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Synthesis and biological activity of 2-anilino-4-(1*H*-pyrrol-3-yl) pyrimidine CDK inhibitors

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Abstract—A series of 2-anilino-4-(1*H*-pyrrol-3-yl)pyrimidines were prepared and evaluated for their ability to inhibit cyclin-dependent kinases (CDKs). A number of analogues were found to be potent CDK2 and CDK4 inhibitors and to exhibit anti-proliferative activity against human tumour cell lines. Structure–activity relationships and biochemical characterization are presented.

Abnormalities in cell cycle regulation are responsible for the majority of human neoplasias.[1–4] Cyclin-dependent kinases (CDKs) are key regulators of cell cycle progression.[5] These enzymes are activated by the formation of periodic complexes with cyclins, proteins that are present at specific stages of the cell cycle. CDK4 and CDK6, coupled with their respective cyclin D partners, are responsible for progression through G1, whereas CDK2 in combination with cyclin E is required for normal progress from G1 into S-phase, where DNA replication takes place. CDK1 and CDK2 (in association with cyclins A and B) functions, on the other hand, are essential for cells to pass through S-phase into G2 and mitosis. The CDK/cyclin complexes are regulated by stoichiometric associations with small proteins, the so-called cyclin-dependent kinase inhibitors (CDKIs), such as p16, p15, p21, and p27, whose loss or inactivation participates in the development of cancer. CDKs phosphorylate and regulate the activity of a variety of cellular proteins that include tumour suppressors (e.g., pRb, p53), transcription factors (e.g., E2F-DP1, RNA pol II), replication factors (e.g., DNA polα, replication protein A), and organizational factors, which influence cellular and chromatin structures (e.g., histone H1, lamin A, MAP4). The recent understanding of the role of CDKs in cell cycle regulations and the discovery that high rates of neoplasias are the result of CDK hyperactivation provide the main impetus to the search for their pharmacological inhibitors.

Our efforts to develop small molecule ATP-antagonistic CDK inhibitors as cancer therapeutics has resulted in the discovery of 2-anilino-4-(thiazol-5-yl)pyrimidine cell cycle inhibitors.[6–8] We now report the discovery of another related class of CDK inhibitors, 2-anilino-4-(1*H*-pyrrol-3-yl)pyrimidines, obtained through structure-guided analogue design and optimization. Here we described the synthesis, SAR analysis, and biochemical characterization of these 2-anilino-4-(1*H*-pyrrol-3-yl)pyrimidine CDK inhibitors.

The chemistry adopted for the synthesis of the target compounds is summarized in Scheme 1. Treatment of 3-acetyl-1*H*-pyrroles **3**–**5** with *tert*-butoxybis(dimethylamino)methane (Bredereck's reagent) afforded the enaminone intermediates **6**. These were then condensed with the appropriate phenyl guanidines **7** to the desired pyrimidines **8** at elevated temperature in alcoholic alkali. The former were derived from the corresponding

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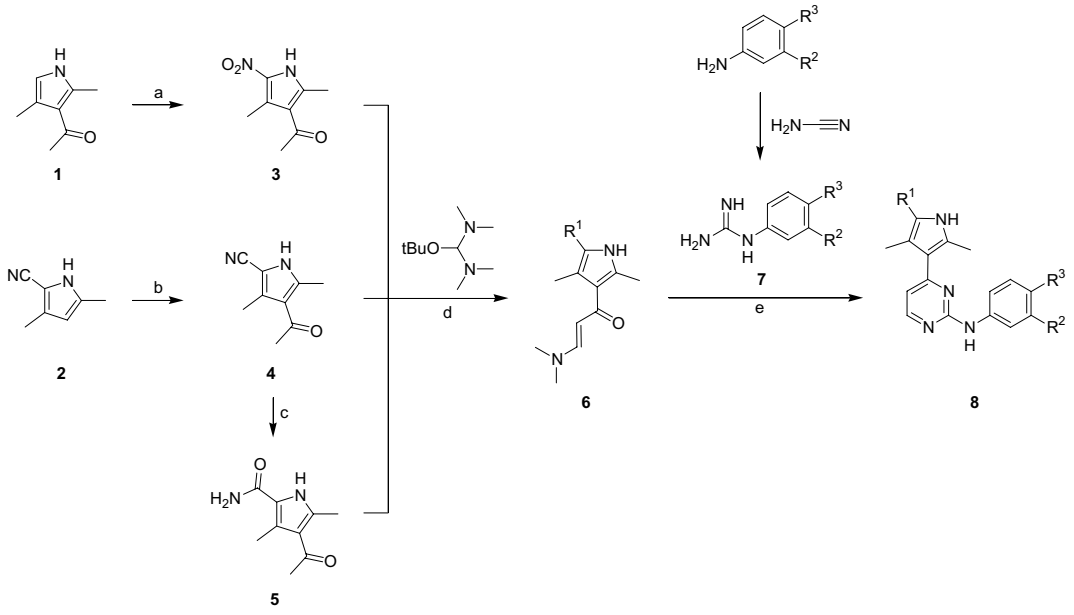

Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4, AcOH/H_2O, DCM; (b) $AlCl_3$, AcCl, DCM, 0 °C–rt; (c) NH_3, MeOH, H_2O_2/H_2O, rt; (d) neat, 75 °C; (e) K_2CO_3, 2-methoxyethanol, 125 °C.

anilines by the treatment of their nitrate or hydrochloride salts with cyanamide.[8]

3-Acetyl-2,4-dimethyl-5-nitro-1*H*-pyrrole **3** was obtained by nitration of 3-acetyl-2,4-dimethylpyrrole in the usual manner. Friedel–Crafts acylation of 3,5-dimethyl-1*H*-pyrrole-2-carbonitrile **2**[9] with acetyl chloride in the presence of aluminium chloride gave 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carbonitrile **4**, whose hydrolysis afforded 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxamide **5**. 2-Halo-3,5-dimethyl-1*H*-pyrrol-4-yl)-pyrimidine analogues **8k** and **8l** (Table 1) were obtained by treatment of [4-(2,4-dimethyl-1*H*-pyrrol-3-yl)pyrimidin-2-yl](4-fluorophenyl)amine with *N*-chloro-succinimide and *N*-bromo-succinimide, respectively.[10]

In the SARs and CDK2 complex crystal structures of our previously described 2-anilino-4-(thiazol-5-yl)pyrimidine cell cycle inhibitors, it was observed that the thiazole ring overlaps roughly with the space occupied by the ribose group of ATP. However, it makes better contacts with adjacent residues, particularly Phe80 in the ATP-binding pocket of CDK2. A number of potent CDK2 inhibitors were obtained through introduction of polar substituents to the thiazole system.[8] One aspect of our analogue design program has therefore been to evaluate the effect of replacing the thiazole ring with other heterocyclic systems, 2,4-dimethyl-1*H*-pyrrol-3-yl in the present case, in the context of the optimal *meta*- or *para*-substituted anilino pyrimidin-2-yl system. A number of analogues were synthesized and screened against CDKs; the SARs are presented in Table 1. The most potently anti-proliferative compound **8b** was also screened in a panel of 16 representative Ser/Thr and Tyr protein kinases. Apart from CDKs, only the closely CDK-related glycogen synthase kinase-3 was inhibited at submicromolar K_i. Of the CDKs not shown in Table 1, CDKs 1, 7, and 9 were

Table 1. CDK inhibitory and in vitro anti-proliferative activities of 2-anilino-4-(1*H*-pyrrol-3-yl)pyrimidines

Compound	R^1	R^2	R^3	Kinase inhibition K_i, (μM)[a]		Anti-proliferative IC_{50}, μM[b]
				CDK2	CDK4	
8a	H	NO_2	H	0.05	4.17	3.30
8b	CN	NO_2	H	0.14	0.96	0.36
8c	CN	NO_2	Me	0.05	0.18	0.38
8d	CN	H	OH	0.05	0.26	1.36
8e	CN	OH	H	0.03	0.18	1.84
8f	CN	OH	Me	0.13	0.22	1.17
8g	CN	Me	F	0.09	0.30	0.89
8h	CN	H	F	0.10	0.90	2.44
8i	$CONH_2$	H	F	0.08	0.42	1.64
8j	NO_2	H	F	0.03	0.12	3.12
8k	Cl	H	F	0.36	1.91	13.03
8l	Br	H	F	0.27	1.60	15.98

[a] Values are means of at least three independent determinations.
[b] Mean values in 72 h MTT assays[11] of A549 and H29 human tumour cell lines.

also inhibited significantly by compound **8b**; the same was true for compounds **8** in general.

Compound **8a** is a potent and comparatively selective CDK2 inhibitor. Despite its potency against the isolated CDK2 enzyme, compound **8a** showed modest cellular activity in cell proliferation assays against A549 lung carcinoma and HT 29 colon cancer cells.

