Synthesis and biological evaluation of 5-substituted O^4-alkylpyrimidines as CDK2 inhibitors†

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CDK2 inhibitory structure–activity relationships have been explored for a range of 5-substituted O^4-alkylpyrimidines. Variation of the 5-substituent in the 2,6-diaminopyrimidine series confirmed the 5-nitroso substituent as optimal, and showed that 5-formyl and 5-acetyl substituents were also tolerated at this position. A series of O^4-alkyl-N^2-aryl-5-substituted-6-aminopyrimidines revealed interesting structure–activity relationships. In the 5-nitroso series, the optimum O^4-alkyl substituents were cyclohexylmethyl or *sec*-butyl, combined with a 2-sulfanilyl group. By contrast, in the N^2-arylsulfonamido-5-formyl series, the cyclohexylmethyl compound showed relatively poor activity compared with the *sec*-butyl derivative (**22j**, (*R*)-4-(4-amino-6-*sec*-butoxy-5-formylpyrimidin-2-ylamino)benzenesulfonamide; CDK2 $IC_{50} = 0.8$ nM). Similarly, in the N^2-arylsulfonamido-5-(hydroxyiminomethyl) series the O^4-*sec*-butyl substituent conferred greater potency than the cyclohexylmethyl (**23c**, (*rac*)-4-(4-amino-6-*sec*-butoxy-5-(hydroxyiminomethyl)pyrimidin-2-ylamino)benzenesulfonamide; CDK2 $IC_{50} = 7.4$ nM). The 5-formyl derivatives show selectivity for CDK2 over other CDK family members, and are growth inhibitory in tumour cells (*e.g.* **22j**, $GI_{50} = 0.57$ μM).

Introduction

Disregulation of the cell-cycle resulting in unscheduled cell division, coupled with genomic and chromosomal instability, is a common feature of cancer.[1] The cell cycle is regulated by the cyclin-dependent kinase (CDK) family of enzymes and their partner cyclins, and mutations resulting in abnormal CDK activity have been found in many tumours.[2]

The 'classical' model of the cell-cycle, developed over the past 20 years, proposed that specific CDK/cyclin combinations were required to be activated to allow progression through checkpoints leading to entry into the critical phases of DNA-synthesis and mitosis. Activation of CDK4 and CDK6 by cyclins D1, D2, and D3 allows progression from G1 into S-phase. The synthesis of cyclin E1 and E2 is also initiated by activation of CDK4 and CDK6, which in turn activates CDK2. Phosphorylation of the Rb protein requires CDK2, thus allowing progression through the early S-phase. Early experiments showed that blocking CDK2 by mutation or antibodies resulted in the inhibition of the cell cycle in tumour cells, suggesting that CDK2 activity was essential for progression through G1 to S-phase.[3,4] Activation of CDK2 by cyclin A2 occurs during the G2-phase and permits the start of mitosis. Finally, CDK1 activation by the B-cyclins drives the cells through mitosis. On this basis, we and many other groups have sought to develop potent and specific inhibitors of CDKs, in particular CDKs 1, 2 and 4, as cancer therapeutic agents.

More recently, however, a series of genetic studies in mice, in which specific CDKs have been disrupted, has demonstrated that CDK2, CDK4 and CDK6 are not essential for the cell cycle in most cells, and that the knockout mice grow with few abnormalities.[2,5] By contrast, the CDK1 knockout is lethal at the two-cell embryo stage. Further experiments with multiple CDK knockouts, showed that specific combinations of CDKs are required to allow differentiation of specialised cell types within the developing embryo, suggesting an additional role for the cyclin-dependent kinases.

The classical model of CDK function in the cell cycle suggested that selective small-molecule CDK inhibitors could be used to restore checkpoint control in many cancers. This suggestion resulted in a large effort from a number of groups worldwide to discover novel therapeutics based on CDK inhibition.[6] Despite unpromising results in clinical trials from the first generation of CDK inhibitors, which lacked potency and selectivity, a number of second generation, ATP-competitive, small-molecule inhibitors are currently in clinical evaluation (Chart 1). Of these, only P1446A-05 (structure not disclosed) is described as selective for a single CDK (CDK4); the others inhibit multiple CDKs, including the essential CDK1, plus CDK7 which regulates the cell cycle CDKs, and CDK9 which is involved in transcription.

We have described previously two series of potent and selective CDK2 inhibitors based on either a purine scaffold *e.g.* (**1**)[7,8] or the purine-mimetic 5-nitrosopyrimidine scaffold (**2**).[9] In this study, we sought to widen the structure activity relationships (SAR) for

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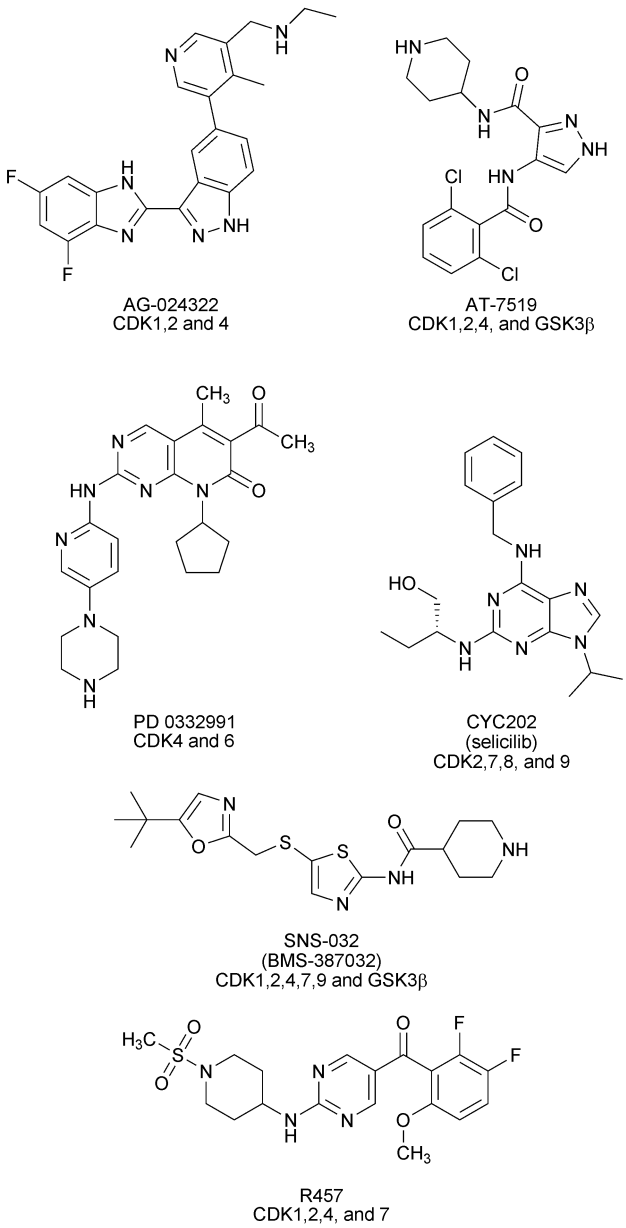
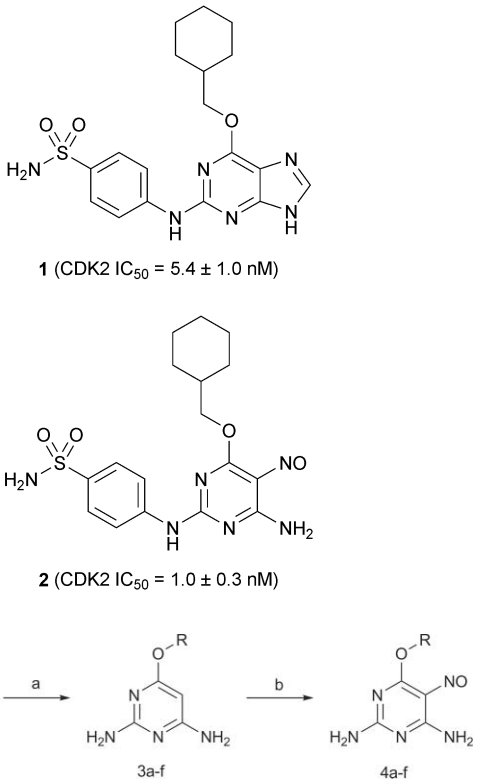


Chart 1



Scheme 1 *Reagents and conditions*: a) Na, ROH, Δ; or NaH, ROH, DMSO, Δ; b) AcOH, $NaNO_2$, H_2O, Δ.

