Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 2741–2752
Bioorganic & Medicinal Chemistry

A new generation of adenosine receptor antagonists: From di- to trisubstituted aminopyrimidines

Jacobus P. D. van Veldhoven, Lisa C. W. Chang, Jacobien K. von Frijtag Drabbe Künzel, Thea Mulder-Krieger, Regina Struensee-Link, Margot W. Beukers, Johannes Brussee and Adriaan P. IJzerman*

Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, PO Box 9502, 2300 RA Leiden, The Netherlands

Received 28 September 2007; revised 3 January 2008; accepted 8 January 2008
Available online 12 January 2008

Abstract—New adenosine receptor ligands were designed as hybrid structures between previously synthesized substituted dicyanopyridines and aminopyrimidines, yielding two series of cyano-substituted diphenylaminopyrimidines. We were interested in assessing the effect of this substitution pattern on both affinity and intrinsic activity, as the dicyanopyridines comprised both agonists and inverse agonists, whereas the original aminopyrimidines were exclusively inverse agonists. It was found that the new compounds were generally selective for adenosine A_1 receptors, although affinity for the adenosine A_{2A} receptor was also noticed for some of the compounds. In a cAMP second messenger assay the compounds behaved as inverse agonists rather than agonists. Among the more A_1 receptor-selective compounds were **5** (LUF6048), **27** (LUF6040) and **53** (LUF6056) with K_i values of 8.1, 1.2 and 5.7 nM, respectively.

1. Introduction

Adenosine receptors belong to the large super family of G protein-coupled receptors. Four subtypes have been cloned and characterized, A_1, A_{2A}, A_{2B} and A_3. Their individual physiological roles and pharmacology have been extensively reviewed.[1] Traditionally, agonists for adenosine receptors were derivatives of adenosine, and antagonists were often xanthines, such as caffeine, theophylline and structural analogues. This simple scenario has changed dramatically, most particularly for the adenosine A_1 receptor. For instance, many different classes of adenosine A_1 receptor antagonists have now been designed, synthesized and tested. These include all kinds of different mono-, bi-, tri- and even quadricyclic mostly nitrogen containing aromatic compounds.[2] Among our own efforts were two classes of substituted pyrimidines, exemplified as **A** in Figure 1.[3] Very interestingly, also agonists have greater structural diversity than originally anticipated. The ribose group in adenosine, long thought to be mandatory for agonistic activity, was shown to be absent in a series of 2-aminopyridine-

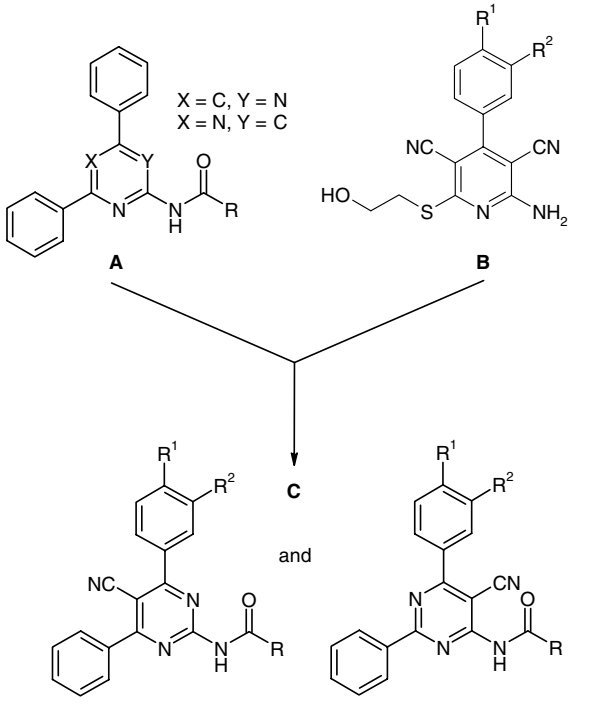


Figure 1. Rationale for the design of the di- and trisubstituted aminopyrimidines (compound **C**). **A**: diphenyl substituted aminopyrimidines as adenosine receptor antagonists;[3] **B**: general structure of non-ribose agonists for the adenosine receptors.[4,5]

Keywords: Adenosine; Adenosine A_1 receptor; Inverse agonist; Cyanopyrimidines.
* Corresponding author. Tel.: +31 0 71 527 4651; fax: +31 0 71 527 4565; e-mail: ijzerman@lacdr.leidenuniv.nl

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.01.013

3,5-dicarbonitriles. Nevertheless, Rosentreter et al. in a series of patents[4] disclosed these compounds to be agonists for adenosine receptors. On these findings we elaborated and synthesized analogous compounds,[5] indeed confirming the agonistic activity on adenosine receptors of some of these derivatives (**B** in Fig. 1). In particular, LUF5853, in which R^1 and R^2 combined into a dioxymethylene bridge, was a potent and selective full agonist for the adenosine A_1 receptor (K_i hA_1 = 11 nM).

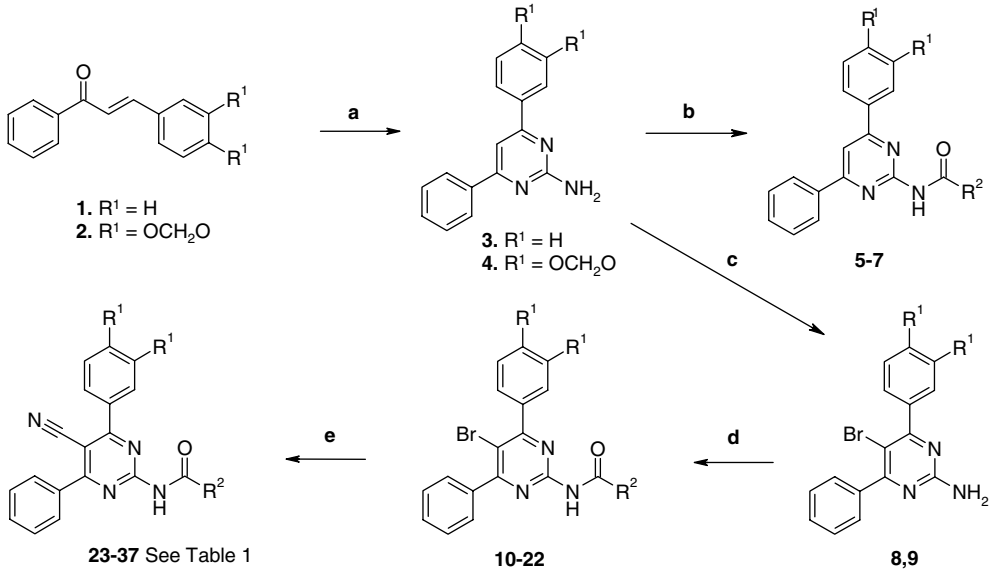
In the present study, hybrid structures of **A** and **B** were designed, predominantly to explore the role of (i) cyano substitution at the pyrimidine core structure and (ii) the introduction of a dioxymethylene bridge on one of the two phenyl rings (compound **C**). We were interested in assessing the effect of this substitution pattern on both affinity and intrinsic activity. It was learned that such compounds were generally selective for adenosine A_1 receptors, although affinity for the adenosine A_{2A} receptor was also noticed for some of the compounds. In a cAMP second messenger assay the compounds behaved as inverse agonists rather than agonists.

2. Results and discussion

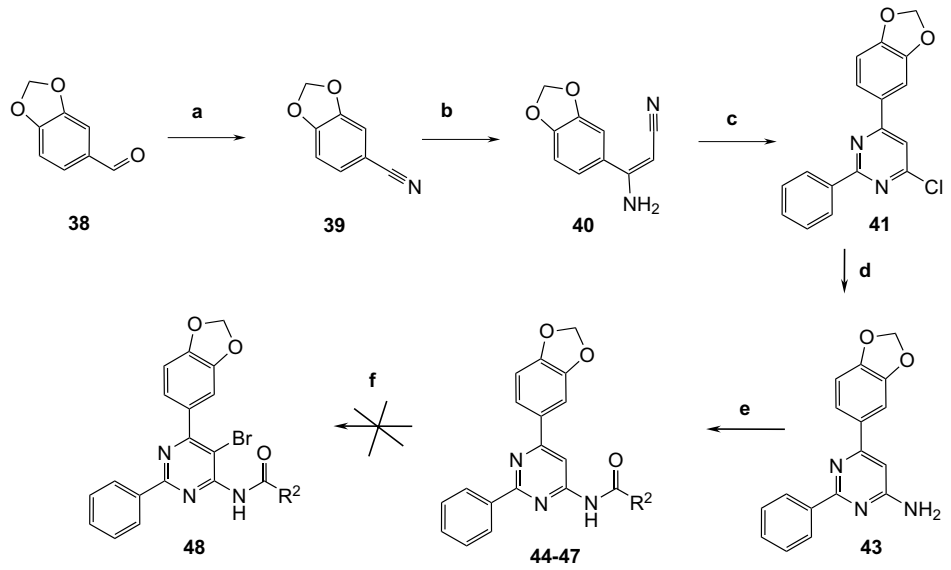
2.1. Chemistry

Compounds **3**–**7** and **23**–**37** were synthesized according to Scheme 1. Chalcone **2** was prepared via an aldol condensation in the presence of NaOH[6] while **1** was commercially available. Guanidine was freed from its hydrochloride salt form with aqueous NaOH and reacted with the appropriate chalcone according to a method described by Al-Hajjar and Sabri.[3,7] The primary amines **3** and **4** were obtained in 29–32% yield. Acid chlorides were reacted with the 2-aminopyrimidines (**3** and **4**) in pyridine to give the desired 2-amidopyrimidines **5**–**7** in 33–73% yield.[8]

