

Identification of Potent Pyrazolo[4,3-*h*]quinazoline-3-carboxamides as Multi-Cyclin-Dependent Kinase Inhibitors[†]

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Abnormal proliferation mediated by disruption of the mechanisms that keep the cell cycle under control is a hallmark of virtually all cancer cells. Compounds targeting complexes between cyclin-dependent kinases (CDKs) and cyclins (Cy) and inhibiting their activity are regarded as promising antitumor agents to complement the existing therapies. An expansion of pyrazolo[4,3-*h*]quinazoline chemical class oriented to the development of three points of variability was undertaken leading to a series of compounds able to inhibit CDKs both *in vitro* and *in vivo*. Starting from the CDK selective but poorly soluble hit compound **1**, we succeeded in obtaining several compounds showing enhanced inhibitory activity both on CDKs and on tumor cells and displaying improved physical properties and pharmacokinetic behavior. Our study led to the identification of compound **59** as a highly potent, orally bioavailable CDK inhibitor that exhibited significant *in vivo* efficacy on the A2780 ovarian carcinoma xenograft model. The demonstrated mechanisms of action of compound **59** on cancer cell lines and its ability to inhibit tumor growth *in vivo* render this compound very interesting as potential antineoplastic agent.

Introduction

Progression through the different phases of the eukaryotic cell cycle has been shown to be critically dependent on a family of proteins known as cyclin-dependent kinases (CDKs⁴), which specifically form heterodimeric complexes with regulatory subunits named cyclins (Cy).¹ Different CDK/Cy complexes finely regulate each cell cycle phase transition: CDK2/CyE, CDK4/CyD, and CDK6/CyD primarily regulate passage through the G1 phase and the transition from G1 to S phase, while CDK2/CyA and CDK1/CyA primarily execute their critical function during the S phase and control progres-

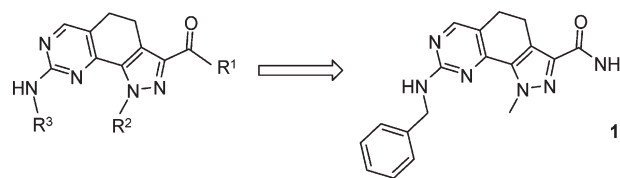


Figure 1. Structure of pyrazolo[4,3-*h*]quinazoline scaffold and hit compound **1**.

sion through the G2 phase. CDK1/CyB complex intervenes then as a key initiator of the M phase or mitosis. These CDKs phosphorylate a wide variety of proteins whose function is required over the cell cycle, thereby modulating their activity and consequently affecting cell growth and survival by several mechanisms.² A second group of CDKs, which include CDK7, CDK8, and CDK9, is involved in transcriptional regulation so that while these CDKs do not directly impinge on the cell cycle, their inhibition can however affect the expression of several cell cycle regulators and mitotic regulatory kinases, as well as of apoptosis mediators. Another member of the family, CDK5, plays a critical role in neuronal and secretory cell function.³ Interphase CDK/Cy complexes cooperate in phosphorylating the retinoblastoma protein (pRb), thereby converting it from a repressor to an activator of transcription factors that mediate the transcription of genes required for DNA synthesis.⁴ pRb function is impaired in most human neoplasms and loss in pRb repressive activity is a consequence of CDK hyperactivation. Aberrant cell proliferation that occurs in the majority of human malignancies can often be attributed to a loss of the correct cell cycle control and in fact a variety of genetic and epigenetic events can cause

[†]Coordinates of the CDK2 complex with compound **59** have been deposited in the Protein Data Bank under accession code 2WXV, together with the corresponding structure factor files.