This was dramatically improved by the introduction of a nitrile group at position 5 of the pyrrole ring and compound **8b** exhibited ~10-fold higher cellular activity. Interestingly, introduction of an additional methyl group at the *para*-position of the anilino moiety in compound **8c** was able to restore CDK2 inhibitory activity (with respect to **8a**), while maintaining the cellular anti-proliferative activity of compound **8b**. While there are clearly different profiles in the CDK2/4 selectivity amongst 1*H*-pyrrol-3-yl nitrile, amide, nitro, and halo pyrimidin-2-yl-aniline analogues **8b–l**, these substitutions are well tolerated as far as CDK activity is concerned. It is notable, however, that the halogenated compounds **8k** and **8l** are much less effective against the tumour cells than other analogues of this series. As is commonly observed with many ATP antagonist kinase inhibitors, the anti-proliferative, that is cellular potency of the 2-anilino-4-(1*H*-pyrrol-3-yl)pyrimidines is significantly lower than the potency against isolated CDK enzymes. In part this is probably due to high intracellular ATP concentrations.

The X-ray crystal structures of CDK2 complexes with **8a** and **8g** were determined; the positions of these inhibitors in the ATP-binding pocket are shown in Figure 1. The binding modes are similar for both ligands. The substituted aniline moiety is positioned at the edge of the ATP binding site and the pyrrole ring is orientated to occupy the deep pocket of the ribose site. In each case, the characteristic H-bonding network involving the aminopyrimidine system with Glu81 and Leu83 is observed. Furthermore, the hydrophobic aniline rings lie against a hydrophobic surface formed by the Ile10, Phe82, and Leu134 side chains. These interactions are consistent with our 2-anilino-4-(thiazol-5-yl)pyrimidine pharmacophore. Interestingly, the pyrrole NH1 forms a strong H-bond interaction with the Asp145 carboxyl group, whereas a polar interaction of the thiazol-2-yl N atom with Asn132 was previously observed for 2-anilino-4-(thiazol-5-yl)pyrimidine ligands. Replacement of the pyrrol-5-yl hydrogen (compound **8a**) with a nitrile (compound **8g**) function does not result in an additional interaction with CDK2, an observation consistent with the similar potency of these two analogues.

The retinoblastoma tumour suppressor protein pRb is the main physiological substrate for the G1/S CDK complexes.[12] The entry of cells into S-phase requires CDK-mediated phosphorylation of pRb, releasing and activating E2F family transcription factors, which are inactive while bound to pRb during the G0 and M-phases of the cell cycle. Cellular CDK inhibitory activity of our inhibitors was confirmed by the examination of pRb phosphorylation status following treatment of A549 lung carcinoma cells with compound **8b** (Fig. 2). A dose- and time-dependent decrease in phosphorylation of pRb at Ser249/Thr252, Ser807/Ser811, and Thr821, the preferential CDK2 and CDK4 phosphorylation sites, were observed. The treatment also resulted in induction of p53 and in decreased phosphorylation at Ser-2 and Ser-5 of RNA polymerase II (data not shown), another substrate of several CDK isoforms, implying a possible transcriptional inhibition with this compound.

In summary, a series of 2-anilino-4-(pyrrol-3-yl)-pyrimidine CDK inhibitors were discovered.[13] Many of them exhibited potent CDK enzyme inhibitory activity and