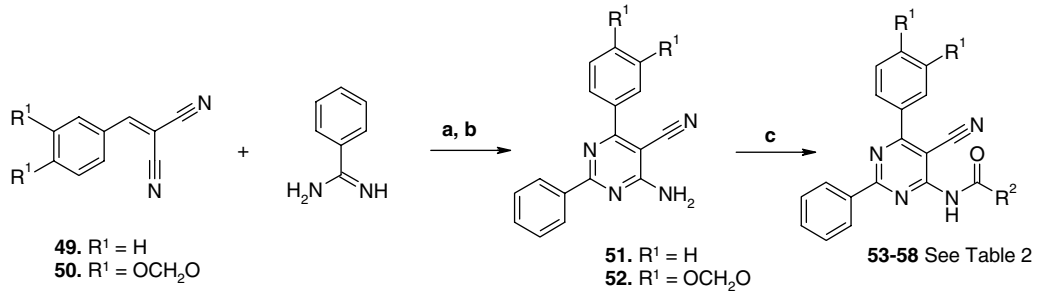
Bromination was done at the 5-position of the 2-aminopyrimidine with Br_2 in the presence of $CaCO_3$ in $CHCl_3$ (**8** and **9**),[9] followed by reaction with acid chlorides in pyridine at room temperature to obtain the corresponding amides (**10**–**22**) in modest to good yields (20–92% yield).[8] The carbonitrile was introduced by substitution of the bromine with CuCN in pyridine in a sealed tube at 250 °C under microwave conditions.[10] This final step gave the 2-amido-5-cyanopyrimidines (**23**–**37**) in 17–66% yield. Compounds **43**–**47** were synthesized according to Scheme 2. The amine **42**, the unsubstituted analogue of compound **43**, was previously described by Chang[3] starting from ethyl benzoylacetate and benzamidine. To synthesize its methylenedioxy analogue **43**, we chose a new pathway starting from piperonal. Nucleophilic addition of hydroxylamine to piperonal (**38**) gave the arylnitrile (**39**) in high yield via dehydration of the oxime intermediate.[11] The enaminonitrile (**40**) was obtained in high yield by reaction of acetonitrile with **39** in the presence of potassium *tert*-butoxide.[12] The pyrimidine ring was created by refluxing **40** and *N*,*N*-dimethylbenzamide in phosphorous oxychloride.[13] Subsequent substitution with ammonia in a sealed vessel gave the aminopyrimidine **43** in a good yield.[3] Pyrimidine **43** was reacted at room temperature with several acid chlorides and gave good yields of the screening compounds **44**–**47**. Unexpectedly the subsequent bromination of **44**–**47** failed with various methods. Analogous to Scheme 1, bromination of **43** followed by microwave-assisted amide formation at 100 °C failed. Hence the procedure of Scheme 2 was not suitable for the synthesis of the cyano products **51**–**58**; these compounds were synthesized according to Scheme 3. Functionalised malononitrile[5] was refluxed in methanol in the presence of benzamidine hydrochloride and K_2CO_3 to yield only 6% of **51**. The yield was improved by an additional oxidation with $KMnO_4$ to oxidize the dihydropyrimidine-intermediate resulting in a 51% yield of the aminopyrimidine–carbonitrile



Scheme 1. Synthetic route to 2-amido-4,6-disubstituted-pyrimidine-5-carbonitriles. Reagents and conditions: (a) guanidine·HCl, NaOH, H_2O, ethanol, 2 h, 130 °C, MW; (b) RCOCl, pyridine; (c) Br_2, $CaCO_3$, $CHCl_3$; (d) RCOCl, pyridine; (e) CuCN, pyridine, 20 min, 250 °C, MW.



Scheme 2. Synthetic route to 4-amido-2,6-disubstituted-pyrimidines. Reagents and conditions: (a) hydroxylamine·HCl, *N*-methylpyrrolidone, 115 °C, 4 h; (b) acetonitrile, *t*-BuOK, benzene, 20 h; (c) *N*,*N*-dimethylbenzamide, $POCl_3$, 120 °C, 1 h; (d) NH_3, ethanol, sealed vessel, 20 h; (e) RCOCl, pyridine, 100 °C, 1 h; (f) Br_2, $CaCO_3$, $CHCl_3$, reflux or Br_2, AcOH, 100 °C.



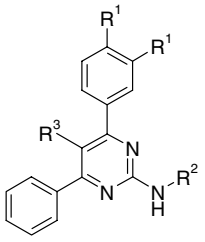
Scheme 3. Synthetic route to 4-amido-2,6-disubstituted-pyrimidine-5-carbonitriles. Reagents and conditions: (a) K_2CO_3, MeOH, reflux, 2 h; (b) $KMnO_4$, acetone, 15 min; (c) RCOCl, pyridine, MW.

51.[14,15] The reaction of **51** and **52** with aliphatic acid chlorides gave only low yields of **53**–**58** and required heating in the microwave synthesizer,[8] whereas aromatic acid chlorides and *tert*-butyryl chloride failed.

2.2. Structure–activity relationships

The two series of pyrimidine derivatives were tested in radioligand binding and second messenger assays, and the results of these experiments are presented in Tables 1 and 2. The unsubstituted aminodiphenylpyrimidine **3** showed appreciable affinity for the adenosine A_1 receptor already, only 2- to 3-fold lower than the standard reference ligands CPA (agonist) and DPCPX (antagonist/inverse agonist). The same held true for position isomer **42**[3] in Table 2. Both compounds also shared a modest selectivity for the A_1 receptor when compared to the A_{2A} receptor. Substitution of one of the phenyl groups with a dioxymethylene moiety (**4** and **43**) did not affect the pharmacological profile very much. Additional substitution of the free amine group to yield aliphatic carboxamides (**5**–**7** and **44**–**47**) generally decreased A_{2A} receptor affinity, except for **47**. The most selective compound in this comparison was **5** with an affinity of 8.1 nM for the adenosine A_1 receptor. As the major focus of this study the introduction of a cyano group on the 5-position in both series (**23**–**37** and **51**–**58**) often led to an increase in both A_1 and A_{2A} receptor affinity, while maintaining the low or negligible affinity for the adenosine A_3 receptor. Fine examples are **23** and **51** when compared to **3** and **42**, respectively. A slight increase in A_1 receptor affinity was paralleled by a more substantial gain in A_{2A} receptor affinity, rendering **23** and **51** less selective overall. Further substitution of **23** and **51** with again the dioxymethylene moiety on one of the phenyl groups, yielding **24** (K_i = 1.8 nM) and **52** (K_i = 4.9 nM), respectively, improved A_1 receptor affinity somewhat without affecting A_{2A} receptor affinity substantially. Substitution of the free amino group of **24** and **52** yielded carboxamido compounds **25**, **26** and **53**–**55**. The latter three compounds showed a remarkable decrease in A_{2A} receptor affinity, while having also little affinity for the adenosine A_3 receptor. As a result cyclopentyl derivative **53** in particular was very selective (>175-fold) for the adenosine A_1 receptor with a K_i value of 5.7 nM. We then returned to the diphenyl substituted pyrimidines, that is, without the dioxymethylene moiety but with the cyano substituent,

Table 1. Affinities of the substituted-pyrimidines **3**–**7** and **23**–**37** in radioligand binding assays of human adenosine receptors



Compound	R^1	R^2	R^3	K_i (nM) or % displacement[a]			% Change in cAMP[e]
				hA_1[b]	hA_{2A}[c]	hA_3[d]	
CPA	—	—	—	10 ± 1.3	1652 ± 344	281 ± 56	51 ± 1
DPCPX	—	—	—	6.1 ± 1.6	129	1700 ± 170	133 ± 1
3	H	H	H	17.7 ± 4.6	405 ± 183	34%	123 ± 8
4	OCH_2O	H	H	34.8 ± 14.1	381 ± 62	48%	118 ± 11
5 LUF6048	OCH_2O	CO-*c*-Pent	H	8.1 ± 1.8	26%	52%	121 ± 3
6	OCH_2O	CO-Pr	H	43.0 ± 15.0	8%	25%	125 ± 3
7	OCH_2O	CO-1-EtPr	H	17.6 ± 8.7	33%	46%	125 ± 5
23	H	H	CN	3.3 ± 1.0	22.7 ± 2.3	8%	123 ± 11
24	OCH_2O	H	CN	1.8 ± 0.1	5.2 ± 0.2	46%	120 ± 5
25	OCH_2O	CO-*c*-Pent	CN	2.8 ± 1.3	27.2 ± 5.8	26%	138 ± 6
26	OCH_2O	CO-Pr	CN	8.3 ± 0.2	21.3 ± 5.2	21%	146 ± 14
27 LUF6040	H	CO-*c*-Pent	CN	1.2 ± 0.3	159 ± 30	26%	121 ± 5
28	H	CO-Pr	CN	2.9 ± 0.3	48.2 ± 6.8	31%	132 ± 3
29	H	CO-1-EtPr	CN	6.4 ± 0.3	54.5 ± 9.4	31%	128 ± 6
30	H	CO-*i*-Pr	CN	2.9 ± 1.0	35.3 ± 12.7	43%	134 ± 4
31	H	CO-*c*-Pr	CN	9.4 ± 3.1	45.5 ± 8.2	45%	121 ± 11
32	H	CO-4-Cl-Ph	CN	92.3 ± 38.2	32.9 ± 19.2	21%	116 ± 10
33	H	CO-2-furan	CN	26%	42%	0%	—
34	H	CO-4-MeO-Ph	CN	102 ± 3.4	300 ± 67	13%	114 ± 6
35	H	CO-4-Me-Ph	CN	115 ± 85	158 ± 25	4%	110 ± 3
36	H	CO-Ph	CN	38%	48%	6%	—
37	H	CO-3,4-diCl-Ph	CN	58%	35%	0%	—

[a] K_i ± SEM (n = 3), % displacement (n = 2).

[b] Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations.

[c] Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations.

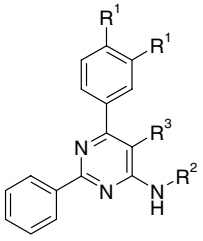
[d] Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.

[e] % Change of cAMP production ± SEM (n = 3) in CHO cell membranes expressing human adenosine A_1 receptors, with 0% as basal cAMP production in the absence of any ligand, 100% representing forskolin-stimulated (10 μM) cAMP production. The forskolin-induced cAMP production was inhibited by CPA (full agonist, reduction from 100% to 51%) and further stimulated by DPCPX (full inverse agonist, increase from 100% to 133%). All compounds were tested at 100× their K_i value in the presence of forskolin, as was the case for CPA and DPCPX (see text).

yielding compounds **27**–**37** (Table 1) and **56**–**58** (Table 2). Various substitution patterns on the amido group were explored. Either introduction of 2-furan (**33**) or aromatic substitution according to Topliss[16] (**32**, **34**–**37**) was not favored by any of the three receptor subtypes. Alkyl, branched alkyl or cycloalkyl substitution was better accommodated by the 5-cyanoamidopyrimidines, as is evident from the radioligand binding data for **27**–**31** and **56**–**58**. Again cyclopentyl substitution appeared favorable, compound **27** displaying nanomolar affinity for the adenosine A_1 receptor (K_i = 1.2 nM) with 132-fold (A_{2A}) and >833-fold (A_3) selectivity. Similarly, although less prominent, **56** was a potent and selective A_1 receptor ligand.