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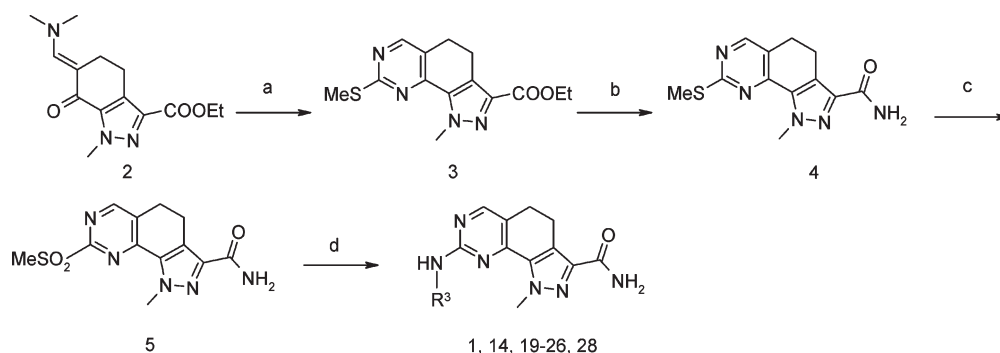
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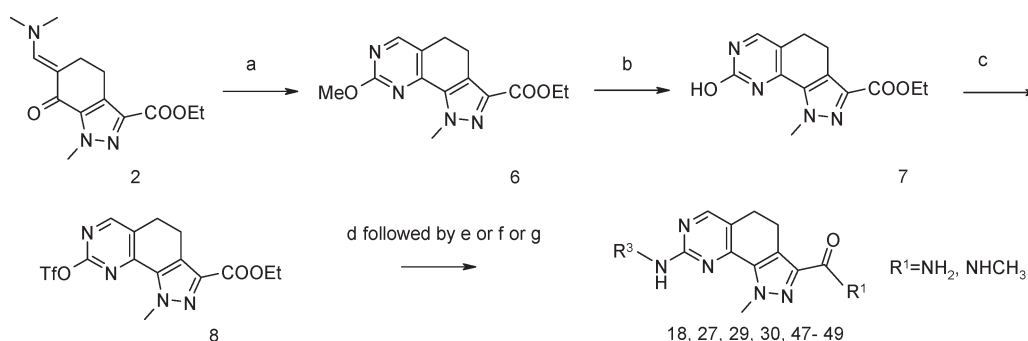
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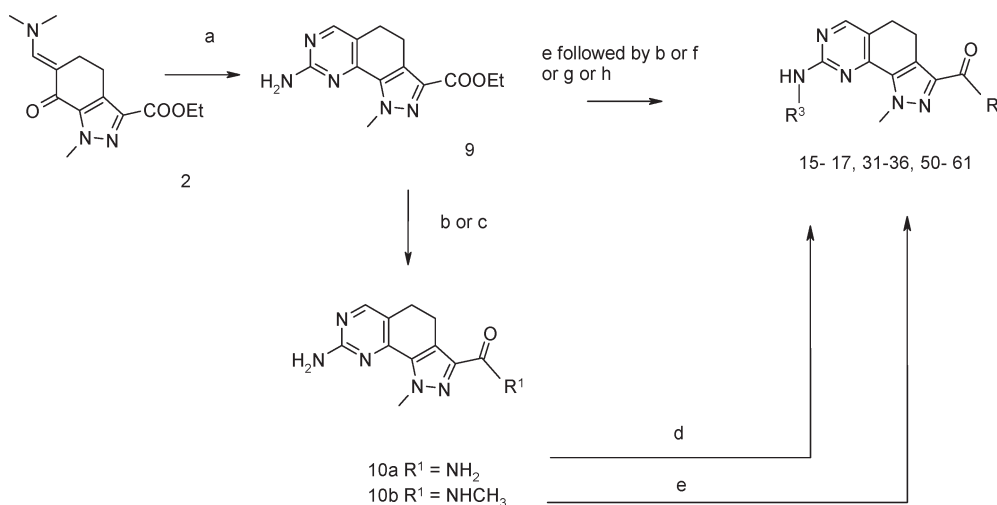
⁴Abbreviations: CDK, cyclin-dependent kinase; Cy, cyclin; CDK2/CyA, (cyclin-dependent kinase 2)/(cyclin A); Aur-A, Aurora A; pRb, retinoblastoma protein; CKI, cyclin-dependent kinase inhibitor; GSK-3 β , glycogen synthase kinase 3 β ; ERK2, extracellular signal-regulated kinase 2; ADME, adsorption-distribution-metabolism-excretion; PK, pharmacokinetics; CL, clearance; V_{ss} , distribution volume; AUC, area under curve; *F*, oral bioavailability; BrdU, bromodeoxyuridine; EDC, *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole; HOBT·NH₃, 1-hydroxybenzotriazole ammonium salt; DIPEA, *N,N*-diisopropyl-*N*-ethylamine; TGI, tumor growth inhibition; PAMPA, parallel artificial membrane permeability assay; ECACC, european collection of cell cultures; FBS, fetal calf serum; PBS, phosphate buffered saline; RNase A, ribonuclease A.

