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# 3-Aminopyrazole Inhibitors of CDK2/Cyclin A as Antitumor Agents. 2. Lead Optimization

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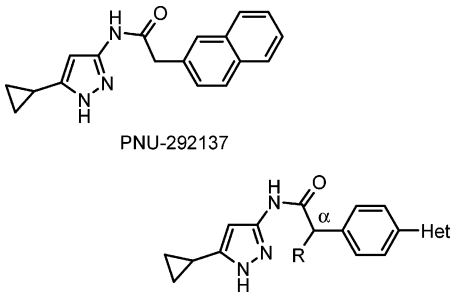
Inhibitors of cyclin-dependent kinases (CDK) such as CDK2/cyclin A−E are currently undergoing clinical trials to verify their potential as new anticancer agents. In a previous article we described the lead discovery process of a 3-aminopyrazole class of CDK2/cyclin A−E inhibitors. The endpoint of this process was PNU-292137, a compound endowed with in vivo antitumor activity in a mouse tumor xenograft model. We optimized this lead compound to improve some physicochemical properties, notably solubility and plasma protein binding. This lead optimization process brought us to the discovery of (2*S*)-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxo-1-pyrrolidinyl)phenyl]propanamide (PHA-533533, **13**), a compound with a balanced activity vs druglike profile. Compound **13** inhibited CDK2/cyclin A with a $K_i$ of 31 nM, counteracting tumor cell proliferation of different cell lines with an $IC_{50}$ in the submicromolar range. Solubility was improved more than 10 times over the starting lead, while plasma protein binding was decreased from 99% to 74%. With exploitation of this globally enhanced in vitro profile, **13** was more active than PNU-292137 in vivo in the A2780 xenograft model showing a tumor growth inhibition of 70%. Proof of mechanism of action was obtained in vivo by immunohistochemical analysis of tumor slices of **13**-treated vs untreated animals.

## Introduction

Abnormal proliferation mediated by disruption of the normal cell cycle mechanisms is a hallmark of virtually all cancer cells.[1] Compounds targeting complexes between cyclin-dependent kinases (CDK) and cyclins, such as CDK2/cyclin A, and inhibiting their kinase activity are regarded as promising antitumor agents to complement the existing therapies, and there is a continuing interest in this field.[2] In a previous paper we described the discovery and the lead finding process of a new class of 3-aminopyrazole CDK2/cyclin A inhibitors.[1] PNU-292137 (Chart 1) was identified as a compound suitable for in vivo evaluation but also endowed with suboptimal physicochemical properties in light of its possible progression into further preclinical development studies.[3] In particular, PNU-292137 displayed a low solubility (in the range 20−50 $\mu$M, depending on its crystalline state) in aqueous buffer (pH 7.0) solubility experiments. While this solubility did not hamper its evaluation in animal models as an oral compound, we deemed it could limit its use in an intravenous continuous infusion administration mode that is still widely practiced in the clinical setting. Furthermore, PNU-292137 was shown to be a strong binder to human serum albumin (HSA) in a preliminary assay. We wanted to avoid potential problems (i.e., abnormal pharmacokinetics (PK) parameters in humans) further down the road to a clinical candidate. We thus embarked on a lead optimization program with the aim of finding new derivatives of PNU-292137 with comparable inhibitory potency but with an improved physicochemical profile consistent with a potential clinical use of a CDK2/cyclin A inhibitor.

**Chart 1**




We describe here the optimization of PNU-292137 to produce a compound ((2*S*)-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxo-1-pyrrolidinyl)phenyl]propanamide (**13**), PHA-533533) retaining potent CDK2 inhibition in biochemical and cellular assays but endowed with a better druglike preclinical profile. Furthermore, an in-depth pharmacological preclinical profile of **13** is also reported.

## Chemistry

All target compounds were obtained by using as a key step the condensation between *tert*-butyl 3-amino-5-

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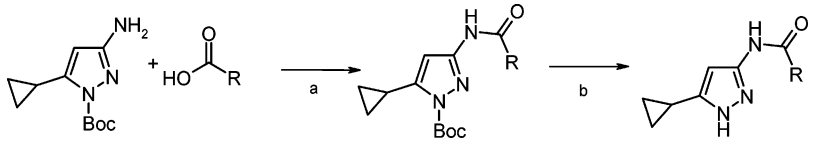
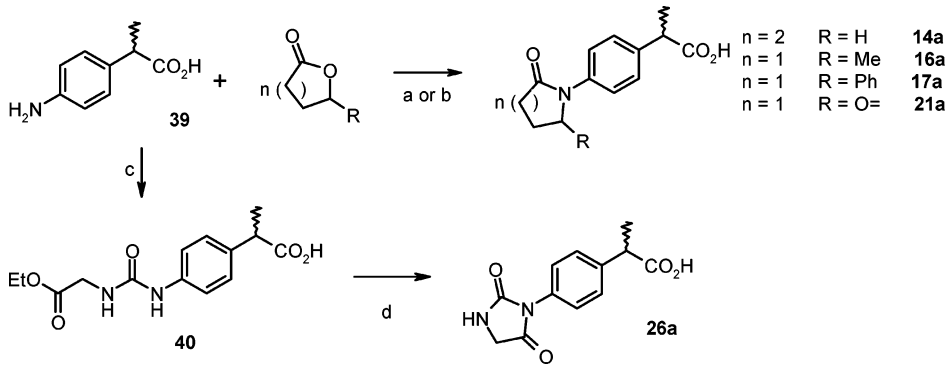
**Scheme 1**[a]



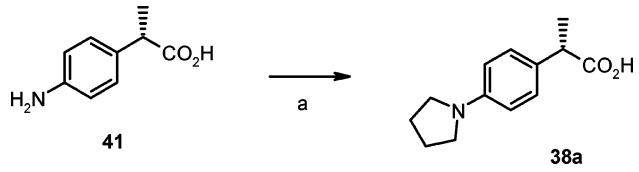
[a] Conditions: (a) EDCI, $CH_2Cl_2$, room temp; (b) TFA/$CH_2Cl_2$ (1/9; v/v), room temp.

**Scheme 2**[a]



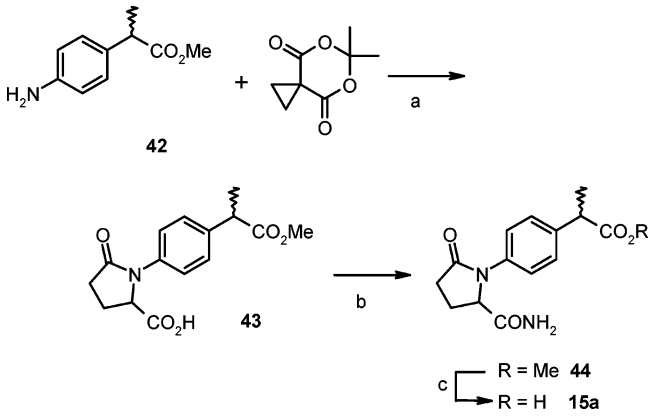

[a] Conditions: (a) HCl, 180 °C; (b) TEA, xylene, reflux; (c) ethyl isocyanateacetate, THF, 0 °C; (d) acetone, HCl, reflux.

**Scheme 3**[a]




[a] Conditions: (a) 1,4-dibromobutane, DIPEA, DMF.

**Scheme 4**[a]




[a] Conditions: (a) toluene, 60 °C; (b) *N*-hydroxybenzotriazole ammonium salt, EDCI, THF/DMF (1:1 v/v), room temp; (c) MeOH, aqueous $Na_2CO_3$, room temp.

cyclopropyl-1*H*-pyrazole-1-carboxylate and the suitable carboxylic acid (Scheme 1) as previously reported by us.[3]

Carboxylic acids employed to obtain final compounds **1**−**13**, **18**−**20**, **22**−**24**, **30**−**35**, and **37** were either commercially available or known from the literature and are reported under the section Registry Numbers, while the synthesis of the remaining ones is described in the Experimental Section (Schemes 2−9).

Carboxylic acids **14a**, **16a**, **17a**, **21a**, and **26a** were prepared starting from racemic 4-aminophenylpropionic acid (**39**) according to Scheme 2,[4] while by using its (*S*)-enantiomer (**41**), we obtained compound **38a** (Scheme 3).[5]

Racemic methyl 4-aminophenylpropionate (**42**) was the starting material to afford carboxylic acids **15a**, **25a**, **27a**, **28a**, and **36a** as outlined in Schemes 4−8.[6]

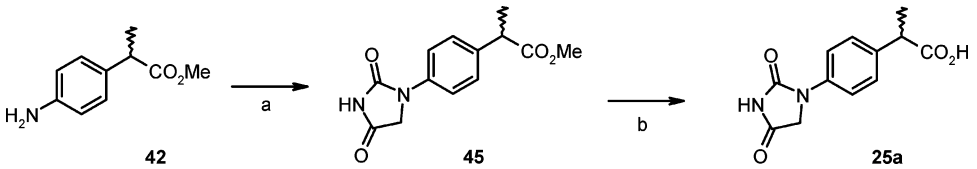
Carboxylic acid **29a** was finally prepared by alkylation of commercially available **52** followed by basic hydrolysis of intermediate **53** (Scheme 9).[7]

## SAR and Structural Studies

For the sake of clarity only a limited yet representative set of compounds is used here to describe the SAR. More compounds were synthesized to support the SAR described below.[8]

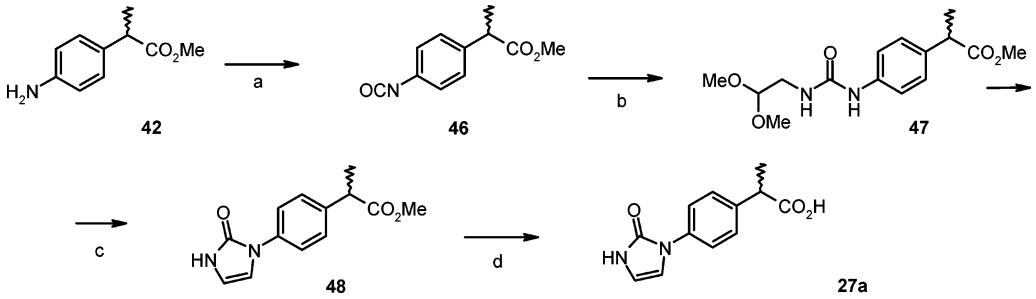
In a previous paper we described our first efforts on the 3-aminopyrazole class to the lead compound PNU-292137 (Chart 1), and we established that a cyclopropyl ring was an optimal substituent for the 5-position because of the efficient packing with the aromatic ring of Phe 80 and other lipophilic residues in the so-called buried region of the ATP-binding pocket of the kinase.[3] On the basis of the SAR learned in this first part of the class expansion, we knew that we could leverage on the variety of substitutions accepted on the 3-amino part of the pyrazole scaffold for achieving a better balance between activity and "druglike" properties. We then turned our attention to the α-position of the arylacetamido group in position 3 (Chart 1, Table 1). Structural inspection of this region based on the X-ray structure[3] of the previous lead compound PNU-292137 suggested that a small group may be tolerated. Figure 1 shows the α-methylphenylacetamido pyrazole compound docked into the CDK2/cyclin A ATP pocket. The accessible surface mesh of the binding site indicates the presence of a small hydrophobic cavity, which can be filled only by a small lipophilic group (e.g., a methyl group with *S* configuration as in Figure 1). Moreover, the docking binding mode shows that there is not much space to accommodate substituents in an *R* configuration. This hypothesis was experimentally verified with the synthesis of a number of α-substituted 3-phenylacetamidopyrazoles (**1**−**6**, **8**−**10**). As shown in Table 1, only a small group, such as a methyl (**8**−**10**) or a fluorine (**4**)

**Scheme 5**[a]



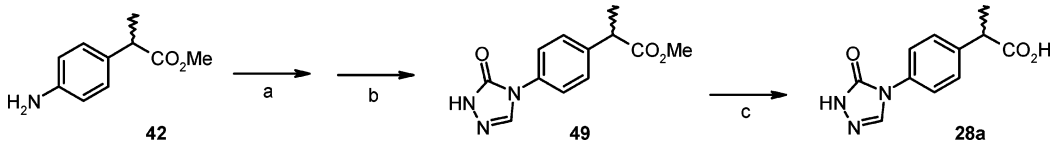

[a] Conditions: (a) chloroacetyl isocyanate, dioxane, room temp; (b) 2 N NaOH, MeOH, reflux.