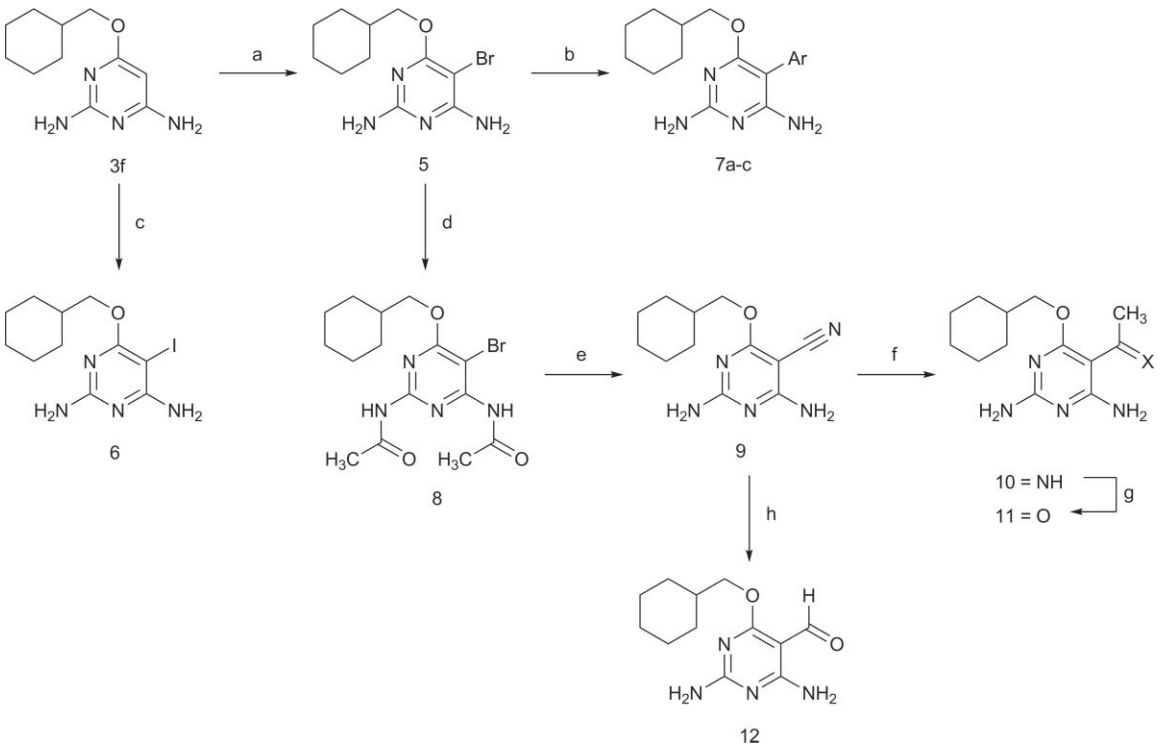
the pyrimidine series. In particular, we intended to improve the drug-like characteristics of this series by replacing the lipophilic cyclohexylmethoxy group with smaller O^4-substituents and by seeking a replacement for the 5-nitroso group, with which there may be toxicity problems. In this paper, we report the discovery of highly potent CDK2 inhibitors, including one compound with sub-nanomolar potency, which retain selectivity against other CDKs.

Results and discussion

Chemical synthesis

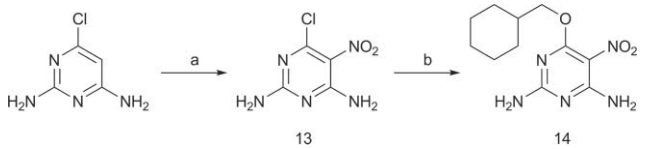
A series of O^4-alkoxy-5-nitrosopyrimidines was prepared using the method described previously (Scheme 1).[9] 4-Chloro-2,6-diaminopyrimidine was heated with a sodium alkoxide in DMSO to give the required 4-alkoxypyrimidine (**3a–f**). 5-Nitrosation was achieved by heating **3a–f** with sodium nitrite in acetic acid giving the corresponding 5-nitrosopyrimidines (**4a–f**) in moderate yields. Reasoning that the low yields may be due to hydrolysis of the 4-alkoxy group under the acidic conditions, a non-aqueous, less acidic medium was sought. The use of sodium nitrite with 1.5 equiv. trifluoroacetic acid in trifluoroethanol gave much improved yields.

A range of 5-substituents was introduced into 4-cyclohexylmethoxy-2,6-diaminopyrimidine (**3f**) using the methods shown in Scheme 2. The 5-bromo derivative (**5**) was prepared in good yield by bromination with *N*-bromosuccinimide.[10] Similarly, the 5-iodo compound (**6**) was prepared using *N*-iodosuccinimide. Bromopyrimidine **5** was used as the substrate for Suzuki-Miyura cross-coupling reactions with the appropriate aryl- or heteroaryl-boronic acid. Elevated temperatures of 170 °C, achieved with microwave heating, were essential for the synthesis of compounds (**7a–d**), albeit in poor yields. Direct cyanation of bromopyrimidine **5** was achievable, but the purification was complicated by the presence of copper complexes. As an alternative, the *N*,*N*-diacetyl compound (**8**) was prepared by heating **5** in acetic anhydride.[11] Treatment of **8** with copper(I)cyanide and ethane-1,2-diamine in DMF gave the 5-cyano derivative (**9**) with concomitant loss of the acetyl groups. The cyano group was converted into the imine (**10**) by treatment of **9** with methylmagnesium bromide at 100 °C in THF. Hydrolysis of **10** to the ketone (**11**) occurred readily on treatment with dilute HCl.[12] For the preparation of the 5-formyl derivative (**12**), the 5-cyano compound (**9**) was



Scheme 2 *Reagents and conditions*: a) NBS, AcOH, 60 °C; b) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, H_2O, DME, MW, 170 °C; c) NIS, AcOH, 60 °C; d) Ac_2O, Δ; e) CuCN, DMF, ethane-1,2-diamine; f) MeMgBr, THF, 0 °C then 100 °C; g) HCl, H_2O, THF; h) H_2SO_4, H_2O, Pd/C, H_2 (1 atmos.).

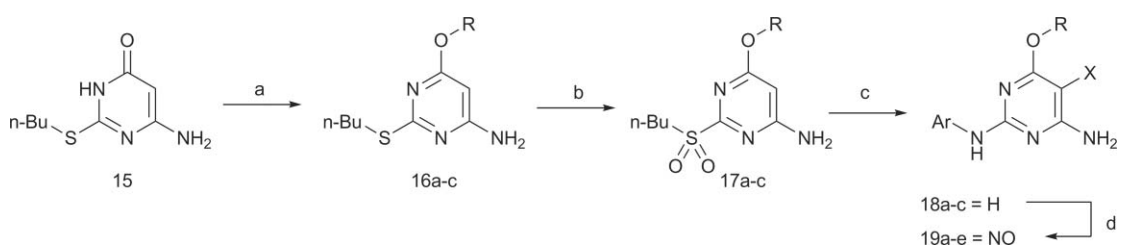
reduced with hydrogen/palladium on charcoal with trapping of the intermediate imine with aqueous sulfuric acid.[13] The 4-cyclohexylmethoxy-5-nitropyrimidine (**14**) was prepared from 4-chloro-2,6-diaminopyrimidine (Scheme 3) by nitration with fuming nitric acid in sulfuric acid to give (**13**), followed by reaction with sodium cyclohexylmethoxide in *t*-butanol.[14]



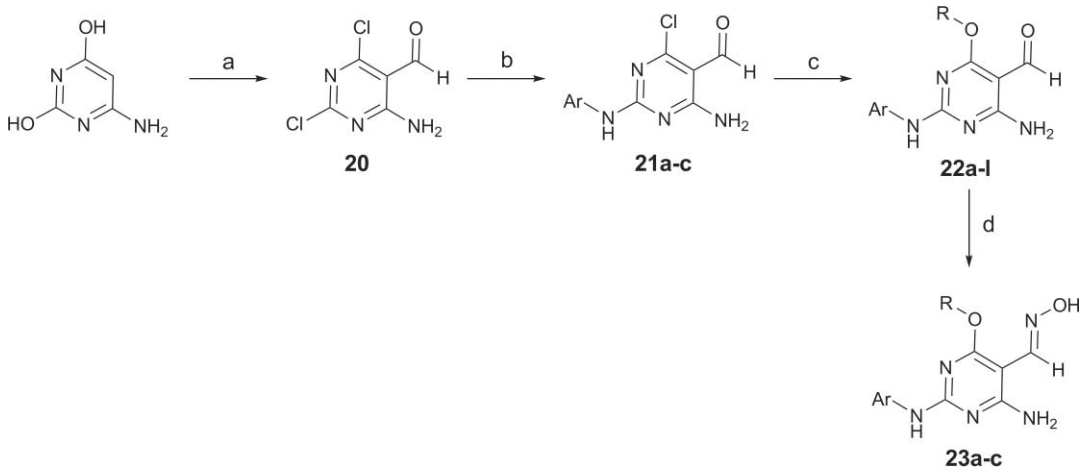
Scheme 3 *Reagents and conditions*: a) HNO_3, H_2SO_4, 35 °C; b) NaO^tBu, ^{t}BuOH, cyclohexylmethanol, 30 °C.

The corresponding O^4-ethoxy- and O^4-*sec*-butoxy- derivatives were prepared for comparison with the O^4-cyclohexylmethoxy compound (**19a**) using the method described previously (Scheme 4).[9] Alkylation of pyrimidinone (**15**) under Mitsunobu conditions gave the O^4-alkoxy derivatives (**16a–c**) good yields. Oxidation of **16a–c** to the corresponding butylsulfones (**17a–c**) with *m*-chloroperbenzoic acid (mCPBA) was followed by displacement of the butanesulfonyl group with the appropriate aniline to give **18a–c**.[15] Subsequent nitrosation at the 5-position gave the desired compounds (**19a–e**).