Scheme 1. Synthesis of Final Compounds **1**, **14**, **19–26**, and **28**^a

^a Reagents and conditions: (a) *S*-methylisothiurea, AcOK, DMF, 90 °C, 74%; (b) NH₄OH, MeOH-DMF, 80 °C, 52%; (c) oxone, DMF, rt, 80%; (d) R³NH₂, DMSO, 50–120 °C, 40–72%.

Scheme 2. Synthesis of Final Compounds **18**, **27**, **29**, **30**, and **47–49**^a

^a Reagents and conditions: (a) *O*-methylisourea, K₂CO₃, acetonitrile, reflux, 16 h, 80%; (b) trimethylsilyl chloride, NaI, rt, 8 h, 78%; (c) trifluoromethanesulfonic anhydride, Et₃N, CH₂Cl₂, from –78 °C to rt, 5 h, 67%; (d) R³NH₂, dioxane, rt, 70–85%; (e) EtOH, KOH, reflux, 75–80%; HOBT·NH₃, EDC, DIPEA, DMF-THF, rt, 61–73%; (f) NH₄OH, MeOH-DMF, 70 °C, 60%; (g) CH₃NH₂, EtOH, 60 °C, 50–78%.

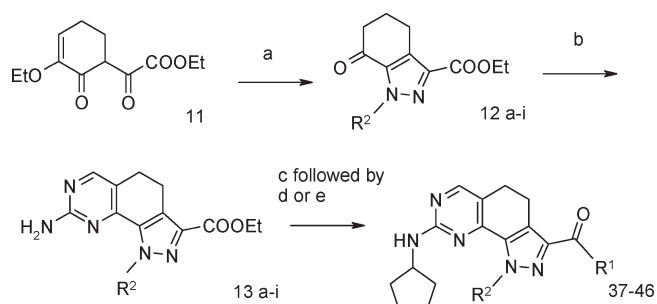
Scheme 3. Synthesis of Final Compounds **15–17**, **31–36**, and **50–61**^a

^a Reagents and conditions: (a) guanidine hydrochloride, EtONa, EtOH, reflux, 12 h, 85%; (b) NH₄OH, MeOH-DMF, 70 °C, 50–65%; (c) CH₃NH₂, EtOH, 60 °C, 78%; (d) ArCHO, NaBH₃CN, AcOH–MeOH–H₂O 1/1/1, rt, 58–62%; (e) cycloalkylketone, NaBH(OAc)₃, TFA, DMF, rt, 50–73%; (f) KOH, EtOH, reflux; R¹NH₂, EDC, HOBT, DIPEA, DMF-THF, rt, 31–51%; (g) KOH, EtOH, reflux; HOBT·NH₃, EDC, DIPEA, DMF-THF, rt, 43–49%; (h) KOH, EtOH, reflux; (COCl)₂, R¹NHOH, rt, 36–62%.

global overactivation of the cell cycle.⁵ Therefore, the development of agents able to modulate CDK activity may have a strong impact on the prevention and therapy of cancer.

The cell produces also specific inhibitors of CDKs known as cyclin-dependent kinase inhibitors (CKIs), which directly compete with ATP for binding to the CDK active site.⁶ In

some cancer types, under-expression of these endogenous CKIs has been demonstrated and these findings have solicited the effort of medicinal chemists aimed at replacing CKIs with synthetic small molecule inhibitors. The first CDK inhibitors evaluated in clinical trials were Flavopiridol (L868275)⁷ and 7-hydroxystaurosporine (UCN-01).⁸ The first one has been

Scheme 4. Synthesis of Final Compounds 37–46^a

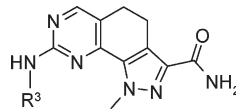
^a Reagents and conditions: (a) $R^2\text{NHNH}_2$, AcOH, rt, 63–80%; (b) dimethylformamide di-*tert*-butylacetal, DMF, 60 °C; guanidine hydrochloride, EtONa, EtOH, reflux, 45–72%; (c) cyclopentylketone, $\text{NaBH}(\text{OAc})_3$, TFA, DMF, rt, 65–80%; (d) NH_4OH , MeOH-DMF, 70 °C, 75–80%; (e) CH_3NH_2 , EtOH, 60 °C, 78%.

