**Scheme 6**[a]



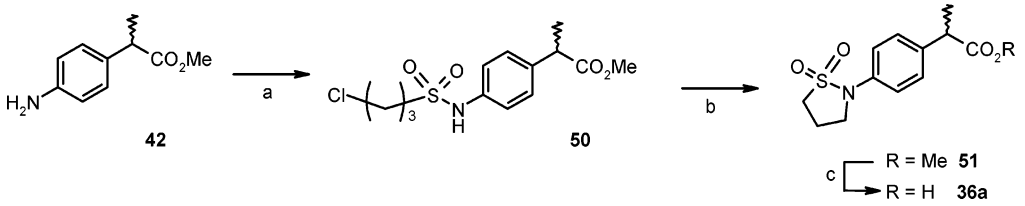

[a] Conditions: (a) triphosgene, TEA, DCM, 0 °C; (b) 2,2-dimethoxyethylamine, DCM; (c) TFA/water, MeCN, room temp; (d) MeOH/NaOH, room temp.

**Scheme 7**[a]



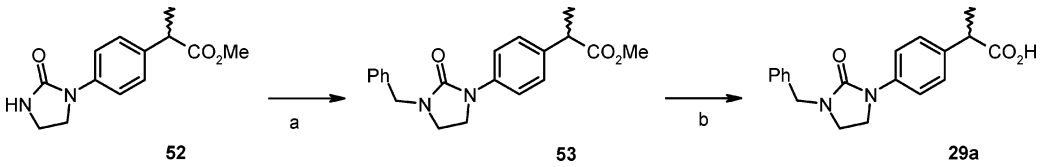

[a] Conditions: (a) methyl hydrazinocarboxylate, trimethylorthoformate; (b) MeONa; (c) MeOH/NaOH, room temp.

**Scheme 8**[a]




[a] Conditions: (a) 3-chloropropane-1-sulfonyl chloride, TEA, DCM; (b) DBU, DMF; (c) aqueous $Na_2CO_3$/MeOH.

**Scheme 9**[a]



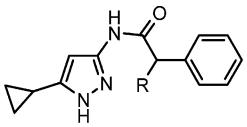

[a] Conditions: (a) benzyl chloride, NaH, DMF; (b) LiOH, THF/water.

could fit into the enzyme cavity; more hydrophilic (**1**−**3**, **5**) or bulkier groups (**6**) were less active CDK2/cyclin A inhibitors. Second, as indicated by the model (Figure 1), there was a clear stereoselectivity, with the *S*-enantiomers (**2**, **9**) more active than the *R*-counterparts (**3**, **10**).

By comparison of similar compounds with or without a α-methyl substitution, a trend was observed toward a better solubility for the α-methyl-substituted compounds, particularly in vehicles containing cosolvents that are commonly used for in vivo administration. A possible explanation for this observation may be that adding an α-substituent to the benzyl carbon atom causes a disruption of the tight package of the molecule in the crystal, bringing about improved solubility. Further expansion of this class retaining an α-methyl substituent in the arylacetamido moiety was then planned, and whenever an interesting racemic product emerged, the *S*-enantiomer (obtained either by stereospecific synthesis or by chiral separation) was evaluated and verified to be the eutomer.

One of the major concerns regarding PNU-292137, besides its rather low solubility in water, was the high plasma protein binding (PPB).[3] We had indications that the naphthyl moiety was likely to play an important role in imparting this property. We reasoned that by decreasing the lipophilic nature of this moiety while retaining the inhibitory activity on CDK2/cyclin A, we could achieve better compounds in terms of balanced activity vs druglike properties. A first set of compounds was devised by replacing the β-naphthyl moiety of PNU-292137 with different 4-lactam-1-ylphenyl moieties. The

**Table 1.** SAR of α-Substituted 3-Phenyl-acetamido-5-cyclopropyl-1*H*-pyrazoles (**1**−**10**)



| Entry | R | Configuration | CDK2/Cyclin A ($IC_{50}$; nM)[a] |
|---|---|---|---|
| **1** | Methoxy | R,S | 5,000 |
| **2** | Methoxy | S | 2,000 |
| **3** | Methoxy | R | >10,000 |
| **4** | Fluoro | R,S | 99 |
| **5** | Amino | S- | 960 |
| **6** | Phenyl | R,S- | >10,000 |
| **7** | Oxo | | 2,600 |
| **8** | Methyl | R,S | 169 |
| **9** | Methyl | S | 84 |
| **10** | Methyl | R | 6,000 |

[a] At least two independent experiments were performed for each compound in order to determine $IC_{50}$ values. Potency is expressed as the mean of $IC_{50}$ values obtained by nonlinear least-squares regression fitting of the data. The coefficient of variation of the mean ranges from 10% to 24%.

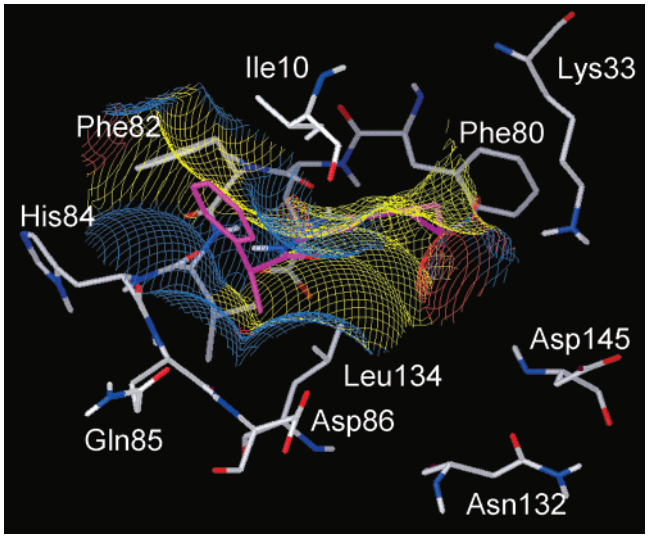
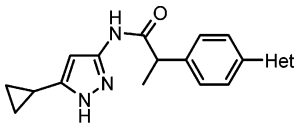


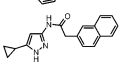

**Figure 1.** Docking of α-methylphenylacetoamidopyrazole (magenta carbon atoms) in CDK2/cyclin A (white carbon atoms). The mesh is the accessible surface of the binding site which is useful for showing steric and chemical complementarity (yellow, hydrophobic; blue, hydrogen bond acceptor; red, hydrogen bond donor).[12]

para position was chosen for the heterocyclic substitution because it allowed the substituent to directly point toward the solvent accessible region. A simple unsubstituted five-membered lactam (**13**) was as active as the parent lead against CDK2/cyclin A (Table 2), while activity was even improved upon benzo condensation of **13** (e.g., **20**) or substitution of the five-membered ring with a suitable group (e.g., **15**), though **15** and **20** turned out to be less active in the cellular assay. Increasing the lactam ring size to a six-membered pyridinone (**14**) was detrimental to activity. Compound **13** was also active in cells in the submicromolar range and scored a >10-fold increase in buffer solubility over PNU-292137. Plasma protein binding (PPB) was consistently reduced from 99% to 74%.

A second set of compounds featuring other five-membered heterocycles is reported in Table 3. The best inhibition in the biochemical assay was seen for compound **24**, an eutomer of **22**, bearing an imidazolidinone heterocyclic moiety. This compound also has a better solubility and improved PPB, although the increase in inhibitory activity is not mirrored in the cellular assay. The structure of this compound in complex with CDK2/cyclin A was elucidated. In the X-ray structure the compound is very well defined; the five-membered imidazolidinone makes favorable hydrophobic interactions with Ile10, as observed for the naphthyl moiety of PNU-292137 (Figure 2). The carbonyl of the imidazolidinone ring interacts with a water molecule that in turn interacts with Lys20. The NH group of this moiety is within hydrogen-bonding distance of Glu8, although from the electron density this latter residue is obviously somewhat mobile. This interaction may explain the further increase in activity over compound **13** and also the reduced activity when an *N*-methyl substitution is made (**30**−**32** vs **22**−**24**). The complex of **13** with CDK2/cyclin A was also solved at a resolution of 2.6 Å (data not shown). In this it was difficult to unambiguously define the orientation of the pyrrolidinone, and hence, it seems that the NH in **23** helps define the orientation of the ring. Bulkier groups such as benzyl (**29**) were detrimental for activity, solubility, and PPB. Introducing an unsaturation into the five-membered ring of imidazolinone did not hamper CDK2/cyclin A inhibitory activity but considerably worsened cellular activity. This finding may be correlated at least in part to the lower cell permeability of **27** and **28** in the Caco-2 cell penetration assay. Adding a second carbonyl moiety on the lactamyl ring of **13** to give **21** gave a moderately

**Table 2.** SAR of 3-(4-Lactam-1-yl)phenyl-acetamido-5-cyclopropyl-1*H*-pyrazoles (**11**−**20**)




| Entry | Het | α-methyl configuration | CDK2/cyclin A ($IC_{50}$; nM)[a] | A2780 ($IC_{50}$; nM)[a] | Solubility (μM; buffer pH 7) | Plasma Protein Binding (%) |
|---|---|---|---|---|---|---|
| **11** |  | R,S | 169 | 630 | >250 | 72 |
| **12** |  | R | 2,500 | 12,200 | >250 | 70 |
| **13** |  | S | 37 | 800 | >250 | 74 |
| **14** |  | R,S | 240 | >10,000 | 240 | 59 |
| **15** |  | R,S | 20 | 5,710 | 120 | 57 |
| **16** |  | R,S | 150 | 6,400 | 222 | 67 |
| **17** |  | R,S | 200 | 15,300 | 50 | 97 |
| **18** |  | R,S | 13 | 4,750 | 12 | ND |
| **19** |  | R | 170 | ND | 51 | ND |
| **20** |  | S | 2 | 1,020 | 5 | ND |
| **54** |  | | 37 | 286 | 22 | 99 |

[a] At least two independent experiments were performed for each compound in order to determine $IC_{50}$ values. Potency is expressed as the mean of $IC_{50}$ values obtained by nonlinear least-squares regression fitting of the data. The coefficient of variation of the mean ranges from 10% to 24%.

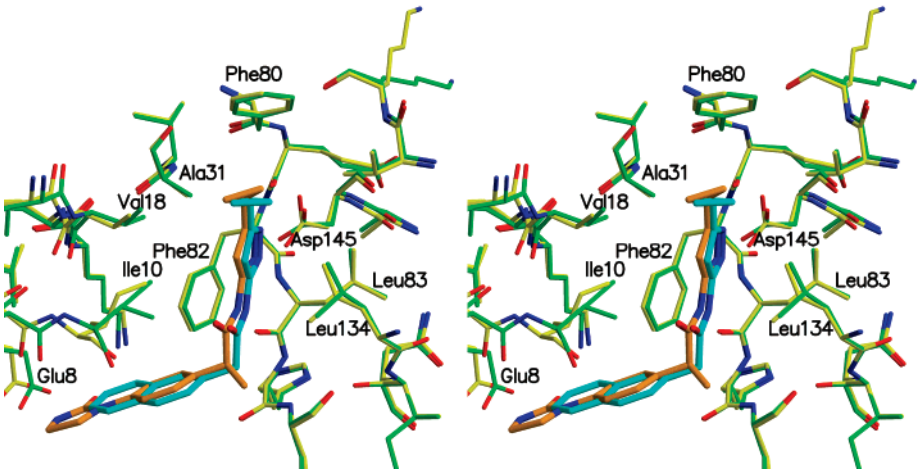
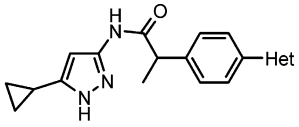
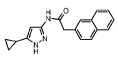



**Figure 2.** Superposition of the CDK2/cyclin A complex with compound **24** (yellow/brown carbon atoms) on the complex with PHA-292137 (green/cyan carbon atoms).[12] It can be seen that the two inhibitors have very similar binding modes. Ile 10 appears to adopt a different conformation in the two complexes. The electron density for this residue is better defined in the complex with PHA-292137 than for that with compound **24**.