An alternative strategy was required for the synthesis of the 2-arylamino-5-formylpyrimidines (Scheme 5). 4-Amino-2,6-dihydroxypyrimidine was reacted with phosphorous oxychloride and DMF to give the 2,6-dichloro-5-formyl pyrimidine (**20**). The N^2-aryl substituent was introduced using the previously optimised conditions for SN_{Ar} displacements at the 2-position of pyrimidines using TFE as solvent with TFA to give the corresponding 4-chloro derivatives (**21a–c**).[15] Displacement of the 4-chloro group with the required sodium alkoxide gave the O^4-alkoxy substituted compounds (**22a–l**) in moderate yields. In the case of the benzoate derivative (**22e**), the carboxylic acid was protected as its neopentyl ester for the alkoxide displacement and removed during the basic work up. Selected 5-formyl compounds were converted into the respective oximes (**23a–c**) on treatment with hydroxylamine hydrochloride in pyridine.

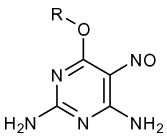


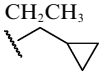
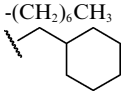
Scheme 4 *Reagents and conditions*: a) ROH, PPh_3, DEAD, THF; b) mCPBA, DCM; c) $ArNH_2$, TFA, TFE, Δ; d) AcOH, $NaNO_2$, H_2O, Δ.



Scheme 5 *Reagents and conditions*: a) DMF, $POCl_3$, 105 °C; b) $ArNH_2$, TFA, TFE, Δ; c) ROH, NaH, THF, Δ; d) $NH_2OH.HCl$, EtOH, pyridine.

Table 1 Inhibition of CDK2 by O^4-substituted-2,6-diamino-5-nitrosopyrimidines



Compound	*R*	IC_{50}/μM
4a	CH_3	79 ± 6
4b	CH_2CH_3	50 ± 1
4c		16 ± 0.3
4d		22[a]
4e	$-(CH_2)_6CH_3$	22%[b]
4f		2.2 ± 0.5[c]

[a] n = 1; [b] % inhibition at 10 μM; [c] ref.[18]

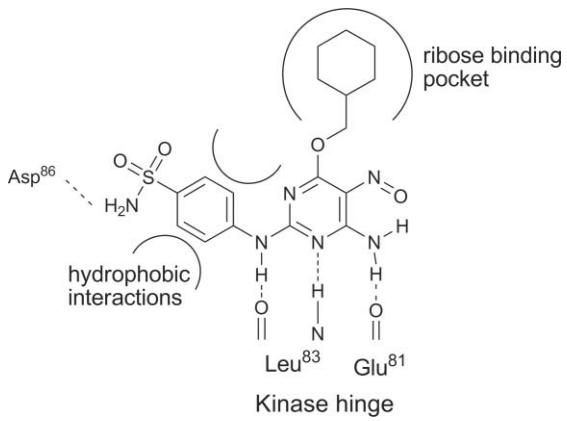


Fig. 1 Binding mode of **NU6027** with CDK2.

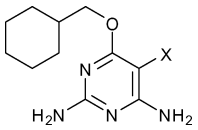
SAR discussion

All compounds were evaluated for CDK inhibitory activity using published procedures.[8,16] Firstly, variation of the O^4-alkoxy substituent was investigated with the simple 2,6-diaminopyrimidine scaffold. The results show that small O^4-alkoxy substituents confer lower potency compared with the cyclohexylmethoxy substituent (Table 1). These results are consistent with previous studies in the O^6-alkylguanine series[17] and the O^4-alkoxypyrimidine series.[18] The efficacy of the cyclohexylmethoxy substituent has been explained by examining the X-ray structure of NU6027 bound to CDK2, which shows the cyclohexyl group occupying the hydrophobic entrance to the ribose binding pocket. The 2-amino group extends close to the hydrophobic selectivity pocket of the enzyme (Fig. 1).

We have previously shown that the 5-nitroso substituent is important for CDK inhibitory activity by forming an intramolecular H-bond with the 6-amino group, which orientates the nitrogen in a pseudo-purine geometry.[19] The nitroso substituent is considered to be unacceptable for pharmaceuticals as it may form toxic and genotoxic metabolites *in vivo*. For this reason, a group of alternative 5-substituents was explored (Table 2). The 5-cyano substituent (**9**), which has similar electronic properties to the nitroso group but is unable to form an intramolecular hydrogen bond due to its linearity, was 30-fold less potent than the parent 5-nitrosopyrimidine **4f**. The 5-nitro substituent (**14**) showed only a 4-fold loss of potency, where in this case an intramolecular hydrogen bond can be formed. The 5-(2-furyl) and 5-(2-thiophenyl) derivatives (**7b** and **7c**) were both 25-fold less active than **4f**, indicating that any hydrogen bonding from a furan oxygen lone pair was insignificant and that hydrophobic bulk at this position is unfavourable. The 5-bromo, 5-iodo and 5-phenyl derivatives (**5**, **6**, and **7a**) lacked both solubility and potency. The 5-acetyl and 5-formyl derivatives **11** and **12**, respectively, were both 10-fold less active than **4f**, whereas the imine derivative **10** suffered a 50-fold drop in potency. Crystal structure analysis of the 5-formyl derivative **12** bound to CDK2 failed to show an intramolecular hydrogen bond, although splitting of the 6-NH_2 proton resonances in **12** in the proton NMR spectrum indicates that hydrogen bonding occurs in DMSO solution.[18]

A series of N^2-aryl compounds was prepared to investigate structural variations within the O^4-cyclohexylmethoxy-N^2-aryl-5-nitrosopyrimidines (Table 3).[9] The aryl substituents were limited

Table 2 Inhibition of CDK2 by 5-substituted-O^4-cyclohexylmethoxy-2,6-diaminopyrimidines



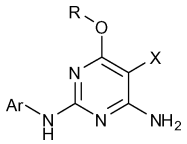
Compound	X	IC_{50}/μM
4f	NO	2.2 ± 0.5[d]
5	Br	2%[b]
6	I	33%[c]
7a	Ph	48%[c]
7b		56.3
7c		55
9	CN	61 ± 10
10		92[a]
11		23[a]
12		24[a,d]
14	NO_2	8.3 ± 5.6

[a] n = 1; [b] percent inhibition at 10 μM; [c] percent inhibition at 100 μM; [d] ref.[18]

to the 4-sulfonamide, 4-methoxy, and the previously unreported 4-carboxylic acid, as representative examples. The 5-substituents explored were the nitroso, formyl, and the methyliminyl groups. The 4-cyclohexylmethoxy-2-(4-methoxybenzyl) derivatives, **19b** and **22a**, were 200-fold and 60-fold less active than the corresponding 5-nitroso- and 5-formyl-2-sulfanilylpyrimidine derivatives, **19a** and **22k**, respectively. The dramatic fall in potency is similar to the >100 fold reduction in activity seen in the corresponding purine series, and is consistent with the key hydrogen bond interactions made by the sulfonamide within the ATP-binding domain of CDK2.[8] Variation was also made at the O^4-position with the introduction of smaller alkyl substituents. In the 2-sulfanilyl-5-nitroso series, the 4-ethoxy derivative **19d** was approximately 100-fold less active than the parent **19a**. By contrast, the racemic *sec*-butoxy derivative **19e** was equipotent with **19a**. A comparison of these results with those observed for the 2-aminopyrimidine series (**4b**, **4d**, and **4f**), again suggests that the 2-sulfanilyl group is a major determinant of CDK2 inhibitory activity for this series.

In the 2-(4-methoxyanilino)-5-formyl series (**22a–d**), the *sec*-butoxy derivative **22d** was >100-fold less active than the 5-nitroso derivative **19e**, consistent with results seen in the 2,6-diamino series (**4f** and **12**). The 4-ethoxy and 4-*iso*-propoxy derivatives, **22b** and **22c**, showed a modest improvement in activity compared with **22a**, and surprisingly, the racemic *sec*-butoxy compound **22d** showed comparable activity to the 4-cyclohexylmethoxy-5-nitroso derivative **19b**. In the 2-sulfanilyl-5-formyl series (**22f–l**), the 4-ethoxy-

Table 3 CDK2 inhibition for O^4-substituted-N^2-aryl-5-substituted-6-aminopyrimidines



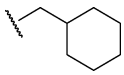
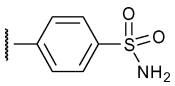
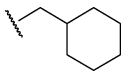
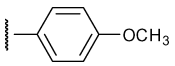
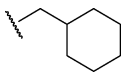
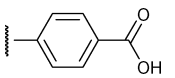
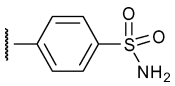
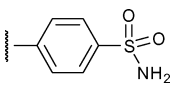
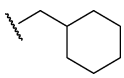
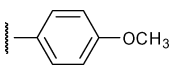
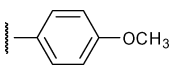
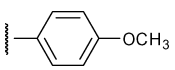
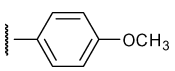
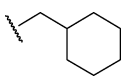
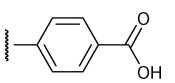
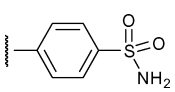
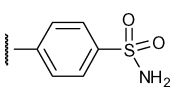
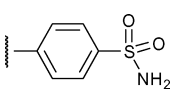
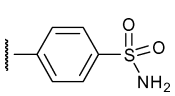
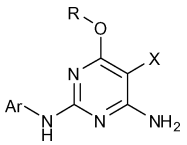
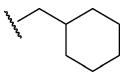
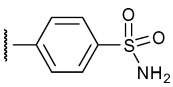
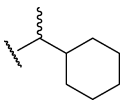
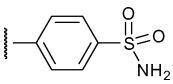
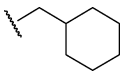
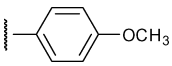
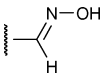
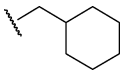
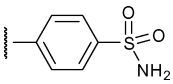
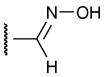
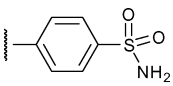
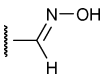
Compound	*R*	Ar	X	IC_{50}/nM
19a			NO	1.0 ± 0.3[b]
19b			NO	215 ± 50[b]
19c			NO	110
19d	CH_2CH_3		NO	93[a]
19e			NO	1.6
22a				2900
22b	CH_2CH_3			1900
22c				1800
22d				225
22e				610
22f	CH_2CH_3			49
22g				26
22h				3.8
22i				13.1
22j				0.77

