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6-Substituted Pyrrolo[3,4-c]pyrazoles: An Improved Class of CDK2 Inhibitors

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Dedicated to Professor S. V. Ley on the occasion of his 60th birthday

We have recently reported a new class of CDK2/cyclin A inhibitors based on a bicyclic tetrahydropyrrolo[3,4-c]pyrazole scaffold. The introduction of small alkyl or cycloalkyl groups in position 6 of this scaffold allowed variation at the other two diversity points. Conventional and polymer-assisted solution phase chemistry provided a way of generating compounds with improved biochemical and cellular activity. Optimization of the physical properties and pharmacokinetic profile led to a compound which exhibited good efficacy in vivo on A2780 human ovarian carcinoma.

Introduction

The cyclin dependent kinases (CDKs) are serine/threonine kinases intimately involved in the regulation of the cell cycle. CDK2 in particular, is a heterodimer composed of a catalytic subunit, CDK2, and one of two activating subunits, cyclin E or cyclin A. The two isoforms of the kinase have distinct roles during the cell cycle. CDK2/cyclin E is mainly involved in progression through G1/S, centrosome duplication, and DNA replication. CDK2/cyclin A is a key regulator of G2/M progression. Many past observations, such as the transient transfection of a catalytically inactive form of CDK2,[1] the inducible expression of a dominant negative form of CDK2[2] and microinjection of antibodies against CDK2 and cyclin A[3] have suggested an essential role for CDK2 in cell proliferation. Recent data from the CDK2 and cyclin E knockout mice[4] indicating that CDK2 is not necessary for normal development has opened up an interesting debate. Some support the hypothesis that selective CDK2 inhibitors will not be toxic. Others argue that CDK2, not being essential for normal cell division, is unlikely involved in tumor cell proliferation. We must not forget, however, that inhibitors render CDK2 inactive rather than prevent its expression and it is not possible to exclude the activation of compensatory mechanisms to overcome the lack of CDK2 or cyclin E genes in mice. In this context, we believe that the pharmacological inhibition of CDK2 activity remains a potential therapeutic strategy for anticancer therapies. Recently, we reported the design and solid-phase generation of potent Aurora-A inhibitors bearing a 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole core structure.[5] This novel adenine mimetic scaffold, which is endowed with high versatility, has also been exploited for the solution-phase synthesis of a series of CDK2/cyclin A inhibitors.[6]

In this study we identified two compounds (**1** and **2**) with nanomolar activity against CDK2/cyclin A in the biochemical assay and able to efficiently inhibit CDK2-mediated cell proliferation although, because of both suboptimal physicochemical properties and an overall unfavorable early ADME profile, further optimization was required (Table 1).

In particular, as the low buffer solubility of these compounds prevented their use in vivo as injectables, the first objective in the optimization of this class of compounds was the improve-

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Table 1. Preliminary expansion of the 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole scaffold.

Compd	R′	R″	IC50 [μM][a,b,c] CDK2/cyclin A	Aurora-A	A2780 cells	Solubility[a,d]	Caco-2-permeability[a]
1			0.033	4.20	0.94	2	Moderate
2			0.036	>10	0.13	23	Moderate

[a] See ref. [7]. [b] See ref. [8]. [c] See ref. [9]. [d] μM; buffer pH 7.

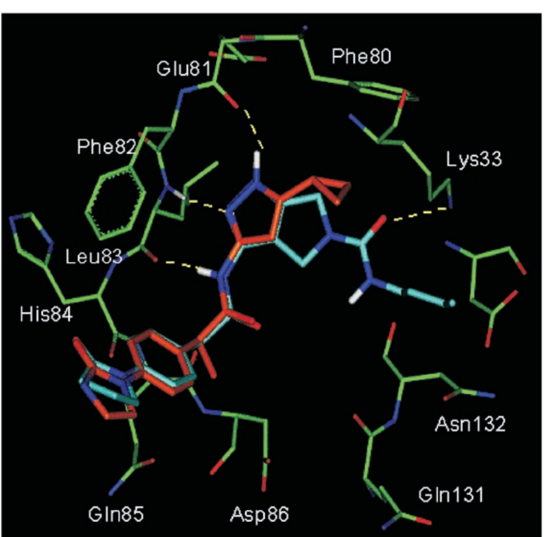
ment of the physicochemical properties yet retaining high pharmacological potency. In the present communication we wish to report the results we have obtained so far.

As we have previously described, this scaffold binds the ATP pocket of Aurora-A and CDK2 kinases with the aminopyrazole making three hydrogen bonds with the Aurora-A and CDK2 hinges, which connect the N- and C-terminal domains (residues 211–214 and residues 81–84, respectively).[5–6] CDK2 has a small cavity at the back of the pocket (also known as the buried region) defined by Ala 31, Val 64, Phe 80, and Ala 144.[10] The size and shape of these cavities vary among the different kinases and thus, are often used for improving the selectivity profile of kinase inhibitors. The buried region of CDK2 is small. That is mainly due to the presence of a big and rigid gatekeeper residue, Phe 80. Structure–activity relationship (SAR) data generated by us[11] and others[12] on multiple chemical classes have shown that occupation of this buried area by small hydrophobic moieties is beneficial to CDK2 binding.

This was also evident during the optimization of our 3-amino-pyrazole class in which a nice packing of the cyclopropyl group at position 5 was important for CDK2 binding.[7,13] Figure 1 shows the overlap between a representative of the 3-aminopyrazole and of the 3-amino-pyrrolo-pyrazole classes. By visual inspection and modeling experiments we identified position 6 of the pyrrolo-pyrazole scaffold as suitable for placing small alkyl or cycloalkyl groups to extend the lipophilic interaction of these groups with the amino acids lining the buried region of CDK2.

Therefore, two novel scaffolds were designed to enhance the hydrophobic contact in the CDK2 buried region: 6-spirocyclopropyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole (**a**) and 6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole (**b**).

a b



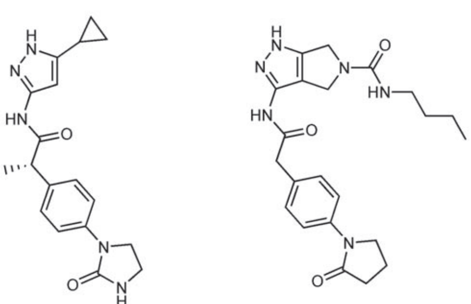


Figure 1. Superimposition of the X-ray structure of CDK2/cyclin A (green carbon atoms) in complex with a pyrazole derivative (orange carbon atoms, PDB 1BPM) on that of the docking model of CDK2/cyclin A in complex with a pyrrolo-pyrazole compound (cyan carbon atoms).

Chemistry

The synthesis of both scaffolds relied on the procedure described in Scheme 1. Treatment of the commercially available amino acid with acrylonitrile under basic conditions (NaOH, H_2O, 4–22 °C, 16 h) followed by Boc-protection of the sterically hindered secondary amine ($[(CH_3)_4NOH]\cdot 5H_2O$, Boc_2O, CH_3CN, 22 °C, 3 days) gave the corresponding intermediates **5 a** and **5 b.**[14] Following esterification, hydride induced ring closing of the cyano ester, and subsequent treatment of the β keto nitrile (**7 a** and **7 b**) with hydrazine in the presence of acetic acid provided the desired core structure (**8 a** and **8 b**). These scaffolds were protected on the pyrazole ring using ethyl chloroformate. In both cases a mixture of 1 and 2-ethylcarbamates was obtained which could either be separated by flash chromatography or used as such in the following steps. The major regioiso-

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Scheme 1. Reagents and conditions: a) acrylonitrile, NaOH, H_2O, 4–22 °C, 16 h; AcOH; b) $[(CH_3)_4NOH]\cdot 5H_2O$, Boc_2O, CH_3CN, 22 °C, 3 days; c) $KHCO_3$, CH_3I, DMF, 22 °C, 12 h; d) 60% NaH, dioxane, reflux, 4 h; e) $NH_2NH_2\cdot H_2O$, AcOH, ethanol, 60 °C, 2 days; f) EtOCOCl, DIEA, THF, 0–5 °C, 1 h; flash chromatography.

mer was for both scaffolds the one bearing the ethylcarbamate group at the nitrogen in position 2 (**9a** and **9b**).

A general synthetic strategy was required to allow the generation of many derivatives using parallel medicinal chemistry. Both conventional and polymer-assisted solution-phase chemistry were employed for this purpose. In particular, by using solid supported reagents and scavenger techniques not only aqueous work-up was completely avoided but also chromatography purification was in most cases unnecessary as the final products were usually obtained in pure form after filtration over a short plug of silica.[15]

Scheme 2 reports a general polymer-assisted solution-phase synthesis for this class of compounds that was applied to obtain the required derivatives. Acylation of the 3-position was accomplished using the corresponding acyl chlorides and polymer-supported triethylamine as a base followed by quenching of excess reagent with a polymer-bound primary amine. Removal of the N-Boc protection and neutralization of the secondary amine with polymer-supported triethylamine were followed by treatment with either an acyl chloride or an isocyanate or triphosgene followed by addition of an amine. A scavenging cocktail of methylisocyanate polystyrene and polymer-bound primary amine were then added to sequester any remaining reagent. Final deprotection of the ethylcarbamate group with macro reticular polymer-bound carbonate in methanol gave compounds **10a**–**28a** and **10b**–**31b** usually with good purities.

Scheme 2. Reagents and conditions: a) R^1COCl, diethylaminomethylpolystyrene; aminomethylated polystyrene; b) TFA/DCM (1:1); aminomethylated polystyrene; c) diethylaminomethylpolystyrene; R^2COCl or R′NCO or triphosgene then NHR′R′′; aminomethylated polystyrene and methylisocyanate polystyrene; d) Amberlite IRA 900 $NaCO_3^-$ form, MeOH.