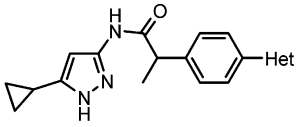
**Table 3.** SAR of 3-(4-Heterocycl-1-yl)phenyl-acetamido-5-cyclopropyl-1*H*-pyrazoles (**21**−**32**)

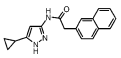



| Entry | Het | α-methyl configuration | CDK2/cyclin A ($IC_{50}$; nM)[a] | A2780 ($IC_{50}$; nM)[a] | Caco-2 Permeability | Solubility (μM; buffer pH 7) | Plasma Protein Binding (%) |
|---|---|---|---|---|---|---|---|
| **21** |  | R,S | 77 | >10,000 | Moderate | 220 | 48 |
| **22** |  | R,S | 12 | 2,250 | Moderate | 224 | 74 |
| **23** |  | R | 455 | 13,200 | Moderate | 220 | 74 |
| **24** |  | S | 2 | 1,270 | Moderate | >225 | 74 |
| **25** |  | R,S | 17 | 4,540 | Low | 201 | 67 |
| **26** |  | R,S | 124 | >10,000 | Low | 218 | 50 |
| **27** |  | R,S | 11 | 6,980 | Low | 233 | 74 |
| **28** |  | R,S | 73 | 8,390 | Low | 109 | 58 |
| **29** |  | R,S | 1,450 | ND | ND | 2 | 100 |
| **30** |  | R,S | 29 | 1,680 | Moderate | 221 | 83 |
| **31** |  | R | 8,500 | ND | Moderate | 218 | 83 |
| **32** |  | S | 17 | 1,400 | Moderate | 210 | 83 |
| **54** |  | | 37 | 286 | High | 22 | 99 |

[a] At least two independent experiments were performed for each compound in order to determine $IC_{50}$ values. Potency is expressed as the mean of $IC_{50}$ values obtained by nonlinear least-squares regression fitting of the data. The coefficient of variation of the mean ranges from 10% to 24%.

**Table 4.** SAR of 3-(4-Heterocycl-1-yl)phenyl-acetamido-5-cyclopropyl-1*H*-pyrazoles (**33**−**38**)




| Entry | Het | α-methyl configuration | CDK2/cyclin A ($IC_{50}$; nM)[a] | A2780 ($IC_{50}$; nM)[a] | Caco-2 Permeability | Solubility (μM; buffer pH 7) | Plasma Protein Binding (%) |
|---|---|---|---|---|---|---|---|
| **33** |  | R,S | 25 | 1,040 | Moderate | 222 | 73 |
| **34** |  | R | 2,500 | ND | Moderate | 220 | 72 |
| **35** |  | S | 20 | 790 | Moderate | 232 | 74 |
| **36** |  | R,S | 13 | 5,240 | Moderate | 135 | 78 |
| **37** |  | R,S | 200 | 370 | Low | 3 | 96 |
| **38** |  | S | 80 | 220 | Low | 24 | 98 |
| **54** |  | | 37 | 286 | High | 22 | 99 |

[a] At least two independent experiments were performed for each compound in order to determine $IC_{50}$ values. Potency is expressed as the mean of $IC_{50}$ values obtained by nonlinear least-squares regression fitting of the data. The coefficient of variation of the mean ranges from 10% to 24%.

active compound but without cellular activity. The position of the carbonyl group into the imidazolidindione ring was important for determining CDK2/cyclin A inhibition, since an imidazolidin-2,4-dione (**25**) was a potent inhibitor ($IC_{50}$ = 17 nM) while the regioisomer imidazolidin-2,5-dione (**26**) was about 10 times less active although there is no apparent structural reason to explain this finding. Again, the stereoselective effect of an α-methyl was confirmed and in the series **22**−**24** and **30**−**32**, the *S*-enantiomer was the eutomer. However, none of the compounds of this set reached sufficient activity in cells to warrant more extensive preclinical studies.

A final set of heterocycles, the oxazolidines **33**−**35**, the sultam **36**, and the pyrrolidines **37** and **38**, was then evaluated (Table 4). The oxazolidine **35** was a potent CDK2/cyclin A inhibitor, and again, solubility and PPB were ameliorated over the parent lead PNU-292137. Also, the pyrrolidine **38**, despite a lower activity in the biochemical assay, behaved very well in the cellular assay but the overall druglike profile was not enhanced over the parent lead.

In general the SAR around the heterocyclic part linked to the phenylacetic moiety shows that (1) it is possible to get compounds with potency up to the single digit nanomolar range using several five-membered heterocycles. Fine-tuned considerations over the relative

**Table 5.** Kinase Selectivity and Tumor Cell Antiproliferative Activity of Compounds **13** and **35**

| | $IC_{50}$, nM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CDK2/A[a] | CDK2/E[a] | CDK1/B[a] | CDK5/p25[a] | CDK4/D1[a] | other kinases[a,b] | A2780 | HT-29 | HCT116 |
| **13** | 37 ($n = 90$) | 55 ($n = 6$) | 208 ($n = 6$) | 65 ($n = 5$) | >10000 ($n = 4$) | GSK-3$\beta$: 732 ($n = 13$) | 744 ± 218 | 639 ± 176 | 1354 ± 326 |
| **35** | 20 ($n = 11$) | 37 ($n = 4$) | 243 ($n = 4$) | 57 ($n = 4$) | >10000 ($n = 2$) | GSK-3$\beta$: 406 ($n = 6$) | 716 ± 134 | 751 ± 230 | 1129 ± 675 |

[a] At least two independent experiments were performed for each compound in order to determine $IC_{50}$ values. Potency is expressed as the mean of $IC_{50}$ values obtained by nonlinear least-squares regression fitting of the data. The coefficient of variation of the mean ranges from 10% to 24%. [b] From a kinase panel of 30 serine−threonine and tyrosine kinases as previously described.[3]

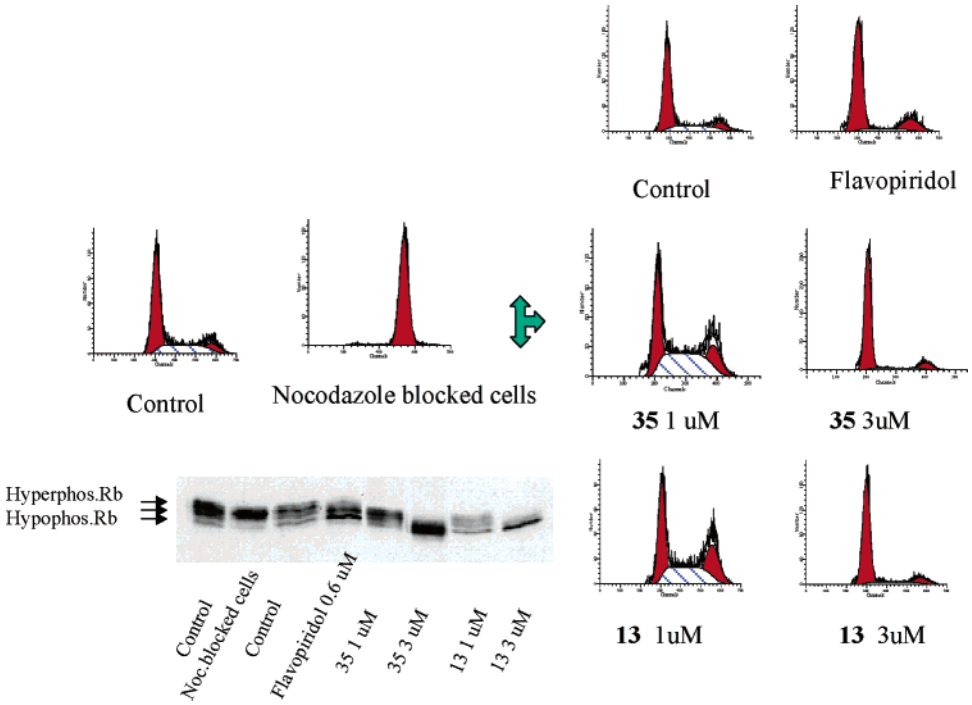



**Figure 3.** HT-29 cells were synchronized in $G_2$\M by a nocodazole treatment of 12 h at 75 ng/mL. $G_2$\M blocked HT-29 cells were recovered by shake-off and reseeded in the presence or absence of compounds at 1 and 3 $\mu$M. Flavopiridol at 0.6 $\mu$M was chosen as a positive reference. HT-29 cells were collected 24 h after the release from the nocodazole block and analyzed by FACS for the cell cycle analysis. Cell lysates obtained from the same cells were used to follow the status of pRb phosphorylation. The data reported in the picture indicate the ability of both **13** and **24** (as well as flavopiridol) to induce an accumulation of the hyposphosphorylated forms of pRb compared to the control and nocodazole blocked cells.

potency of the compounds still remain difficult to make because groups are pointing toward the solvent accessible region and a high degree of conformational motion is possible both for the protein and the inhibitor. (2) Physicochemical properties such as solubility and PPB can be definitely improved on converting a lipophilic naphthyl into a suitable heteroaryl moiety.

At the end of this optimization process two viable compounds (**13** and **35**) were further evaluated in order to choose a suitable compound to be tested in vivo.

## Biological and Pharmacological Evaluation

By comparison of **13** and **35** across a panel of kinases, a slightly different kinase profile for the two compounds was observed (Table 5). Both products exhibited a double-digit nanomolar inhibition of CDK2/cyclin A, CDK2/cyclin E, and CDK5/p25. CDK1/cyclin B was inhibited at low-micromolar concentrations, while either **13** or **35** were inactive on CDK4/cyclin D1 below 10 $\mu$M concentrations. Of a panel of 30 kinases only GSK-3$\beta$ was inhibited by either of the two lead compounds; compound **13** showed an $IC_{50}$ of 0.73 $\mu$M and **35** of 0.41 $\mu$M. On the basis of this selectivity profile, we would not expect a dramatically different outcome for the two leads in vivo.

**Table 6.** Percentage of BrdU Incorporation in Treated and Untreated HT-29 Cells

| | control | flavopiridol | **35** (1 $\mu$M) | **35** (3 $\mu$M) | **13** (1 $\mu$M) | **13** (1 $\mu$M) |
|---|---|---|---|---|---|---|
| % Brdu positive cells | 40 | 1 | 65 | 0.7 | 25 | 4 |

Both compounds were tested on a panel of tumor cell lines including an ovarian carcinoma cell line (A2780) and two colon carcinoma cell lines (HT-29 and HCT116). Their activities ($EC_{50}$) ranged between 0.6 and 1.3 $\mu$M (Table 5).

## Cell Cycle Analysis and CDK Substrate Phosphorylation

Confirmation that these compounds were inhibiting CDK2 enzyme activity in cells was obtained through examination of the phosphorylation status of a CDK substrate (pRb) by Western blotting (WB), cell cycle profile, and DNA replication (analyzing the incorporation of BrdU by immunofluorescence).

