acetonitrile, isolated as a white solid (0.59 g, 55%). mp 106-107 °C. IR (ν_{max}/cm^{-1}): 3440, 3327, 3008, 1560, 1141, 828, 791. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.13 (1H, t, *J* 1.8 Hz), 7.03 (2H, d, *J* 1.8 Hz), 5.98 (2H, s), 5.82 (2H, s), 5.04 (1H, s), 4.15-4.10 (2H, m), 4.06-4.00 (2H, m), 1.83-1.68 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.1, 165.9, 163.0, 160.1, 134.5 (2C), 120.1, 113.8 (2C), 76.1, 68.2, 25.2, 25.1, HRMS (ESI): found $[M + H]^+$ 343.0724, $C_{14}H_{17}Cl_2N_4O_2$ requires 343.0730.

6-(4-(3-fluorophenoxy)butoxy)pyrimidine-2,4-diamine (7d). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.27 g, 2.14 mmol), potassium carbonate (0.44 g, 3.22 mmol) and **9d** (0.53 g, 2.14 mmol) in dry acetonitrile, isolated as an off-white solid (0.25 g, 40%). mp 98-99 °C. IR (ν_{max}/cm^{-1}): 3512, 3344, 2939, 1569, 1145, 1016. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.34-7.26 (1H, m), 6.83-6.71 (3H, m), 6.02 (2H, s), 5.86 (2H, s), 5.04 (1H, s), 4.15 (2H, t, *J* 6.0 Hz), 4.01 (2H, t, *J* 3.7 Hz), 1.79-1.77 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.1, 165.9, 163.0 (d, $J_{C\text{-}F}$ 245.3 Hz), 162.8, 160.1 (d, $J_{C\text{-}F}$ 10.8 Hz), 130.6 (d, $J_{C\text{-}F}$ 10.0 Hz), 110.8 (d, $J_{C\text{-}F}$ 2.9 Hz), 106.9 (d, $J_{C\text{-}F}$ 21.3 Hz), 101.9 (d, $J_{C\text{-}F}$ 24.8 Hz), 76.1, 67.6, 64.2, 25.33, 25.31. HRMS (ESI): found $[M + H]^+$ 293.1413, $C_{14}H_{18}FN_4O_2$ requires 293.1416.

6-(4-(4-Fluorophenoxy)butoxy)pyrimidine-2,4-diamine (7e). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.23 g, 1.86 mmol), potassium carbonate (0.39 g, 2.79 mmol) and **9e** (0.46 g, 1.86 mmol) in dry acetonitrile, isolated as a white solid (0.18 g, 33%). mp 138-139 °C. IR (ν_{max}/cm^{-1}): 3478, 2957, 1577, 1216, 1199, 1075. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.12-7.06 (2H, m), 6.99-6.84 (2H, m), 5.99 (2H, s), 5.81 (2H, s), 5.06 (1H, s), 4.13 (2H, t, *J* 3.1 Hz), 3.98-3.94 (2H, m), 1.78-1.74 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.1, 165.9, 162.9, 156.4 (d, $J_{C\text{-}F}$ 227.9 Hz), 154.8 (d, $J_{C\text{-}F}$ 2.0 Hz), 115.8 (d, $J_{C\text{-}F}$ 23.1 Hz), 115.6 (d, $J_{C\text{-}F}$ 8.0 Hz), 76.1, 67.7, 64.2, 25.4, 25.3. HRMS (ESI): found $[M + H]^+$ 293.1411, $C_{14}H_{18}FN_4O_2$ requires 293.1416.

6-(4-(3-(Trifluoromethyl)phenoxy)butoxy)pyrimidine-2,4-diamine (7f). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.51 g, 4.04 mmol), potassium carbonate (0.84 g, 6.06 mmol) and **9f** (1.20 g, 4.04 mmol) in dry acetonitrile, isolated as a white solid (0.64 g, 46%). mp 100-101 °C. IR (ν_{max}/cm^{-1}): 3512, 2939, 1569, 1216, 1141, 1015. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.35 (1H, t, *J* 8.0 Hz), 7.17 (1H, d, *J* 7.8 Hz), 7.11 (1H, t, *J* 2.1 Hz), 7.04 (1H, dd, *J* 8.3, 2.5 Hz), 5.27-5.13 (2H, m), 4.97 (2H, s), 4.26-4.13 (2H, m), 4.00 (2H, t, *J* 3.8 Hz), 1.97-1.74 (4H, m). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 170.6, 166.4, 163.4, 159.4, 130.8 (q, *J* 31.7 Hz), 124.5 (q, $J_{C\text{-}F}$ 272.4 Hz), 119.2, 117.4 (q, $J_{C\text{-}F}$ = 3.8 Hz), 111.4 (q, *J* 3.7 Hz), 76.6, 68.2, 64.6, 25.8 (2C). HRMS (ESI): found $[M + H]^+$ 343.1377, $C_{15}H_{18}F_3N_4O_2$ requires 343.1384.