Results and Discussion

For the sake of clarity only a limited representative set of compounds is used here to describe the SAR. More compounds were synthesized to support the SAR described below.[16]

In a previous paper we demonstrated that 3-benzamido substituents on the 6-unsubstituted tetrahydropyrrole[3,4-c]pyrazole scaffold inhibit CDK2 being often equipotent to Aurora-A, whereas 3-arylacetamido compounds are more potent against CDK2/cyclin A and selective against Aurora-A.[6]

The preference of CDK2 for arylacetamido substituents at position 3 and their incompatibility with significant Aurora-A inhibition is maintained in the presence of the substitution at position 6 (see **10a** and **10b**, Table 2). Remarkably, both substituents reduce the activity towards Aurora-A even in the presence of an Aurora-A oriented substituent in position 3 such as benzamido moieties. In particular, the presence of the 6,6-*gem*-dimethyl substitution renders compound **11b** inactive against Aurora-A, whereas the spirocyclopropyl analogue **11a** maintains a low micromolar activity.

As previously described the substituents at position 6 occupy the buried region formed in CDK2 by Val 18 (Val 147 in Aurora-A), Val 64 (Leu 194 in Aurora-A), Phe 80 (Leu 210 in Aurora-A), and Ala 144 (Ala 273 in Aurora-A) (Figure 2).

By docking compounds **11a** and **11b** into CDK2 and Aurora-A ATP pockets it seems that the larger size of Aurora-A-Leu 194 versus CDK2-Val 64 causes a steric clash with the methyl of the 6,6-*gem*-dimethyl substituent of **11b** pointing toward the C-terminal lobe (Figure 3). The 6,6-*gem*-dimethyl is more cumbersome than the spirocyclopropyl because of its greater C-C6-C angle.

Remarkably, solubility in neutral buffer was not decreased by C6 substitution; actually, the

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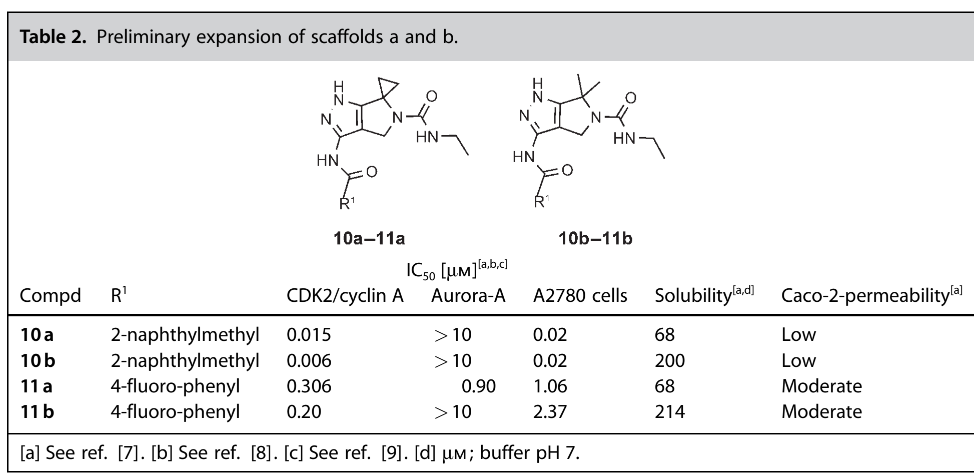
Table 2. Preliminary expansion of scaffolds a and b.



Compd	R^1	IC$_{50}$ [µM][a,b,c] CDK2/cyclin A	Aurora-A	A2780 cells	Solubility[a,d]	Caco-2-permeability[a]
10a	2-naphthylmethyl	0.015	>10	0.02	68	Low
10b	2-naphthylmethyl	0.006	>10	0.02	200	Low
11a	4-fluoro-phenyl	0.306	0.90	1.06	68	Moderate
11b	4-fluoro-phenyl	0.20	>10	2.37	214	Moderate

[a] See ref. [7]. [b] See ref. [8]. [c] See ref. [9]. [d] µM; buffer pH 7.

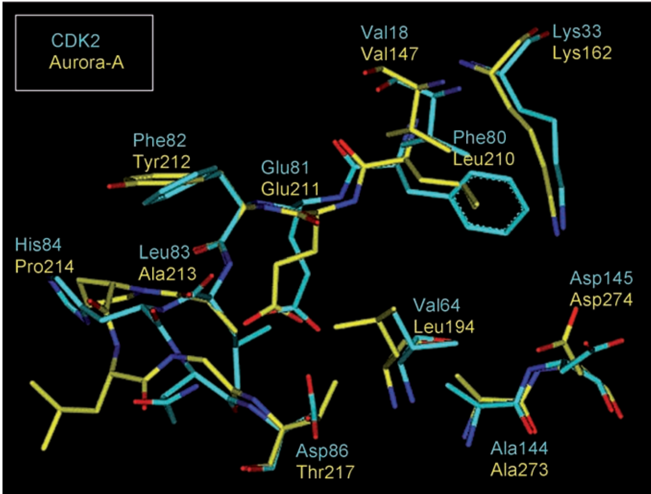


Figure 2. Close up of the ATP binding pocket of CDK2 (cyan) and Aurora-A (yellow).

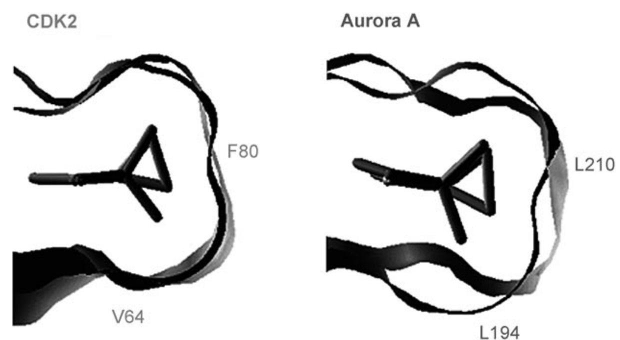


Figure 3. Compounds **11a** and **11b** docked into CDK2 and Aurora-A. The slice through the CDK2 (gray in the structure on the left) and Aurora-A (gray in the structure on the right) surface taken perpendicular to the plane of the pyrrolo-pyrazole scaffold and passing through the carbon at position 6 is shown. The 6,6-*gem*-dimethyl surface of compound **11b** taken on the same plane is also shown in magenta.

6,6-*gem*-dimethyl series showed a distinct advantage in this respect (compare **2** of Table 1 with **10a** and **10b** of Table 2).

These encouraging results prompted us to vary substitution at position 5 (pointing towards the relatively spacious phosphate binding region) while keeping unvaried a 4-fluoro benzamido group at position 3 (Table 3). To further improve both potency and physicochemical properties, several ureas were prepared.

With the introduction of cyclic residues (**12a**–**15a** and **12b**–**15b**) a remarkable increase of potency was observed on the 6,6-*gem*-dimethylsubstituted pyrrolo-pyrazole series, on the spirocyclopropyl series the effect was only moderate. Interestingly, both series showed increased selectivity versus Aurora-A. Moreover, compounds **12b**–**13b** and particularly **15b** also displayed increased antiproliferative activity with respect to the parent compound **11b**. As far as buffer solubility is concerned, a distinct advantage of the 6,6-*gem*-dimethyl series was confirmed.

Focusing our attention on **15b**, the best compound found in this series, we compared several *N*-methylpiperazino ureas (**16a**–**19a** and **16b**–**19b**) bearing different amido substituents at position 3 of the scaffold (Table 4). These data confirmed the trend observed above as far as activity, selectivity versus Aurora-A, and buffer solubility are concerned but no particular advantages on changing the nature of R^1 were found and compound **15b** was chosen as lead compound in the 5-urea series.

To improve permeability, functionalization of position 5 by means of an amide instead of a urea was taken into account. For this purpose, a rapid expansion on the 6,6-*gem*-dimethyl-substituted pyrrolo-pyrazole scaffold b was performed choosing linear/branched alkyl and aryl groups for position 5. A small library was generated, keeping the 3-substituent fixed as 4-fluorophenyl. Four representative compounds, **20b**–**23b**, are reported in Table 5.

The *tert*-butyl group (see compound **23b**), because of its lipophilic character, emerged as the potential best moiety prompting us to expand this subclass. Thus, we prepared a series of 5-pivaloyl 6-substituted pyrrolo-pyrazoles to further explore the SAR of the 3-amido substitutions (Table 6).