More specifically, the cell cycle profile and phosphorylation status of pRb were analyzed on HT-29 colon carcinoma cell line released from a nocodazole block in

**Table 7.** In Vitro and in Vivo ADME Parameters for Compounds **13** and **35**

| | in vitro ADME parameters[3] | | | | in vivo ADME parameters (CD-1 mice)[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | solubility ($\mu$M) | Caco-2 cell permeability | CYP4503A4 (% remaining) | PPB (%) | clearance (mL min$^{-1}$ kg$^{-1}$) | $t_{1/2}$ (min) | $V_{ss}$ (mL kg$^{-1}$) | $F_{os}$ (%) |
| **13** | 239 | high | 96 | 74 | 5.8 | 0.8 (iv)<br>3.7 (os) | 280 | 100 |
| **35** | 225 | moderate | 92 | 73 | 4.7 | 2.8 (iv)<br>3.1 (os) | 573 | 63 |

[a] Pharmacokinetic analysis was performed as previously reported.[3]

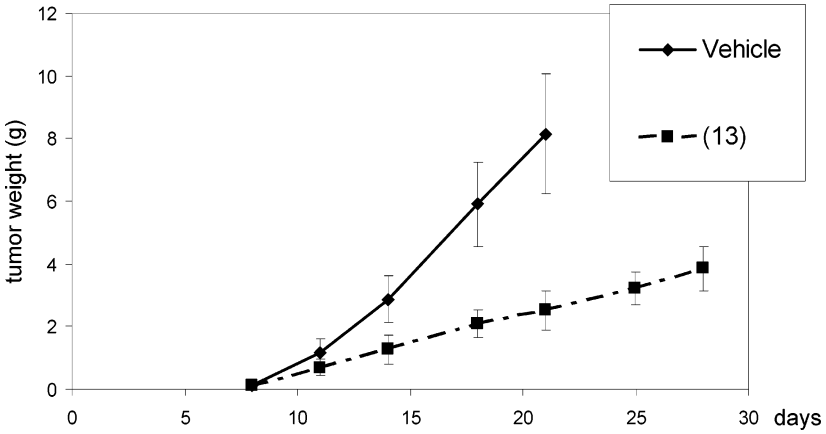



**Figure 4.** **13** shows 73% tumor growth inhibition (TGI) against a human ovarian cancer model (A2780) transplanted into nude mice when administered at 7.5 mg/kg twice a day for 20 consecutive days.

the presence or absence of compounds at the indicated concentrations (Figure 3). It was evident that under these conditions, at a dose of 3 $\mu$M, both compounds blocked the cells in $G_0\backslash G_1$, hampering the progression of cells in the S phase as highlighted by both the cell cycle profile and BrdU incorporation data (Table 6). Regarding the phosphorylation status of pRb, it was possible to note a clear reduction of the hyperphosphorylated form of pRb in the samples obtained by cells treated with the compounds versus the samples of cells treated with the vehicle, indicative of an effect on the activity of CDK2. All together, these data confirmed that **13** and **35** were hitting the target in the cells where they displayed an antiproliferative effect.

## Pharmacokinetics and Metabolism in Vitro and in Vivo

On the right-hand side of Table 7 some in vitro parameters that we used to prioritize compounds for in vivo testing are reported. The increase in solubility for both compounds was about 10-fold over that of the previous lead PNU-292137. Caco-2 permeability assay was more favorable for **13** (high permeability) than for **35** (moderate permeability). The plasma protein binding (PPB) in a high-throughput assay was nearly the same for the two compounds. In the left part of Table 7 some in vivo pharmacokinetic parameters are shown (CD1 mice). Both **13** and **35** were characterized by a low clearance together with a moderate volume of distribution ($V_{ss}$). The terminal half-life after oral administration was comparable for **35** and **13**. Although the terminal half-life in mice was shorter than optimal, it turned out to be sufficiently long after oral administration of **13** to elicit an in vivo response (vide infra). Oral bioavailability ($F_{os}$%) was complete for **13** and lower but still satisfactory for **35**. After an oral dose (10 mg/kg) of **13** a $C_{max}$ of 21 $\mu$M and an AUC of 96 $\mu$M/h were attained.

## In Vivo Antitumor Activity

On the basis of the data presented above, **13** was prioritized for further in vivo characterization in a mouse xenograft model to assess its preclinical antitumor activity.

The human ovarian A2780 xenograft mouse model was used (see Experimental Section), and on the basis of the plasma levels reached in the preliminary in vivo PK study, we selected a dose of 7.5 mg/kg, oral, twice a day. Figure 4 reports the results from this study. At this dose and scheduling, a 73% tumor growth inhibition (TGI) was obtained with a maximal body weight loss of 7% and without any signs of toxicity.

## Study of the Molecular Mechanism of Action of 13 in the Tumors

The in vivo efficacy result in the A2780 model boosted an interest in **13** as a candidate for further preclinical study, but first, we tried to establish a relationship between the antitumor activity observed in vivo in the xenograft model and markers of CDK2 inhibition in tumor sections taken from treated animals.

BrdU incorporation and phosphorylation of pRb on Ser 795 (this site being phospho-specific for CDK2 and CDK4) were chosen as markers of CDK2 inhibition. Sections of A2780 xenograft tumors were collected after 6 days of treatment at 7.5 mg/kg, oral, twice a day, and processed for the immunohistochemistry (IHC) analysis. The IHC analysis of BrdU staining indicates a significant reduction in the percentage of positive cells in the tumor sections obtained from the treated group compared to the section obtained from the vehicle group (Figure 5A), thus demonstrating a clear effect on DNA replication.

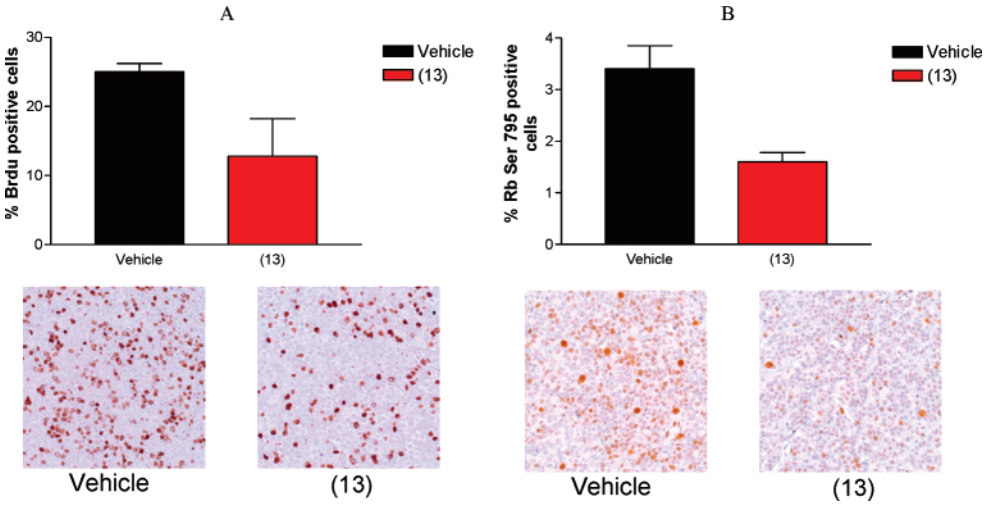



**Figure 5.** Analysis by IHC of the BrdU incorporation (A) and pRb staining (B) of the sections of A2780 xenograft tumors at the end of treatment. Tumor sections were fixed in formalin, paraffin-embedded, and stained for BrdU incorporation and phospho-pRb expression. The analysis shows a significant reduction of both BrdU and phospo-pRb positive cells in the treated vs control tumors. Representative examples of the stainings are also reported.

A comparative analysis of the phospho-Rb positive cells between the tumor sections obtained from the vehicle and the treated groups indicates a significant reduction of 50% (Figure 5B), confirming that the tumor growth inhibition obtained was related to the inhibition of the target CDK2.

## Conclusion

A pharmacological potent lead had been identified at a previous stage of lead finding.[3] The lead compound PNU-292137 was systematically modified in the part of molecule pointing toward the solvent-accessible region of the CDK2 ATP pocket in order to improve druglike properties while maintaining good activity in models predictive of antitumor efficacy. By introducing a methyl group in the *S*-configuration on the benzylic position of PNU-292137 and by changing the naphthyl moiety into a 4-lactam-1-ylphenyl group, we obtained **13**, a candidate for further preclinical investigation with an improved solubility and lower plasma protein binding. At a dose of 7.5 mg/kg twice a day for 20 days, **13** was endowed with a tumor growth inhibition (TGI) of more than 70% without significant toxicity in a human ovarian A2780 xenograft model in nude mice. A relationship between the antitumor activity observed in vivo in the xenograft model and markers of CDK2 inhibition in tumor sections taken from treated animals was found.

## Experimental Section

**Chemistry.** Melting points were determined in open glass capillaries with a Buchi 535 melting point apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1110 instrument, and C, H, and N results were within ±0.4% of theoretical values. $^1$H NMR spectra were recorded on a Varian Mercury 400 spectrometer, using the solvent as internal standard. Chemical shifts are expressed in ppm ($\delta$). Electron impact (EI) mass spectra (MS) were obtained on a Finnigan-MAT TSQ 700 triple quadrupole instrument. (ESI) mass spectra were obtained on an LCQ ion trap thermo Finnigan.[9] HPLC−MS spectra were obtained combining the MS instrument with HPLC system SSP4000 (Thermo Separation) with an autosampler Lc Pal (CTC Analytics) and UV6000LP PDA detector (UV detection 215−400 nm). Exact mass data ESI(+) were obtained on Micromass Q-Tof Ultima directly connected with micro HPLC 1100 Agilent. Organic solutions, where applicable, were dried over anhydrous $Na_2SO_4$ and evaporated using a Heidolph WB 2001 rotary evaporator at 15−20 mmHg. Flash column chromatographic separations were carried out on 40/60 $\mu$m silica gel (Carlo Erba). Thin-layer chromatography was performed on Whatman silica gel 60 plates coated with 250 $\mu$m layer with fluorescent indicator. Components were visualized by UV light ($\lambda$ = 254 nm) and by iodine vapor. Dichloromethane was distilled from $P_2O_5$ and stored over 4 Å molecular sieves. All experiments dealing with moisture-sensitive compounds were conducted under dry nitrogen. Starting materials, unless otherwise specified, were commercially available (Aldrich, Fluka) and of the best grade and were used without further purification.

**2-[4-(2-Oxopiperidin-1-yl)phenyl]propanoic Acid 14a.** To a solution of 5 g (30.3 mmol) of **39** in 2 mL of 37% HCl, 4.1 mL (45.5 mmol) of $\delta$-valerolactone were added under stirring. The resulting solution was heated at 150 °C overnight. The reaction mixture was diluted with water and extracted several times with dichloromethane. The organic layer was then dried over $Na_2SO_4$ and evaporated to dryness. The residue was finally purified by chromatography on a silica gel column by eluting with a mixture $CH_2Cl_2$/EtOH 93/7, affording 2.6 g (35% yield) of **14a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 4 H), 2.4 (t, $J$ = 6.4 Hz, 2 H), 3.4 (t, $J$ = 5.7 Hz, 2 H), 3.9 (m, 1 H), 7.2 (d, $J$ = 8.5 Hz, 2 H), 7.3 (d, $J$ = 8.4 Hz, 2 H); MS (ESI+) *m/z* 248 ($MH^+$).

The following intermediates were analogously obtained.

**2-[4-(2-Methyl-5-oxopyrrolidin-1-yl)phenyl]propanoic Acid 16a.** Yield 41%; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.0 (d, $J$ = 6.1 Hz, 3 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.7 (m, 1 H), 2.4 (m, 3 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 7.2 (s, 4 H); MS (ESI+) *m/z* 265 ($MNH_4^+$), 248 ($MH^+$).

**2-[4-(2-Oxo-5-phenylpyrrolidin-1-yl)phenyl]propanoic Acid 17a.** Yield 33%; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.3 (d, $J$ = 7.2 Hz, 3 H), 1.8 (m, 1 H), 2.6 (m, 3 H), 3.5 (q, $J$ = 7.1 Hz, 1 H), 5.4 (dd, $J$ = 6.8, 5.2 Hz, 1 H), 7.1 (m, 2 H), 7.2 (m, 1 H), 7.2 (s, 2 H), 7.3 (m, 2 H), 7.4 (d, $J$ = 8.7 Hz, 2 H), 12.2 (s, 1 H); MS (ESI+) *m/z* 327 ($MNH_4^+$), 310 ($MH^+$).