The recent discoveries of non-ribose agonists[4,5] required our attention to also focus on performing functional assays at the adenosine A_1 receptor with these compounds. In fact the current two series of compounds were designed as hybrids between these non-ribose agonists and pyrimidine antagonists as detailed in Figure 1. Production of cAMP was determined in the same cells as used for the preparation of membranes for our radioligand binding studies on the A_1 receptor. In these cells the reference agonist N^6-cyclopentyladenosine (CPA) inhibited the (forskolin-stimulated) cAMP production by almost 50% (Tables 1 and 2). In contrast, the reference inverse agonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) further increased the forskolin-induced cAMP production by approximately 30%. When we tested all compounds in this setup at a single concentration of 100× their K_i value (to ensure full receptor occupancy), we learned that all compounds resembled DPCPX much more than CPA. In all cases a further enhancement of cAMP production was observed compared to the cAMP concentrations in the presence of

Table 2. Affinities of the substituted-pyrimidines **42**–**58** in radioligand binding assays of human adenosine receptors



Compound	R^1	R^2	R^3	K_i (nM) or % displacement[a]			% Change in cAMP[e]
				hA_1[b]	hA_{2A}[c]	hA_3[d]	
CPA	—	—	—	10 ± 1.3	1652 ± 344	281 ± 56	51 ± 1
DPCPX	—	—	—	6.1 ± 1.6	129	1700 ± 170	133 ± 1
42	H	H	H	25.2 ± 12.3	169 ± 15	42%	123 ± 4
43	OCH_2O	H	H	15.6 ± 5.7	557 ± 92	15%	131 ± 9
44	OCH_2O	CO-*c*-Pent	H	17.3 ± 5.6	339 ± 31	186 ± 25	136 ± 7
45	OCH_2O	CO-Pr	H	6.8 ± 2.6	222 ± 58	53.9 ± 2.4	135 ± 6
46	OCH_2O	CO-1-EtPr	H	27.9 ± 11.6	574 ± 49	634 ± 34	139 ± 14
47	OCH_2O	CO-*c*-Pr	H	13.0 ± 6.8	69.2 ± 12.7	30.7 ± 2.7	142 ± 16
51	H	H	CN	10.8 ± 6.0	19.0 ± 3.6	58%	122 ± 8
52	OCH_2O	H	CN	4.9 ± 1.6	44.3 ± 2.5	50%	128 ± 5
53 LUF 6056	OCH_2O	CO-*c*-Pent	CN	5.7 ± 1.6	14%	27%	126 ± 4
54	OCH_2O	CO-Pr	CN	13.4 ± 6.1	23%	50%	131 ± 4
55	OCH_2O	CO-1-EtPr	CN	18.4 ± 0.6	0%	6%	140 ± 7
56	H	CO-*c*-Pent	CN	5.3 ± 2.5	280 ± 35	22%	131 ± 9
57	H	CO-Pr	CN	12.6 ± 1.3	69.5 ± 7.2	38%	125 ± 12
58	H	CO-*c*-Pr	CN	27.9 ± 9.3	49.8 ± 2.6	27%	125 ± 11

[a] K_i ± SEM (n = 3), % displacement (n = 2).

[b] Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations.

[c] Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations.

[d] Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.

[e] % Change of cAMP production ± SEM (n = 3) in CHO cell membranes expressing human adenosine A_1 receptors, with 0% as basal cAMP production in the absence of any ligand, 100% representing forskolin-stimulated (10 μM) cAMP production. The forskolin-induced cAMP production was inhibited by CPA (full agonist, reduction from 100% to 51%) and further stimulated by DPCPX (full inverse agonist, increase from 100% to 133%). All compounds were tested at 100× their K_i value in the presence of forskolin, as was the case for CPA and DPCPX (see text).

forskolin only. These results suggest that the compounds are all antagonists/inverse agonists for the adenosine A_1 receptor. This claim was substantiated with a more detailed analysis of the behavior of two of the more potent compounds, **27** and **53**. Concentration–effect curves were recorded for both compounds in the presence of 10 μM forskolin and 100 nM CPA (Fig. 2), yielding IC_{50} values (n = 3) of 0.15 ± 0.02 μM for **27** and 1.4 ± 0.2 μM for **53**. This corresponds to apparent affinities (K_B-values) of approximately 3 and 28 nM, respectively, quite comparable to the K_i values of the two compounds obtained in radioligand binding studies.

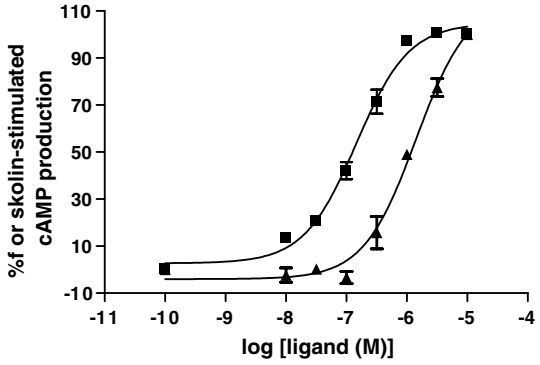


Figure 2. Concentration–effect curves for compounds **27** (■) and **53** (▲) inhibiting the CPA (100 nM)-induced decrease in forskolin (10 μM)-stimulated cAMP production in CHO cells expressing the human adenosine A_1 receptor (n = 3).

3. Conclusion

This series of pyrimidine derivatives with affinity for adenosine receptors are hybrid structures based on cyanopyridine agonists and pyrimidine antagonists/inverse agonists. All compounds synthesized were inverse agonists rather than agonists. Among the more A_1 receptor selective compounds were **5**, **27** and **53** with K_i values of 8.1, 1.2 and 5.7 nM, respectively.

4. Experimental

4.1. Chemistry

All reagents used were obtained from commercial sources and all solvents were of analytical grade. ^{1}H

and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad; ar, aromatic protons. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4% of the theoretical values unless otherwise stated. Reactions were routinely monitored on TLC using Merck silica gel F_{254} plates. Microwave reactions were performed in an Emrys Optimizer (Biotage AB, formerly Personal Chemistry). Wattage was automatically adjusted so as to maintain the desired temperature. The yields of all products were not optimized. All the final products (Tables 1 and 2) were purified by column chromatography followed by recrystallization.

4.1.1. Synthesis of 3,4-methylenedioxychalcone (2). Prepared as described in the literature.[6]

4.1.2. General procedure for the synthesis of 2-amino-4,6-disubstituted-pyrimidines.[3] To a mixture of the chalcone (4 mmol, 1 equiv) and guanidine hydrochloride (4.1 mmol, 1.05 equiv) in EtOH (3 mL) was added an aqueous solution (1 mL) of NaOH (12 mmol, 3.05 equiv). This mixture was heated at 130 °C in a sealed tube in the microwave for 2 h. The solvent was removed in vacuo and the organics were extracted with hot acetone. The crude product was purified by recrystallization from methanol or column chromatography on SiO_2, eluting with a methanol–dichloromethane solvent system. The same system was used for TLC evaluation, usually yielding R_f values in between 0.3 and 0.5.

4.1.2.1. 2-Amino-4,6-diphenyl-pyrimidine (3). Prepared as described in the literature.[3]

4.1.2.2. 2-Amino-4-benzo[1,3]dioxol-5yl-6-phenyl-pyrimidine (4).[7] Yield 32%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.08–7.99 (m, 2H, phenyl-*H*), 7.65–7.59 (m, 2H, Ar-*H*), 7.52–7.45 (m, 3H, phenyl-*H*), 7.36 (s, 1H, pyrimidinyl-*H*), 6.91 (d, 1H, Ar-*H*), 6.04 (s, 2H, –OC*H*$_2$O–), 5.13 (br s, 2H, N*H*$_2$). ^{13}C NMR δ (DMSO): 164.8, 164.2, 163.9, 149.3, 147.9, 137.5, 131.6, 130.4, 128.6, 127.0, 121.7, 108.3, 107.0, 101.6. Anal. Calcd for $C_{17}H_{13}N_3O_2 \cdot 0.2\,H_2O$: C, 69.26; H, 4.57; N, 14.25. Found: C, 69.20; H, 4.46; N, 14.49.

4.1.3. General procedure for the synthesis of 2-amido-4-benzo[1,3]dioxol-5yl-6-phenyl-pyrimidines.[8] To a solution of the 2-amino-4-benzo[1,3]dioxol-5yl-6-phenylpyrimidine (1.4 mmol, 1.0 equiv) in anhydrous pyridine (3.5 mL) was added the appropriate acid chloride (2.1 mmol, 1.5 equiv) and stirred at room temperature for 2 h, after which according to TLC no starting material was visible. The solvent was evaporated under reduced pressure and the crude product purified by column chromatography, eluting with dichloromethane. The same solvent was used for TLC evaluation, usually yielding R_f values in between 0.3 and 0.5. Recrystallization from methanol gave the corresponding amide in crystalline form.

4.1.3.1. Cyclopentane carboxylic acid (4-benzo[1,3]dioxol-5yl-6-phenyl-pyrimidin-2-yl)-amide (5) LUF 6048. Yield 51%; white solid. ^{1}H NMR δ (DMSO): 8.13–8.08 (m, 3H, phenyl-*H* + N*H*), 7.70 (dd, 1H, J^1 = 4.9 Hz, J^2 = 1.8 Hz, Ar-*H*), 7.70 (s, 1H, pyrimidinyl-*H*), 7.64 (d, 1H, J = 1.5 Hz, Ar-*H*), 7.55–7.50 (m, 3H, phenyl-*H*), 6.93 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.06 (s, 2H, –OC*H2*O–), 3.54–3.47 (m, 1H, C*H*-cyclopentyl), 2.04–1.58 (m, 8H, C*H*$_2$-cyclopentyl). ^{13}C NMR δ ($CDCl_3$): 176.1, 165.5, 165.0, 157.5, 149.9, 148.2, 136.5, 130.7, 128.6, 126.9, 121.7, 108.2, 107.1, 106.4, 101.4, 45.6, 29.9, 25.8. Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.24; H, 5.61; N, 11.21.