A phenyl ring bearing halogen substitutents (**20a**–**24a** and **23b**–**27b**) gave compounds with strong activity against CDK2/cyclin A and in the A2780 cell proliferation assay in both series. The *p*-trifluoromethyl phenyl derivatives (**25a** and **28b**) were less potent inhibitors of CDK2/cyclin A than the previous ones (**20a**–**24a** and **23b**–**27b**). As seen before, 6,6-*gem*-dimethyl substitution was detrimental for Aurora-A whereas the spirocyclopropyl substituent was partially tolerated. The 2-thienyl residue, chosen as a generic phenyl bioisoster, gave compounds which were potent against CDK2/cyclin A but not completely Aurora-A selective (**26a** and **29b**). The cyclopropyl and the *tert*-butyl groups also gave potent CDK2/cyclin A inhibitors in both series (**27a**–**28a** and **30b**–**31b**). Interestingly, the *tert*-

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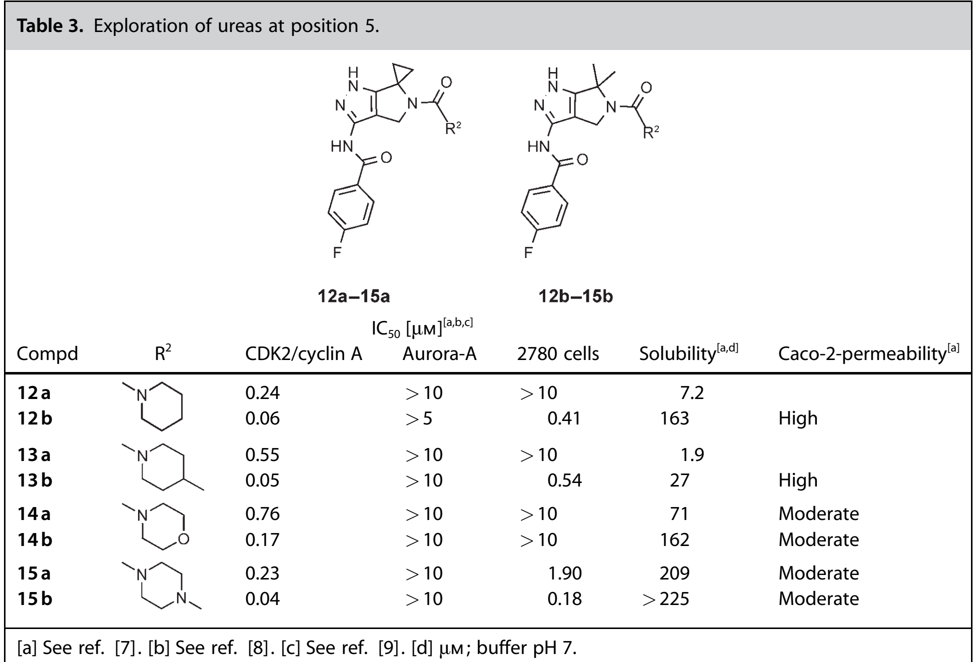
Table 3. Exploration of ureas at position 5.



12a–15a 12b–15b

Compd	R^2	IC$_{50}$ [μM][a,b,c] CDK2/cyclin A	Aurora-A	2780 cells	Solubility[a,d]	Caco-2-permeability[a]
12a		0.24	>10	>10	7.2	
12b		0.06	>5	0.41	163	High
13a		0.55	>10	>10	1.9	
13b		0.05	>10	0.54	27	High
14a		0.76	>10	>10	71	Moderate
14b		0.17	>10	>10	162	Moderate
15a		0.23	>10	1.90	209	Moderate
15b		0.04	>10	0.18	>225	Moderate

[a] See ref. [7]. [b] See ref. [8]. [c] See ref. [9]. [d] μM; buffer pH 7.

Table 4. Exploration of amides at position 3.

16a–19a 16b–19b

Compd	R^1	IC$_{50}$ [μM][a,b,c] CDK2/cyclin A	A2780 cells	Solubility[a,d]	Caco-2-permeability[a]
16a	2,4-difluoro-phenyl	0.26	3.10	198	Moderate
16b		0.04	0.23	>225	Moderate
17a	4-chloro-phenyl	0.14	4.60	192	Moderate
17b		0.04	0.12	210	Low
18a	4-CF_3-phenyl	0.33	3.89	166	Moderate
18b		0.05	0.58	196	Moderate
19a	cyclopropyl	0.13	2.88	>225	Low
19b		0.03	1.38	69	Low

[a] See ref. [7]. [b] See ref. [8]. [c] See ref. [9]. [d] μM; buffer pH 7.

butyl group gave a completely CDK2 selective compound with the spirocyclopropyl pyrrolo-pyrazole scaffold most likely as a result of steric clash of the *tert*-butyl group in the solvent accessible region of the Aurora-A enzyme. It is known that the solvent accessible region of Aurora-A differs from CDK2 by the presence of an inserted glycine (Gly 216) that is absent in CDK2. This additional residue in Aurora-A causes a different protein conformation between the hinge region and the beginning of the C-terminal domain that tends to disfavor inhibitors, such as 3-pivaloylamino pyrrolo-pyrazoles, that do not protrude out of the ATP pocket in a planar fashion.

Based on the biochemical assay, cellular potency, and preliminary physicochemical properties compounds **15b**, **20a**, **23b**, and **26b** were selected for further assessment.

Table 7 reports their selectivity profile on a panel of 33 serine/threonine and tyrosine kinases.[7]

Among the members of the CDK family that were tested, they were found to inhibit CDK2/cyclin A and CDK2/cyclin E at comparable levels. A slight difference was observed in the inhibition of CDK1/cyclin B: compounds **20a** and **23b** were more potent than **15b** and **26b.** In addition, compound **26b** was less potent against CDK5/p25 than **15b, 20a**, and **23b**. Among all the other enzymes in the panel only Aurora-A and GSK-3β were inhibited in the low micromolar range by compound **20a** whereas compounds **15b**, **23b**, and **26b** inhibited less potently only GSK-3β. As we previously observed when optimizing a different class, the buried region of GSK-3β can be exploited to improve the inhibitors selectivity profile in favor of CDK2 against GSK-3β.[11,17] The larger size of GSK3β-Cys 198 versus CDK2-Ala 144 may be responsible for the differences in binding of **20a** compared to **15b**, **23b**, and **26b**.

The effects of the compounds on the cell cycle progression and DNA synthesis were studied using FACS analysis and BrdU incorporation, respectively. In addition, the effect on the phosphorylation status of the known CDK substrate pRb was analyzed. More specifically, the cell cycle profile, BrdU incorporation, and phosphorylation status of pRb were analyzed on a mid-log population of A2780 ovarian carcinoma cell line in the presence or absence of compounds, for 24 h, at the concentrations of 1 and 3 μM. At the concentration of 3 μM, all the compounds were able to determine a clear reduction of S phase population, which was associated with an increase of G0/G1 and G2/M population for compounds **26b** and **15b** and to an increase of G2/M population for compounds **20a** and **23b** (Table 8). The reduction of S phase population was linked to a strong reduction in the percentage of BrdU-incorporating cells, meaning that DNA synthesis in these cells was impaired. The higher increase in G2/M population observed for compounds

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Table 5. Exploration of amides at position **5**.

20b–23b

Compd	R^2	IC$_{50}$ [μM][a,b,c] CDK2/cyclin A	Aurora-A	A2780 cells	Solubility[a,d]	Caco-2-permeability[a]
20b	Me	1.24	> 10	> 10	217	High
21b	*n*Pr	0.18	> 10	1.45	194	High
22b	Ph	0.19	> 10	3.43	136	High
23b	*t*Bu	0.03	> 10	0.27	235	High

[a] See ref. [7]. [b] See ref. [8]. [c] See ref. [9]. [d] μM; buffer pH 7.

Table 6. 5-Pivaloylamides: exploration at position 3.

20a–28a **23b–31b**

Compd	R^1	IC$_{50}$ [μM][a,b,c] CDK2/cyclin A	Aurora-A	A2780 cells	Solubility[a,d]	Caco-2-permeability[a]
20a	4-fluorophenyl	0.04	0.34	0.67	127	High
23b		0.03	> 10	0.27	235	High
21a	3-fluorophenyl	0.06	0.85	0.95	95	High
24b		0.05	> 10	0.71	198	High
22a	2,4-difluorophenyl	0.14	3.72	1.30	138	High
25b		0.06	> 10	0.48	57	High
23a	3,5-difluorophenyl	0.12	3.15	2.18	91	High
26b		0.06	> 10	0.31	186	High
24a	4-chlorophenyl	0.16	0.77	1.29	28	High
27b		0.05	> 10	0.34	142	High
25a	4-CF_3-phenyl	0.5	1.98	1.76	2.5	
28b		0.19	> 10	0.30	73	High
26a	2-thienyl	0.02	0.08	0.75	153	High
29b		0.02	0.92	0.23	184	High
27a	cyclopropyl	0.09	0.24	0.72	194	Moderate
30b		0.11	> 10	4.55		
28a	*t*Bu	0.02	> 10	0.46	197	High
31b		0.01	> 10	0.35	185	High

[a] See ref. [7]. [b] See ref. [8]. [c] See ref. [9]. [d] μM; buffer pH 7.

20a and **23b** can be ascribed to higher potency of these compounds in inhibiting CDK1/cyclin B (Table 7).

Regarding the phosphorylation status of pRb, it was possible to note a clear reduction of the hyperphosphorylated form of pRb and an accumulation of the hypophosphorylated form of pRb at the concentration of 3 μM in the samples obtained by cells treated with all the compounds versus the samples of cells treated with the vehicle, indicative of an effect on the activity of CDK2 (Figure 4).

Table 9 reports preliminary in vitro and in vivo pharmacokinetic parameters.[7] All the compounds displayed good buffer solubility, stability to human CYP3A4, and good permeability in the Caco-2 permeability assay. The plasma protein binding was relatively low. In all compounds, the in vivo results in healthy nude mouse indicated a volume of distribution higher than the total body water suggesting tissue distribution. Whereas the in vivo clearance was relatively high for **15b**, accounting for about 60% of the hepatic blood flow, the other three compounds showed a low clearance in the range of 6% (**26b**), 8% (**20a**), and 19% (**23b**) of the hepatic blood flow.

On the basis of the data presented above, **26b** was prioritized for further in vivo characterization in a mouse xenograft model.[7] The human ovarian A2780 xenograft mouse model was used, and on the basis of

Table 7. Selectivity profile of compounds **15b**, **20a**, **23b**, and **26b**.

Compd	IC$_{50}$ [μM][a,b] CDK2/A	CDK2/E	CDK1/B	CDK5/p25	Others
15b	0.040	0.060	0.27	0.050	GSK-3β: 0.98
20a	0.040	0.020	0.083	0.040	GSK-3β: 0.13 Aurora-A: 0.34
23b	0.030	0.012	0.082	0.040	GSK-3β: 1.0
26b	0.060	0.090	0.45	0.14	GSK-3β: 3.63

[a] See ref. [7]. [b] See ref. [8].