**2-[4-(2,5-Dioxopyrrolidin-1-yl)phenyl]propanoic Acid 21a.** A 825 mg sample of **39** (5 mmol) and 600 mg (6 mmol) of succinic anhydride were dissolved in 30 mL of xylene and 1 mL of triethylamine. The mixture was heated at 150 °C for 3 h under vigorous stirring. After evaporation of the solvent, the crude was chromatographed on a silica gel column by eluting with EtOAc to give 956 mg (77% yield) of **21a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.2 Hz, 3 H), 2.8 (s, 4 H), 3.7 (q, $J$ = 7.2 Hz, 1 H), 7.2 (d, $J$ = 8.5 Hz, 2 H), 7.4 (d, $J$ = 8.3 Hz, 2 H), 12.3 (s, 1 H); MS (ESI+) *m/z* 265 ($MNH_4^+$), 248 ($MH^+$).

**2-(4-{[(2-Ethoxy-2-oxoethylamino)carbonyl]amino}-phenyl)propanoic Acid 40.** A 4 g (0.02 mol) sample of **39** was dissolved in 100 mL of THF. After the mixture was cooled to 0 °C, 2.96 mL of ethyl isocyanatoacetate (0.026 mol) were added. The mixture was stirred at room temperature for 24 h. The solvent was then evaporated, and the crude was redissolved in water. The pH was then adjusted to 4 by adding aqueous $KHSO_4$. The crude product was extracted with ethyl acetate and finally crystallized from a mixture AcOEt/acetone, giving 3.5 g (60% yield) of **40**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.2 (t, $J$ = 7.1 Hz, 3 H), 1.3 (d, $J$ = 7.2 Hz, 3 H), 3.5 (q, $J$ = 7.1 Hz, 1 H), 3.8 (d, $J$ = 5.9 Hz, 2 H), 4.1 (q, $J$ = 7.1 Hz, 2 H), 6.4 (t, $J$ = 6.0 Hz, 1 H), 7.1 (d, $J$ = 8.7 Hz, 2 H), 7.3 (d, $J$ = 8.5 Hz, 2 H), 8.7 (s, 1 H), 12.1 (s, 1 H); MS (ESI+) *m/z* 312 ($MNH_4^+$), 295 ($MH^+$).

**2-[4-(2,5-Dioxoimidazolidin-1-yl)phenyl]propanoic Acid 26a.** To a solution of 500 mg (1.7 mmol) of **40** in 20 mL of acetone, 20 mL of 6 N HCl were added. The resulting solution was refluxed for 2 h, and the solvent was removed under reduced pressure. The residue was taken up with $CH_2Cl_2$ and washed with water. The organic phase was dried over $Na_2SO_4$ and evaporated, and 350 mg (84% yield) of **26a** were obtained by crystallization from diethyl ether. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.1 Hz, 3 H), 3.7 (q, $J$ = 7.1 Hz, 1 H), 4.0 (s, 2 H), 7.3 (d, $J$ = 8.5 Hz, 2 H), 7.4 (m, 2 H), 8.2 (s, 1 H), 12.3 (s, 1 H); MS (ESI+) *m/z* 266 ($MNH_4^+$), 249 ($MH^+$).

**(2*S*)-2-[4-(1-Pyrrolidinyl)phenyl]propanoic Acid 38a.** A 16.5 g (0.1 mol) sample of **41** was dissolved in 450 mL of *N*,*N*-dimethylformamide, and 15.3 mL (0.13 mol) of 1,4-dibromobutane and 69.6 mL (0.4 mol) of *N*,*N*-diisopropylethylamine were added. The mixture was heated under stirring at 90 °C for 2 h. The solvent was then evaporated under vacuum, the residue was redissolved in water, and the resulting solution was extracted several times with AcOEt. The organic phase was then dried over anhydrous sodium sulfate and concentrated. The crude was finally purified on a Florisil (200−300 mesh) column by using *n*-hexane/AcOEt 7/3 as eluant, leading to 13.2 g (60% yield) of **38a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.3 (d, $J$ = 7.1 Hz, 3H), 1.9 (m, 4 H), 3.2 (m, 4 H), 3.5 (q, $J$ = 7.1 Hz, 1 H), 6.5 (d, $J$ = 8.8 Hz, 2 H), 7.0 (d, $J$ = 8.4 Hz, 2 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 220 ($MH^+$).

**1-[4-(2-Methoxy-1-methyl-2-oxoethyl)phenyl]-5-oxoproline 43.** A 3 g (16.74 mmol) sample of **42** was dissolved in 60 mL of toluene, and 2.85 g (16.74 mmol) of 6,6-dimethyl-5,7-dioxaspiro[2.5]octane-4,8-dione were added. The mixture was heated at 60 °C for 12 h under stirring. After cooling to room temperature, the precipitated product was collected by filtration and washed with a mixture of toluene and cyclohexane (3.9 g, 80% yield). $^1$H NMR (DMSO-$d_6$) $\delta$ 1.33 (d, $J$ = 7.0 Hz, 3 H), 2.25 (m, 2 H), 3.46 (dd, $J$ = 9.1, 7.9 Hz, 1 H), 3.60 (s, 3H), 3.78 (m, 3H), 7.33 (d, $J$ = 8.6 Hz, 2 H), 7.53 (d, $J$ = 8.3 Hz, 2 H); MS (ESI+) *m/z* 292 ($MH^+$).

**1-[4-(2-Methoxy-1-methyl-2-oxoethyl)phenyl]-5-oxoprolinamide 15a.** A 3 g (10.3 mmol) sample of **43** was dissolved in 100 mL of 1/1 DMF/THF mixture. The resulting solution was cooled to 0 °C, and 3.94 g (20.6 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 3.59 mL (20.6 mmol) of *N*-ethyl-*N*′,*N*′-diisopropylamine were added. After 30 min under stirring at the same temperature 3.13 g (20.6 mmol) of *N*-hydroxybenzotriazole ammonium salt were added. The mixture was then maintained at room temperature overnight, the solvent was removed under reduced pressure, and the residue was redissolved with $CH_2Cl_2$. After being washed with water, the organic layer was dried over $Na_2SO_4$ and evaporated to dryness, affording intermediate **44**, which was submitted to the next step without any further purification. The crude was dissolved in 200 mL of methanol, and an aqueous solution of 2.18 g (20.6 mmol) of $Na_2CO_3$ was added. After being stirred overnight, the solution was made acidic with diluted HCl and extracted with ethyl acetate, affording 1.42 g (50% yield overall) of **15a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.34 (d, $J$ = 7.1 Hz, 3 H), 2.25 (m, 2 H), 3.41 (dd, $J$ = 9.2, 8.1 Hz, 1 H), 3.78 (m, 3H), 7.13 (s, 1 H), 7.30 (d, $J$ = 8.7 Hz, 2 H), 7.51 (m, 3 H); MS (ESI+) *m/z* 277 ($MH^+$).

**Methyl 2-[4-(2,4-Dioxoimidazolidin-1-yl)phenyl]propanoate 45.** A 1 g (5.6 mmol) sample of **42** and 0.5 mL (5.6 mmol) of 2-chloroacetylisocyanate were dissolved in 60 mL of dioxane. After 3 h at room temperature 1.8 mL (11.2 mmol) of DBU were added together with an additional 40 mL of dioxane. The resulting solution was stirred overnight at room temperature. The solvent was then removed under vacuum, and the residue was redissolved in $CH_2Cl_2$ and washed with diluted HCl. The organic phase was dried over $Na_2SO_4$, concentrated, and chromatographed on a silica gel column by eluting with a mixture $CH_2Cl_2$/MeOH 97/3, affording 367 mg (25% yield) of **45**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.1 Hz, 3 H), 3.6 (s, 3 H), 3.8 (q, $J$ = 7.1 Hz, 1 H), 4.4 (s, 2 H), 7.2 (d, $J$ = 8.5 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 11.1 (s, 1 H); MS (ESI−) *m/z* 261 (M − H).

**2-[4-(2,4-Dioxoimidazolidin-1-yl)phenyl]propanoic Acid 25a.** A 330 mg (1.26 mmol) sample of **45** was dissolved in 15 mL of MeOH and 2 mL (4 mmol) of 2 N NaOH. The resulting solution was refluxed for 2 h and cooled to room temperature, and 2 mL of 2 N HCl were added. The solvent was removed under vacuum, and the residue was redissolved in AcOEt and washed with water. The organic layer was dried with $Na_2SO_4$ and evaporated to dryness, affording 300 mg (96% yield) of **25a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.35 (d, $J$ = 7.11 Hz, 3 H), 3.79 (m, 1 H), 4.42 (s, 2 H), 7.33 (d, $J$ = 8.80 Hz, 2 H), 7.49 (d, $J$ = 8.65, 2 H), 11.15 (s, 1 H); MS (ESI+) *m/z* 249 ($MH^+$).

**Methyl 2-[4-(2-Oxo-2,3-dihydro-1*H*-imidazol-1-yl)phenyl]propanoate 48.** A 1 g (5.58 mmol) sample of **42** was dissolved in 10 mL of $CH_2Cl_2$, and 0.9 mL of triethylamine was added in a nitrogen atmosphere. The solution was cooled to 0 °C, and 1.78 g (5.58 mmol) of triphosgene were cautiously added portionwise. The reaction mixture was then stirred at room temperature for 6 h. After this time the solution was rapidly washed with ice/water, dried over $Na_2SO_4$, and evaporated to dryness, giving **46**, which was employed for the next step without any purification. The crude product was dissolved in 10 mL of $CH_2Cl_2$, and 0.69 mL (5.58 mmol) of aminoacetaldehyde dimethylacetal was added. The reaction mixture was stirred at room temperature for 5 h, washed with aqueous $NaHCO_3$, dried over $Na_2SO_4$, and evaporated in vacuo, affording **47** as a crude intermediate. Compound **47** was then dissolved in 5 mL of MeCN, 2 mL of water, and 2 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 6 h. The solvent was finally removed under reduced pressure, and the residue was diluted with $CH_2Cl_2$ and washed with aqueous $NaHCO_3$. The organic phase was dried over $Na_2SO_4$ and concentrated. The crude was purified by chromatography on a silica gel column (cyclohexane/AcOEt 4/1), affording 411 mg (30% overall yield) of **48**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.3 (d, $J$ = 7.2 Hz, 3 H), 3.6 (s, 3H), 3.8 (q, $J$ = 7.2 Hz, 1 H), 6.6 (dd, $J$ = 3.0, 2.5 Hz, 1 H), 6.9 (dd, $J$ = 3.1, 2.2 Hz, 1 H), 7.3 (d, $J$ = 8.5 Hz, 2 H), 7.6 (d, $J$ = 8.7 Hz, 2 H), 10.2 (s, 1 H); MS (ESI+) *m/z* 247 ($MH^+$).

**2-[4-(2-Oxo-2,3-dihydro-1*H*-imidazol-1-yl)phenyl]propanoic Acid 27a.** A 246 mg (1.0 mmol) sample of **48** was dissolved in 10 mL of MeOH, and 1 mL of 2 N NaOH (2 mmol) was added. After 6 h at room temperature MeOH was removed and water was added to the residue. The solution was acidified with 2 N HCl and extacted with a mixture $CH_2Cl_2$/MeOH 95/5. The organic layer was dried over $Na_2SO_4$ and evaporated in vacuo to give 200 mg (86% yield) of **27a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.2 Hz, 3 H), 3.7 (q, $J$ = 7.2 Hz, 1 H), 6.6 (dd, $J$ = 3.0, 2.5 Hz, 1 H), 6.9 (dd, $J$ = 3.1, 2.2 Hz, 1 H), 7.3 (d, $J$ = 8.5 Hz, 2 H), 7.6 (d, $J$ = 8.7 Hz, 2 H), 10.2 (s, 1 H), 12.3 (s, 1 H); MS (ESI+) *m/z* 250 ($MNH_4^+$), 233 ($MH^+$).