4.1.3.2. *N*-(4-Benzo[1,3]dioxol-5yl-6-phenyl-pyrimidin-2-yl)-butyramide (6). Yield 33%; off-white solid. ^{1}H NMR δ ($CDCl_3$): 8.14–8.08 (m, 2H, phenyl-*H*), 8.04 (br s, 1H, N*H*), 7.70 (dd, 1H, J^1 = 6.4 Hz, J^2 = 1.8 Hz, Ar-*H*), 7.70 (s, 1H, pyrimidinyl-*H*), 7.64 (d, 1H, J = 1.8 Hz, Ar-*H*), 7.56–7.50 (m, 3H, phenyl-*H*), 6.94 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.07 (s, 2H, –OC*H*$_2$O–), 3.00 (t, 2H, J = 8.9 Hz, –C*H*$_2$CH$_2$CH$_3$), 1.94–1.75 (m, 2H, –CH$_2$C*H*$_2$CH$_3$), 1.07 (t, 3H, J = 7.3 Hz, –CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 173.7, 165.6, 165.1, 157.5, 150.1, 148.2, 136.5, 130.8, 128.6, 126.9, 121.7, 108.3, 107.1, 106.4, 101.5, 39.1, 18.1, 13.7. Anal. Calcd for $C_{21}H_{19}N_3O_3$: C, 69.79; H, 5.30; N, 11.63. Found: C, 70.16; H, 5.43; N, 12.01.

4.1.3.3. *N*-(4-Benzo[1,3]dioxol-5yl-6-phenyl-pyrimidin-2-yl)-2-ethyl-butyramide (7). Yield 73%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.15–8.10 (m, 2H, phenyl-*H*), 8.08 (br s, 1H, N*H*), 7.78–7.70 (m, 2H, Ar-*H* + pyrimidinyl-*H*), 7.67 (d, 1H, J = 1.8 Hz, Ar-*H*), 7.55–7.50 (m, 3H, phenyl-*H*), 6.94 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.06 (s, 2H, –OC*H*$_2$O–), 3.07–2.84 (m, 1H, –C*H*(CH$_2$CH$_3$)$_2$), 1.96–1.55 (m, 4H, –CH(C*H*$_2$CH$_3$)$_2$), 1.00 (t, 6H, J = 7.3 Hz, –CH(CH$_2$C*H*$_3$)$_2$). ^{13}C NMR δ ($CDCl_3$): 175.3, 165.6, 165.1, 157.5, 150.0, 148.2, 136.6, 130.7, 128.7, 127.0, 121.8, 108.3, 107.2, 106.7, 101.4, 49.9, 24.8, 11.7. Anal. Calcd for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 70.72; H, 6.06; N, 10.97.

4.1.4. General procedure for the synthesis of 2-amino-5-bromo-4,6-disubstituted-pyrimidines.[9] To a mixture of the 2-amino-4,6-disubstituted-pyrimidine (14.96 mmol, 1.0 equiv) and $CaCO_3$ (8.98 mmol, 0.6 equiv) in $CHCl_3$ (70 mL) was added drop wise to a solution of bromine (16.47 mmol, 1.1 equiv) in $CHCl_3$ (10 mL). The reaction mixture was stirred at room temperature overnight. Upon completion as judged by TLC (ethylacetate/petroleum ether, 1:3) the reaction mixture was separated between water (pH 9 with 2 M NaOH solution) and dichloromethane (3× 50 mL). The combined organic layers were dried over $MgSO_4$, concentrated in vacuo and recrystallized from ethanol resulting in the corresponding crystalline form.

4.1.4.1. 2-Amino-5-bromo-4,6-phenyl-pyrimidine (8). Yield 70%; white solid. ^{1}H NMR δ ($CDCl_3$): 7.70–7.61 (m, 4H, phenyl-*H*), 7.52–7.42 (m, 6H, phenyl-*H*), 5.49 (br s, 2H, NH_2). MS (ESI): 327.0.

4.1.4.2. 2-Amino-4-benzo[1,3]dioxol-5yl-5-bromo-6-phenyl-pyrimidine (9). Yield 70%; white solid. ^{1}H NMR δ (DMSO): 7.62–7.59 (m, 2H, phenyl-*H*), 7.51–7.47 (m, 3H, phenyl-*H*), 7.22–7.16 (m, 2H, Ar-*H*), 7.03 (d, 2H, Ar-*H*), 7.01 (br s, 2H, NH_2), 6.12 (s, 2H, –OCH_2O–). ^{13}C NMR δ (DMSO): 167.0, 166.1, 148.1, 146.7, 139.1, 132.6, 129.1, 128.9, 127.8, 123.4, 109.5, 107.8, 103.0, 101.5.

4.1.5. General procedure for the synthesis of 2-amido-5-bromo-4,6-disubstituted-pyrimidines.[8] Starting from compound **8**. The experimental procedure was identical to the general procedure of compounds **5**–**7**.

4.1.5.1. Cyclopentane carboxylic acid (4-benzo-[1,3]dioxol-5-yl)-5-bromo-6-phenyl-pyrimidin-2-yl-amide (10). Yield 65%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.00 (br s, 1H, N*H*), 7.75–7.71 (m, 2H, phenyl-*H*), 7.51–7.47 (m, 3H, phenyl-*H*), 7.39 (dd, 1H, J^1 = 8.0 Hz J^2 = 1.8 Hz, Ar-*H*), 7.32 (d, 1H, J = 1.8 Hz, Ar-*H*), 6.92 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.05 (s, 1H, –OCH_2O–), 3.38–3.20 (m, 1H, C*H*-cyclopentyl), 2.05–1.51 (m, 8H, CH_2-cyclopentyl). ^{13}C NMR δ ($CDCl_3$): 175.4, 167.8, 166.7, 155.1, 148.9, 147.2, 137.9, 131.5, 129.7, 129.1, 127.8, 124.3, 110.1, 109.9, 107.7, 101.3, 45.6, 29.9, 25.8.

4.1.5.2. *N*-(4-Benzo[1,3]dioxol-5yl)-5-bromo-6-phenyl-pyrimidin-2-yl-butyramide (11). Yield 79%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.02 (br s, 1H, N*H*), 7.75–7.70 (m, 2H, phenyl-*H*), 7.53–7.48 (m, 3H, phenyl-*H*), 7.39–7.30 (m, 2H, Ar-*H*), 6.92 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.06 (s, 1H, –OCH_2O–), 2.79 (t, 2H, J = 7.7 Hz, –CH_2CH$_2$CH$_3$), 1.85–1.60 (m, 2H, –CH$_2$CH_2CH$_3$), 0.97 (t, 3H, J = 7.3 Hz, –CH$_2$CH$_2$CH_3).

4.1.5.3. Cyclopentane carboxylic acid (5-bromo-4,6-diphenyl-pyrimidin-2-yl)-amide (12). Yield 20%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.02 (br s, 1H, N*H*), 7.80–7.74 (m, 4H, phenyl-*H*), 7.51–7.48 (m, 6H, phenyl-*H*), 3.32–3.24 (m, 1H, C*H*-cyclopentyl), 1.99–1.50 (m, 8H, CH_2-cyclopentyl). ^{13}C NMR δ (DMSO): 175.5, 167.6, 155.2, 137.8, 129.7, 129.2, 127.8, 45.6, 29.9, 25.8. MS (ESI): 423.0.

4.1.5.4. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-butyramide (13). Yield 63%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.03 (br s, 1H, N*H*), 7.82–7.73 (m, 4H, phenyl-*H*), 7.55–7.47 (m, 6H, phenyl-*H*), 2.79 (t, 1H, J = 7.3 Hz, –CH_2CH$_2$CH$_3$), 1.87–1.70 (m, 2H, –CH$_2$CH_2CH$_3$), 0.97 (t, 3H, J = 7.7 Hz, –CH$_2$CH$_2$CH_3).

4.1.5.5. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-2-ethyl-butyramide (14). Yield 46%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.01 (br s, 1H, N*H*), 7.83–7.75 (m, 4H, phenyl-*H*), 7.53–7.48 (m, 6H, phenyl-*H*), 2.87–2.60 (m, 1H, –C*H*(CH$_2$CH$_3$)$_2$), 1.85–1.47 (m, 4H, –CH(CH_2CH$_3$)$_2$), 0.95 (t, 6H, J = 7.2 Hz, –CH(CH$_2$CH_3)$_2$).

4.1.5.6. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-iso-butyramide (15). Yield 36%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.05 (br s, 1H, N*H*), 7.83–7.76 (m, 4H, phenyl-*H*), 7.54–7.48 (m, 6H, phenyl-*H*), 3.18–3.04 (m, 1H, –C*H*(CH$_3$)$_2$), 1.27 (s, 3H, –CH(CH_3)$_2$), 1.24 (s, 3H, –CH(CH_3)$_2$). ^{13}C NMR δ ($CDCl_3$): 176.5, 167.6, 155.3, 137.8, 129.8, 129.3, 127.8, 110.6, 35.2, 18.9.

4.1.5.7. Cyclopropane carboxylic acid (5-bromo-4,6-diphenyl-pyrimidin-2-yl)-amide (16). Yield 71%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.25 (br s, 1H, N*H*), 7.78–7.73 (m, 4H, phenyl-*H*), 7.56–7.45 (m, 6H, phenyl-*H*), 2.58–2.40 (m, 1H, –C*H*CH$_2$CH$_2$–), 1.25–1.14 (m, 2H, –CHCH_2CH$_2$–), 0.96–0.87 (m, 2H, –CHCH$_2$CH_2).

4.1.5.8. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-4-chloro-benzamide (17). Yield 92%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.92 (br s, 1H, N*H*), 7.81–7.69 (m, 6H, phenyl-*H* + Ar-*H*), 7.47–7.23 (m, 8H, phenyl-*H* + Ar-*H*).