Table 8. Effects on cell cycle progression and DNA synthesis.[18]

Compd	G0/G1[a] % increase	S[a] % decrease	G2/M[a] % increase	BrdU incorporation[a] % reduction
15b	16.9	44	37	98
20a	3.7	39.2	59	92
23b	0	36.7	63.4	64.8
26b	22	52	26	96

[a] versus control cells.

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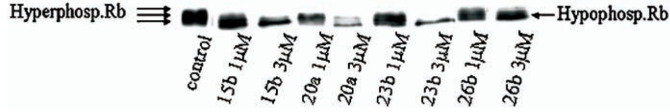

Figure 4. A2780 (ovarian carcinoma) cells were exposed to the compounds for 24 h at the concentration of 1 and 3 μM. Cell lysates obtained from the same cells were used to follow the status of pRb phosphorylation.

Table 9. In vitro ADME and in vivo pharmacokinetic parameters.[7]

	In vitro ADME parameters				In vivo ADME parameters (IV 5 mg mL^{-1})		
Compd	Solubility [μM]	Caco-2 cell permeability	CYP3A4 (% remaining)	PPB (%)	Clearance (mL min^{-1}/Kg)	$t_{1/2}$ (hs)	V_{ss} (mL kg^{-1})
15 b	>225	Moderate	89	79	51.7	0.9	1784
20 a	127	High	88	92	7.7	3.9	1223
23 b	235	High	97	76	16.4	1.2	939
26 b	186	High	80	83	4.8	2.2	1034

the plasma levels reached in the preliminary in vivo PK study, we selected the doses of 20 and 30 mg kg^{-1}, intravenous, once a day.

Figure 5 reports the results from this study. Compound **26 b** caused a dose-dependent inhibition of A2780 tumor growth, significant (70 %) at a dose of 30 mg kg^{-1}.

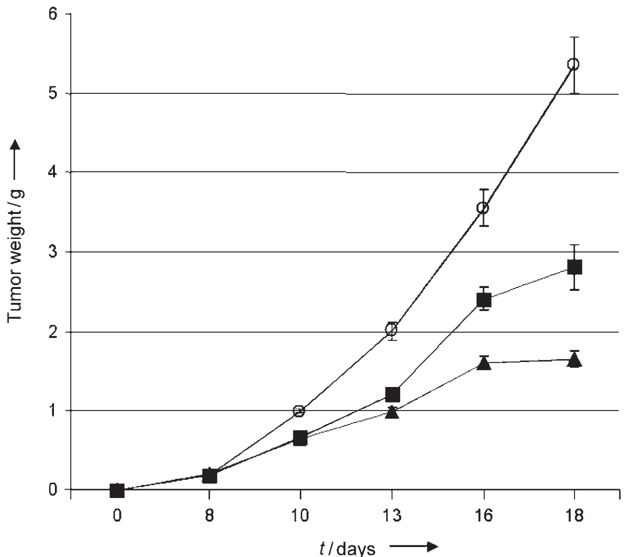

Figure 5. Compound **26 b** shows 47 %and 70 % tumor growth inhibition (TGI) versus control animals (open circle) against a human ovarian cancer model (A2780) transplanted into nude mice when administered respectively at doses of 20 (square) and 30 (triangle) mg kg^{-1} once a day for 10 consecutive days.

Conclusions

The introduction of *gem*-dimethyl or cyclopropyl groups on position 6 of the bicyclic pyrrolo-pyrazole scaffold allowed us to vary substitutions at the other two diversity points. Conventional and polymer-assisted solution phase chemistry provided a way of generating compounds with improved biochemical characteristics. Many analogues exhibited potent CDK2/cyclin A inhibitory activity with improved selectivity versus Aurora-A and other kinases. Many of these compounds showed antiproliferative activity in the A2780 assay. Analysis of the cell cycle profile and CDK2 substrate phosphorylation status of selected compounds indicated an antiproliferative effect that is mediated by CDK2 inhibition. Optimization of the physical properties such as solubility, permeability, and pharmacokinetic profile led to compound **26 b**, which demonstrated good efficacy in vivo in A2780 human ovarian carcinoma by IV administration.

Experimental Section

All solvents and reagents, unless otherwise stated, were commercially available (Aldrich, Fluka), of the best grade, and were used without further purification. All experiments dealing with moisture-sensitive compounds were conducted under dry nitrogen. Organic solutions were evaporated using a Heidolph WB 2001 rotary evaporator at 15–20 mmHg. Thin-layer chromatography was performed on Merck silica gel 60 F_{254} pre-coated plates. Flash chromatography was performed on 40–60 μm silica gel (Carlo Erba). Components were visualized by UV light (λ: 254 nm) and by iodine vapor. Elemental analyses were performed on a Carlo Erba 1110 instrument, and C, H, and N results were within ±0.4 % of theoretical values unless specified. ^{1}H NMR spectra were recorded on a Varian Inova 400 spectrometer, using the solvent as internal standard. Chemical shifts (δ) are reported in parts per million (ppm). The following abbreviations are used for multiplicities: s = singlet; bs = broad singlet; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets, and coupling constant *J*-values are quoted in Hz. Electron impact (EI) mass spectra (MS) were obtained on a Finnigan TSQ 700 triple quadrupole instrument operating at 70 eV. Samples were introduced by direct-inlet probe and heated in the range 25–250 °C until a constant sample evaporation rate was reached. Electrospray (ESI) mass spectra were obtained on a Finnigan LCQ ion trap. HPLC–UV-MS analyses, used to assess compound purity, were carried out combining the ion trap MS instrument with HPLC system SSP4000 (Thermo Separation Products) equipped with an autosampler LC Pal (CTC Analytics) and UV6000 LP diode array detector (UV detection 215–400 nm). Instrument control, data acquisition, and processing were performed by using Xcalibur 1.2 software (Finnigan). HPLC chromatography was run at room temperature, and 1 mL min^{-1} flow rate, using a Waters XTerra RP18 column (4.6×50 mm; 3.5 μm). Mobile phase A was ammonium acetate 5 mM buffer (pH 5.5 with acetic acid): acetonitrile 90:10, and mobile phase B was ammonium acetate 5 mM buffer (pH 5.5 with acetic acid): acetonitrile 10:90; the gradient was from 0 to 100 % B in 7 min then hold 100 % B for 2 min before requilibration. Exact mass data ESI(+) were obtained on a Waters Q-Tof Ultima directly connected with micro HPLC 1100 Agilent as previously described[19].

1-[(2-Cyanoethyl)amino]cyclopropanecarboxylic acid (4 a). 1-Amino-cyclopropanecarboxylic acid (3.03 g, 30 mmol) suspended in 6 mL of water at +4 °C was treated with a solution of NaOH (1.2 g, 30 mmol) in water (6 mL). To the resulting clear solution,

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acrylonitrile (2.094 mL, 31.805 mmol) was added upon cooling. The mixture was allowed to warm to room temperature overnight then treated at +4 °C with acetic acid (1.7 mL). Precipitation of a white solid occurred which was taken up with 95% ethanol (25 mL), kept at +4 °C for a few hours, and finally filtered and washed with ethanol (7 mL×2). After drying at 40 °C under vacuum, 3.97 g of desired product were obtained. The mother liquors were concentrated, taken up with EtOH (15 mL) to afford a second crop of 200 mg (overall yield 90%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.47 (s, 1H), 2.86 (m, 2H), 2.48 (m, 2H), 1.09 (m, 2H), 0.85 ppm (m, 2H); MS (EI): *m/z* 154 [$M^{+\bullet}$].

Analogously, starting from 2-amino-2-methyl-propionic acid the following compound was prepared:

***N*-(2-Cyanoethyl)-2-methylalanine (4b)**: yield 95%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.47 (s, 1H), 2.71 (m, 2H), 2.57 (m, 2H), 1.17 ppm (s, 6H); MS (ESI): *m/z* 157 [MH^+]; Anal. ($C_7H_{12}N_2O_2$) C (calcd 53.83, found 53.71), H (calcd 7.74, found 7.78), N (calcd 17.94, found 17.85).

1-[(*tert*-Butoxycarbonyl)(2-cyanoethyl)amino]cyclopropanecarboxylic acid (5a). 1-[(2-Cyanoethyl)amino]cyclopropanecarboxylic acid (4.974 g, 32 mmol) in acetonitrile (320 mL) was treated with tetramethylammonium hydroxide pentahydrate (5.8 g, 32 mmol) and stirred for 2 h at room temperature. The cloudy solution was then treated with di-*tert*-butyl dicarbonate (10.5 g, 48 mmol) and stirred for two days. An additional portion of di-*tert*-butyl dicarbonate was added (3.49 g, 16 mmol) and stirring was continued for another day. The solvent was evaporated, the residue taken up with water (100 mL), and washed twice with ethyl ether (50 mL). The aqueous phase was acidified to pH 3–4 with solid citric acid (5 g) and extracted twice with ethyl acetate (50 mL). The combined organic fractions were washed with brine, dried over sodium sulfate, and concentrated to afford 5.78 g of the title compound as a white solid (yield 71%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.55 (bs, 1H), 3.33 (m, 2H), 2.71 (m, 2H), 0.97–1.63 ppm (m, 13H); MS (ESI): *m/z* 272 [MNH_4^+].

Analogously, the following compound was prepared:

***N*-(*tert*-Butoxycarbonyl)-*N*-(2-cyanoethyl)-2-methylalanine (5b)**: yield 72%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.18 (bs, 1H), 3.52 (t, J = 6.71 Hz, 2H), 2.67 (t, J = 6.71 Hz, 2H), 1.38 (s, 6H), 1.36 ppm (s, 9H); MS (ESI): *m/z* 274 [MNH_4^+]; Anal. ($C_{12}H_{20}N_2O_4$) C (calcd 56.24, found 56.22), H (calcd 7.87, found 7.92), N (calcd 10.93, found 10.84).