Table 3 *(Contd.)*



Compound	*R*	Ar	X	IC_{50}/nM
22k				49
22l				92[b]
23a				8300
23b				117
23c				7.4

[a] n = 2; [b] ref. [9]

derivative **22f** showed a surprising 2-fold improvement in activity over the corresponding nitroso derivative **19d**. Improved potency was also observed for the racemic *sec*-butoxy derivative **22h**, which displayed potent activity (CDK2; $IC_{50} = 3.8$ nM). The enantiomers of **22h** were prepared from the respective chiral *sec*-butanols, and the *R*-enantiomer **22j** showed sub-nanomolar potency against CDK2. By contrast, the 4-cyclohexylmethoxy and the *rac*-1-cyclohexylethoxy derivatives (**22k** and **22l**) were 49 and 92-fold less active than **19a**, respectively. The 4-cyclohexylmethoxy-5-methyliminyl derivatives, **23a** and **23b**, were significantly less potent than the corresponding 5-nitroso derivatives (**19a** and **19b**). The racemic *sec*-butoxy-5-methylimine **23c**, however, retained potency but was less active than the corresponding 5-formyl derivative **22h**.

Three compounds were selected for profiling in a panel of CDKs (Table 4). The 5-nitrososulfonamide **19a** showed 100-fold selectivity for CDK2 over CDK1/cyclinB, 740-fold selectivity against CDK9/cyclinT, and > 1000-fold selectivity *vs.* CDK4/cyclinD and CDK7/cyclinH. Interestingly, the (*R*)-*sec*-butoxy-5-formylsulfonamide **22j** retained excellent selectivity for CDK2 over CDK4/cyclinD and CDK7/cyclinH, but displayed only 10-fold selectivity against CDK1/cyclinB and 50-fold selectivity *vs.* CDK9/cyclinT. The same compounds were evaluated for growth inhibitory activity using an Rb-positive SKUT1B cancer cell line.[5] As has been demonstrated previously, the CDK2 inhibitors showed only modest growth inhibitory activity (GI_{50} ~1 μM) in comparison to their potency as inhibitors of CDK2 (IC_{50} ~1 nM).

Conclusions

In summary, we have synthesised a series of 5-substituted O^4-alkylpyrimidine CDK2 inhibitors, with a view to investigating structure activity relationships for this chemotype. The results are consistent with those determined previously for the analogous purine series, and support the key role played by the 2-sulfanilyl substituent. In addition, we have demonstrated that a 4-*sec*-butoxy substituent can replace the cyclohexylmethoxy group at this position without loss of potency, and evidence of stereospecificity of binding was observed. At the pyrimidine 5-position, a small electron withdrawing group capable of making an intramolecular hydrogen bond with the 6-amino group is favoured. Of the substituents explored the 5-formyl was preferred. Thus, the combination of 4-(*R*)-*sec*-butoxy-5-formyl-2-sulfanilylpyrimidine conferred excellent CDK2 inhibitory activity (**22j**; $IC_{50} = 0.8$ nM) and is one of the most potent CDK2 inhibitors reported to date.

Experimental

Reagents were purchased from fine chemicals vendors, and used as received unless otherwise stated. Solvents were purified and stored according to standard procedures. Petrol refers to that fraction in the boiling range 40–60 °C. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. Thin layer chromatography was performed using silica gel plates (Kieselgel $60F_{254}$; 0.2 mm), and visualized with UV light or potassium permanganate. Chromatography was conducted under medium pressure in glass columns or using a Biotage SP4 instrument in prepacked columns (FLASH+ Silica columns (40–63 μm, 60 Å), NH FLASH+ cartridges (40–75 μm, 100 Å) and C18 FLASH+ cartridges (40–63 μm, 90 Å)). Semi-preparative HPLC was conducted using a Varian Prostar instrument equipped with a Waters XTerra column (RP_{18} OBD 10 μm, 150 × 30 mm) or Phenomenex Synergi 4u Fusion-RP (80 Å, 250 × 21.2 mm) as indicated, monitoring by UV at $\lambda = 270$ nm. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin AC 300E (^{1}H at 300 MHz, ^{13}C

Table 4 CDK selectivity and cell growth inhibition for compounds **6189**, **6353**, and **6407**

	IC_{50}/nM					
Compound	CDK1/B	CDK2/A	CDK4/D	CDK7/H	CDK9/T	GI_{50}/μM[a]
19a	100 ± 10	1 ± 0.3	1500 ± 500	2800 ± 1300	740 ± 100	2.1
22h	73	3.8	3200	890	87	0.93
22j	9	0.77	1400	710	51	0.57

[a] Concentration required to inhibit the growth of SKUT1B tumour cells by 50%

at 75 MHz) or a Jeol JNM-LA500 spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz) employing the solvent as internal standard. Coupling constants *J* values are given in Hz. NH signals appeared as broad singlets (br s) exchangeable with D_2O. Mass spectra were determined on a Micromass Autospec M spectrometer in electron impact (EI) mode. Liquid Chromatography-Mass Spectrometry (LCMS) was carried out on a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing either a Waters Symmetry (30 × 4.6 mm; C18; 5 min run) or Waters Atlantis (50 × 4.6 mm; C18; 12 min run) column with 0.05% aqueous formic acid and acetonitrile (5 to 95% organic). Elemental combustion analyses were either recorded on Carlo-Erba instrument 1106 analyser, in-house, or were performed either by Medac Ltd (Brunel Science Centre, Egham, Surrey, TW20 0JZ), or by the School of Pharmacy at London University (29–39 Brunswick Square, London, WC1N 1AX). Accurate mass analyses were measured using a Finnigan MAR 95 XP or a Finnigan MAR 900 XLT at Swansea EPSRC National Mass Spectrometry Service Centre (EPSRC, Chemistry Department, University of Wales Swansea, Wales, SA2 8PP). Final compounds were found to be >97% purity by LCMS and NMR analysis or gave satisfactory CNH analysis. Experimental details for additional compounds are found in the ESI.†

4-Chloro-5-nitropyrimidine-2,6-diamine (13)

4-Chloro-2,6-diaminopyrimidine (0.5 g, 3.5 mmol) was added to a mixture of fuming HNO_3 (0.5 mL) and conc. H_2SO_4 (2.5 mL). The resulting mixture was stirred at 35 °C for 30 min, then poured slowly onto crushed ice with vigorous stirring yielding a yellow solid. The mixture was adjusted to pH 8–9 by the dropwise addition of conc. aq. NH_3, at −10 °C, then filtered, washed with copious amounts of water and dried *in vacuo* over P_2O_5 (0.28 g, 42%): m.p. 218–220 °C; (Lit. 220–222 °C);[14] δ_H (300 MHz d_6-DMSO) 7.46 (1H, s, NH_2, ex), 7.63 (1H, s, NH_2, ex), 8.13 (2H, s br, NH_2, ex). MS (ESI+) *m/z* 190 $[M+H]^+$.

4-Cyclohexylmethoxy-5-nitropyrimidine-2,6-diamine (14)

Compound **13** (0.11 g, 0.58 mmol) was added to a mixture of sodium *tert*-butoxide (0.07 g, 0.70 mmol), in *tert*-butanol (10 mL) and cyclohexylmethanol (0.11 mL, 0.87 mmol) and stirred at 30 °C for 22 h, then concentrated *in vacuo* yielding a yellow solid. Chromatography (silica; 40% EtOAc, petrol), gave **14** as a yellow solid (0.04 g, 25%): m.p. 177–178 °C. UV λ_{max} = 328 and 211 nm, IR ν_{max} 1340, 1544, 1618, 2850–2921, 3152, 3360–3481 cm^{-1}. δ_H (300 MHz d_6-DMSO) 1.00–1.26 (5H, m, C_6H_{11}), 1.68–1.80 (6H, m, C_6H_{11}), 4.10 (2H, d, *J* = 6.0 Hz, CH_2O), 7.20 (2H, s, NH_2, ex), 7.89 (2H, s, NH_2, ex) ppm; δ_C (75 MHz d_6-DMSO) 25.6 (**C**$_6$H$_{11}$), 26.3 (**C**$_6$H$_{11}$), 29.4 (**C**$_6$H$_{11}$), 37.0 (**C**$_6$H$_{11}$), 72.2 (O**C**H$_2$), 83.0 (**C**5), 160.3 (**C**6), 161.2 (**C**4), 165.0 (**C**2). MS (ESI+) *m/z* 268 $[M+H]^+$; $C_{11}H_{17}N_5O_3 \cdot 0.2$ EtOAc requires: C, 49.75; H, 6.58; N, 24.58; found: C, 49.58; H, 6.38; N, 24.43.