**Methyl 2-[4-(5-Oxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)-phenyl]propanoate 49.** A 1 g (5.58 mmol) sample of **42** was dissolved in 10 mL of methanol, and 0.7 mL (5.3 mmol) of trimethylorthoformate together with 531 mg (5.3 mmol) of methyl hydrazinocarboxylate and 20 mg (0.1 mmol) of *p*-toluenesulfonic acid were added. The resulting solution was stirred at 65 °C for 2 h, then 858 mg of sodium methoxide dissolved in methanol were added. After the mixture was stirred for 24 h at room temperature, the solvent was removed

under vacuum and water was added to the residue. The resulting solution was acidified with 23% HCl and extracted with $CH_2Cl_2$. The organic layer was dried over $Na_2SO_4$ and evaporated to afford 827 mg (60% overall yield) of **49**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.1 Hz, 3 H), 3.6 (s, 3 H), 3.9 (q, $J$ = 7.1 Hz, 1 H), 7.4 (d, $J$ = 8.4 Hz, 2 H), 7.6 (d, $J$ = 8.5 Hz, 2 H), 8.3 (d, $J$ = 1.4 Hz, 1 H), 11.9 (s, 1 H); MS (ESI+) $m/z$ 265 ($MNH_4^+$), 248 ($MH^+$).

**2-[4-(5-Oxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)phenyl]-propanoic Acid 28a.** A solution of 800 mg (3.25 mmol) of **49** and 3.25 mL of 1 N NaOH in 20 mL of MeOH was stirred at room temperature for 5 h. The solvent was then removed, and the residue was diluted with water, acidified with 1 N HCl, and extracted with $CH_2Cl_2$. The organic layer was dried over $Na_2SO_4$ and evaporated to afford 643 mg (85% yield) of **28a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.4 (d, $J$ = 7.3 Hz, 3 H), 3.7 (q, $J$ = 7.1 Hz, 1 H), 7.4 (d, $J$ = 8.4 Hz, 2 H), 7.6 (d, $J$ = 8.4 Hz, 2 H), 8.3 (d, $J$ = 1.3 Hz, 1 H), 11.9 (s, 1 H), 12.3 (s, 1 H); MS (ESI+) $m/z$ 251 ($MNH_4^+$), 234 ($MH^+$).

**2-[4-(1,1-Dioxidoisothiazolidin-2-yl)phenyl]propanoic Acid 36a.** To a solution of 1 g (5.58 mmol) of **42** in 10 mL of $CH_2Cl_2$ and 1.5 mL (10 4 mmol) of $Et_3N$, 0.9 mL (7.4 mmol) of 3-chloropropane-1-sulfonyl chloride was added. The mixture was stirred overnight at room temperature, washed with 1 N HCl, and evaporated to dryness. The resulting crude **50** was dissolved in 8 mL of DMF, and 1 mL (6.7 mmol) of DBU was added. After being stirred for 3 h at room temperature, the reaction mixture was poured into 250 mL of hexane/AcOEt 1/1 and washed with 1 N HCl and brine. The organic layer was dried over $Na_2SO_4$ and evaporated to give 1.2 g of crude **51**, which was employed without any further purification. Compound **51** was dissolved in 30 mL of MeOH, and 10 mL of saturated aqueous $Na_2CO_3$ were added. After the mixture was stirred at room temperature for 16 h, MeOH was removed in vacuo and the residue was diluted with water and washed with AcOEt. The aqueous layer was then acidified with 1 N HCl and extracted with AcOEt. The organic phase was dried over $Na_2SO_4$ and evaporated to afford 900 mg (60% overall yield) of **36a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.3 (d, $J$ = 7.2 Hz, 3 H), 2.4 (m, 2 H), 3.5 (t, $J$ = 7.5 Hz, 2 H), 3.6 (q, $J$ = 7.1 Hz, 1 H), 3.7 (t, $J$ = 6.5 Hz, 2 H), 7.1 (d, $J$ = 8.7 Hz, 2 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 12.2 (s, 1 H); MS (ESI+) $m/z$ 287 ($MNH_4^+$), 270 ($MH^+$), 178.

**2-[4-(3-Benzyl-2-oxoimidazolidin-1-yl)phenyl]propanoic Acid 29a.** To a solution of 248 mg (1 mmol) of **52** in 5 mL of dry DMF cooled to 0 °C, 48 mg (1.2 mmol) of NaH 60% in mineral oil were added. After 30 min, the reaction mixture was treated with 0.13 mL (1.1 mmol) of benzylbromide, and the temperature was allowed to reach room temperature. After 2 h under stirring the mixture was poured into ice/water, acidified with 2 N HCl, and extracted with AcOEt/hexane 1/1. The organic layer was dried over $Na_2SO_4$ and evaporated under vacuum, giving crude **53** that, without any further purification, was dissolved in 10 mL of THF/water 1/1. The resulting solution was treated with 40 mg (1.58 mmol) of LiOH and stirred for 3 h at room temperature. The reaction mixture was diluted with AcOEt and water. The aqueous phase was acidified with 2 N HCl and extracted with $CH_2Cl_2$. The organic phase was then dried over $Na_2SO_4$ and concentrated under reduced pressure to afford 180 mg (55% overall yield) of **29a**. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.3 (d, $J$ = 7.2 Hz, 3 H), 3.3 (m, 2 H), 3.6 (q, $J$ = 7.1 Hz, 1 H), 3.8 (m, 2 H), 4.4 (s, 2 H), 7.2 (d, $J$ = 8.7 Hz, 2 H), 7.3 (m, 1 H), 7.3 (m, 2 H), 7.3 (m, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 12.2 (s, 1 H); MS (ESI+) $m/z$ 342 ($MNH_4^+$), 325 ($MH^+$).

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2,4-dioxoimidazolidin-1-yl)phenyl]propanamide 25.** A 300 mg (1.21 mmol) sample of **25a** was dissolved in 20 mL of $CH_2Cl_2$ and 12 mL of DMF, 460 mg (2.40 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide were added, and the resulting solution was cooled to 0°C. After the mixture was stirred for 1 h at the same temperature, 225 mg (1 mmol) of *tert*-butyl 3-amino-5-cyclopropyl-1*H*-pyrazole-1-carboxylate in 4 mL of $CH_2Cl_2$ were added. The mixture was allowed to reach room temperature overnight. After this time $CH_2Cl_2$ was added and the solution was washed with 5% citric acid, aqueous $NaHCO_3$, and brine. The organic layer was dried over $Na_2SO_4$ and evaporated to dryness.

The crude was dissolved in 16 mL of $CH_2Cl_2$ and 1.6 mL of trifluoroacetic acid and stirred at room temperature for 3 h. The solvent was then removed under reduced pressure, and the residue was redissolved in $CH_2Cl_2$ and washed with aqueous $NaHCO_3$. The organic layer was dried over $Na_2SO_4$ and evaporated to dryness. The crude was purified by column chromatography ($CH_2Cl_2$/MeOH 95/5), affording 110 mg (31% overall yield) of **25**. $^1$H NMR (DMSO-$d_6$) $\delta$ 0.60 (m, 2 H), 0.86 (m, 2 H), 1.33 (d, $J$ = 7.09 Hz, 3 H), 1.80 (m, 1 H), 3.79 (m, 1 H), 4.39 (s, 2 H), 6.10 (s, 1 H), 7.32 (d, $J$ = 8.79 Hz, 2 H), 7.48 (m, 2 H), 10.30 (s, 1 H), 11.11 (s, 1 H), 11.96 (s, 1 H); MS (ESI+) $m/z$ 354 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{19}N_5O_3$ + H 354.1561, found 354.1558. HPLC purity (as area %): 100.

By using the suitable carboxylic acid, the following compounds were analogously prepared.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-methoxy-2-phenylacetamide 1.** Yield 65%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.60 (m, 2 H), 0.86 (ddd, $J$ = 8.36, 6.46, 3.96 Hz, 2 H), 1.80 (tt, $J$ = 8.44, 5.06 Hz, 1 H), 3.28 (s, 3 H), 4.82 (s, 1 H), 6.07 (s, 1 H), 7.35 (m, 5 H), 10.20 (s, 1 H), 12.10 (s, 1 H); MS (ESI+) $m/z$ 272 ($MH^+$), 240, 212; HRMS (ESI+) calcd for $C_{15}H_{17}N_3O$ + H 272.1393, found 272.1396. HPLC purity (as area %): 100.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-methoxy-2-phenylacetamide 2.** Yield 60%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.61 (m, 2 H), 0.86 (m, 2 H), 1.81 (m, 1 H), 3.28 (s, 3 H), 4.82 (s, 1 H), 6.08 (s, 1 H), 7.38 (m, 5 H), 10.23 (s, 1 H); MS (ESI+) $m/z$ 272 ($MH^+$), 240; HRMS (ESI+) calcd for $C_{15}H_{17}N_3O_2$ + H 272.1393, found 272.1405. HPLC purity (as area %): 95.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-methoxy-2-phenylacetamide 3.** Yield 66%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.61 (m, 2 H), 0.86 (m, 2 H), 1.81 (m, 1 H), 3.28 (s, 3 H), 4.83 (s, 1 H), 6.08 (s, 1 H), 7.38 (m, 5 H), 10.23 (s, 1 H); MS (ESI+) $m/z$ 272 ($MH^+$), 240; HRMS (ESI+) calcd for $C_{15}H_{17}N_3O_2$ + H 272.1393, found 272.1393. HPLC purity (as area %): 95.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-fluoro-2-phenylacetamide 4.** Yield 71%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.8 (m, 1 H), 6.0 (d, $J$ = 47.4 Hz, 1 H), 6.1 (s, 1 H), 7.4 (m, 3 H), 7.5 (m, 2 H), 10.7 (s, 1 H), 12.1 (s, 1 H); MS (ESI+) $m/z$ 260 ($MH^+$), 240, 212; HRMS (ESI+) calcd for $C_{14}H_{14}FN_3O$ + H 260.1194, found 260.1190. HPLC purity (as area %): 95.