Methyl 1-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]cyclopropanecarboxylate (6a). 1-[(*tert*-Butoxycarbonyl)(2-cyanoethyl)amino]-cyclopropanecarboxylic acid (5.766 g, 22.70 mmol) in DMF (33 mL) was treated with solid $KHCO_3$ (4.55 g, 45.40 mmol) and after a few minutes with MeI (2.24 mL). After stirring for a couple of hours, additional MeI was added (2 mL). The mixture was left at room temperature overnight. After dilution with water (100 mL), extraction with 1:1 hexane/ethyl acetate (200 mL and 100 mL×2), drying of the combined organic extracts, and evaporation of the volatiles, the title compound was obtained as a yellow oil in quantitative yield. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.61 (s, 3H), 3.42 (t, J = 6.66 Hz, 2H), 2.71 (m, 2H), 1.07–1.62 ppm (m, 13H); MS (ESI): *m/z* 286 [MNH_4^+].

Analogously, the following compound was prepared:

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-2-methylalaninate (6b) yield 96%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.56 (s, 3H), 3.53 (t, J = 6.71 Hz, 2H) 2.69 (t, J = 6.71 Hz, 2H), 1.40 (s, 6H), 1.35 ppm (s, 9H); MS (ESI): *m/z* 288 [MNH_4^+]; Anal. ($C_{13}H_{22}N_2O_4$) C (calcd 56.24, found 56.22), H (calcd 8.20, found 8.26), N (calcd 10.36, found 10.25).

***tert*-Butyl 6-cyano-7-oxo-4-azaspiro[2.4]heptane-4-carboxylate (7a)**. Methyl 1-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]cyclopropanecarboxylate (6.83 g, 22.70 mmol) was dissolved in dioxane (32 mL) under argon and treated with 60% NaH (1.089 g, 27.24 mmol). The mixture was heated, with stirring, at 100 °C (oil bath temperature) for 4 h. Formation of a white precipitate occurred. The solvent was evaporated and the solid dissolved in water (50 mL) and diluted with ethyl acetate (100 mL). The solution was acidified to pH 3–4 with citric acid (approximately 4 g) while stirring. The organic phase was separated and the aqueous one was further extracted with ethyl acetate. The combined organic extracts were then washed with brine until neutral, dried and evaporated leaving an off white solid in quantitative yield. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 4.63 (t, J = 9.87 Hz, 1H), 4.24 (t, J = 10.2 Hz, 1H), 3.74 (t, J = 10.2 Hz, 1H), 1.67–2.16 (m, 2H), 1.36 and 1.38 (2 s, 9H), 0.93–1.20 ppm (m, 2H); MS (ESI): *m/z* 235 [$M-H^-$].

Analogously, the following compound was prepared:

***tert*-Butyl 4-cyano-3-hydroxy-2,2-dimethyl-2,5-dihydro-1*H*-pyrrole-1-carboxylate (7b)**: yield 85%. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 4.01 and 3.97 (2 s, 2H), 1.48 (s, 6H), 1.47 ppm (s, 9H). MS (ESI): *m/z* 237 [$M-H^-$]; Anal. ($C_{12}H_{18}N_2O_3$) C, (calcd 60.49, found: 60.66), H (calcd 7.61, found 7.52), N (calcd 11.76, found 11.58).

***tert*-Butyl 3-amino-4,6-dihydropyrrolo[3,4-c]pyrazole-6-spirocyclopropane-5(1*H*)-carboxylate (8a)**. *tert*-Butyl 6-cyano-7-oxo-4-azaspiro[2.4]heptane-4-carboxylate (5.36 g, 22.70 mmol) was dissolved in absolute ethanol (73 mL) by warming to 60 °C, and was then treated, at room temperature, with hydrazine hydrate (1.54 mL, 31.78 mmol) followed by glacial acetic acid (1.95 mL, 34.05 mmol). The mixture was stirred at 60 °C for 3 days. The ethanol was removed by evaporation; the residue was taken up with ethyl acetate (200 mL), and washed with a saturated aqueous solution of sodium bicarbonate (100 mL). The organic phase was dried and evaporated. Purification of the crude product by flash chromatography (97:3 ethyl acetate/methanol) afforded 4.2 g of the title compound as yellow foam (yield 75%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.03 (bs, 1H), 4.98 (bs, 2H), 4.26 and 4.22 (m, 2H, rotamers), 1.84–1.64 (m, 2H), 1.38 (s, 9H), 0.76–0.71 ppm (m, 2H); MS (ESI): *m/z* 251 [MH^+].

Analogously, the following compound was prepared:

***tert*-Butyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-5(1*H*)-carboxylate (8b)**: yield 88%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.09 (bs, 1H), 4.93 (bs, 2H), 4.10 and 4.06 (2 s, 2H, rotamers), 1.48 and 1.47 (2 s, 6H, rotamers), 1.43 and 1.40 ppm (2 s, 9H, rotamers); MS (ESI): *m/z* 253 [MH^+].

5-*tert*-Butyl 2-ethyl 3-amino-pyrrolo[3,4-c]pyrazole-6-spirocyclopropane-2,5(4*H*,6*H*)-dicarboxylate (9a). *tert*-Butyl 3-amino-2,6-dihydropyrrolo[3,4-c]pyrazole-6-spirocyclopropane-5(4H)-carboxylate (4.14 g, 16.57 mmol) was dissolved in anhydrous THF (46 mL) and treated at +4 °C, under an argon atmosphere, with *N,N*-diisopropylethylamine (11.5 mL, 66.29 mmol) followed by dropwise addition of ethyl chloroformate (1.58 mL, 16.57 mmol) in THF (15 mL). After 1 h the solvent was evaporated and the crude product subjected to flash chromatography purification (7:3 dichloromethane/ethyl acetate) to afford 4 g of title compound (yield 73%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.55 (s, 2H), 4.30 (q, J = 7.07 Hz, 2H), 4.27 (bs, 2H), 1.65 and 2.01 (m, 2H), 1.38 (s, 9H) 1.27 (t, J = 7.07 Hz, 3H), 0.82–0.96 ppm (m, 2H); MS (ESI): *m/z* 323 [MH^+].

Analogously, the following compound was prepared:

5-*tert*-Butyl 2-ethyl 3-amino-6,6-dimethylpyrrolo[3,4-c]pyrazole-2,5(4*H*,6*H*)-dicarboxylate (9b): yield 60%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.53 and 6.51 (2bs, 2H, rotamers), 4.32 (q, J = 7.07 Hz, 2H), 4.12 and 4.08 (2 s, 2H, rotamers), 1.51 and 1.50 (2 s, 6H, rotamers), 1.43 and 1.41 (2 s, 9H, rotamers), 1.29 ppm (t, J = 7.07 Hz, 3H); MS (ESI): *m/z* 325 [MH^+]; Anal. ($C_{15}H_{24}N_4O_4$) C, (calcd 55.54, found: 55.26), H (calcd 7.46, found 7.48), N (calcd 17.27, found 17.26).

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Method A

The general procedure for the preparation of **10a**–**11a**, **10b**–**11b** is illustrated below for the preparation of **10a**.

3-(2-naphthalen-2-yl-acetylamino)-4,6-dihydro-1*H*-pyrrolo[3,4-c]pyrazole-6-spirocyclopropane-5-carboxylic acid ethylamide (10a). Compound 9a (966 mg, 3 mmol) was dissolved in dichloromethane (30 mL), treated first with diethylaminomethyl-polystyrene (Fluka, loading: 3.2 $mmol g^{-1}$, 1.4 g) and then, while stirring, with naphthalen-2-yl-acetyl chloride (673 mg, 3.3 mmol). After two days tris-(2-aminoethyl)-amine polystyrene (Novabiochem, loading: 3.2 $mmol g^{-1}$, 1 g) was added to sequester excess of acid chloride. The resins were then filtered, washed with dichloromethane; the filtrate was evaporated to afford 1440 mg of compound in 98% yield, and 100% HPLC purity (measured at 254 nm); MS (ESI): *m/z* 491 [MH^+]. This intermediate (800 mg, 1.66 mmol) was treated with 1:1 dichloromethane/trifluoroacetic acid (8 mL). After 20 min the volatiles were evaporated and the residue was dissolved in dichloromethane (10 mL), treated with aminomethylated polystyrene (Novabiochem, loading: 1.17 $mmol g^{-1}$, 1 g) and stirred overnight. The resin was filtered, washed with dichloromethane, and the filtrate was evaporated to afford the trifluoroacetate as an amorphous solid in 93% yield and 100% HPLC purity (measured at 254 nm); MS (ESI): *m/z* 397 [MH^+]. This intermediate (462 mg, 0.92 mmol) in dichloromethane (10 mL) was then treated with diethylaminomethyl-polystyrene (Fluka, loading: 3.2 $mmol g^{-1}$, 400 mg) and ethyl isocyanate (112 μL, 1.42 mmol). When reaction was completed, tris-(2-aminoethyl)-amine polystyrene (Novabiochem, loading: 3.2 $mmol g^{-1}$, 100 mg) was added. After stirring for a few hours the resins were filtered, washed with dichloromethane, and the filtrate evaporated to leave 421 mg of urea derivative as a foam in quantitative yield and 97% HPLC purity (measured at 254 nm); MS (ESI): *m/z* 462 [MH^+]. Finally, this intermediate (420 mg, 0.91 mmol) was dissolved in methanol (10 mL), treated with Amberlite IRA 900 $NaCO_3^-$ form (loading: ~3.5 $mmol g^{-1}$, 1 g) and stirred overnight at room temperature. The resin was filtered, washed thoroughly with methanol and dichloromethane and the filtrate evaporated. The crude was purified by filtration over a short plug of silica gel (9:1 dichloromethane/methanol) and 213 mg of title compound were obtained as off white solid (yield 60%); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.34 (bs, 1H), 7.83–7.92 (m, 4H), 7.46–7.53 (m, 3H), 6.22 (bt, *J* = 10.6 Hz, 1H), 4.53 (bs, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 4.01 (s, 2H), 2.91–3.01 (m, 2H), 2.10 (dd, *J* = 6.9 and 4.1 Hz, 2H), 1.29 (t, *J* = 7.2 Hz, 3H), 0.96 (t, *J* = 7.2 Hz, 3H), 0.84 ppm (dd, *J* = 6.9 and 4.1 Hz, 2H). MS (ESI): *m/z* 390 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{22}H_{24}N_5O_2^+$ 390.1924 [MH^+], found 390.1926; HPLC purity (as area, %): 100.