5-Bromo-6-cyclohexylmethoxypyrimidine-2,4-diamine (5)[10]

To 6-cyclohexylmethoxypyrimidine-2,4-diamine (**3f**) (2.00 g, 9.01 mmol) in AcOH (10.0 mL) was added NBS (1.60 g, 9.01 mmol) slowly, in small portions. The temperature was increased to 60 °C and the reaction mixture was allowed to stir for 1 h, then allowed cool to rt, a white precipitate was formed. Water (30 mL) was added and the mixture was neutralised with a 2.5 M NaOH solution, extracted with EtOAc (4 × 40 mL), and the combined organic layers were washed with water (40 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Recrystallisation (EtOAc,Petrol) gave **5** as a white solid (1.92 g, 71%). M.p. 193–194 °C; (Lit 195–196 °C). UV λ_{max} (EtOH) 274 and 234 nm; IR ν_{max} 3471, 3337, 3140, 2923, 2848, 1618, 1559, 1433, 1192 cm^{-1}. δ_H (300 MHz, d_6-DMSO): 0.96–1.25 (5H, m, C_6H_{11}), 1.67–1.76 (6H, m, C_6H_{11}), 3.98 (2H, d, *J* = 6.0 Hz, OCH_2), 6.11 (2H, s, NH_2, ex), 6.28 (2H, s, NH_2, ex). δ_C (75 MHz, d_6-DMSO): 25.6 (**C**$_6$H$_{11}$), 26.4 (**C**$_6$H$_{11}$), 29.4 (**C**$_6$H$_{11}$), 37.2 (**C**$_6$H$_{11}$), 71.1 (O**C**H$_2$), 71.9 (**C**5), 161.4 (**C**6), 162.0 (**C**4), 165.1 (**C**2). MS (ESI+) *m/z* 301 and 303 $[M+H]^+$,HRMS (ESI+) *m/z*: Calc. for $C_{11}H_{17}{}^{79}BrN_4O$: 301.0659 $[M+H]^+$. Found 301.0655 $[M+H]^+$. $C_{11}H_{17}BrN_4O$: requires C, 43.87; H, 5.69; N,18.59; found: C, 43.98; H, 5.72; N, 18.55%.

N,*N*′-(5-Bromo-6-cyclohexylmethoxypyrimidine-2,4-diyl)diacetamide (8)

5-Bromo-6-cyclohexylmethoxypyrimidine-2,4-diamine (**5**) (2.00 g, 6.64 mmol) in a mixture of acetic anhydride (16 mL) and acetic acid (16 mL) was heated at reflux for 18 h. The mixture was cooled to rt, poured into ice and water, basified with conc. NH_3, and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with aq. sodium bicarbonate (sat; 30 mL), water (30 mL), dried (Na_2SO_4) and then concentrated *in vacuo* giving **8** as an off-white solid (2.35 g, 95%). M.p.: 179–182 °C. UV λ_{max} (EtOH): 253, 276 and 299 nm; IR υ_{max} 3391, 3253, 2921, 2853, 1716, 1672, 1577, 1508, 1424, 1190 cm^{-1}; δ_H (300 MHz, d_6-DMSO) 1.02–1.23 (5H, m, C_6H_{11}), 1.69–1.75 (6H, m, C_6H_{11}), 2.17 (3H, s, $COCH_3$), 2.20 (3H, s, $COCH_3$), 4.19 (2H, d, *J* = 6.1 Hz, OCH_2), 9.96 (1H, s, NH, ex), 10.5 (1H, s, NH, ex); δ_C (75 MHz, d_6-DMSO) 24.1, 25.2, 25.5, 26.3, 29.3, 36.9, 72.9, 90.5, 155.1, 157.5, 166.7, 169.3, 169.6. MS (ESI+) *m/z* 385 and 387 $[M+H]^+$; HRMS (ESI+) *m/z*: Calc. for $C_{15}H_{21}{}^{79}BrN_4O_3$ 385.0870 $[M+H]^+$. Found 385.0873 $[M+H]^+$. $C_{15}H_{21}BrN_4O_3$: requires C, 46.76; H, 5.49; N, 14.54%. Found C, 46.52; H, 5.79; N, 14.23%.

2,4-Diamino-5-cyano-6-cyclohexylmethoxypyrimidine (9)[13]

Compound **8** (0.4 g, 1.04 mmol) and CuCN (0.112 g, 1.25 mmol) were heated at reflux in DMF (8.0 mL) for 5 h. Ethane-1,2-diamine (20 mL) was added and the reaction mixture was stirred for further 2 h. The mixture was filtered through Celite and the filter pad was washed with DMF (20 mL). The filtrate was extracted with EtOAc (60 mL) and washed with saturated aq. sodium chloride (30 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography (silica; 20–80% EtOAc, petrol) gave **9** as a pale yellow solid (0.212 g, 82%). M.p.: 186–188 °C. UV λ_{max} (EtOH): 252 and 266 nm. IR υ_{max} 3317, 3233, 2919, 2848, 2208, 1607, 1573, 1547, 1348, 1232 cm^{-1}. δ_H (300 MHz, d_6-DMSO): 0.96–1.26 (5H, m, C_6H_{11}), 1.64–1.75 (6H, m, C_6H_{11}), 4.06 (2H, d, *J* = 6.0 Hz, OCH_2), 6.88 (2H, s, NH_2 ex), 6.93 (2H, s, NH_2 ex); δ_C (75 MHz, d_6-DMSO) 25.5, 26.3, 29.3, 37.0, 63.1, 71.1, 116.4, 163.3, 166.0, 171.4; MS (ESI+) *m/z* 248 $[M+H]^+$; HRMS (ESI+) *m/z*: Calc. for $C_{12}H_{17}N_5O$: 248.1506 $[M+H]^+$. Found 248.1504 $[M+H]^+$.

2,4-Diamino-6-cyclohexylmethoxy-5-formylpyrimidine (12)[13,20]

A solution of 2,4-diamino-5-cyano-6-cyclohexylmethoxypyrimidine (**9**) (0.20 g, 0.81 mmol) in water (7.2 mL) and sulfuric acid (1.2 mL) was stirred with 5% palladium on charcoal (0.094 g) under H_2 (1 atm) at rt for 48 h. The mixture was filtered through Celite and the filter pad was washed with hot water (200 mL) and hot methanol (200 mL). The filtrate was neutralised (NaOH, 2M), concentrated *in vacuo*, and the residues extracted with EtOAc (4 × 50 mL). The combined organic layers were washed with water (20 mL) and dried (Na_2SO_4) and concentrated *in vacuo* giving white solid. Chromatography (Biotage C18; 50-25% H_2O, MeOH, 1% formic acid) and recrystallisation (methanol) gave **12** as a white solid (0.151 g, 75%). M.p.: 160–163 °C; (Lit: 118–120 °C).[20] UV λ_{max} (EtOH): 291.6 nm; IR ν_{max} 3327, 3194, 3147, 2916, 2846, 1662, 1586, 1528, 1342, 1259 cm^{-1}; δ_H (300 MHz, d_6-DMSO) 0.92–1.35 (5H, m, C_6H_{11}), 1.74–1.82 (6H, m, C_6H_{11}), 4.16 (2H, d, J = 6.0 Hz, OCH_2), 7.15 (2H, s, NH_2 ex), 7.45 (1H, s, NH_2 ex), 8.36 (1H, s, NH_2 ex), 9.89 (1H, s, CHO) ppm; δ_C (75 MHz, d_6-DMSO) 25.2, 25.9, 29.1, 36.7, 70.5, 91.7, 163.8, 164.4, 171.6, 184.4 ppm. MS (ESI+) m/z 251.02 $[M+H]^+$. HRMS (ESI+) m/z: Calc. for $C_{12}H_{18}N_4O_2$: 251.1503 $[M+H]^+$. Found 251.1506 $[M+H]^+$.

6-Cyclohexylmethoxy-5-(1-iminoethyl)pyrimidine-2,4-diamine (10)[12]

To a solution of **9** (0.50 g, 2.02 mmol) in THF (50 mL), under N_2, in an ice bath was added dropwise methyl magnesium bromide (3.0 M in THF, 3.37 mL, 10.1 mmol), over 10 min while stirring at 0 °C. The mixture was stirred 2 h at 0 °C, then heated at reflux for 24 h. Further methyl magnesium bromide (3.0 M in THF, 6.74 mL, 20.2 mmol) was added over the next 48 h with continued heating, until the reaction came to completion. The mixture was cooled to rt, quenched with aq. ammonium chloride (sat, 30 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (40 mL), dried (Na_2SO_4) and concentrated *in vacuo* yielding a yellow powder. Recrystallisation (EtOAc, petrol) gave **10** as yellow solid (2.35 g, 95%), m.p.: 141–143 °C. UV λ_{max} (EtOH): 240, 312 nm; IR ν_{max} 3365, 3210, 3127, 2984, 2914, 1655, 1554, 1418, 1253 cm^{-1}; δ_H (300 MHz, d_4-MeOD): 0.99–1.32 (5H, m, C_6H_{11}), 1.71–180 (6H, m, C_6H_{11}), 2.45 (3H, s, CH_3), 4.08 (2H, d, J = 6.0 Hz, OCH_2); δ_C (75 MHz, $CDCl_3$) 26.1, 26.8, 30.4, 33.1, 37.9, 72.1, 91.4, 161.4, 165.8, 170.2, 175.3. MS (ESI+) m/z 264.25 $[M+H]^+$. HRMS (ESI+) m/z: Calc. for $C_{13}H_{21}N_5O$: 264.1819 $[M+H]^+$. Found 264.1821 $[M+H]^+$.