**(2*S*)-2-Amino-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-phenylacetamide 5.** Yield 48%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.63 (m, 2 H), 0.89 (m, 2 H), 1.83 (m, 1 H), 5.00 (m, 1 H), 6.15 (s, 1 H), 7.70 (m, 5 H), 8.68 (s, 2 H), 10.95 (s, 1 H); MS (ESI+) $m/z$ 257 ($MH^+$), 240; HRMS (ESI+) calcd for $C_{14}H_{16}N_4O$ + H 257.1397, found 257.1401. HPLC purity (as area %): 95.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2,2-diphenylacetamide 6.** Yield 70%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.8 (ddd, $J$ = 13.5, 8.5, 5.1 Hz, 1 H), 5.2 (s, 1 H), 6.2 (s, 1 H), 7.2 (m, 2 H), 7.3 (m, 8 H), 10.7 (s, 1 H), 12.0 (s, 1 H); MS (EI) $m/z$ 317 ($M^+$); HRMS (ESI+) calcd for $C_{20}H_{19}N_3O$ + H 318.1601, found 318.1605. Anal. ($C_{20}H_{19}N_3O$) C, H, N. HPLC purity (as area %): 95.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-oxo-2-phenylacetamide 7.** Yield 66%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.67−0.72 (m, 2 H), 0.90−0.97 (m, 2 H), 1.86−1.94 (m, 1 H), 6.27 (s, 1 H), 7.59 (t, $J$ = 7.76 Hz, 2 H), 7.74 (t, $J$ = 7.46 Hz, 1 H), 7.94 (d, $J$ = 7.46 Hz, 2 H), 11.14 (s, 1 H), 12.28 (s, 1 H); MS (ESI) $m/z$ 256 ($MH^+$); HRMS (ESI+) calcd for $C_{14}H_{13}N_3O_2$ + H 256.1080, found 256.1086. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-phenylpropanamide 8.** Yield 75%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.59 (m, 2 H), 0.86 (m, 2 H), 1.34 (d, $J$ = 7.04 Hz, 3 H), 1.80 (ddd, $J$ = 13.50, 8.51, 4.99 Hz, 1 H), 3.81 (q, $J$ = 7.04 Hz, 1 H), 6.11 (s, 1 H), 7.19 (t, $J$ = 7.04 Hz, 1 H), 7.28 (t, $J$ = 7.48 Hz, 2 H), 7.33 (m, 2 H), 10.31 (s, 1 H), 11.98 (s, 1 H); MS (ESI+) $m/z$ 256 ($MH^+$), 124; HRMS (ESI+) calcd for $C_{15}H_{17}N_3O$ + H 256.1444, found 256.1443. HPLC purity (as area %): 100.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-phenylpropanamide 9.** Yield 72%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, $J$ = 8.5, 8.5, 5.1, 5.0 Hz, 1 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 6.1 (s, 1 H), 7.2 (m, 1 H), 7.3 (m, 2 H), 7.4 (m, 2 H), 10.4 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 256 ($MH^+$); HRMS (ESI+) calcd for $C_{15}H_{17}N_3O$ + H 256.1444, found 256.1438. Anal. ($C_{15}H_{17}N_3O$) C, H, N. HPLC purity (as area %): 100.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-phenylpropanamide 10.** Yield 78%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 6.1 (s, 1 H), 7.2 (tt, 1 H), 7.3 (m, 2 H), 7.4 (m, 2 H), 10.4 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 256 ($MH^+$); HRMS (ESI+) calcd for $C_{15}H_{17}N_3O_2$ + H 256.1444, found 256.1432. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxopyrrolidin-1-yl)phenyl]propanamide 11.** Yield 74%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 2.0 (m, 2 H), 2.4 (t, $J$ = 8.0 Hz, 2 H), 3.8 (m, 1 H), 3.8 (t, $J$ = 7.1 Hz, 2 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (d, $J$ = 8.7 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 339 ($MH^+$); HRMS (ESI+) calcd for $C_{19}H_{22}N_4O_2$ + H 339.1815, found 339.1827. Anal. ($C_{19}H_{22}N_4O_2$) C (calcd 67.44, found 66.80), H, N. HPLC purity (as area %): 100.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxopyrrolidin-1-yl)phenyl]propanamide 12.** Yield 73%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 2.0 (m, 2 H), 2.4 (t, $J$ = 8.0 Hz, 2 H), 3.8 (m, 1 H), 3.8 (t, $J$ = 7.0 Hz, 2 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 339 (MH+); HRMS (ESI+) calcd for $C_{19}H_{22}N_4O_2$ + H 339.1815, found 339.1816. HPLC purity (as area %): 100.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxopyrrolidin-1-yl)phenyl]propanamide 13.** Yield 76%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 2.0 (m, 2 H), 2.4 (t, $J$ = 8.1 Hz, 2 H), 3.8 (m, 1 H), 3.8 (t, $J$ = 7.0 Hz, 2 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); $^{13}$C NMR (DMSO-$d_6$): 7.2 (CH-6), 8.1 (CH2-7, CH2-8), 17.9 (CH2-22), 18.8 (CH3-13), 32.7 (CH2-23), 44.8 (CH-12), 48.5 (CH2-21), 92.8 (CH-4), 119.8 (CH-16, CH-18), 127.9 (CH-15, CH-19), 138.0 (C-17), 138.6 (C-14), 145.8 (C-3), 147.8 (C-5), 171.6 (C-10), 174.0 (C-24); MS (ESI+) *m/z* 339 ($MH^+$); HRMS (ESI+) calcd for $C_{19}H_{22}N_4O_2$ + H 339.1815, found 339.1804. Anal. ($C_{19}H_{22}N_4O_2$) C, H, N. $[\alpha]_D$ +115.0° (*c* 1.0, MeOH). HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxopiperidin-1-yl)phenyl]propanamide 14.** Yield 67%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 5 H), 2.3 (t, $J$ = 6.3 Hz, 2 H), 3.5 (t, $J$ = 5.8 Hz, 2 H), 3.8 (m, 1 H), 6.1 (s, 1 H), 7.2 (d, $J$ = 8.5 Hz, 2 H), 7.3 (d, $J$ = 8.4 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 353 ($MH^+$), 202; HRMS (ESI+) calcd for $C_{20}H_{24}N_4O_2$ + H 353.1972, found 353.1969. HPLC purity (as area %): 100.

**1-(4-{2-[(5-Cyclopropyl-1*H*-pyrazol-3-yl)amino]-1-methyl-2-oxoethyl}phenyl)-2-oxopyrrolidine-3-carboxamide 15.** Yield 52%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.59 (m, 2 H), 0.86 (m, 2 H), 1.33 (d, $J$ = 6.96 Hz, 3 H), 1.79 (m, 1 H), 2.25 (m, 2 H), 3.46 (dd, $J$ = 9.12, 7.94 Hz, 1 H), 3.78 (m, 3 H), 6.10 (s, 1 H), 7.13 (s, 1 H), 7.33 (d, $J$ = 8.66 Hz, 2 H), 7.53 (m, 3 H), 10.31 (s, 1 H), 11.97 (s, 1 H); MS (ESI+) *m/z* 382 ($MH^+$); HRMS (ESI+) calcd for $C_{20}H_{23}N_5O_3$ + H 382.1874, found 382.1889. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-methyl-5-oxopyrrolidin-1-yl)phenyl]propanamide 16.** Yield 58%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.1 (d, $J$ = 6.2 Hz, 3 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.6 (m, 1 H), 1.8 (m, 1 H), 2.3 (m, 1 H), 2.4 (m, 1 H), 2.5 (m, 1 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 6.1 (s, 1 H), 7.3 (s, 4 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 353 ($MH^+$), 202; HRMS (ESI+) calcd for $C_{20}H_{24}N_4O_2$ + H 353.1972, found 353.1984. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-phenyl-5-oxopyrrolidin-1-yl)phenyl]propanamide 17.** Yield 60%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.8 (m, 2 H), 1.2 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 2 H), 2.6 (m, 3 H), 3.7 (m, 1 H), 5.4 (dd, $J$ = 7.0, 5.4 Hz, 1 H), 6.1 (s, 1 H), 7.2 (m, 7 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 10.2 (m, 1 H), 11.9 (s, 1 H); MS (ESI+) *m/z* 415 ($MH^+$); HRMS (ESI+) calcd for $C_{25}H_{26}N_4O_2$ + H 415.2128, found 415.2144. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]propanamide 18.** Yield 50%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.1 Hz, 3 H), 1.7 (m, 1 H), 3.8 (m, 1 H), 5.0 (s, 2 H), 6.1 (s, 1 H), 7.4 (d, $J$ = 8.7 Hz, 2 H), 7.5 (m, 1 H), 7.6 (m, 2 H), 7.8 (d, $J$ = 7.5 Hz, 1 H), 7.8 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 387 ($MH^+$), 236; HRMS (ESI+) calcd for $C_{23}H_{22}N_4O_2$ + H 387.1815, found 387.1811. HPLC purity (as area %): 95.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]propanamide 19.** Yield 49%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.1 Hz, 3 H), 1.7 (m, 1 H), 3.8 (m, 1 H), 5.0 (s, 2 H), 6.1 (s, 1 H), 7.4 (d, $J$ = 8.7 Hz, 2 H), 7.5 (m, 1 H), 7.7 (m, 2 H), 7.8 (d, $J$ = 7.6 Hz, 1 H), 7.8 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 387 ($MH^+$), 236; HRMS (ESI+) calcd for $C_{23}H_{22}N_4O_2$ + H 387.1815, found 387.1818. HPLC purity (as area %): 95.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]propanamide 20.** Yield 52%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 3.8 (m, 1 H), 5.0 (s, 2 H), 6.1 (s, 1 H), 7.4 (d, $J$ = 7.0 Hz, 2 H), 7.5 (m, 1 H), 7.7 (m, 2 H), 7.8 (d, $J$ = 7.6 Hz, 1 H), 7.8 (d, $J$ = 8.7 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 387 ($MH^+$), 236; HRMS (ESI+) calcd for $C_{23}H_{22}N_4O_2$ + H 387.1815, found 387.1819. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2,5-dioxopyrrolidin-1-yl)phenyl]propanamide 21.** Yield 63%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.61 (m, 2 H), 0.86 (ddd, $J$ = 8.50, 6.40, 3.87 Hz, 2 H), 1.37 (d, $J$ = 7.09 Hz, 3 H), 1.80 (tt, $J$ = 8.48, 5.04 Hz, 1 H), 2.75 (s, 4 H), 3.87 (q, $J$ = 7.00 Hz, 1 H), 6.11 (s, 1 H), 7.16 (d, $J$ = 8.53 Hz, 2 H), 7.43 (d, $J$ = 8.53 Hz, 2 H), 10.40 (s, 1 H), 11.98 (s, 1 H); MS (ESI+) *m/z* 353 ($MH^+$); HRMS (ESI+) calcd for $C_{19}H_{20}N_4O_3$ + H 353.1608, found 353.1614. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanamide 22.** Yield 65%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 3.4 (m, 2 H), 3.8 (m, 1 H), 3.8 (m, 2 H), 6.1 (s, 1 H), 6.8 (s, 1 H), 7.3 (d, $J$ = 8.8 Hz, 2 H), 7.4 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 340 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{21}N_5O_2$ + H 340.1768, found 340.1762. HPLC purity (as area %): 100.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanamide 23.** Yield 68%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 3.4 (m, 2 H), 3.8 (m, 1 H), 3.8 (m, 2 H), 6.1 (s, 1 H), 6.8 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.4 (d, $J$ = 8.7 Hz, 2 H), 10.3 (s, 1 H), 11.9 (s, 1 H); MS (ESI+) *m/z* 340 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{21}N_5O_2$ + H 340.1768, found 340.1775. HPLC purity (as area %): 100.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanamide 24.** Yield 69%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 6.8 Hz, 3 H), 1.8 (tt, $J$ = 8.4, 5.0 Hz, 1 H), 3.4 (m, 2 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 3.8 (m, 2 H), 6.1 (s, 1 H), 6.9 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (d, $J$ = 8.7 Hz, 2 H), 10.3 (s, 1 H), 11.9 (s, 1 H); MS (ESI+) *m/z* 340 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{21}N_5O_2$ + H 340.1768, found 340.1767. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2,5-dioxoimidazolidin-1-yl)phenyl]propanamide 26.** Yield 42%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 3.9 (q, $J$ = 7.3 Hz, 1 H), 4.0 (d, $J$ = 1.1 Hz, 2 H), 6.1 (s, 1 H), 7.2 (d, $J$ = 8.5 Hz, 2 H), 7.4 (d, $J$ = 8.4 Hz, 2 H), 8.2 (s, 1 H), 10.4 (s, 1 H), 12.0 (s, 1 H); MS (ESI+)

*m/z* 354 ($MH^+$), 203; HRMS (ESI+) calcd for $C_{18}H_{19}N_5O_3$ + H 354.1561, found 354.1570. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxo-2,3-dihydro-1*H*-imidazol-1-yl)phenyl]propanamide 27.** Yield 58%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.8 (tt, $J$ = 8.4, 5.0 Hz, 1 H), 3.8 (q, $J$ = 6.9 Hz, 1 H), 6.1 (s, 1 H), 6.5 (dd, $J$ = 3.0, 2.6 Hz, 1 H), 6.9 (dd, $J$ = 3.0, 2.2 Hz, 1 H), 7.4 (d, $J$ = 8.5 Hz, 2 H), 7.6 (d, $J$ = 8.7 Hz, 2 H), 10.2 (s, 1 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 338 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{19}N_5O_2$ + H 338.1611, found 338.1615. HPLC purity (as area %): 95.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(5-oxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)phenyl]propanamide 28.** Yield 34%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.4 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 3.9 (q, $J$ = 7.1 Hz, 1 H), 6.1 (s, 1 H), 7.4 (d, $J$ = 8.7 Hz, 2 H), 7.6 (d, $J$ = 8.7 Hz, 2 H), 8.3 (d, $J$ = 1.4 Hz, 1 H), 10.4 (s, 1 H), 11.9 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 339 ($MH^+$); HRMS (ESI+) calcd for $C_{17}H_{18}N_6O_2$ + H 339.1564, found 339.1571. HPLC purity (as area %): 100.