6-(3-(4-Chlorophenoxy)propoxy)pyrimidine-2,4-diamine (7g). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.51 g, 4.01 mmol), potassium carbonate (0.83 g, 6.01 mmol) and **12a** (1.00 g, 4.01 mmol) in dry acetonitrile, isolated as a white solid (0.41 g, 35%). mp 81-82 °C. IR (ν_{max}/cm^{-1}): 3520, 3359, 3011, 1576, 1125, 791. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.31 (2H, d, *J* 9.0 Hz), 6.96 (2H, d, *J* 9.0 Hz), 6.00 (2H, s), 5.85 (2H, s), 5.06 (1H, s), 4.22 (2H, t, *J* 6.4 Hz), 4.05 (2H, t, *J* 6.3 Hz), 2.07 (2H, p, *J* 6.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.0,

165.9, 163.0, 157.3, 129.3, 124.2, 116.2, 76.1, 64.8, 61.4, 28.5. HRMS (ESI): found $[M + H]^+$ 295.0956, $C_{13}H_{16}ClN_4O_2$ requires 295.0964.

6-(3-(3,4-Dichlorophenoxy)propoxy)pyrimidine-2,4-diamine (7h). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.44 g, 3.52 mmol), potassium carbonate (0.73 g, 5.28 mmol) and 12b (1.00 g, 3.52 mmol) in dry acetonitrile, isolated as a light yellow solid (0.43 g, 37%). mp 134-135 °C. IR (ν_{max}/cm^{-1}): 3529, 3343, 3010, 1581, 1101. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.50 (1H, d, *J* 8.9 Hz), 7.22 (1H, d, *J* 2.9 Hz), 6.95 (1H, dd, *J* 8.9, 2.9 Hz), 5.99 (2H, s), 5.82 (2H, s), 5.04 (1H, s), 4.22 (2H, t, *J* 6.4 Hz), 4.12 (2H, t, *J* 6.2 Hz), 2.06 (2H, p, *J* 6.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ_H 170.4, 166.5, 163.4, 158.5, 132.1, 131.4, 122.8, 116.8, 115.9, 76.6, 65.8, 61.8, 28.9. HRMS (ESI): found $[M + H]^+$ 329.0565, $C_{13}H_{15}Cl_2N_4O_2$ requires 329.0574.

6-(3-(3,5-Dichlorophenoxy)propoxy)pyrimidine-2,4-diamine (7i). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.89 g, 7.04 mmol), potassium carbonate (1.46 g, 10.6 mmol) and 12c (2.00 g, 7.04 mmol) in dry acetonitrile, isolated as a creamy white solid (1.26 g, 54%). mp 105-107 °C. IR (ν_{max}/cm^{-1}): 3541, 3362, 3035, 1588, 1144, 799. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.14 (1H, t, *J* 1.8 Hz), 7.06 (2H, d, *J* 1.8 Hz), 6.00 (2H, s), 5.84 (2H, s), 5.05 (1H, s), 4.21 (2H, t, *J* 6.4 Hz), 4.12 (2H, t, *J* 6.2 Hz), 2.11-2.02 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.0, 166.0, 163.0, 160.0, 134.6 (2C), 120.3, 113.9 (2C), 76.1, 65.5, 61.2, 28.3, HRMS (ESI): found $[M + H]^+$ 329.0572, $C_{13}H_{15}Cl_2N_4O_2$ requires 329.0574.

6-(3-(4-Fluorophenoxy)propoxy)pyrimidine-2,4-diamine (7j). Prepared from 2,4-diamino-6-hydroxy pyrimidine (0.27 g, 2.15 mmol), potassium carbonate (0.44 g, 3.22 mmol) and compound 12d (0.50 g, 2.15 mmol) in dry acetonitrile, isolated as a white solid (0.20 g, 34%). mp 69-71 °C. IR (ν_{max}/cm^{-1}): 3539, 3332, 3032, 1586, 1153. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.18-7.01 (2H, m), 6.99-6.87 (2H, m), 5.99 (2H, s), 5.85 (2H, s), 5.04 (1H, s), 4.22 (2H, t, *J* 6.3 Hz), 4.03 (2H, t, *J* 6.3 Hz), 2.06 (2H, p, *J* 6.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.0, 166.0, 163.0, 159.7 (d, $J_{C\text{-}F}$ 225.5 Hz), 154.8 (d, $J_{C\text{-}F}$ 3.0 Hz), 115.8 (d, $J_{C\text{-}F}$ 15.7 Hz), 115.6, 76.1, 65.0, 61.4, 28.6. HRMS (ESI): found $[M + H]^+$ 279.1252, $C_{13}H_{16}FN_4O_2$ requires 279.1310.

Biology

SYBR green assay against Gambian FCR-3 strain

P. falciparum (FCR-3) strain was cultured *in vitro* at 37 °C in 3% O_2, 5% CO_2, 92% N_2 and adjusted to a 0.625% parasitaemia/1.25% haematocrit before being incubated along with the analogues for 72 h. The plates were frozen overnight and, once thawed, incubated in the dark for 1 h at room temperature with buffered SYBR green I. The fluorescence was read in a microplate reader with excitation and emission wavelength bands centred at 485 and 528 nm, respectively. The percentage inhibition was calculated taking the untreated and dihydroartemisinin control into account. Dihydroartemisinin (DHA) and methotrexate (MTX) were used as positive controls. At least three independent experiments were conducted for each analogue. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined from the log sigmoid dose response curves generated by GraphPad Prism® software. Each experiment was repeated in triplicate.

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Supplementary Material

Readers will be able to access supporting information (^{1}H and ^{13}C NMR spectra of synthesized compounds) using the link "Supplementary Material" in the journal issue contents.

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