The following compounds **11a**, **10b**–**11b** were prepared according to the general procedure described above.

6,6-Dimethyl-3-(2-naphthalen-2-yl-acetylamino)-4,6-dihydro-1*H*-pyrrolo[3,4-c]pyrazole-5-carboxylic acid ethylamide (10b): ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.23 (s, 1H), 10.63 (s, 1H), 7.43–7.87 (m, 7H), 5.92 (m, 1H), 4.29 (s, 2H), 3.76 (s, 2H), 2.97 (m, 2H), 1.56 (s, 6H), 0.95 ppm (t, *J* = 7.1 Hz, 3H); MS (ESI) *m/z:* 392 [MH^+]; Anal. ($C_{22}H_{25}N_5O_2$) C (calcd 67.50, found 66.65), H (calcd 6.44, found 6.60), N (calcd 17.89, found 18.02); HRMS (ESI): *m/z* calcd for $C_{22}H_{26}N_5O_2^+$ 392.2081 [MH^+], found 392.2071; HPLC purity (as area, %): 100.

3-(4-Fluoro-benzoylamino)-4,6-dihydro-1*H*-pyrrolo[3,4-c]pyrazole-6-spirocyclopropane-5-carboxylic acid ethylamide (11a): ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 11.45–12.71 (bs, 1H), 10.89 (bs, 1H), 8.01–8.11 (m, 2H), 7.27–7.38 (m, 2H), 6.14 (m, 1H), 4.56 (s, 2H), 2.92–3.05 (m, 2H), 2.07 (dd, *J* = 6.6 and 4.1 Hz, 2H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.79 ppm (dd, *J* = 6.6 and 4.1 Hz, 2H); MS (ESI): *m/z* 344 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{17}H_{19}FN_5O_2^+$ 344.1517 [MH^+], found 344.1516; HPLC purity (as area, %): 100.

3-(4-Fluoro-benzoylamino)-6,6-dimethyl-4,6-dihydro-1*H*-pyrrolo-[3,4-c]pyrazole-5-carboxylic acid ethylamide (11b): ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.38 (bs, 1H), 10.85 (s, 1H), 7.99–8.13 (m, 2H), 7.21–7.42 (m, 2H), 6.01 (bt, *J* = 5.4 Hz, 1H), 4.42 (s, 2H), 2.95–3.08 (m, 2H), 1.62 (s, 6H), 1.01 ppm (t, *J* = 7.1 Hz, 3H); MS (ESI): *m/z* 346 [MH^+]; HRMS (ESI) calcd for $C_{17}H_{21}FN_5O_2^+$ 346.1674 [MH^+], found 346.1672; HPLC purity (as area, %): 100.

Method B

The general procedure for the preparation of **12a**–**19a**, **12b**–**19b** is illustrated below in the synthesis of **15b.**

***N*-[6,6-Dimethyl-5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-fluoro-benzamide (15b)**: A solution of ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c]pyrazole-2(4H)-carboxylate hydrochloride (442 mg, 1.15 mmol) in DCM (30 mL), prepared as described in Method A but employing HCl 4 M in dioxane instead of TFA, followed by *N*,*N*-diisopropylethylamine (760 μL, 4.31 mmol) was added to a solution of triphosgene (195 mg, 0.65 mmol, 0.56 eq) in DCM (15 mL). After 3 h, a solution of *N*-methylpiperazine (195 μL, 1.72 mmol) and *N*,*N*-diisopropylethylamine (300 μL, 1.72 mmol) in DCM (8 mL) was added. The reaction was stirred overnight at room temperature. The solution was washed with brine, the organic phase was dried over sodium sulfate, and concentrated. Deprotection of the carbamate, as described for **10a**, purification by flash chromatography (eluent: CH_2Cl_2/MeOH 90/10) followed by treatment of the solid with diisopropylether and filtration afforded 0.294 g of the title compound in 64% yield. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.39 (bs, 1H), 10.89 (s, 1H), 8.04 (m, 2H), 7.31 (m, 2H), 4.53 (s, 2H), 3.04 (m, 4H), 2.40 (m, 4H), 2.22 (bs, 3H), 1.60 ppm (s, 6H); MS (ESI): *m/z* 401 [MH^+]; Anal. ($C_{20}H_{25}FN_6O_2$) C (calc 59.99, found 58.70), H (calcd 6.29, found 6.40), N (calcd 20.99, found 19.66); HRMS (ESI): *m/z* calcd for $C_{20}H_{26}FN_6O_2^+$ 401.2096 [MH^+], found 401.2091; HPLC purity (as area, %): 100.

The following compounds **12a**–**19a**, **12b**–**14b**, and **16b**–**19b** were prepared according to the general procedure described above.

***N*-[5-(piperidine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-fluoro-benzamide (12a)**: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.13 (bs, 1H), 10.93 (s, 1H), 8.09 (m, 2H), 7.36 (m, 2H), 4.65 (bs, 2H), 3.08 (m, 4H), 1.87 (m, 2H), 1.53 (m, 6H), 0.96 ppm (m, 2H); MS (ESI): *m/z* 384 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{20}H_{23}FN_5O_2^+$ 384.1830 [MH^+], found 384.1836; HPLC purity (as area, %): 100.

***N*-[6,6-Dimethyl-5-(piperidine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-fluoro-benzamide (12b)**: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.37 (bs, 1H), 10.88 (s, 1H), 8.04 (m, 2H), 7.31 (m, 2H), 4.51 (bs, 2H), 2.97 (m, 4H), 1.59 (bs, 6H), 1.50 ppm (m, 6H); MS (ESI): *m/z* 386 [MH^+]; Anal. ($C_{20}H_{24}FN_5O_2$) C (calcd 62.32, found 62.47), H (calcd 6.28, found 6.20), N (calcd 18.17, found 17.81); HRMS (ESI): *m/z* calcd for $C_{20}H_{25}FN_5O_2^+$ 386.1987 [MH^+], found 386.1974; HPLC purity (as area, %): 100.

***N*-[5-(4-methyl-piperidine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo-[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-fluoro-benzamide (13a)**: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.15 (bs, 1H), 10.93 (s, 1H), 8.09 (m, 2H), 7.36 (m, 2H), 4.65 (bs, 2H), 3.54 (m, 2H), 2.65 (m, 2H), 1.87 (m, 2H), 1.61 (m, 2H), 1.50 (m, 1H), 1.12 (m, 2H), 0.94 ppm (m, 5H); MS (ESI): *m/z* 398 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{21}H_{25}FN_5O_2^+$ 398.1987 [MH^+], found 398.1984; HPLC purity (as area, %): 100.

***N*-[6,6-Dimethyl-5-(4-methyl-piperidine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-fluoro-benzamide (13b)**: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.52 (bs, 1H), 10.91 (s, 1H), 8.07

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(m, 2H), 7.35 (m, 2H), 4.54 (bs, 2H), 3.33 (m, 2H), 2.63 (m, 2H), 1.63 (m, 8H), 1.49 (m, 1H), 1.15 (m, 2H), 0.94 ppm (d, $J=6.5$ Hz, 3H); MS (ESI): m/z 400 [MH^+]; Anal. ($C_{21}H_{26}FN_5O_2$) C (calc 63.14, found 62.56), H (calcd 6.56, found 6.51), N (calcd 17.53, found 17.25); HRMS (ESI): m/z calcd for $C_{21}H_{27}FN_5O_2^+$ 400.2143 [MH^+], found 400.2145; HPLC purity (as area, %): 100.

***N*-[5-(morpholine-4-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-fluoro-benzamide (14a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.10$ (bs, 1H), 10.95 (s, 1H), 8.09 (m, 2H), 7.34 (m, 2H), 4.70 (bs, 2H), 3.62 (m, 4H), 3.12 (m, 4H), 1.93 (m, 2H), 0.96 ppm (m, 2H); MS (ESI): m/z 386 [MH^+]; HRMS (ESI): m/z calcd for $C_{19}H_{21}FN_5O_3^+$ 386.1623 [MH^+], found 386.1631; HPLC purity (as area, %): 100.

***N*-[6,6-Dimethyl-5-(morpholine-4-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-fluoro-benzamide (14b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.41$ (bs, 1H), 10.89 (s, 1H), 8.04 (m, 2H), 7.30 (m, 2H), 4.56 (bs, 2H), 3.59 (m, 4H), 3.01 (m, 4H), 1.61 ppm (bs, 6H); MS (ESI): m/z 388 [MH^+]; HPLC purity (as area, %): 100.

***N*-[5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo-[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-fluoro-benzamide (15a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.19$ (bs, 1H), 10.95 (s, 1H), 8.09 (m, 2H), 7.35 (m, 2H), 4.70 (s, 2H), 3.18 (m, 2H), 2.53 (m, 5H), 2.34 (m, 2H), 1.92 (m, 2H), 0.97 ppm (m, 2H); MS (ESI): m/z 399 [MH^+]; HRMS (ESI): m/z calcd for $C_{20}H_{24}FN_6O_2^+$ 399.1939 [MH^+], found 399.1942; HPLC purity (as area, %): 100.