4.1.5.9. Furan-2-carboxylic acid (5-bromo-4,6-diphenyl-pyrimidin-2-yl)-amide (18). Yield 54%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.92 (br s, 1H, N*H*), 7.85–7.81 (m, 4H, phenyl-*H*), 7.52–7.47 (m, 7H, phenyl-*H* + furan-*H*), 7.32 (d, 1H, J = 3.7 Hz, furan-*H*), 6.57 (dd, 1H, J^1 = 1.8 Hz J^2 = 1.8 Hz, furan-*H*). ^{13}C NMR δ ($CDCl_3$): 167.7, 155.0, 154.6, 147.1, 144.7, 137.7, 129.8, 129.4, 127.9, 116.4, 112.6, 111.2.

4.1.5.10. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-4-methoxy-benzamide (19). Yield 29%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.61 (br s, 1H, N*H*), 7.92–7.80 (m, 6H, phenyl-*H* + Ar-*H*), 7.52–7.46 (m, 6H, phenyl-*H*), 6.97 (d, 2H, J = 5.1 Hz, Ar-*H*), 3.87 (s, 3H, –OCH_3). ^{13}C NMR δ ($CDCl_3$): 167.6, 164.2, 162.8, 155.7, 137.8, 129.8, 129.4, 129.3, 127.9, 126.0, 113.8, 111.0, 55.2.

4.1.5.11. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-4-methyl-benzamide (20). Yield 63%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.63 (br s, 1H, N*H*), 7.86–7.81 (m, 6H, phenyl-*H* + Ar-*H*), 7.52–7.48 (m, 6H, phenyl-*H*), 7.28 (d, 2H, J = 7.6 Hz, Ar-*H*), 2.42 (s, 3H, CH_3). ^{13}C NMR δ ($CDCl_3$): 167.7, 164.7, 162.8, 155.7, 142.8, 137.8, 131.0, 129.8, 129.3, 129.2, 127.9, 127.4, 111.0, 21.3.

4.1.5.12. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-benzamide (21). Yield 26%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.66 (br s, 1H, N*H*), 7.94–7.90 (m, 2H, phenyl-*H*), 7.88–7.79 (m, 4H, phenyl-*H*), 7.58–7.44 (m, 9H, phenyl-*H*). ^{13}C NMR δ ($CDCl_3$): 167.7, 164.8, 155.6, 137.7, 133.9, 132.2, 129.8, 129.3, 128.6, 127.9, 127.3, 111.2.

4.1.5.13. *N*-(5-Bromo-4,6-diphenyl-pyrimidin-2-yl)-3,4-dichloro-benzamide (22). Yield 37%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.65 (br s, 1H, N*H*), 7.99 (d, 1H, J = 1.8 Hz, Ar-*H*), 7.81–7.77 (m, 4H, phenyl-*H*), 7.71 (dd, 1H, J^1 = 6.2 Hz J^2 = 2.2 Hz, Ar-*H*), 7.56 (s, 1H, Ar-*H*), 7.51–7.48 (m, 6H, phenyl-*H*). ^{13}C NMR δ

($CDCl_3$): 167.7, 162.9, 155.3, 137.5, 136.5, 133.5, 132.8, 130.4, 129.8, 129.7, 129.3, 128.0, 126.7, 111.6.

4.1.6. General procedure for the synthesis of 2-amido-4,6-disubstituted-pyrimidin-5-carbonitriles. To a solution of 2-amido-5-bromo-disubstituted-pyrimidine (0.9 mmol, 1.0 equiv) in pyridine (3 mL) was added CuCN (1.0 mmol, 1.1 equiv). This was heated at 250 °C under microwave conditions[10] for 20 min, after which no starting material was visible according to TLC (a mixture of petroleum ether/tetrahydrofuran/dichloromethane, 4:1:1). The solvent was evaporated under reduced pressure, dissolved in dichloromethane (75 mL) and backwashed with a 5% NH_4OH solution in water (3× 20 mL) and water (1× 20 mL). The organic layer was dried over $MgSO_4$ and the mixture was concentrated in vacuo. This crude product was purified by column chromatography, eluting with a mixture of petroleum ether/tetrahydrofuran/dichloromethane (4:1:1) and recrystallized from methanol or *n*-heptane.

4.1.6.1. 2-Amino-4,6-diphenyl-pyrimidin-5-carbonitrile (23). Yield 32%; off-white solid. ^{1}H NMR δ ($CDCl_3$): 8.00–7.90 (m, 4H, phenyl-*H*), 7.55–7.48 (m, 6H, phenyl-*H*), 5.86 (br s, 2H, NH_2). ^{13}C NMR δ (acetone-d_6): 206.1, 172.0, 164.1, 137.8, 131.6, 129.8, 129.1, 118.9, 93.1.

MS (ESI): 271.7. Anal. Calcd for $C_{17}H_{12}N_4 \cdot 1.2\ H_2O$: C, 69.33; H, 4.95; N, 19.02. Found: C, 69.32; H, 5.33; N, 19.18.

4.1.6.2. 2-Amino-4-benzo[1,3]dioxol-5yl-6-phenyl-pyrimidin-5-carbonitrile (24). Yield 64%; off-white solid. ^{1}H NMR δ (DMSO-d_6): 7.91–7.86 (m, 4H, phenyl-*H* + NH_2), 7.62–7.52 (m, 4H, phenyl-*H* + Ar-*H*), 7.47 (dd, 1H, J_1 = 2.6 Hz, J_2 = 1.8 Hz, Ar-*H*), 7.12 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.17 (s, 2H, –OCH_2O–). ^{13}C NMR δ (DMSO-d_6): 170.9, 169.6, 162.7, 149.7, 147.4, 136.6, 130.8, 130.4, 128.8, 128.3, 123.9, 118.8, 108.9, 108.2, 101.9, 90.7. Anal. Calcd for $C_{18}H_{12}N_4O_2 \cdot 0.2\ H_2O$: C, 67.61; H, 3.90; N, 17.52. Found: C, 67.58; H, 3.67; N, 17.54.

4.1.6.3. Cyclopentane carboxylic acid (4-benzo-[1,3]dioxol-5yl-5-carbonitrile-6-phenyl-pyrimidin-2-yl)-amide (25). Yield 66%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.15 (br s, 1H, N*H*), 8.04–8.00 (m, 2H, phenyl-*H*), 7.72 (dd, 1H, J_1 = 6.6 Hz, J_2 = 1.8 Hz, Ar-*H*), 7.60–7.50 (m, 4H, phenyl-*H* + Ar-*H*), 6.98 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.05 (s, 2H, –OCH_2O–), 3.50–3.35 (m, 1H, C*H*-cyclopentyl), 2.03–1.60 (m, 8H, CH_2-cyclopentyl). ^{13}C NMR δ ($CDCl_3$): 175.8, 171.3, 169.9, 156.8, 150.7, 147.9, 135.3, 131.5, 129.0, 128.4, 124.6, 117.3, 109.1, 108.1, 101.7, 96.4, 45.8, 29.9, 25.8. Anal. Calcd for $C_{24}H_{20}N_4O_3$: C, 69.89; H, 4.89; N, 13.58. Found: C, 70.20; H, 4.95; N, 13.78.

4.1.6.4. *N*-(4-Benzo[1,3]dioxol-5yl-5-carbonitrile-6-phenyl-pyrimidin-2-yl)-butyramide (26). Yield 59%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.16 (br s, 1H, N*H*), 8.03–7.99 (m, 2H, phenyl-*H*), 7.71 (dd, 1H, J_1 = 6.2 Hz, J_2 = 1.8 Hz, Ar-*H*), 7.61–7.50 (m, 4H, phenyl-*H* + Ar-*H*), 6.97 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.09 (s, 2H, –OCH_2O–), 2.91 (t, 2H, J = 7.3 Hz, –CH_2CH$_2$CH$_3$), 1.89–1.71 (m, 2H, –CH$_2$CH_2CH$_3$), 1.03 (t, 3H, J = 7.7 Hz, –CH$_2$CH$_2$CH_3). ^{13}C NMR δ (DMSO-d_6): 172.1, 170.7, 169.2, 157.5, 150.4, 147.6, 135.9, 131.5, 129.5, 129.3, 128.6, 124.7, 117.7, 109.3, 108.4, 102.1, 96.8, 40.9, 17.9, 13.6. Anal. Calcd for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50. Found: C, 68.28; H, 4.70; N, 14.45.

4.1.6.5. Cyclopentane carboxylic acid (5-carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-amide (27) LUF6040. Yield 51%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.22 (br s, 1H, N*H*), 8.11–8.00 (m, 4H, phenyl-*H*), 7.65–7.51 (m, 6H, phenyl-*H*), 3.50–3.36 (m, 1H, –C*H*-cyclopentyl), 2.03–1.54 (m, 8H, CH_2-cyclopentyl). ^{13}C NMR δ ($CDCl_3$): 177.7, 172.9, 158.8, 137.0, 133.4, 130.8, 130.3, 118.8, 98.9, 47.6, 31.7, 27.6. Anal. Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 74.61; H, 5.49; N, 15.19.

4.1.6.6. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-butyramide (28). Yield 25%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.24 (br s, 1H, N*H*), 8.06–8.02 (m, 4H, phenyl-*H*), 7.64–7.51 (m, 6H, phenyl-*H*), 2.92 (t, 1H, J = 7.3 Hz, –CH_2CH$_2$CH$_3$), 1.86–1.75 (m, 2H, –CH$_2$CH_2CH$_3$), 1.02 (t, 3H, J = 7.3 Hz, –CH$_2$CH$_2$CH_3). ^{13}*C NMR* δ ($CDCl_3$): 173.2, 171.3, 156.9, 135.2, 131.6, 129.0, 128.5, 116.9, 39.4, 17.9, 13.5. MS (ESI): 342.0. Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36. Found: C, 73.37; H, 5.16; N, 16.67.

4.1.6.7. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-2-ethyl-butyramide (29). Yield 42%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.23 (br s, 1H, N*H*), 8.09–8.04 (m, 4H, phenyl-*H*), 7.64–7.51 (m, 6H, phenyl-*H*), 2.93–2.75 (m, 1H, –C*H*(CH$_2$CH$_3$)$_2$), 1.93–1.52 (m, 4H, –CH(CH_2CH$_3$)$_2$), 0.98 (t, 6H, J = 7.3 Hz, –CH(CH$_2$CH_3)$_2$). ^{13}C NMR δ ($CDCl_3$): 175.2, 171.2, 156.9, 135.2, 131.6, 129.1, 128.5, 116.9, 97.4, 50.2, 24.7, 11,6. MS (ESI): 370.0. Anal. Calcd for $C_{23}H_{22}N_4O$: C, 74.57; H, 5.99; N, 15.12. Found: C, 74.47; H, 5.94; N, 15.46.