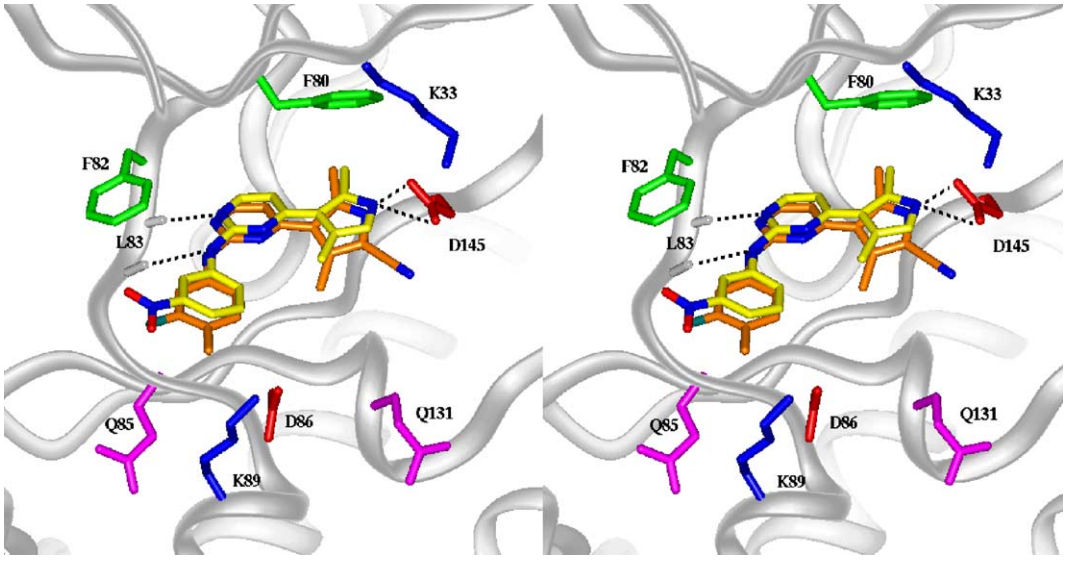

Figure 1. A stereo view of the X-ray crystal structure superimpositions of the CDK2/**8a** (yellow) and CDK2/**8g** (orange) complexes in the ATP binding site. H-bonds are indicated with broken lines. Monomeric CDK2 crystals were used for complex formation and structure calculations.[6,8]

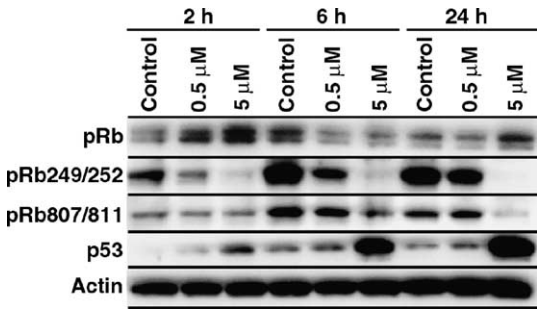

Figure 2. Western blot analysis of A549 cells treated with compound **8b** at 0.5 and 5 μM for 2, 6, and 24 h. DMSO treatment as control. Experimental procedures were as described previously.[8]

anti-proliferative activity in tumour cells. Cellular CDK2/4 inhibitory activities were confirmed.