***N*-[5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo-[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-2,4-difluoro-benzamide (16a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.16$ (bs, 1H), 10.84 (s, 1H), 7.76 (m, 3H), 4.69 (bs, 2H), 3.12 (m, 4H), 2.35 (m, 4H), 2.21 (bs, 3H), 1.90 (m, 2H), 0.97 ppm (m, 2H); MS (ESI): m/z 417 [MH^+]; HRMS (ESI): m/z calcd for $C_{20}H_{23}F_2N_6O_2^+$ 417.1845 [MH^+], found 417.1841; HPLC purity (as area, %): 100.

***N*-[6,6-Dimethyl-5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-2,4-difluoro-benzamide (16b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.44$ (bs, 1H), 10.85 (s, 1H), 7.74 (m, 1H), 7.18 (m, 1H), 7.20 (s, 1H), 4.59 (bs, 2H), 3.06 (m, 4H), 2.38 (m, 4H), 2.22 (bs, 3H), 1.64 ppm (bs, 6H); MS (ESI): m/z 419 [MH^+]; Anal. ($C_{20}H_{24}F_2N_6O_2$) C (calc 57.41, found 57.11), H (calcd 5.78, found 5.71), N (calcd 20.08, found 19.70); HRMS (ESI): m/z calcd for $C_{20}H_{25}F_2N_6O_2^+$ 419.2002 [MH^+], found 419.1999; HPLC purity (as area, %): 100.

4-Chloro-*N*-[5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-benzamide (17a): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.20$ (bs, 1H), 11.00 (s, 1H), 8.02 (m, 2H), 7.59 (m, 2H), 4.70 (bs, 2H), 3.15 (m, 4H), 2.51 (m, 4H), 2.29 (bs, 3H), 1.92 (m, 2H), 0.89 ppm (m, 2H); MS (ESI): m/z 415 [MH^+]; HRMS (ESI): m/z calcd for $C_{20}H_{24}ClN_6O_2^+$ 415.1644 [MH^+], found 415.1646; HPLC purity (as area, %): 100.

4-Chloro-*N*-[6,6-dimethyl-5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-benzamide (17b): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.46$ (bs, 1H), 10.99 (s, 1H), 8.01 (m, 2H), 7.59 (m, 2H), 4.59 (bs, 2H), 3.07 (m, 4H), 2.40 (m, 4H), 2.25 (bs, 3H), 1.64 ppm (bs, 6H); MS (ESI): m/z 417 [MH^+]; Anal. ($C_{20}H_{25}ClN_6O_2$) C (calc 57.62, found 53.11), H (calcd 6.04, found 6.11), N (calcd 20.16, found 17.93); HRMS (ESI): m/z calcd for $C_{20}H_{26}ClN_6O_2^+$ 417.1800 [MH^+], found 417.1802; HPLC purity (as area, %): 100.

***N*-[5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo-[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-trifluoromethyl-benzamide (18a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.24$ (bs, 1H), 11.17 (s, 1H), 8.19 (m, 2H), 7.89 (m, 2H), 4.72 (bs, 2H), 3.14 (m, 4H), 2.41 (m, 4H), 2.26 (bs, 3H), 1.92 (m, 2H), 0.98 ppm (m, 2H); MS (ESI): m/z 449 [MH^+]; HRMS (ESI): m/z calcd for $C_{21}H_{24}F_3N_6O_2^+$ 449.1907 [MH^+], found 449.1892; HPLC purity (as area, %): 100.

***N*-[6,6-Dimethyl-5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-trifluoromethyl-benzamide (18b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.49$ (bs, 1H), 11.16 (s, 1H), 8.19 (d, $J=7.80$ Hz, 2H), 7.88 (d, $J=7.80$ Hz, 2H), 4.60 (s, 2H), 3.06 (m, 4H), 2.38 (m, 4H), 2.22 (s, 3H), 1.64 ppm (bs, 6H); MS (ESI): m/z 451 [MH^+]; Anal. ($C_{21}H_{25}F_3N_6O_2$) C (calc 55.99, found 55.12), H (calcd 5.57, found 5.59), N (calcd 18.66, found 18.16); HRMS (ESI): m/z calcd for $C_{21}H_{26}F_3N_6O_2^+$ 451.2064 [MH^+], found 451.2057; HPLC purity (as area, %): 100.

Cyclopropanecarboxylic acid [5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-amide (19a): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.98$ (bs, 1H), 10.66 (s, 1H), 4.56 (bs, 2H), 3.10 (m, 4H), 2.34 (m, 4H), 2.21 (bs, 3H), 1.87 (m, 2H), 1.86 (m, 1H), 0.92 (m, 2H), 0.82 ppm (m, 4H); MS (ESI): m/z 345 [MH^+]; HRMS (ESI): m/z calcd for $C_{17}H_{25}N_6O_2^+$ 345.2033 [MH^+], found 345.2031; HPLC purity (as area, %): 100.

Cyclopropanecarboxylic acid [6,6-dimethyl-5-(4-methyl-piperazine-1-carbonyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-amide (19b): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.23$ (bs, 1H), 10.66 (s, 1H), 4.44 (bs, 2H), 3.02 (m, 4H), 2.33 (m, 4H), 2.19 (s, 3H), 1.82 (m, 1H), 1.59 (bs, 6H), 0.78 ppm (m, 4H); MS (ESI): m/z 347 [MH^+]; HPLC purity (as area, %): 100.

Method C.

The general procedure for the preparation of **20a**–**28a**, **20b–31b,** is illustrated below in the synthesis of **20a**.

***N*-[5-(2,2-dimethylpropanoyl)-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-fluorobenzamide (20a)**. Ethyl 3-(4-fluoro-benzoylamino)-5,6-dihydropyrrolo[3,4-c]pyrazole-6-spirocyclopropane-2(4*H*)-carboxylate trifluoroacetate (150 mg, 0.32 mmol), prepared according to Method A, in dichloromethane (10 mL) was treated with diethylaminomethyl-polystyrene (Fluka, loading: 3.2 mmol g^{-1}, 400 mg) and pivaloyl chloride (44 μL, 0.36 mmol). After a couple of hours, tris-(2-aminoethyl)-amine polystyrene (Novabiochem, loading: 3.2 mmol g^{-1}, 100 mg) and methylisocyanate polystyrene (Argonaut, loading 1.51 mmol g^{-1}) were added to sequester any unreacted reagent. After stirring overnight the resins were filtered, washed with dichloromethane, and the filtrate evaporated to leave 137 mg of title compound as a foam in 98% yield and 100% HPLC purity (measured at 254 nm); MS (ESI): m/z 456 [MH^+]. Deprotection of the carbamate, as described for **10a**, led to the title compound in 53% yield. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.18$ (bs, 1H), 10.94 (s, 1H), 8.06 (m, 2H), 7.32 (m, 2H), 4.99 (s, 2H), 2.26 (dd, $J=6.46$ and 4.02 Hz, 2H), 1.20 (s, 9H), 0.79 ppm (dd, $J=6.46$ and 4.02 Hz, 2H);); MS (ESI): m/z 357 [MH^+]; Anal. ($C_{19}H_{21}FN_4O_2$) C (calc 64.03, found 63.64), H (calcd 5.94, found 6.26), N (calcd 15.72, found 15.50); HRMS (ESI): m/z calcd for $C_{19}H_{22}FN_4O_2^+$ 357.1721 [MH^+], found 357.1717; HPLC purity (as area, %): 100.

The following compounds **21a**–**28a**, **20b**–**31b** were prepared according to the general procedure described above.

***N*-(5-Acetyl-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl)-4-fluoro-benzamide (20b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.40$ and 12.14 (bs, 1H), 10.95 (bs, 1H), 8.10 (m, 2H), 7.33 (m, 2H), 4.69 and 4.61 (bs, 2H), 2.02 (s, 3H), 1.68 ppm (s, 6H); MS (ESI): m/z 317 [MH^+]; HRMS (ESI): m/z calcd for $C_{16}H_{18}FN_4O_2^+$ 317.1408 [MH^+], found 317.1421; HPLC purity (as area, %): 100.

***N*-(5-Butyryl-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl)-4-fluoro-benzamide (21b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.45$ and 12.12 (bs, 1H), 10.94 (bs, 1H), 8.08 (m, 2H), 7.32 (m, 2H), 4.67 and 4.59 (bs, 2H), 2.27 (t, $J=7.19$ Hz, 2H)

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1.68 (s, 6H) 1.54 (m, 2H) 0.92 ppm (t, $J=7.32$ Hz, 3H); MS (ESI): *m/z* 345 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{18}H_{22}FN_4O_2^+$ 345.1721 [MH^+], found 345.1734; HPLC purity (as area, %): 100.