4.1.6.8. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-isobutyramide (30). Yield 26%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.22 (br s, 1H, N*H*), 8.09–8.00 (m, 4H, phenyl-*H*), 7.65–7.51 (m, 6H, phenyl-*H*), 3.33–3.19 (m, 1H, –C*H*(CH$_3$)$_2$), 1.32 (s, 3H, –CH(CH_3)$_2$), 1.29 (s, 3H, –CH(CH_3)$_2$). ^{13}C NMR δ ($CDCl_3$): 176.5, 171.2, 157.0, 135.2, 131.6, 129.1, 128.5, 117.0, 97.3, 35.5, 24.7, 18.9. MS (ESI): 342.0 Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36. Found: C, 73.34; H, 5.16; N, 16.69.

4.1.6.9. Cyclopropane carboxalic acid (5-carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-amide (31). Yield 29%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.33 (br s, 1H, N*H*), 8.07–8.02 (m, 4H, phenyl-*H*), 7.78–7.51 (m, 6H, phenyl-*H*), 2.71–2.58 (m, 1H, –C*H*CH$_2$CH$_2$–), 1.29–1.21 (m, 2H, –CHCH_2CH$_2$–), 1.06–0.96 (m, 2H, –CHCH$_2$CH_2–). ^{13}C NMR δ ($CDCl_3$): 173.8, 171.2, 157.1, 135.2, 131.6, 129.0, 128.5, 116.9, 97.3, 15.0, 9.9.

MS (ESI): 340.1. Anal. Calcd for $C_{21}H_{16}N_4O \cdot 0.7\ H_2O$: C, 71.47; H, 4.97; N, 15.88. Found: C, 71.46; H, 4.92; N, 15.94.

4.1.6.10. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-4-chloro-benzamide (32). Yield 27%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.75 (br s, 1H, N*H*), 8.12–8.07 (m, 4H, phenyl-*H*), 7.90 (d, 2H, J = 8.4 Hz, Ar-*H*), 7.65–7.54 (m, 6H, phenyl-*H*), 7.51 (d, 2H, J = 12.4 Hz, Ar-*H*). ^{13}C NMR δ ($CDCl_3$): 171.2, 163.8, 157.3, 139.0, 135.0, 131.9, 131.7, 129.1, 128.9, 128.5, 116.8, 97.9. MS (ESI): 410.1. Anal. Calcd for $C_{24}H_{15}ClN_4O \cdot 1.1\ H_2O$: C, 66.95; H, 4.02; N, 13.01. Found: C, 66.92; H, 4.04; N, 13.15.

4.1.6.11. Furan-2-carboxylic acid (5-carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-amide (33). Yield 35%; white solid. ^{1}H NMR δ ($CDCl_3$): 9.09 (br s, 1H, N*H*), 8.17–8.12 (m, 4H, phenyl-*H*), 7.62–7.54 (m, 7H, phenyl-*H* + furan-*H*), 7.39 (d, 1H, J = 3.3 Hz, furan-*H*), 6.62 (dd, 1H, J^1 = 1.8 Hz J^2 = 1.5 Hz, furan-*H*). ^{13}C NMR δ ($CDCl_3$): 171.3, 156.9, 154.3, 146.7, 145.0, 135.2, 131.7, 129.2, 128.6, 117.1, 117.0, 112.9, 97.9. Anal. Calcd for $C_{22}H_{14}N_4O_2 \cdot 1.0\ H_2O$: C, 68.59; H, 3.76; N, 14.36. Found: C, 68.56; H, 3.88; N, 14.48.

4.1.6.12. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-4-methoxy-benzamide (34). Yield 21%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.76 (br s, 1H, N*H*), 8.11–8.08 (m, 4H, phenyl-*H*), 7.94 (dd, 2H, J^1 = 4.7 Hz J^2 = 1.8 Hz, Ar-*H*), 7.62–7.52 (m, 6H, phenyl-*H*), 7.00 (dd, 2H, J^1 = 4.8 Hz J^2 = 1.8 Hz, Ar-*H*), 3.90 (s, 3H, –OC*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 171.1, 164.0, 163.1, 157.6, 135.2, 131.6, 129.6, 129.2, 128.5, 125.6, 117.0, 113.9, 97.5, 55.3. Anal. Calcd for $C_{25}H_{18}N_4O_2 \cdot 0.5\ H_2O$: C, 72.29; H, 4.61; N, 13.49. Found: C, 72.27; H, 4.27; N, 13.45.

4.1.6.13. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-4-methyl-benzamide (35). Yield 17%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.81 (br s, 1H, N*H*), 8.13–8.09 (m, 4H, phenyl-*H*), 7.86 (d, 2H, J = 8.0 Hz, Ar-*H*), 7.62–7.54 (m, 6H, phenyl-*H*), 7.32 (d, 2H, J = 8.0 Hz, Ar-*H*), 2.45 (s, 3H, C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 171.2, 164.5, 157.5, 143.5, 135.2, 131.6, 130.6, 129.4, 129.2, 128.5, 127.5, 116.9, 97.7, 21.4. Anal. Calcd for $C_{25}H_{18}N_4O \cdot 0.7\ H_2O$: C, 74.60; H, 4.84; N, 13.92. Found: C, 74.67; H, 5.15; N, 13.67.

4.1.6.14. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-benzamide (36). Yield 60%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.85 (br s, 1H, N*H*), 8.13–8.08 (m, 4H, phenyl-*H*), 7.95 (d, 2H, J = 6.6 Hz, phenyl-*H*), 7.66–7.48 (m, 9H, phenyl-*H*). ^{13}C NMR δ ($CDCl_3$): 171.2, 164.7, 157.4, 135.2, 133.5, 132.6, 131.7, 129.2, 128.7, 128.6, 127.5, 116.9, 97.7. Anal. Calcd for $C_{24}H_{16}N_4O \cdot 0.4\ H_2O$: C, 74.98; H, 4.43; N, 14.57. Found: C, 75.00; H, 4.47; N, 14.54.

4.1.6.15. *N*-(5-Carbonitrile-4,6-diphenyl-pyrimidin-2-yl)-3,4-dichloro-benzamide (37). Yield 25%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.77 (br s, 1H, N*H*), 8.10–8.03 (m, 5H, phenyl-*H* + Ar-*H*), 7.75 (dd, 1H, J^1 = 6.2 Hz J^2 = 2.2 Hz, Ar-*H*), 7.65–7.51 (m, 7H, phenyl-*H* + Ar-*H*). ^{13}C NMR δ ($CDCl_3$): 171.3, 162.8, 157.1, 137.2, 134.9, 133.2, 131.8, 130.7, 129.6, 129.1, 128.6, 126.6, 116.7, 98.2. Anal. Calcd for $C_{24}H_{14}Cl_2N_4O \cdot 0.6\ H_2O$: C, 63.20; H, 3.36; N, 12.28. Found: C, 63.21; H, 3.32; N, 12.23.

4.1.7. Benzo[1,3]dioxole-5-carbonitrile (39). Prepared as described in the literature.[11]

4.1.8. 3-Amino-3-benzo[1,3]dioxol-5-yl-acrylonitrile (40). Prepared as described in the literature.[12]

4.1.9. 4-Benzo[1,3]dioxol-5-yl-6-chloro-2-phenyl-pyrimidine (41). Prepared as described in the literature.[13]

4.1.10. 2,6-Diphenyl-pyrimidin-4-ylamine (42).[3] Yield 68%, white solid. ^{1}H NMR δ ($CDCl_3$): 8.52–8.47 (m, 2H, phenyl-*H*), 8.10–8,05 (m, 2H, phenyl-*H*), 7.52–7.42 (m, 6H, phenyl-*H*), 6.63 (s, 1H, pyrimidine-*H*), 5.08 (br s, 2H, N*H*$_2$). ^{13}C NMR δ ($CDCl_3$): 163.9, 162.9, 138.0, 137.2, 130.1, 129.9, 128.3, 128.1, 127.9, 126.6, 98.2. MS (ESI): 248.4. Anal. Calcd for $C_{16}H_{13}N_3$: C, 77.71; H, 5.29; N, 16.99. Found: C, 77.39; H, 5.09; N, 17.33.

4.1.11. 6-Benzo[1,3]dioxol-5-yl-2-phenyl-pyrimidin-4-ylamine (43).[3] Yield 64%; white solid. ^{1}H NMR δ (CDCl3): 8.50–8.44 (m, 2H, phenyl-*H*), 7.74 (s, 1H, Ar-*H*), 7.66 (d, 1H, J = 5.4 Hz, Ar-*H*), 7.53–7.43 (m, 3H, phenyl-*H*), 6.95 (dd, 1H, J^1 = 8.8 Hz J^2 = 1.5 Hz, Ar-*H*), 6.64 (s, 1H, pyrimidine-*H*), 6.04 (s, 2H, –OC*H*$_2$O–). ^{13}C NMR δ (DMSO): 164.9, 162.9, 160.8, 149.0, 148.0, 138.4, 131.9, 130.1, 128.3, 127.7, 120.9, 108.5, 106.6, 101.5, 97.4. Anal. Calcd for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42. Found: C, 69.86; H, 4.64; N, 14.39.

4.1.12. General procedure for the synthesis of 2,6-disubstituted-pyrimidin-4-yl-amides. Procedure identical to compounds **5**–**7**, starting from **43**.

4.1.12.1. Cyclopentane carboxylic acid (6-benzo-[1,3]dioxol-5-yl-2-phenyl-pyrimidin-4-yl)-amide (44). Yield 48%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.51–8.46 (m, 3H, phenyl-*H* + pyrimidinyl-*H*), 8.06 (br s, 1H, N*H*), 7.83–7.78 (m, 2H, Ar-*H*), 7.51–7.46 (m, 3H, phenyl-*H*), 6.92 (d, 1H, J = 8.8 Hz, Ar-*H*), 6.05 (s, 2H, –OC*H*$_2$O–), 2.83–2.71 (m, 1H, C*H*-cyclopentane), 1.98–1.60 (m, 8H, C*H*$_2$-cyclopentane). ^{13}C NMR δ ($CDCl_3$): 176.9, 163.8, 163.0, 159.3, 149.8, 148.2, 137.4, 131.0, 130.8, 128.6, 127.9, 121.7, 108.7, 106.8, 102.2, 101.8, 89.7, 88.7. Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 70.99; H, 5.70; N, 10.83.