granted orphan drug status for the treatment of chronic lymphocytic leukemia. A second generation of CDK inhibitors endowed with higher selectivity have also entered clinical trials, such as Roscovitine (CYC-202)⁹ and BMS-387032,¹⁰ targeting primarily CDK2, but also CDK7 and 9 and PD0332991,¹¹ targeting CDK4 and 6. Recently, several studies have suggested that CDKs and their cyclin partners can substitute and compensate for one another, leading to the notion that the inhibition of more than one CDK might be necessary to sustain the suppression of tumor growth.¹² For example, CDK1 is able to replace CDK2 in the case of its absence or inhibition. Indeed, more recent products under clinical study are endowed with a multi-CDK inhibitor activity, such as AT7519¹³ (CDK1, 2, 4, and 5 inhibitor), R547¹⁴ (CDK1, 2, and 4 inhibitor), SCH-727965¹⁵ (CDK1, 2, 5, and 9 inhibitor), and AZD5597¹⁶ (CDK1, 2, and 9 inhibitor).

We recently reported on a series of pyrazolo[4,3-*h*]quinazoline-3-carboxamides as CDK inhibitors.^{17,18} Previous expansion of this class led to the identification of PHA-848125 as a potent, orally available compound, mainly targeting CDK2 and TRKA, currently undergoing phase II clinical trials. Further exploring this class (Figure 1) with the objective to obtain a multi-CDK inhibitor, we identified compound **1**, endowed with an interesting activity profile: CDK2/CyA $\text{IC}_{50} = 0.051 \mu\text{M}$, CDK2/CyE $\text{IC}_{50} = 0.074 \mu\text{M}$, CDK1/CyB $\text{IC}_{50} = 0.120 \mu\text{M}$, CDK5 $\text{IC}_{50} = 0.049 \mu\text{M}$. Compound **1** presented a significant selectivity when evaluated on a panel of 35 serine-threonine and tyrosine kinases (data not shown) but only a moderate antiproliferative activity in A2780 human ovarian carcinoma cells ($\text{IC}_{50} = 0.933 \mu\text{M}$) and very low solubility ($< 1.0 \mu\text{M}$). An expansion program based on the development of three diversity points of the scaffold, R^1 , R^2 , and R^3 , was then undertaken aimed at obtaining more potent compounds both on CDKs and on tumor cells, with improved physical properties and pharmacokinetic profile.

Chemistry

The synthetic route to prepare the new pyrazolo[4,3-*h*]quinazoline-3-carboxamide series started from compound **2** (Scheme 1), a key intermediate for the development of this chemical class, whose synthesis we have previously described.^{17,18} In a first approach, the pyrimidine moiety was built by condensation of compound **2** with *S*-methylisothiourea, affording 2-thiomethylpyrimidino derivative **3**, that was submitted to ammonolysis to give carboxamide **4**. The latter was then oxidized with oxone to the corresponding sulfonyl

Table 1. R^3 Preliminary Exploration^a


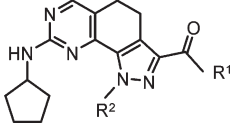
Compd	R^3	CDK2/CyA $\text{IC}_{50}, \mu\text{M}$	A2780 $\text{IC}_{50}, \mu\text{M}$	Aur-A $\text{IC}_{50}, \mu\text{M}$	Solubility pH=7, μM
1	PhCH ₂	0.051	0.933	1.004	<1.0
10a	H	>10	2.200	>10	>225
14		0.066	1.980	1.144	4.4
15		0.058	4.137	1.447	<1.0
16		0.081	0.567	1.688	<1.0
17		0.013	0.903	0.672	10
18		0.038	2.359	0.890	138
19		0.104	0.920	2.136	210
20		0.017	1.585	1.287	28
21		0.021	1.244	1.923	222
22		0.021	1.387	2.306	138
23		0.087	5.125	4.643	123
24		0.209	8.665	>10	214
25		0.019	2.550	4.597	>225
26		2.490	7.604	>10	182
27		0.004	0.410	>10	<1.0
28		0.149	2.460	>10	221
29		0.002	0.245	>10	13

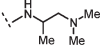
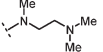
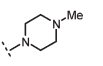

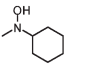
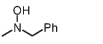






^a Values are the means of two or more experiments.

derivative **5**, suitable for introducing diversity point R^3 by nucleophilic substitution, thus, affording final compounds **1**, **14**, **19–26**, and **28**.