**2-[4-(3-Benzyl-2-oxoimidazolidin-1-yl)phenyl]-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)propanamide 29.** Yield 56%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 3.3 (m, 2 H), 3.8 (m, 3 H), 4.4 (s, 2 H), 6.1 (s, 1 H), 7.3 (m, 5 H), 7.3 (m, 2 H), 7.5 (m, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 430 ($MH^+$); HRMS (ESI+) calcd for $C_{25}H_{27}N_5O_2$ + H 430.2237, found 430.2250. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl]propanamide 30.** Yield 60%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 2.7 (s, 3 H), 3.4 (m, 2 H), 3.7 (dd, $J$ = 9.3, 6.6 Hz, 2 H), 3.8 (m, 1 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (m, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 354 ($MH^+$); HRMS (ESI+) calcd for $C_{19}H_{23}N_5O_2$ + H 354.1924, found 354.1920. HPLC purity (as area %): 100.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl]propanamide 31.** Yield 65%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (tt, $J$ = 8.4, 5.0 Hz, 1 H), 2.7 (s, 3 H), 3.4 (m, 2 H), 3.7 (dd, 2 H), 3.8 (q, $J$ = 7.1 Hz, 1 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.8 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 354 ($MH^+$), 203; HRMS (ESI+) calcd for $C_{19}H_{23}N_5O_2$ + H 354.1924, found 354.1921. HPLC purity (as area %): 100.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl]propanamide 32.** Yield 58%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (tt, $J$ = 8.3, 5.2 Hz, 1 H), 2.7 (s, 3 H), 3.4 (m, 2 H), 3.7 (m, 2 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 354 ($MH^+$), 203; HRMS (ESI+) calcd for $C_{19}H_{23}N_5O_2$ + H 354.1924, found 354.1918. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxo-1,3-oxazolidin-3-yl)phenyl]propanamide 33.** Yield 70%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 4.0 (dd, $J$ = 8.9, 7.2 Hz, 2 H), 4.4 (m, 2 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.8 Hz, 2 H), 7.5 (d, $J$ = 8.0 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 341 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{20}N_4O_3$ + H 341.1608, found 341.1598. HPLC purity (as area %): 100.

**(2*R*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxo-1,3-oxazolidin-3-yl)phenyl]propanamide 34.** Yield 72%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 3.8 (q, $J$ = 6.9 Hz, 1 H), 4.0 (m, 2 H), 4.4 (m, 2 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.8 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 341 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{20}N_4O_3$ + H 341.1608, found 341.1597. HPLC purity (as area %): 100.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(2-oxo-1,3-oxazolidin-3-yl)phenyl]propanamide 35.** Yield 73%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (tt, $J$ = 8.4, 5.1 Hz, 1 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 4.0 (m, 2 H), 4.4 (m, 2 H), 6.1 (s, 1 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 7.5 (d, $J$ = 8.8 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 7.2 (CH-23), 8.1 (CH2−24, CH2-25), 18.8 (CH3-16), 44.7 (CH-13), 45.3 (CH2-3), 61.9 (CH2-2), 92.8 (CH-22), 118.5 (CH-7, CH-11), 128.1 (CH-8, CH-10), 137.6 (C-6, C-9), 145.8 (C-21), 147.8 (C-18), 155.4 (C-5), 171.6 (C-14); MS (ESI+) *m/z* 341 ($MH^+$); HRMS (ESI+) calcd for $C_{18}H_{20}N_4O_3$ + H 341.1608, found 341.1597. Anal. ($C_{18}H_{20}N_4O_3$) C, H, N. $[\alpha]_D$ +109.0° (*c* 1.0, MeOH). HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-[4-(1,1-dioxidoisothiazolidin-2-yl)phenyl]propanamide 36.** Yield 55%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.1 Hz, 3 H), 1.8 (m, 1 H), 2.4 (m, 2 H), 3.5 (m, 2 H), 3.7 (t, $J$ = 6.6 Hz, 2 H), 3.8 (q, $J$ = 7.0 Hz, 1 H), 6.1 (s, 1 H), 7.1 (d, $J$ = 8.7 Hz, 2 H), 7.3 (d, $J$ = 8.7 Hz, 2 H), 10.3 (s, 1 H), 12.0 (s, 1 H); MS (ESI+) *m/z* 375 ($MH^+$), 224; HRMS (ESI+) calcd for $C_{18}H_{22}N_4O_3S$ + H 375.1485, found 375.1488. HPLC purity (as area %): 100.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-(4-pyrrolidin-1-ylphenyl)propanamide 37.** Yield 30%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.6 (m, 2 H), 0.9 (m, 2 H), 1.3 (d, $J$ = 7.0 Hz, 3 H), 1.8 (m, 1 H), 1.9 (m, 4 H), 3.2 (m, 4 H), 3.7 (q, $J$ = 7.0 Hz, 1 H), 6.1 (s, 1 H), 6.4 (d, $J$ = 8.8 Hz, 2 H), 7.1 (d, $J$ = 8.7 Hz, 2 H), 10.1 (s, 1 H), 11.9 (s, 1 H); MS (ESI+) *m/z* 325 ($MH^+$), 174; HRMS (ESI+) calcd for $C_{19}H_{24}N_4O$ + H 325.2023, found 325.2029. Anal. ($C_{19}H_{24}N_4O$) C, H, N. HPLC purity (as area %): 100.

**(2*S*)-*N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-(4-pyrrolidin-1-ylphenyl)propanamide 38.** Yield 32%; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.59 (m, 2 H), 0.86 (m, 2 H), 1.28 (d, $J$ = 6.96 Hz, 3 H), 1.80 (m, 1 H), 1.90 (m, 4 H), 3.15 (m, 4 H), 3.65 (q, $J$ = 7.48 Hz, 1 H), 6.10 (s, 1 H), 6.44 (d, $J$ = 8.79 Hz, 2 H), 7.12 (d, $J$ = 8.66 Hz, 2 H), 10.12 (s, 1 H), 11.93 (s, 1 H); MS (ESI+) *m/z* 325 ($MH^+$), 174; HRMS (ESI+) calcd for $C_{19}H_{24}N_4O$ + H 325.2023, found 325.2027. Anal. ($C_{19}H_{24}N_4O$) C, H, N. HPLC purity (as area %): 100.

**Registry Numbers.** Methoxy(phenyl)acetic acid (RN, 7021-09-2), (2*R*)-methoxy(phenyl)acetic acid (RN, 3966-32-3), (2*S*)-methoxy(phenyl)acetic acid (RN, 26164-26-1), fluoro(phenyl)-acetic acid (RN, 1578-63-8), (2*S*)-[(*tert*-butoxycarbonyl)-amino](phenyl)acetic acid (RN, 2900-27-8), diphenylacetic acid (RN, 117-34-0), oxo(phenyl)acetic acid (RN, 611-73-4), 2-phenylpropanoic acid (RN, 492-37-5), (2*R*)-2-phenylpropanoic acid (RN, 7782-26-5), (2*S*)-2-phenylpropanoic acid (RN, 7782-24-3), 2-[4-(2-oxopyrrolidin-1-yl)phenyl]propanoic acid (RN, 437982-74-6), (2*R*)-2-[4-(2-oxopyrrolidin-1-yl)phenyl]propanoic acid (RN, 492445-55-3), (2*S*)-2-[4-(2-oxopyrrolidin-1-yl)phenyl]propanoic acid (RN, 492445-57-5), 2-[4-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]propanoic acid (RN, 31842-01-0), (2*R*)-2-[4-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]propanoic acid (RN, 53086-14-9), (2*S*)-2-[4-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]-propanoic acid (53086-13-8), 2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanoic acid (RN, 492445-87-1), (2*R*)-2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanoic acid (RN, 492445-88-2), (2*S*)-2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanoic acid (RN, 492445-90-6), 2-[4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl]propanoic acid (RN, 492445-98-4), (2*R*)-2-[4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl]propanoic acid (RN, 492445-94-0), (2*S*)-2-[4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl]propanoic acid (RN, 492445-96-2), 2-[4-(2-oxo-1,3-oxazolidin-3-yl)phenyl]propanoic acid (RN, 437982-65-5), (2*R*)-2-[4-(2-oxo-1,3-oxazolidin-3-yl)-phenyl]propanoic acid (RN, 492445-70-2), (2*S*)-2-[4-(2-oxo-1,3-oxazolidin-3-yl)phenyl]propanoic acid (RN, 492445-72-4), 2-(4-pyrrolidin-1-ylphenyl)propanoic acid (RN, 26586-38-9), 2-(4-nitrophenyl)propanoic acid (RN, 19910-33-9), 2-(4-aminophenyl)propanoic acid (RN, 59430-62-5), (2*S*)-2-(4-aminophenyl)propanoic acid (RN, 118417-73-5), methyl 2-(4-aminophenyl)propanoate (RN, 39718-97-3), methyl 2-[4-(2-oxoimidazolidin-1-yl)phenyl]propanoate (RN, 492445-82-6)

**Kinase Assays.** Kinase assays were performed as previously described.[3]

**Crystal Structure of CDK2/Cyclin A with Compound 24.** Expression, purification, and crystallization of the CDK2/cyclin A complex were carried out as previously described.[3] A crystal of CDK2/cyclin A was then soaked with compound **24** in a manner analogous to that described previously for the

**Table 8**

| | |
|---|---|
| data collection | |
| space group | $P6_222$ |
| cell dimensions (Å) | $a = b = 183.6$, $c = 214.1$ |
| resolution (Å) | 30−2.4 (2.49−2−4)[a] |
| no. of measured reflections | 673 208 |
| no. of unique reflections | 82 891 |
| completeness (%) | 99.9 (100) |
| $I/\sigma I$ | 16.9 (2.9) |
| $R_{merge}$ (%) | 7.3 (59.7) |
| refinement | |
| $R_{factor}$ (%) | 23.0 |
| $R_{free}$ (%) | 27.6 |
| no. of protein atoms | 8978 |
| no. of nonprotein atoms | 456 |
| rmsd from ideal values | |
| bonds (Å) | 0.007 |
| angles (deg) | 1.27 |
| % of residues in disallowed regions of Ramachandran plot[b] (%) | 2.4 |
| average $B$ (protein) | 50 |
| average $B$ (inhibitor) | 45 |

[a] Values in parentheses are for the highest-resolution shells. [b] As defined by Kleywegt and Jones.[12]

PHA-292137 complex.[3] Data were collected at the ESRF ID14-1 and processed using the HKL suite of programs.[10] Refinement was carried out in CNX[11] using the structure of the complex with PHA-292137 as the starting model. The results are shown in Table 8.

**In Vitro Pharmacokinetics.** High-throughput solubility, cell permeability, plasma protein binding, and metabolic stability (rat hepatocytes) data were acquired according to previously reported procedures.[3]

**In Vivo Pharmacokinetics.** The pharmacokinetics of **13** and **35** was investigated in nu/nu mice as described earlier.[3] For both the iv and oral legs of the preliminary PK experiments, compounds **13** and **35** were administered using PEG 400/glucosate as a vehicle.

**In Vitro and in Vivo Pharmacology.** Cell culture, inhibition of cell proliferation, cell treatment after synchronization with nocodazole, DNA content determination, Western blot analysis, and microscopic analysis of BrdU incorporation were performed as previously described.[3] Evaluation of the in vivo efficacy was performed as follows: A2780 human ovary carcinoma (from American Type Culture Collection) was maintained by subcutaneous (sc) transplantation in athymic mice using 20−30 mg of tumor brei. For the experiment, tumors were excised and fragments were implanted SC into the left flank, 10 mice/group. The treatment started when the tumors were measurable; the mean tumor weight for all the groups was 0.13 g (day 8). Compound **13** was administered after dissolution in 50% PEG 400/glucosate. Data are from a single experiment, but efficacy was confirmed in similar experiments using different doses and administration schedules.

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**Supporting Information Available:** Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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