4.1.12.2. *N*-(6-Benzo[1,3]dioxol-5-yl-2-phenyl-pyrimidin-4-yl)-butyramide (45). Yield 46%; white solid. Anal. ^{1}H NMR δ ($CDCl_3$): 8.50–8.45 (m, 3H, phenyl-*H* + pyrimidinyl-*H*), 8.05 (br s, 1H, N*H*), 7.84–7.79 (m, 2H, Ar-*H*), 7.51–7.48 (m, 3H, phenyl-*H*), 6.93 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.06 (s, 2H, –OC*H*$_2$O–), 2.44 (t, 2H, J = 7.3 Hz, –C*H*$_2$CH$_2$CH$_3$), 1.85–1.74 (m, 2H, –CH$_2$C*H*$_2$CH$_3$), 1.04 (t, 3H, J = 7.7 Hz, –CH$_2$CH$_2$C*H*$_3$). ^{13}*C NMR* δ ($CDCl_3$): 172.4, 164.9, 163.5, 158.0, 149.8,

148.1, 137.3, 131.3, 130.5, 128.3, 127.9, 121.9, 108.2, 107.4, 102.2, 101.3, 39.3, 18.2, 13.3. Anal. Calcd for $C_{21}H_{19}N_3O_3$: C, 69.79; H, 5.30; N, 11.63. Found: C, 69.72; H, 5.50; N, 11.65.

4.1.12.3. *N*-(6-Benzo[1,3]dioxol-5-yl-2-phenyl-pyrimidin-4-yl)-2-ethyl-butyramide (46). Yield 52%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.55 (s, 1H, pyrimidinyl-*H*), 8.50–8.45 (m, 2H, phenyl-*H*), 8.16 (br s, 1H, N*H*), 7.85–7.80 (m, 2H, Ar-*H*), 7.51–7.46 (m, 3H, phenyl-*H*), 6.92 (d, 1H, J = 8.8 Hz, Ar-*H*), 6.05 (s, 2H, –OCH_2O–), 2.18–2.07 (m, 1H, –C*H*(CH_2CH_3)$_2$), 1.82–1.53 (m, 4H, –CH(C$H_2$$CH_3$)$_2$), 0.95 (t, 6H, J = 7.3 Hz, –CH(CH_2CH_3)$_2$). ^{13}C NMR δ ($CDCl_3$): 175.8, 165.1, 163.6, 158.1, 149.9, 148.1, 137.3, 131.3, 130.6, 128.2, 127.9, 122.0, 108.2, 107.4, 102.3, 101.3, 51.9, 25.2, 11.6. Anal. Calcd for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 70.60; H, 5.83; N, 10.75.

4.1.12.4. Cyclopropane carboxylic acid (6-benzo[1,3]dioxol-5-yl-2-phenyl-pyrimidin-4-yl)-amide (47). Yield 51%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.51–8.47 (m, 2H, phenyl-*H*), 8.42 (s, 1H, pyrimidinyl-*H*), 8.32 (br s, 1H, N*H*), 7.82–7.77 (m, 2H, Ar-*H*), 7.51–7.47 (m, 3H, phenyl-*H*), 6.91 (d, 1H, J = 7.7 Hz, Ar-*H*), 6.05 (s, 2H, –OCH_2O–), 1.67–1.55 (m, 1H, C*H*-cyclopropane), 1.19–1.12 (m, 2H, CH_2-cyclopropane), 0.98–0.90 (m, 2H, CH_2-cyclopropane). ^{13}C NMR δ ($CDCl_3$): 173.1, 164.9, 163.6, 158.0, 149.8, 148.1, 137.3, 131.3, 130.5, 128.2, 127.9, 121.9, 108.2, 107.4, 102.2, 101.3, 15.8, 8.6. Anal. Calcd for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 69.81; H, 4.50; N, 11.67.

4.1.13. 2-Benzylidene-malononitrile (49). Prepared as described in the literature.[5]

4.1.14. 2-Benzo[1,3]dioxol-5-ylmethylene-malononitrile (50). Yield 97%, yellow solid. ^{1}H NMR δ ($CDCl_3$): 7.61 (s, 1H, Ar-*H*), 7.60 (s, 1H, C*H*C(CN)$_2$), 7.32 (dd, 1H, J^1 = 6.6 Hz J^2 = 1.5 Hz, Ar-*H*), 6.93 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.13 (s, 2H, OCH_2O).

4.1.15. General procedure for the synthesis of 4-amino-2,6-disubstituted-pyrimidine-5-carbonitriles.[14] A mixture of the functionalized malononitrile (45 mmol, 1 equiv), benzamidin hydrochloride (45 mmol, 1 equiv) and K_2CO_3 (95 mmol, 2.05 equiv) was refluxed in methanol (35 mL) for 2 h. The methanol was removed in vacuo and the residue was slurried in hot acetone. $KMnO_4$ (2.5 g, 16 mmol) was portion wise added and monitored by TLC (dichloromethane). The reaction mixture was filtered over Celite, the excess of $KMnO_4$ in the filtrate was reduced with $NaHSO_3$ and followed by a second filtration over Celite. The filtrate was concentrated in vacuo. Recrystallization from ethyl acetate–petroleum ether or a methanol–acetone mixture gave white crystals.

4.1.15.1. 4-Amino-2,6-diphenyl-pyrimidine-5-carbonitrile (51). Yield 51%, off-white solid. ^{1}H NMR δ ($CDCl_3$): 8.51–8.48 (m, 2H, phenyl-*H*), 8.15–8.10 (m, 2H, phenyl-*H*), 7.60–7.44 (m, 6H, phenyl-*H*), 5.70 (br s, 2H, NH_2). ^{13}C NMR δ (DMSO): 168.2, 164.7, 164.1, 136.7, 136.7, 131.6, 130.9, 128.6, 116.5, 84.5. MS (ESI): 272.0. Anal. Calcd for $C_{17}H_{12}N_4$·0.5 H_2O: C, 72.31; H, 4.68; N, 19.84. Found: C, 72.33; H, 4.49; N, 19.69.

4.1.15.2. 4-Amino-6-benzo[1,3]dioxol-5-yl-2-phenyl-pyrimidine-5-carbonitrile (52). Yield 39%, white solid. ^{1}H NMR δ ($CDCl_3$): 8.50–8.46 (m, 2H, phenyl-*H*), 7.76 (dd, 1H, J^1 = 6.6 Hz, J^2 = 1.8 Hz, Ar-*H*), 7.60 (d, 1H, J = 1.8 Hz, Ar-*H*), 7.54–7.45 (m, 3H, phenyl-*H*), 6.96 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.08 (s, 1H, –OCH_2O–), 5.68 (br s, 2H, NH_2). ^{13}C NMR δ (DMSO): 167.1, 164.9, 163.9, 149.8, 147.6, 136.7, 131.5, 130.5, 128.5, 123.7, 116.7, 108.8, 108.2, 101.9, 83.7. Anal. Calcd for $C_{18}H_{12}N_4O_2$: C, 68.35; H, 3.82; N, 17.71. Found: C, 68.33; H, 3.88; N, 17.91.

4.1.16. General procedure for the synthesis of 4-amido-2,6-disubstituted-pyrimidine-5-carbonitriles.[8] To a solution of the 4-amino-2,6-disubstituted-pyrimidine (1 mmol, 1 equiv) in anhydrous pyridine (4 mL) was added the appropriate acid chloride (5 mmol, 5 equiv) and heated at 100 °C in a sealed tube in the microwave for 20 min. After completion (TLC, dichloromethane), the solvent was evaporated under reduced pressure, the crude product was purified by column chromatography, eluting with dichloromethane and crystallized from methanol or acetone.

4.1.16.1. Cyclopentane carboxylic acid (6-benzo[1,3]dioxol-5yl-5-carbonitrile-2-phenyl-pyrimidin-4-yl)-amide (53) LUF6056. Yield 33%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.53–8.48 (m, 2H, phenyl-*H*), 8.07 (br s, 1H, N*H*), 7.80 (dd, 1H, J^1 = 5.8 Hz, J^2 = 1.8 Hz, Ar-*H*), 7.68 (d, 1H, J = 1.8 Hz, Ar-*H*), 7.60–7.46 (m, 3H, phenyl-*H*), 6.98 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.09 (s, 2H, –OCH_2O–), 3.45–3.29 (m, 1H, C*H*-cyclopentyl), 2.06–1.62 (m, 8H, CH_2-cyclopentyl). ^{13}C NMR δ ($CDCl_3$): 174.8, 167.7, 164.6, 159.7, 150.7, 148.0, 135.7, 132.0, 129.3, 128.8, 128.4, 124.5, 115.4, 109.0, 108.2, 101.7, 91.4, 46.1, 29.9, 25.7. Anal. Calcd for $C_{24}H_{20}N_4O_3$: C, 69.89; H, 4.89; N, 13.58. Found: C, 69.68; H, 4.87; N, 13.88.

4.1.16.2. *N*-(6-Benzo[1,3]dioxol-5-yl-5-cyano-2-phenyl-pyrimidin-4-yl)-butyramide (54). Yield 49%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.50–8.47 (m, 2H, phenyl-*H*), 8.07 (br s, 1H, N*H*), 7.78 (dd, 1H, J^1 = 6.2 Hz, J^2 = 2.2 Hz, Ar-*H*), 7.66 (d, 1H, J = 1.8 Hz, Ar-*H*), 7.60–7.46 (m, 3H, phenyl-*H*), 6.97 (d, 1H, J = 8.0 Hz, Ar-*H*), 6.09 (s, 2H, –OCH_2O–), 2.95 (t, 2H, J = 6.9 Hz, C$H_2$$CH_2CH_3$), 1.95–1.77 (m, 2H, CH_2C$H_2$$CH_3$), 1.09 (t, 3H, J = 7.3 Hz, CH_2CH_2CH_3). ^{13}C NMR δ ($CDCl_3$): 172.2, 167.7, 164.5, 159.6, 150.8, 148.0, 135.7, 132.1, 129.3, 128.9, 128.4, 124.4, 115.3, 109.0, 108.2, 101.7, 90.1, 39.6, 17.9, 13.5. Anal. Calcd for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50. Found: C, 68.78; H, 4.74; N, 14.83.