The low solubility of intermediate **5**, together with the moderate reactivity of the methansulfonyl group toward nucleophilic substitution, prompted us to carry out step d in dimethylsulfoxide at high temperatures. In some cases, partial aromatization of the scaffold central ring occurred in these conditions, lowering yields and complicating the product purification process.

An alternative route was also designed to overcome these issues exploiting the trifluoromethanesulfonyl moiety, which being a better leaving group than the methansulfonyl group, would have allowed to carry out the reaction more efficiently already at room temperature, thus, avoiding the central ring oxidation side reaction. The synthetic pathway to obtain

Table 2. R² and R¹ Modification^a


Compd	R ²	R ¹	CDK2/CyA IC ₅₀ , μM	A2780 IC ₅₀ , μM	Aur-A IC ₅₀ , μM	Solubility pH=7, μM
30	Me	NHMe	0.097	>10	>10	36
31	Me		1.759	>10	>10	171
32	Me		>10	5.173	>10	189
33	Me		>10	0.506	>10	83
34	Me		0.175	5.532	3.057	29
35	Me		0.296	3.966	3.839	<1.0
36	Me		1.372	6.137	4.564	<1.0
37	PhCH ₂	NH ₂	0.023	0.840	>10	<1.0
38	Ph	NH ₂	0.020	0.480	4.050	<1.0
39		NH ₂	0.052	2.164	>10	4.9
40		NH ₂	0.006	0.557	2.312	<1.0
41		NH ₂	0.005	1.003	0.442	108
42	HOCH ₂ CH ₂	NH ₂	0.008	0.330	6.04	27
43	CF ₃ CH ₂	NH ₂	0.005	0.370	>10	<1.0
44		NH ₂	0.006	0.046	>10	181
45		NHMe	0.059	1.592	>10	182
46		NH ₂	0.007	0.070	1.97	179

^a Values are the means of two or more experiments.

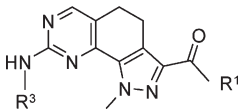
the proper intermediate entailed the condensation of **2** with *O*-methylisourea, providing derivative **6** (Scheme 2), which afforded, through methoxy group hydrolysis, intermediate **7**. The triflate **8** was finally obtained by reaction with trifluoromethanesulfonic anhydride. Substituted amino groups were introduced in intermediate **8** by nucleophilic substitution with the proper amine, followed by transformation of the ester group into amide. This step was performed by hydrolysis of the carboxy group under basic conditions to the corresponding potassium carboxylate, followed by condensation with *N*-hydroxy-benzotriazole ammonium salt, as a synthetic equivalent of ammonia, to give the final amides **18** and **29**. Alternatively, final compounds were obtained by ammonolysis with ammonium hydroxide (**27**, **48**) or by aminolysis with methylamine (**30**, **47**, **49**).
















Diversity point R³ could also be introduced in the scaffold by condensing the versatile intermediate **2** with guanidine to give compound **9** (Scheme 3) that allowed the preparation of **10a** and **10b**, by ammonolysis or by aminolysis with methylamine, respectively. R³ was introduced either starting from **10a**, by reductive amination with the suitable arylaldehyde giving final compounds **15–17**, or

starting from **10b** by reductive amination with the proper cycloalkylketone, thus, affording final compounds **51**, **53**, **55**, **57**, **59**, and **61**.

In some cases, for reactivity reasons, it was profitable to invert the order of the sequence steps so that R³ was first introduced into the scaffold from intermediate **9** via reductive amination with the suitable cycloalkylketone, then the R¹ diversity point was constructed by several methodologies. Thus, ammonolysis with ammonium hydroxide afforded compounds **54** and **58**, while basic hydrolysis to the corresponding carboxylic acids followed either by condensation with the suitable amine or with HOBT·NH₃ provided compounds **31–33** and **50**, **52**, **56**, and **60**, respectively. Alternatively, a sequence of basic hydrolysis, acyl chloride formation and reaction with the proper substituted hydroxylamine afforded compounds **34–36**.