1-(2,4-Diamino-6-cyclohexylmethoxypyrimidin-5-yl)ethanone (11)

A solution of HCl (2M; 10 mL) was added dropwise to a solution of **10** (0.075 g, 0.285 mmol) in THF (10 mL). The resulting mixture was stirred at rt for 16 h, then neutralised by dropwise addition of NaOH (2M), and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (30 mL) and dried (Na_2SO_4) and concentrated *in vacuo* giving a yellow powder. Recrystallisation (EtOAc, petrol), gave **11** as yellow solid (0.063 g, 84%), m.p. 149–151 °C. UV λ_{max} (EtOH): 283 nm. IR ν_{max} 3340, 3221, 2923, 2849, 1683, 1548, 1524, 1431, 1244 cm^{-1}; δ_H (300 MHz, $CDCl_3$): 0.93–1.28 (5H, m, C_6H_{11}), 1.62–1.77 (6H, m, C_6H_{11}), 2.47 (3H, s, CH_3), 4.08 (2H, d, J = 6.0 Hz, OCH_2), 4.93 (2H, br, NH_2 ex), 5.38 (1H, br, NH ex), 9.27 (1H, br, NH ex); δ_C (75 MHz, $CDCl_3$): 23.9, 24.6, 28.2, 31.2, 35.6, 70.4, 91.8, 160.3, 164.8, 169.4, 195.1. MS (ESI+) m/z 265.28 $[M+H]^+$; HRMS (ESI+) m/z: Calc. for $C_{13}H_{20}N_4O_2$: 265.1659 $[M+H]^+$. Found 265.1656 $[M+H]^+$.

5-Iodo-6-(cyclohexylmethoxy)pyrimidine-2,4-diamine. (6)

To a solution of 6-cyclohexylmethoxypyrimidine-2,4-diamine (**3f**) (0.20 g, 0.90 mmol, 1 eq.) in AcOH (2.0 mL) was added NIS (0.24 g, 1.08 mmol). The resulting mixture was stirred for 1 h at 60 °C, then allowed to cool to rt, water (5 mL) was added, then neutralised NaOH (2.5 M), and extracted with DCM (3 × 30 mL). The combined organic layers were washed with water (30 mL) and dried (Na_2SO_4) and concentrated *in vacuo* giving a solid residue. Recrystallisation (EtOAc, Petrol) gave **6** as an off-white solid (0.23 g, 73%) m.p.: 177–178 °C. UV λ_{max} (EtOH): 325 and 228 nm; IR ν_{max} 3477, 3332, 3214, 2914, 2848, 1619, 1582, 1541, 1423, 1167 cm^{-1}; δ_H (300 MHz, d_6-DMSO): 0.95–1.25 (5H, m, C_6H_{11}), 1.61–1.78 (6H, m, C_6H_{11}), 3.96 (2H, d, J = 6.4 Hz, OCH_2), 6.08 (4H, s, NH_2 ex); δ_C (75 MHz, d_6-DMSO): 25.6, 26.4, 29.5, 37.3, 43.8, 71.4, 162.9, 164.7, 167.9. MS (ESI+) m/z 349.12 $[M+H]^+$; HRMS (ESI+) m/z: Calc. for $C_{11}H_{17}IN_4O$: 349.0522 $[M+H]^+$. Found 349.0522 $[M+H]^+$. $C_{11}H_{17}IN_4O.0.2\ CHCl_3$: requires C, 59.51; H, 6.71; N, 18.24%; found C, 59.18; H, 6.47, N, 17.96%.

4-Amino-2,6-dichloro-5-formylpyrimidine (20)[12]

Dry N,N-dimethylformamide (7 mL) was added slowly with stirring over 15 min to phosphorous oxychloride (22 mL, 0.24 mol), at ~ 5 °C. The reaction mixture was warmed gently to dissolve the resulting precipitate, and 4-amino-2,6-dihydroxypyrimidine (5.6 g, 0.044 mmol) was added in small portions over 10 min, then heated for 5 h at 105 °C, producing a dark red–brown solution. Remaining phosphorous oxychloride was removed by distillation *in vacuo*, leaving a viscous dark brown oil, which was mixed with crushed ice and allowed to stand at rt overnight giving a red–orange solution and a dark yellow solid. The solid was collected by filtration and the filtrate was treated with ammonium hydroxide in portions to pH ~ 7 and left to stand overnight. The yellow–brown precipitate, was collected by filtration. The combined precipitates were dried *in vacuo* over P_2O_5 overnight and then extracted with hot EtOAc (6 × 200 ml) leaving a brown insoluble residue which was discarded. The solvent was removed *in vacuo* to give a bright yellow solid, which after recrystallisation (EtOAc) gave **20** (5.3 g, 63%) m.p.: 187–188 °C. UV λ_{max} (EtOH): 250 and 316 nm. IR ν_{max} 3354, 3188, 2877, 1728, 1630, 1521 cm^{-1}. δ_H (300 MHz, d_6-DMSO): 8.74 (1H, s, NH exchangeable with D_2O), 9.11 (1H, s, NH exchangeable with D_2O), 10.18 (1H, s, CHO). δ_C (75 MHz, d_6-DMSO): 90.6, 149.6, 157.9, 165.4, 186.7. MS (ESI+) m/z 191.92 $[M+H]^+$. HRMS (EI) m/z: Calc. for $C_5H_3Cl_2N_3O$, 190.9659. Found 190.9661. $C_5H_3Cl_2N_3O$ requires C, 31.28; H, 1.57; N, 21.89%. Found C, 31.50; H, 1.58, N, 21.84%.

General procedure A

Sodium (1.5 eq.) was added to the appropriate alcohol (20 mL) and the resulting mixture was stirred and heated for 2 h, then 4-chloro-2,6-diaminopyrimidine (1 eq.) was added and stirring continued 5 h with heating to the specified temperature. After cooling, the mixture was neutralised with glacial acetic acid and concentrated

in vacuo to yield a yellow oil, which was diluted with water (25 mL) and extracted with EtOAc (4 × 25 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* giving the desired product.

6-Methoxypyrimidine-2,4-diamine (3a)

General procedure A. Sodium (0.38 g, 50 mmol), methanol (50 mL), 4-chloro-2,6-diaminopyrimidine (5.00 g, 34.6 mmol), at reflux for 1 h. After cooling, the mixture was neutralised with glacial acetic acid and concentrated *in vacuo* to yield a yellow oil, which was diluted with water (25 mL) and extracted with EtOAc (4 × 25 mL). The combined organic layers were dried ($NaSO_4$) and concentrated *in vacuo* giving **3a** as a white solid (4.64 g, 96%). m.p. 165.9–167.8 °C. λ_{max} (CH_3OH) 264.5 nm; IR ν_{max}/cm^{-1}: 3485, 3322, 3142, 1391, 1620, 1568, 1445. δ_H (300 MHz, d_6-DMSO) $\delta =$ 3.67 (3H, s, CH_3), 5.04 (1H, s, H^5), 5.90 (2H, br s, D_2O exch, NH_2), 6.02 (2H, br s, D_2O exch, NH_2); δ_C (75 MHz, d_6-DMSO) $\delta = 52.7$, 76.6, 163.4, 166.4, 171.0; HRMS (ESI+) *m/z*: Calc. for $C_5H_8N_4O$: 141.0771 $[M+H]^+$. Found 141.0770 $[M+H]^+$.

General procedure B. A mixture of 5-bromo-6-cyclohexylmethoxypyrimidine-2,4-diamine (**5**) (0.6 g, 1.99 mmol, 1.0 eq.), the appropriate aryl or heteroaryl boronic acid (2.39 mmol, 1.2 eq.), tetrakis(triphenylphosphine)palladium(0) (0.115 g, 0.1 mmol, 5% mol eq.), K_2CO_3 solution (4 M, 2 mL, 8 mmol, 4.0 eq.), and 1,2-dimethoxyethane (10 mL) was heated under microwave irradiation at 170 °C, for 1–2 h. The resulting mixture was filtered through Celite, washed successively with MeOH (4 × 30 mL) and DCM (4 × 30 mL). The combined fractions were concentrated *in vacuo*. To remove further palladium residues, a thiol-functionalized resin PL-Thiol SPE Tube (Stratospheres™ SPE) was employed. The SPE tube was preconditioned with methanol, then the compound sample, previously dissolved in the smallest amount possible of methanol, was loaded and allowed to pass through the SPE media under gravity and the filtrate concentrated *in vacuo.* The residues were dissolved in water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography (silica; EtOAc, petrol) followed by HPLC, Waters XTerra column, gave the desired product **7a–c** as a white solid.