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References and notes

1. Hartwell, L. H.; Kastan, M. B. *Science* **1994**, *266*, 1821–1828.
2. Webster, K. R.; Kimball, S. D. *Emerg. Drugs* **2000**, *5*, 45–59.
3. Toogood, P. L. *Med. Res. Rev.* **2001**, *21*, 487–498.
4. Sherr, C. J. *Science* **1996**, *274*, 1672–1677.
5. Senderowicz, A. M. *Oncogene* **2003**, *22*, 6609–6620.
6. Wu, S. Y.; McNae, I.; Kontopidis, G.; McClue, S. J.; McInnes, C.; Stewart, K. J.; Wang, S.; Zheleva, D. I.; Marriage, H.; Lane, D. P.; Taylor, P.; Fischer, P. M.; Walkinshaw, M. D. *Structure* **2003**, *11*, 399–410.
7. Wang, S.; Anderson, S.; Clarke, R.; Duncan, K.; Eland, S.; Jackson, W.; Lyon, C.; McClue, S.; Meades, C.; Osnowski, A.; Perry, A.; Renachowski, S.; Stuart, I.; Thomas, M.; Westwood, B.; Wood, G.; Yuill, R.; Lane, D.; Jackson, R.; Fischer, P. *Proc. Am. Assoc. Cancer Res.* **2003**, *44*, Abs. 3974.
8. Wang, S.; Meades, C.; Wood, G.; Osnowski, A.; Anderson, S.; Yuill, R.; Thomas, M.; Mezna, M.; Jackson, W.; Midgley, C.; Griffiths, G.; Fleming, I.; Green, S.; McNae, I.; Wu, S.-Y.; McInnes, C.; Zheleva, D.; Walkinshaw, M. D.; Fischer, P. M. *J. Med. Chem.* **2004**, *47*, 1662–1675.
9. Cheng, L. J.; Lightner, D. A. *Synthesis* **1999**, 46–48.
10. Fischer, P. M.; Wang, S.; Wood, G. In *PCT Intl. Patent Appl. Publ. WO 02/079193*; Cyclacel Limited, UK, 2002.
11. Haselsberger, K.; Peterson, D. C.; Thomas, D. G.; Darling, J. L. *Anti-Cancer Drugs* **1996**, *7*, 331–338.
12. Ortega, S.; Malumbres, M.; Barbacid, M. *Curr. Genomics* **2002**, *3*, 245–263.
13. Analytical data for representative compounds. **8a**: yellow solid, mp 198–200 °C; anal. RP-HPLC (Vydac 218TP54, 1 mL/min, H_2O/MeCN solvent system containing 0.1% CF_3COOH) t_R 14.3 min (10–70% MeCN linear gradient over 20 min, purity 99%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.19 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.48 (s, 1H, pyrrole-H), 6.87 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 7.52 (m, 1H, Ph-H), 7.73 (m, 1H, Ph-H), 8.08 (m, 1H, Ph-H), 8.38 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 8.93 (s, 1H, Ph-H), 9.83 (br s, 1H, NH), 10.76 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 13.34, 14.21, 112.02, 112.89, 115.80, 115.82, 117.65, 117.90, 125.25, 130.25, 130.86, 143.01, 148.82, 157.62, 160.05, 164.42; MS (ESI$^+$) m/z 310.07 $(M+H)^+$; anal. ($C_{16}H_{15}N_5O_2$) C, H, N. Compound **8b**: pale yellow solid, mp 258–259 °C; anal. RP-HPLC t_R 17.1 min (purity 100%); ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 6.96 (d, 1H, $J = 4.5$ Hz, pyrimidinyl-H), 7.55 (t, 1H, $J = 8.3$ Hz, Ph-H), 7.76 (m, 1H, Ph-H), 8.05 (m, 1H, Ph-H), 8.51 (d, 1H, $J = 5.0$ Hz, pyrimidinyl-H), 8.90 (s, 1H, Ph-H), 10.01 (s, 1H, NH), 12.25 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 12.32, 13.94, 98.87, 112.66, 112.99, 115.09, 116.18, 119.51, 125.39, 130.41, 131.18, 136.34, 142.68, 148.81, 158.65, 160.12, 162.18; MS (ESI$^+$) m/z 334.22. Compound **8d**: pale tan solid, mp 272–276 °C; anal. RP-HPLC t_R 10.8 min (purity 97%); ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 6.67 (m, 2H, Ph-H), 6.73 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 7.43 (d, 2H, $J = 8.5$ Hz, Ph-H), 8.32 (d, 1H, $J = 5.4$ Hz, pyrimidinyl-H), 9.01 (s, 1H, NH), 12.16 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 12.44, 14.05, 98.57, 110.61, 115.21, 115.52, 122.31, 131.09, 132.71, 136.03, 153.03, 158.30, 161.01, 162.20; MS (ESI$^+$) m/z 305.80. Compound **8e**: off-white solid, anal. RP-HPLC t_R 16.4 min (0–60% MeCN, purity 97%); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.83 (d, 1H, $J = 5.0$ Hz, pyrimidinyl-H), 7.11 (t, 1H, $J = 8.1$ Hz, Ph-H), 7.73 (m, 1H, Ph-H), 8.40 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 9.46 (s, 1H, NH), 12.20 (br s, 1H, NH); MS (ESI$^+$) m/z 307.70. Compound **8i**: light yellow solid, mp 94–96 °C; anal. RP-HPLC t_R 10.64 min (10–70% MeCN, purity 100%); ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 6.76 (d, 1H, $J = 6.0$ Hz, pyrimidinyl-H), 7.09 (m, 2H, Ph-H), 7.77 (m, 2H, Ph-H), 8.37 (d, 1H, $J = 6.0$ Hz, pyrimidinyl-H), 9.41 (s, 1H, NH), 11.25 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 12.58, 13.57, 112.20, 115.42, 115.60, 120.49, 121.12, 121.18, 121.19, 122.15, 122.89, 131.99, 137.86, 137.88, 157.94, 160.53, 163.55; MS (ESI$^+$) m/z 325.87. Compound **8k**: yellow solid, mp 200–203 °C; anal. RP-HPLC: t_R 15.4 min (purity 98%); ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.77 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 7.10 (m, 2H, Ph-H), 7.74 (m, 2H, Ph-H), 8.32 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 9.42 (s, 1H, NH), 11.54 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 11.41, 13.82, 110.80, 111.00, 113.55, 115.49, 115.66, 121.67, 121.73, 129.61, 137.50, 156.93, 156.94, 158.82, 159.96, 163.62; MS (ESI$^+$) m/z 317.72 $(M+H)^+$. **8l**: yellow solid, mp 181–183 °C; anal. RP-HPLC t_R 15.8 min (purity 95%); ^{1}H NMR (DMSO-d_6) δ 2.08 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.77 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 7.11 (m, 2H, Ph-H), 7.74 (m, 2H, Ph-H), 8.33 (d, 1H, $J = 5.5$ Hz, pyrimidinyl-H), 9.39 (s, 1H, NH), 11.50 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 12.56, 13.87, 110.00, 111.12, 115.46, 115.64, 116.75, 119.06, 121.49, 121.51, 131.34, 137.63, 157.41, 158.74, 160.15, 163.41; MS (ESI$^+$) m/z 362.90 $(M+H)^+$.