***N*-(5-Benzoyl-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl)-4-fluoro-benzamide (22b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.53$ and 12.23 (bs, 1H), 10.93 (bs, 1H), 8.00 (m, 2H), 7.46 (m, 5H), 7.27 (m, 2H) 4.48 and 4.37 (bs, 2H) 1.84 ppm (s, 6H); MS (ESI): *m/z* 379 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{21}H_{20}FN_4O_2^+$ 379.1565 [MH^+], found 379.1568; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-fluoro-benzamide (23b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.41$ (bs, 1H), 10.91 (bs, 1H), 8.05 (m, 2H), 7.29 (m, 2H), 4.85 (bs, 2H), 1.64 (s, 6H), 1.21 ppm (s, 9H); MS (ESI): *m/z* 359 [MH^+]; Anal. ($C_{19}H_{23}FN_4O_2$) C (calcd 63.67, found 63.83), H (calcd 6.47, found 6.62), N (calcd 15.63, found 15.4); HRMS (ESI): *m/z* calcd for $C_{19}H_{24}FN_4O_2^+$ 359.1878 [MH^+], found 359.1889; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-3-fluoro-benzamide (21a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.29$ (bs, 1H), 11.05 (s, 1H), 7.9–7.35 (m, 3H), 5.03 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 ppm (m, 2H); MS (ESI): *m/z* 357 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{19}H_{22}FN_4O_2^+$ 357.1721 [MH^+], found 357.1716; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-3-fluoro-benzamide (24b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.45$ (bs, 1H), 10.99 (s, 1H), 7.84 (m, 2H), 7.52 (m, 1H), 7.40 (m, 1H), 4.86 (s, 2H), 1.65 (s, 6H), 1.21 ppm (s, 9H); MS (ESI): *m/z* 359 [MH^+]; Anal. ($C_{19}H_{23}FN_4O_2$) C (calcd 63.67, found 62.85), H (calcd 6.47, found 6.78), N (calcd 15.63 found 14.76); HRMS (ESI): *m/z* calcd for $C_{19}H_{24}FN_4O_2^+$ 359.1878 [MH^+], found 359.1874; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-2,4-difluoro-benzamide (22a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.21$ (bs, 1H), 10.90 (s, 1H), 7.79 (m, 1H), 7.40 (m, 1H), 7.21 (m, 1H), 5.03 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.83 ppm (m, 2H); MS (ESI): *m/z* 375 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{19}H_{21}F_2N_4O_2^+$ 375.1627 [MH^+], found 375.1622; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-2,4-difluoro-benzamide (25b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.45$ (bs, 1H), 10.89 (s, 1H), 7.7 (m, 1H), 7.38 (m, 1H), 7.20 (s, 1H), 4.89 (s, 2H), 1.68 (s, 6H), 1.24 ppm (s, 9H); MS (ESI): *m/z* 377 [MH^+]; Anal. ($C_{19}H_{22}F_2N_4O_2$) C (calcd 60.63, found 60.59), H (calcd 5.89, found 6.02), N (calcd 14.88, found 14.71); HRMS (ESI): *m/z* calcd for $C_{19}H_{23}F_2N_4O_2^+$ 377.1784 [MH^+], found 377.1794; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-3,5-difluoro-benzamide (23a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.30$ (bs, 1H), 11.17 (bs, 1H), 7.77 (m, 2H), 7.51 (m, 1H), 5.04 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 ppm (m, 2H); MS (ESI): *m/z* 375 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{19}H_{21}F_2N_4O_2^+$ 375.1627 [MH^+], found 375.1626; HPLC purity (as area, %): 90.

***N*-[5-(2,2-Dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-3,5-difluoro-benzamide (26b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.54$ (bs, 1H), 11.12 (bs, 1H), 7.73 (m, 2H), 7.51 (m, 1H), 4.89 (s, 2H), 1.68 (s, 6H), 1.25 ppm (s, 9H); MS (ESI): *m/z* 377 [MH^+]; Anal. ($C_{19}H_{22}F_2N_4O_2$) C (calcd 60.63, found 60.56), H (calcd 5.89, found 5.91), N (calcd 14.88, found 14.76); HRMS (ESI): *m/z* calcd for $C_{19}H_{23}F_2N_4O_2^+$ 377.1784 [MH^+], found 377.1786; HPLC purity (as area, %): 100.

4-Chloro-*N*-[5-(2,2-dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-benzamide (24a): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.28$ (bs, 1H), 11.04 (s, 1H), 8.03 (d, $J=8.0$ Hz, 2H), 7.61 (d, $J=8.0$ Hz, 2H), 5.03 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.83 ppm (m, 2H); MS (ESI): *m/z* 373 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{19}H_{22}ClN_4O_2^+$ 373.1426 [MH^+], found 373.1425; HPLC purity (as area, %): 100.

4-Chloro-*N*-[5-(2,2-dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-benzamide (27b): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.43$ (bs, 1H), 10.98 (s, 1H), 7.98 (d, $J=8.0$ Hz, 2H), 7.54 (d, $J=8.0$ Hz, 2H), 4.86 (s, 2H), 1.65 (s, 6H), 1.21 ppm (s, 9H); MS (ESI): *m/z* 375 [MH^+]; Anal. ($C_{19}H_{23}ClN_4O_2$) C (calcd 60.88, found 60.79), H (calcd 6.18, found 6.16), N (calcd 14.95, found 14.85); HRMS (ESI): *m/z* calcd for $C_{19}H_{24}ClN_4O_2^+$ 375.1582 [MH^+], found 375.1584; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-4-trifluoromethyl-benzamide (25a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.32$ (bs, 1H), 11.22 (s, 1H), 8.21 (d, $J=7.9$ Hz, 2H), 7.91 (d, $J=7.9$ Hz, 2H), 5.05 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 ppm (m, 2H); MS (ESI): *m/z* 407 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{20}H_{22}F_3N_4O_2^+$ 407.1689 [MH^+], found 407.1683; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-4-trifluoromethyl-benzamide (28b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.47$ (bs, 1H), 11.16 (s, 1H), 8.16 (d, $J=7.9$ Hz, 2H), 7.85 (d, $J=7.9$ Hz, 2H), 4.88 (s, 2H), 1.65 (s, 6H), 1.21 ppm (s, 9H); MS (ESI): *m/z* 409 [MH^+]; Anal. ($C_{20}H_{23}F_3N_4O_2$) C (calcd 58.82, found 59.03), H (calcd 5.68, found 5.76), N (calcd 13.72, found 13.54); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}F_3N_4O_2^+$ 409.1846 [MH^+], found 409.1838; HPLC purity (as area, %): 100.

Thiophene-2-carboxylic acid [5-(2,2-dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-amide (26a): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.23$ (bs, 1H), 11.02 (s, 1H), 8.10 (m, 1H), 7.87 (m, 1H), 7.22 (m, 1H), 5.01 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 ppm (m, 2H); MS (ESI): *m/z* 345 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{17}H_{21}N_4O_2S^+$ 345.1380 [MH^+], found 345.1370; HPLC purity (as area, %): 100.

Thiophene-2-carboxylic acid [5-(2,2-dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-amide (29b): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.47$ (bs, 1H), 11.00 (s, 1H), 8.12 (m, 1H), 7.86 (m, 1H), 7.12 (m, 1H), 4.86 (s, 2H), 1.68 (s, 6H), 1.25 ppm (s, 9H); MS (ESI): *m/z* 347 [MH^+]; Anal. ($C_{17}H_{22}N_4O_2S$) C (calcd 58.94, found 56.20), H (calcd 6.40, found 6.76), N (calcd 16.17, found 15.24); HRMS (ESI): *m/z* calcd for $C_{17}H_{23}N_4O_2S^+$ 347.1536 [MH^+], found 347.1535; HPLC purity (as area, %): 100.

Cyclopropanecarboxylic acid [5-(2,2-dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-amide (27a): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.03$ (bs, 1H), 10.72 (bs, 1H), 4.91 (s, 2H), 2.25 (m, 2H), 1.82 (m, 1H), 1.20 (s, 9H), 0.80 ppm (m, 6H); MS (ESI): *m/z* 303 [MH^+]; Anal. ($C_{16}H_{22}N_4O_2$) C (calcd 63.56, found 59.03), H (calcd 7.33, found 7.67), N (calcd 18.53, found 17.16); HRMS (ESI): *m/z* calcd for $C_{16}H_{23}N_4O_2^+$ 303.1815 [MH^+], found 303.1805; HPLC purity (as area, %): 100.

Cyclopropanecarboxylic acid [5-(2,2-dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-amide (30b): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.35$ (bs, 1H), 10.38 (bs, 1H), 4.75 (s, 2H), 1.82 (m, 1H), 1.60 (s, 6H), 1.20 (s, 9H), 0.78 ppm (m, 4H); MS (ESI): *m/z* 305 [MH^+]; HPLC purity (as area, %): 100.

***N*-[5-(2,2-Dimethyl-propionyl)-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole-6-spirocyclopropan-3-yl]-2,2-dimethyl-propionamide (28a)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.01$ (bs, 1H), 9.92 (s, 1H), 4.94 (s, 2H), 2.25 (m, 2H), 1.22 (s, 18H), 0.78 ppm (m, 2H); MS (ESI): *m/z* 319 [MH^+]; HRMS (ESI): *m/z* calcd for $C_{17}H_{27}N_4O_2^+$ 319.2128 [MH^+], found 319.2121; HPLC purity (as area, %): 100.

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***N*-[5-(2,2-Dimethyl-propionyl)-6,6-dimethyl-1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazol-3-yl]-2,2-dimethyl-propionamide (31 b)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 12.3$ (bs, 1H), 9.89 (s, 1H), 4.80 (s, 2H), 1.64 (s, 6H), 1.23 (s, 9H), 1.21 ppm (s, 9H); MS (ESI): *m/z* 321 [MH^+]; Anal. ($C_{17}H_{28}N_4O_2$) C (calcd 63.72, found 63.43), H (calcd 8.81, found 8.87), N (calcd 17.48, found 17.02); HRMS (ESI): *m/z* calcd for $C_{17}H_{29}N_4O_2^+$ 321.2285 [MH^+], found 321.2279; HPLC purity (as area, %): 100.

Keywords: antitumor therapy · cyclin dependent kinases · kinase selectivity · solution-phase synthesis · structure–activity relationships

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[9] A2780 cells proliferation assay. Cells were seeded into 96 or 384 wells plates at final concentration ranging from 10000 to 30000 cells per cm^2 in appropriate medium plus 10% FCS. After 24 h cells were treated using serial dilution of compounds in two replicates. 72 h after the treatment the amount of cells were evaluated using the Cell Titer_Glo Assay (Promega). IC_{50}s were calculated using a Sigmoidal fitting (Assay Explorer MDL). Experiments were replicated at least twice.
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