6-Cyclohexylmethoxy-5-phenylpyrimidine-2,4-diamine (7a)

General procedure B. Phenyl boronic acid (0.73 g, 6.0 mmol, 3 eq.). Chromatography and HPLC, Waters XTerra column, gave **7a** as an off-white solid (0.126 g, 23%), m.p.: 103–105 °C. UV λ_{max} (EtOH): 271 and 206 nm; IR ν_{max} 3311, 3170, 3057, 2919, 2856, 1611, 1554 cm^{-1}; δ_H (300 MHz, $CDCl_3$): 0.77–1.18 (6H, m, C_6H_{11}), 1.58–1.78(5H, m, C_6H_{11}), 3.93 (2H, d, $J = 6.0$ Hz, OCH_2), 4.48 (2H, s, NH_2 ex), 4.62 (2H, s, NH_2 ex), 7.21–7.35 (5H, m, ArH); δ_C (75 MHz, $CDCl_3$) 26.1, 26.9, 30.1, 37.8, 71.4, 94.2, 127.1, 128.8, 131.1, 133.9, 161.6, 162.9, 167.9; MS (ESI+) *m/z* 299 $[M+H]^+$. $C_{17}H_{22}N_4O$: requires C, 68.43; H, 7.43; N,18.78%. Found C, 68.53; H, 7.45; N, 18.79%.

General procedure C. A mixture of 6-amino-2-(n-butylsulfanyl)-4(3H)-pyrimidinone (**15**) (1 eq.), the appropriate alcohol (1.5 eq.), and triphenylphosphine (1.5 eq.) in THF (anh) was stirred at 5 °C in an ice-bath and diethyl azodicarboxylate (1.5 eq.) was added dropwise. The resulting mixture was allowed to stir at rt for 72 h then concentrated *in vacuo* to yield a yellow oil, which was triturated with Et_2O in an ice-bath at 0 °C, giving a white precipitate which was removed by filtration. The filtrate was concentrated *in vacuo*, water (30 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography (silica; 20–80% EtOAc, petrol) and recrystallisation (MeOH) gave the desired product **16a–c**.

2-Butylsulfanyl-6-cyclohexylmethoxypyrimidin-4-amine (16a)

General procedure C. **15** (9.00 g, 45.2 mmol), cyclohexylmethanol (8.34 mL, 67.8 mmol), triphenylphosphine (17.76 g, 67.8 mmol), diethyl azodicarboxylate (10.7 mL, 67.8 mmol) gave **16a** as a white solid (10.1 g, 76%) m.p.: 81–83 °C. UV λ_{max} (EtOH): 257 nm; IR ν_{max} 3438, 3283, 3167, 2961, 2929, 1631, 1573, 1542, 1347, 1243, 1019 cm^{-1}. δ_H (300 MHz, $CDCl_3$): 0.93 (3H, t, $J =$ 7.3 Hz, $CH_3CH_2CH_2CH_2S$), 1.02–1.32 (7H, m, 5H C_6H_{11} + 2H $CH_3CH_2CH_2CH_2S$), 1.38–1.51 (2H, m, $CH_3CH_2CH_2CH_2S$), 1.65–1.80 (6H, m, C_6H_{11}), 3.06 (2H, t, $J = 7.1$ Hz, $CH_3CH_2CH_2CH_2S$), 4.07 (2H, d, $J = 6.3$ Hz, OCH_2), 4.75 (2H, br, NH_2 ex), 5.44 (1H, s, H-5); δ_C (75 MHz, $CDCl_3$): 13.9, 22.4, 26.1, 26.8, 30.2, 30.8, 32.2, 37.9, 71.7, 83.4, 164.5, 170.5, 171.3. MS (ESI+) *m/z* 296.35 $[M+H]^+$; HRMS (ESI+) *m/z*: Calc. for $C_{15}H_{25}N_3OS$: 296.1791 $[M+H]^+$. Found 296.1792 $[M+H]^+$.

General procedure D. To a solution of the appropriate 2-(n-butylsulfanyl)-6-alkoxyoxypyrimidin-4-amine (**16a–c**) (1 eq.) in DCM under N_2, *m*-CPBA (2.5–3 eq.) was added slowly. The resulting mixture was stirred at rt for 24 h, then concentrated *in vacuo*. The residue was extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with aq. sodium sulfite (sat.; 30 mL), aq. sodium bicarbonate (sat.; 2 × 30 mL) and water (2 × 30 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid. Chromatography (silica; 20–80% EtOAc, petrol) gave **17a–c** as a white solid.

2-Butylsulfonyl-6-(cyclohexylmethoxy)pyrimidin-4-amine (17a)

General procedure D. **16a** (8.0 g, 27.1 mmol), DCM (150 mL), *m*-CPBA (18.7 g, 0.11 mol). Recrystallisation (EtOAc, petrol) gave **17a** as a white solid (7.18 g, 81%), m.p.: 142–144 °C. UV λ_{max} (EtOH): 279 nm; IR ν_{max}/cm^{-1}: 3416, 3323, 3121, 2962, 2927, 2880, 1636, 1595, 1533, 1460, 1379, 1231; δ_H (300 MHz, $CDCl_3$): 0.93 (3H, t, $J = 7.3$ Hz, $CH_3CH_2CH_2CH_2SO_2$), 1.01–1.27 (7H, m, 12H C_6H_{11} and $CH_3CH_2CH_2CH_2SO_2$), 1.39–1.52 (2H, m, $CH_3CH_2CH_2CH_2SO_2$), 1.71–1.83 (2H, m, C_6H_{11}), 3.39 (2H, t, $J =$ 7.8 Hz, $CH_3CH_2CH_2CH_2SO_2$), 4.08 (2H, d, $J = 6.1$ Hz, OCH_2), 5.80 (2H, br, NH_2, ex), 5.90 (1H, s, H-5). δ_C (75 MHz, $CDCl_3$): 13.7, 22.1, 24.5, 26.0, 26.7, 30.1, 37.8, 51.2, 72.9, 89.1, 165.2, 165.6, 171.4; MS (ESI+) *m/z* 328.36 $[M+H]^+$; HRMS (ESI+) *m/z*: Calc. for $C_{15}H_{25}N_3O_3S$: 328.1689 $[M+H]^+$. Found 328.1684 $[M+H]^+$.

General procedure E. A solution of the required pyrimidine (0.50 g, 2.62 mmol, 1 eq.) and the chosen aniline (5.76 mmol, 2.2 eq.) in trifluoroethanol (10 mL) with trifluoroacetic acid (1.0 mL, 13.1 mmol, 5 eq.) was stirred for 48 h at rt or heated at reflux 24 h as specified. The solvents were removed *in vacuo*, water (30 mL) was added and the pH was adjusted to neutral with saturated aq. sodium bicarbonate and the mixture was extracted with EtOAc

(3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL) and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The compounds were purified as specified.

4-(4-Amino-6-chloro-5-formylpyrimidin-2-ylamino)benzenesulfonamide (21a)

General procedure E. 4-amino-2,6-dichloro-5-formylpyrimidine (**20**) (0.5 g, 2.62 mmol), sulfanilamide (0.54 g, 3.13 mmol), trifluoroacetic acid (1.0 mL, 13.1 mmol) and trifluoroethanol (10 mL). Recrystallisation (methanol) gave **21a** as a yellow solid (0.63 g, 74%) m.p.: 244 –246 °C. UV λ_{max} (EtOH): 332 and 262 nm. IR ν_{max}/cm^{-1}: 3325, 3194, 3155, 2852, 1713, 1574, 1503, 1342, 1163, 1061; δ_H (300 MHz, d_6-DMSO): 7.28 (2H, s, NH_2 exchangeable with D_2O), 7.74 (2H, d, J = 8.2 Hz, ArH(2,6)), 8.01 (2H, d, J = 8.2 Hz, ArH(3,5)), 8.51 (1H, br, NH exchangeable with D_2O), 8.64 (1H, br, NH exchangeable with D_2O), 10.04 (1H, s, CHO), 10.58 (1H, br, NH exchangeable with D_2O). δ_C (75 MHz, d_6-DMSO): 102.8, 120.2, 126.7, 138.6, 142.4, 159.0, 163.7, 165.0, 188.3. MS (ESI+) m/z 328.10 $[M+H]^+$. HRMS (ESI+) m/z: Calc for $C_{11}H_{10}ClN_5O_3S$: 328.0266 $[M+H]^+$. Found 328.0264 $[M+H]^+$. $C_{11}H_{10}ClN_5O_3S{\cdot}0.1CH_2Cl_2$: requires C, 39.65; H, 3.06; N, 20.83%. Found C, 39.58; H, 2.98; N, 20.95%.

General procedure F. The chosen alcohol (10 eq.) was added dropwise to a suspension of sodium hydride (5 eq.) in THF (anh; 5 mL), and the mixture was stirred at rt under N_2 for 30 min. The chosen 4-amino-6-chloro-2-(arylamino)-5-formylpyrimidine (1 eq.) was added slowly, in small portions and the reaction mixture was heated at reflux 16 h, then allowed to cool to rt, quenched with water (10 mL), and acidified (2 M HCl) to pH < 2, then THF (10 mL) was added, the resulting mixture was stirred overnight, neutralised (saturated aq. sodium bicarbonate), and concentrated *in vacuo*. The residue was extracted with EtOAc (4 × 30 mL) and the combined organic layers were dried (Na_2SO_4), and concentrated *in vacuo* to give a yellow–orange oil. Chromatography (silica; 40–100% EtOAc, petrol, or 10% MeOH, DCM) and recrystallisation (methanol) or HPLC purification, gave the product as a white solid.