4.1.16.3. *N*-(6-Benzo[1,3]dioxol-5-yl-5-cyano-2-phenyl-pyrimidin-4-yl)-2-ethyl-butyramide (55). Yield 65%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.53–8.50 (m, 2H, phenyl-*H*), 8.05 (br s, 1H, N*H*), 7.81 (dd, 1H, J^1 = 6.2 Hz,

J^2 = 1.8 Hz, Ar-*H*), 7.68 (d, 1H, *J* = 1.8 Hz, Ar-*H*), 7.60–7.46 (m, 3H, phenyl-*H*), 6.98 (d, 1H, *J* = 8.4 Hz, Ar-*H*), 6.09 (s, 2H, –OCH_2O–), 2.71 (m, 1H, C*H*(CH_2CH_3)$_2$), 1.83–1.59 (m, 4H, CH(CH_2CH$_3$)$_2$), 1.05 (t, 3H, *J* = 7.3 Hz, CH(CH_2CH_3)$_2$). ^{13}C NMR δ ($CDCl_3$): 174.2, 164.8, 159.6, 150.8, 148.0, 135.7, 132.0, 129.4, 128.9, 128.4, 124.7, 115.4, 109.2, 108.2, 101.6, 92.0, 50.8, 24.9, 11.5. Anal. Calcd for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52. Found: C, 69.46; H, 5.43; N, 13.31.

4.1.16.4. Cyclopentane carboxylic acid (5-carbonitrile-2,6-diphenyl-pyrimidin-4-yl)-amide (56). Yield 25%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.60–8.50 (m, 2H, phenyl-*H*), 8.17–8.13 (m, 2H, phenyl-*H*), 8.08 (s, 1H, N*H*), 7.62–7.47 (m, 6H, phenyl-*H*), 3.47–3.32 (m, 1H, C*H*-cyclopentyl), 2.20–1.95 (m, 4H, CH_2-cyclopentyl), 1.95–1.60 (m, 4H, CH_2-cyclopentyl).

MS (ESI): 369.2. Anal. Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 74.67; H, 5.42; N, 15.15.

4.1.16.5. Cyclopropane carboxylic acid (5-carbonitrile-2,6-diphenyl-pyrimidin-4-yl)-amide (57). Yield 18%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.56–8.51 (m, 4H, phenyl-*H*), 8.24 (br s, 1H, N*H*), 7.17–8.13 (m, 6H, phenyl-*H*), 2.53–2.41 (m, 1H, C*H*-cyclopropyl), 1.37–1.29 (m, 2H, CH_2-cyclopropyl), 1.14–1.06 (m, 2H, CH_2-cyclopropyl). Anal. Calcd for $C_{21}H_{16}N_4O{\cdot}0.2\ H_2O$: C, 73.29; H, 4.81; N, 16.28. Found: C, 73.28; H, 4.74; N, 16.24.

4.1.16.6. *N*-(5-Carbonitrile-2,6-diphenyl-pyrimidin-4-yl)-butyramide (58). Yield 37%; white solid. ^{1}H NMR δ ($CDCl_3$): 8.60–8.45 (m, 2H, phenyl-*H*), 8.16–8.12 (m, 2H, phenyl-*H*), 8.10 (br s, 1H, N*H*), 7.64–7.48 (m, 6H, phenyl-*H*), 3.29 (t, 2H, *J* = 7.3 Hz, CH_2CH$_2$CH$_3$), 1.97–1.79 (m, 2H, CH$_2$CH_2CH$_3$), 1.10 (t, 3H, *J* = 7.3 Hz, CH$_2$CH$_2$CH_3). ^{13}C NMR δ (DMSO): 172.5, 169.1, 165.1, 159.7, 135.9, 135.6, 132.4, 131.9, 129.2, 129.1, 128.8, 128.7, 115.2, 91.1, 39.9, 18.1, 13.7. MS (ESI): 343.0. Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.66; H, 5.29; N, 16.36. Found: C, 73.34; H, 5.16; N, 16.74.

4.2. Biology

4.2.1. Materials and methods. [^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]ZM 241385 was obtained from Tocris Cookson, Ltd, UK. CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College London, UK. HEK 293 cells stably expressing the human adenosine A_{2A} and A_3 receptor were gifts from Dr. J. Wang (Biogen, USA) and Dr. K.-N. Klotz (University of Würzburg, Germany), respectively.

4.2.1.1. Radioligand binding studies. All compounds were tested in radioligand binding assays to determine their affinities at the human adenosine A_1, A_{2A} and the A_3 receptors as described previously[3] with the exception of non-specific binding on the A_{2A} receptor, which was determined in the presence of 10 μM CGS21680 instead of 100 μM CPA. The human A_1 receptors were expressed in CHO cells, and [^{3}H]DPCPX was used as the radioligand. The A_{2A} and A_3 receptors were expressed in HEK 293 cells, and [^{3}H]ZM 241385 and [^{125}I]AB-MECA were used as the respective radioligands.

4.2.1.2. cAMP determinations. A number of compounds specified in the text were tested in functional assays for their ability to influence the levels of cAMP in the test system under various experimental conditions, (i) in the presence of forskolin (10 μM, Tables 1 and 2), and (ii) in the presence of both forskolin (10 μM) and CPA (100 nM, Fig. 2).

A suspension in PBS buffer (supplemented with 5 mM Hepes and 0.1% BSA) of CHO cells expressing the human adenosine A_1 receptor was seeded into a 384-well plate (white Optiplates, Perkin Elmer, NL; approximately 2500 cells/well). To each well were added the compounds of interest, adenosine deaminase (0.8 IU/mL), rolipram (50 μM), cilostamide (50 μM) in a total volume of 7.5 μl, after which the plate was centrifuged at 600*g* for 1 min. This mixture was then incubated for 15 min at room temperature followed by the addition (2.5 μl) of forskolin (10 μM final concentration). After a subsequent 45 min incubation at room temperature, cAMP antibody solution (5 μl) and detection mix (5 μl) were added, and the plate was centrifuged once more. After a further incubation of at least 2 h the amount of cAMP was determined in a TR-FRET assay on a Victor spectrometer (Perkin-Elmer, NL) according to the instructions of the supplier (Lance cAMP assay, Perkin-Elmer, NL). All data reflect at least three independent experiments performed in duplicate.

4.2.1.3. Data analysis. K_i values were calculated using a non-linear regression curve-fitting program (GraphPad Prism 4, GraphPad Software Inc., San Diego, CA, USA). K_D values of the radioligands were 1.6, 1.0 and 5.0 nM for [^{3}H]DPCPX (A_1 receptor), [^{3}H]ZM 241385 (A_{2A} receptor) and [^{125}I]AB-MECA (A_3 receptor), respectively. The data from the functional assays with compounds **27** and **53** were also analyzed with GraphPad Prism and Figure 2 was generated by evaluating the data to relate to the reference agonist CPA (set at 0%) and the compounds' maximum effects (set at 100%).

References and notes

1. (a) Jacobson, K. A.; Gao, Z. G. *Nat. Rev. Drug Disc.* **2006**, *5*, 247; (b) Fredholm, B. B.; Chen, J. F.; Masino, S. A.; Vaugeois, J. M. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 385; (c) Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. *Pharmacol. Rev.* **2001**, *53*, 527.
2. Chang, L. C. W.; Brussee, J.; IJzerman, A. P. *Chem. Biodivers.* **2004**, *1*, 1591.
3. Chang, L. C. W.; Spanjersberg, R. F.; von Frijtag Drabbe Künzel, J. K.; Mulder-Krieger, T.; van den Hout, G.; Beukers, M. W.; Brussee, J.; IJzerman, A. P. *J. Med. Chem.* **2004**, *47*, 6529.

4. Bayer Aktiengesellschaft WO0125210, 2001; Bayer Aktiengesellschaft DE10115922A1, 2002.
5. Chang, L. C. W.; von Frijtag Drabbe Künzel, J. K.; Mulder-Krieger, T.; Spanjersberg, R. F.; Roerink, S. F.; van den Hout, G.; Beukers, M. W.; Brussee, J.; IJzerman, A. P. *J. Med. Chem.* **2005**, *48*, 2045.
6. Doherty, A. M.; Patt, W. C.; Edmunds, J. J.; Berryman, K. A.; Reisdorph, B. R.; Plummer, M. S.; Shahripour, A.; Lee, C.; Cheng, X.-M., et al. *J. Med. Chem.* **1995**, *38*, 1259.
7. Al-Hajjar, F. H.; Sabri, S. S. *J. Heterocycl. Chem.* **1982**, *19*, 1087.
8. Kondal, K.; Kamala, K.; Rao, P. J.; Reddy, K. K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3791.
9. Nasielski, J.; Standaert, A.; Nasielski-Hinkens, R. *Synth. Commun.* **1991**, *21*, 901.
10. Brown, D. J.; Paddon-Row, M. N. *J. Chem. Soc.* **1967**, 903.
11. Sampath Kumar, H. M.; Subba Reddy, B. V.; Tirupathi Reddy, P.; Yadav, J. S. *Synthesis* **1999**, *4*, 586.
12. Yamaguchi, Y.; Katsuyama, I.; Funabiki, K.; Matui, M.; Shibata, K. *J. Heterocycl. Chem.* **1998**, *35*, 805.
13. Harris, R. L. N.; Huppatz, J. L.; Teitei, T. *Aust. J. Chem.* **1979**, *32*, 669.
14. Peters, J.-U.; Weber, S.; Kritter, S.; Weiss, P.; Wallier, A.; Boehringer, M.; Hennig, M.; Kuhn, B.; Loeffler, B. *Bioorg. Med. Chem. Lett.* **2004**, *6*, 1491.
15. Abd-Elfattah, A. M.; Hussain, S. M.; El-Reedy, A. M. *Tetrahedron* **1983**, *39*, 3197.
16. Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006.