The diversity point R² was regioselectively introduced into the pyrazolo moiety of the scaffold by condensation of compound **11**, prepared as previously described,^{17,18} with the suitable substituted hydrazine, providing intermediates **12a–i** (Scheme 4); reaction of **12a–i** with *N,N*-dimethylformamide di-*tert*-butyl acetal, followed by condensation with

Table 3. R³ and R¹ Optimization^a


Compd	R ³	R ¹	CDK2/CyA IC ₅₀ , μM	A2780 IC ₅₀ , μM	Aur-A IC ₅₀ , μM	Solubility pH=7, μM
47		NHMe	0.039	8.600	>10	50
48		NH ₂	0.351	2.000	>10	>225
49		NHMe	4.585	>10	>10	220
50		NH ₂	0.002	0.200	>10	216
51		NHMe	0.032	>10	>10	>225
52		NH ₂	0.003	0.032	0.67	186
53		NHMe	0.025	0.822	>10	168
54		NH ₂	0.012	1.300	4.708	>225
55		NHMe	1.122	>10	>10	210
56		NH ₂	0.001	0.020	1.308	23
57		NHMe	0.058	4.112	>10	179
58		NH ₂	0.001	0.024	1.237	17
59		NHMe	0.002	0.388	3.5	220
60		NH ₂	0.004	0.019	1.288	<1.0
61		NHMe	0.005	0.117	3.112	<1.0

^a Values are the means of two or more experiments.

guanidine hydrochloride afforded compounds **13a–i**. R³ was then introduced in the scaffold from **13a–i** by reductive amination with cyclopentyl ketone and R¹ diversity point was finally built by ammonolysis with ammonium hydroxide (**37–44** and **46**) or by aminolysis with methylamine (**45**).

Results and Discussion

We initially focused our attention on R³ modifications by pursuing a synthetic strategy that allowed the introduction of this group in a late step, thus, enabling a convenient parallel approach (Table 1). While the unsubstituted compound **10a** did not show any biological activity, benzyl derivative **1** showed an interesting activity profile, suggesting that the involved diversity point deserved a more in-depth investigation. In this perspective a series of substituted arylmethyl and heteroarylmethyl derivatives (**14–23**) were synthesized. Within this series several compounds (**14–18** and **20–23**) showed good activity on CDK2/CyA that was used as a sensor of compound activity toward the CDK family and, in several cases, also good selectivity, based on their potency ratio

relative to Aur-A being > 20×. Indeed, Aur-A, consistently with its similarity to CDK2 in its ATP binding pocket, was the most frequently cross-affected kinase in the selectivity panel used to characterize our compounds and, therefore, in the SAR tables, Aur-A IC₅₀ will be reported as an index of selectivity. Unfortunately, good activity on CDK2/CyA and good selectivity were generally accompanied by poor solubility (**14–17**) and low antiproliferative activity on cells, with the exception of compounds **16**, **17**, and **19**, which showed an IC₅₀ < 1 μM. To improve the solubility, some alkyl derivatives bearing hydrophilic or basic groups were also prepared (**24–26**), which still displayed, with the exception of **26**, good inhibitory potency on the enzyme and selectivity versus Aur-A, but their activity on cells was poor. Conversely, exploration of the subclass of cycloalkyl derivatives (**27–29**) was quite promising. Good solubility was achieved with compound **28**, but its potency against CDK2/CyA was the lowest in this subclass; instead, derivatives **27** and **29**, even though scarcely soluble, were potent enzyme inhibitors and, in addition, showed a significant improvement in the antiproliferative activity in A2780 human ovarian carcinoma cells and an

excellent selectivity. Based on its potency on the enzyme and on cells, we selected compound **29**, bearing a cyclopentyl group as R³, as an interesting hit from which to start further expansion. An investigation of the R¹ and R² role on a series of cyclopentyl derivatives was then accomplished (Table 2). We could observe that activity on CDK2/CyA was negatively affected upon increasing R¹ steric hindrance. In fact, secondary amides bearing small substituents such as methyl (**30**) displayed interesting activity as CDK2/CyA inhibitors,