4-Amino-6-cyclohexylmethoxy-2-(4-methoxyphenylamino)-5-formylpyrimidine (22a)

General procedure F. 4-amino-6-chloro-2-(4-methoxyphenylamino)-5-formylpyrimidine (**21b**) (0.20 g, 0.72 mmol), cyclohexylmethanol (0.88 mL, 7.2 mmol). Chromatography (silica; 40–100% EtOAc, Petrol) and recrystallisation (methanol) gave **22a** as an off-white solid (0.159 g, 62%) m.p. 94–97 °C. UV λ_{max} (EtOH): 325 nm. IR ν_{max}/cm^{-1}: 3311, 3070, 2920, 1663, 1550, 1491, 1236. δ_H (300 MHz, d_4-MeOD): 0.81–1.32 (5H, m, C_6H_{11}), 1.64–1.78 (6H, m, C_6H_{11}), 3.72 (3H, s, OCH_3), 4.15 (2H, d, J = 6.0 Hz, OCH_2), 6.81 (2H, d, J = 8.9 Hz, ArH(3,5)), 7.48 (2H, d, J = 8.9 Hz, ArH(2,6)), 9.83 (1H, s, CHO); δ_C (75 MHz, d_4-MeOD): 26.8, 27.6, 30.9, 38.8, 56.1, 72.9, 94.3, 115.1, 124.3, 133.6, 157.8, 162.7, 166.3, 173.6, 187.3. MS (ESI+) m/z 357.33 $[M+H]^+$. HRMS (ESI+) m/z: Calc. for $C_{19}H_{24}N_4O_3$: 357.1921 $[M+H]^+$. Found 357.1922 $[M+H]^+$. $C_{19}H_{24}N_4O_3{\cdot}0.4$ M MeOH requires C, 63.02; H, 6.96; N, 15.23%; found C, 63.49; H, 6.46, N, 14.78%.

rac-4-(4-Amino-6-*sec*-butoxy-5-formylpyrimidin-2-ylamino)benzenesulfonamide (22h)

General procedure F. 4-(4-amino-6-chloro-5-formylpyrimidin-2-ylamino)benzenesulfonamide (**21a**) (0.40 g, 1.22 mmol), *sec*-butanol (2.3 mL, 24.5 mmol). Chromatography (silica; 2–15% MeOH, DCM) and HPLC, Phenomenex Synergi (35–100% MeCN, water) gave **22h** as a white solid (97 mg, 11%), m.p. 265–267 °C. UV λ_{max} (EtOH): 325 nm; IR ν_{max} 3278, 3215, 2923, 2852, 1645, 1564, 1321, 1149 cm^{-1}; δ_H (300 MHz, d_4-MeOD) 1.00 (3H, t, J = 7.5 Hz, $CH_3CHORCH_2C\mathbf{H}_3$), 1.38 (3H, d, J = 6.2 Hz, $C\mathbf{H}_3CHORCH_2CH_3$), 1.65–1.86 (2H, m, $CH_3CHORC\mathbf{H}_2CH_3$), 5.30–5.41 (1H, m, $CH_3C\mathbf{H}ORCH_2CH_3$), 7.82 (2H, d, J = 8.9 Hz, ArH(2,6)), 7.95 (2H, d, J = 8.9 Hz, ArH(3,5)), 9.97 (1H, s, CHO); δ_C (75 MHz, d_4-MeOD): 10.1, 19.8, 30.6, 75.9, 96.1, 120.6, 128.0, 138.1, 144.7, 156.7, 162.1, 170.4, 188.0. MS (ESI+) m/z 366.31 $[M+H]^+$; HRMS (ESI+) m/z: Calc. for $C_{15}H_{19}N_5O_4S$: 366.1231 $[M+H]^+$. Found 366.1237 $[M+H]^+$.

(*S*)-4-(4-Amino-6-*sec*-butoxy-5-formylpyrimidin-2-ylamino)benzenesulfonamide (22j)

General procedure F. 4-(4-amino-6-chloro-5-formylpyrimidin-2-ylamino)benzenesulfonamide (**21a**) (0.40 g, 1.22 mmol), (*S*)-*sec*-butanol (1.1 mL, 12.2 mmol). Chromatography (silica; 2–15% MeOH–DCM) and HPLC, Phenomenex Synergi (35–100% MeCN, water), gave **22j** as a white solid (31 mg, 7%), m.p.: 269–270 °C. UV λ_{max} (EtOH): 319 nm; IR ν_{max} 3275, 3230, 2959, 2924, 2861, 1650, 1558, 1377, 1156 cm^{-1}; δ_H (300 MHz, d_3-MeCN) 0.74 (3H, t, J = 7.4 Hz, $CH_3CHORCH_2C\mathbf{H}_3$), 1.13 (3H, d, J = 6.2 Hz, $C\mathbf{H}_3CHORCH_2CH_3$), 1.39–1.61 (2H, m, $CH_3CHORC\mathbf{H}_2CH_3$), 5.01–5.09 (1H, m, $CH_3C\mathbf{H}ORCH_2CH_3$), 5.36 (2H, s, NH_2), 6.06 (1H, br, NH), 7.56 (2H, d, J = 8.9 Hz, ArH(2,6)), 7.68 (2H, d, J = 8.9 Hz, ArH(3,5)), 7.97 (1H, br, NH), 8.34 (1H, s, NH), 9.80 (1H, s, CHO); δ_C (75 MHz, d_4-MeOD) 10.2, 20.0, 30.4, 76.4, 90.4, 121.3, 128.3, 137.0, 144.7, 155.9, 162.5, 173.2, 188.4. MS (ESI+) m/z 366.24 $[M+H]^+$. HRMS (ESI+) m/z: Calc. for $C_{15}H_{19}N_5O_4S$: 366.1231 $[M+H]^+$. Found 366.1236 $[M+H]^+$.

General procedure G. The appropriate O^4-substituted pyrimidine (1 eq.) was dissolved in 30% aq. acetic acid (1 ml–4 ml) at rt. The temperature was increased to 80 °C and sodium nitrite (1.3 eq.) in water (1 ml–3 ml) was added dropwise to give a purple precipitate. The reaction was maintained at 80 °C for 40 min, allowed to cool to rt and the precipitate was collected by filtration. The crude product was purified by recrystallisation from the appropriate solvent.

6-Methoxy-5-nitrosopyrimidine-2,4-diamine (4a)

General procedure G. 6-methoxypyrimidine-2,6-diamine (**3a**)(0.25 g, 1.78 mmol), 30% aq. acetic acid (5 mL), sodium nitrite (160 mg, 2.32 mmol) in water (2 mL). The purple precipitate was filtered off and dried (P_2O_5) giving **4a**. (246 mg, 82%). m.p. 249.2–251.8 °C; UV (EtOH): λ_{max1} = 330 nm; IR ν_{max} 3508, 3282, 1566, 1492, 1441, 1372, 1311 cm^{-1}; δ_H (300 MHz, d^6-DMSO) δ 4.05 (3H, s, CH_3), 7.81 (1H, br s, NH_2), 7.87 (1H, br s, NH_2), 8.03 (1H, br s, NH_2), 10.07 (1H, br s, NH_2); HRMS (ESI+) m/z: Calc. for $C_5H_7N_5O_2$: 141.0771 $[M+H]^+$. Found 141.0770 $[M+H]^+$.

General procedure H. To a solution of formylpyrimidine (**22**)(0.17 mmol) in EtOH (anh; 3 mL) was added hydroxylamine hydrochloride (0.014 g, 0.202 mmol), followed by pyridine (anh., 0.016 mL, 0.202 mmol) and the mixture was stirred 16 h at rt under N_2, then concentrated *in vacuo*. The residues were diluted with water (30 mL) and extracted with EtOAc (3 × 40 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*.

4-Amino-6-(cyclohexylmethoxy)-2-(4-methoxyphenylamino)-pyrimidine-5-carbaldehyde oxime (23a)

General procedure H. 4-amino-6-cyclohexylmethoxy-2-(4-methoxyphenylamino)-5-formylpyrimidine (**22a**) (0.06 g, 0.17 mmol). Recrystallisation (EtOAc, petrol) gave **23a** as a white solid (0.047 g, 75%), m.p. 158–160 °C. UV λ_{max} (EtOH): 310 and 219 nm. IR ν_{max} 3481, 3343, 3145, 2922, 1580, 1497, 1238, 952 cm^{-1}; δ_H (300 MHz, $CDCl_3$): 0.96–1.34 (6H, m, C_6H_{11}), 1.68–1.82 (5H, m, C_6H_{11}), 3.79 (3H, s, OCH_3), 4.12 (2H, d, J = 6.1 Hz, OCH_2), 6.85 (2H, d, J = 9.0 Hz, ArH(3,5)), 7.07 (1H, br, NH), 7.44 (2H, d, J = 9.0 Hz, ArH(2,6)), 7.64 (1H, br, NH), 8.47 (1H, s, C**H**NOH), 10.02 (1H, s, CHNO**H**); δ_C (75 MHz, $CDCl_3$): 26.1, 26.8, 29.9, 37.8, 55.9, 72.1, 85.7, 114.5, 122.3, 133.1, 146.9, 156.2, 159.1, 162.5, 168.9; MS (ESI+) m/z 372.40 $[M+H]^+$; HRMS (ESI+) m/z: Calc. for $C_{19}H_{25}N_5O_3$: 372.2030 $[M+H]^+$. Found 372.2034 $[M+H]^+$.

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