Table 4. Selectivity Profile of Compounds **50**, **52**, and **59**^a

kinase	50	52	59
CDK2/CyA	0.002	0.003	0.002
CDK2/CyE	0.007	0.016	0.008
CDK1/CyB	0.005	0.006	0.006
CDK5/p25	0.009	0.010	0.006
CDK4/CyD1	nd	0.350	0.176
GSK-3 β	0.028	0.048	0.358
ERK2	0.212	0.226	0.622
Aur-A	> 10	0.67	3.565
other kinases ^b	> 10	> 10	> 10

^a Values are the means of two or more experiments. ^b c-ABL, ACK1, ALK, AKT1, Aur-B, BRK, BUB1, CDC7/DBF4, CHK1, CK2, EEF2K, EGFR, FAK, FGFR1, FLT3, IGF1R, IKK1, IKK2, IR, JAK1, JAK3, C-KIT, LCK, LYN, MAPKAPK2, MET, MNK2, MST4, NEK6, NIM, PAK4, PDGFR, PDK1, PERK, PIM1, PIM2, PKA α , PKC β , PLK1, RET, SYK, SULU1, TYK, TRKA, VEGFR2, VEGFR3, ZAP70.

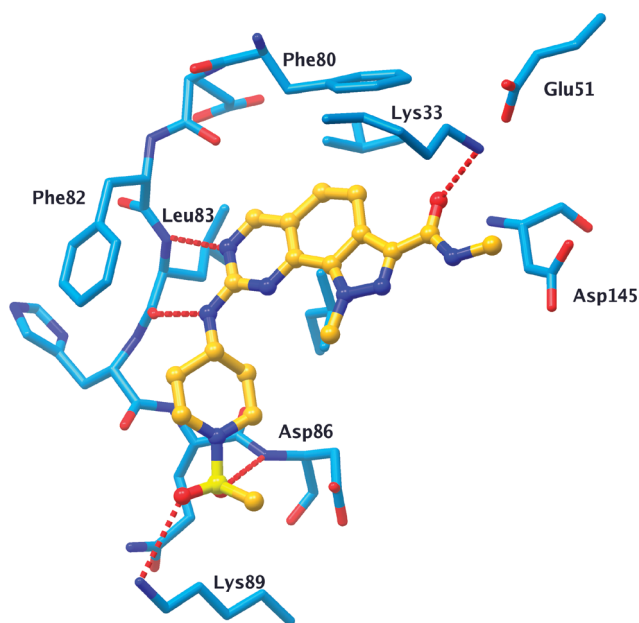


Figure 2. Crystal structure of selected compound **59** in complex with CDK2/CyA.

whereas bulkier residues (**31–33**) caused a marked decrease in activity on the enzyme complex, even though an improvement in the solubility in neutral buffer was observed. Hydroxamate derivatives (**34–36**) conserved a certain affinity for the enzyme, but poor activity on cells and low solubility were observed for these compounds.

R² modulation provided compounds endowed with significant activity on the enzyme (**37–46**) and also on cells (**37–38**, **40**, **42–44**, and **46**) and good selectivity versus Aur-A. Some derivatives showed a very poor solubility (**37–40**, **42**, and **43**), while the solubility parameter was improved for compounds bearing hydrophilic or basic substituents (**41** and **44–46**). Nevertheless, we observed that R² modification caused a general loss of selectivity within the kinase panel (data not shown). We could conclude that a bulky R² was tolerated and was suitable to modulate the solubility but could be critical for the selectivity and moreover did not represent a significant advantage with respect to methyl, in terms of atom economy and synthetic complexity. A more focused study of the R³ substituent role was then undertaken by a further expansion of the cycloalkyl derivative subclass. In this perspective, compounds with R¹ as either hydrogen or methyl and R² as methyl (Table 3) were synthesized. We could observe that secondary methylamides (**49**, **51**, **53**, **55**, and **57**), although having good solubility, were less potent than the corresponding primary amides (**48**, **50**, **52**, **54**, and **56**) both on enzyme and on cells. Interestingly, compounds **58–61** showed good activity against CDK2/CyA and in A2780 cell proliferation assay. As far as buffer solubility is concerned, compound **59**, albeit less potent than compound **58** on cells, displayed higher solubility. Compounds **50**, **52**, and **59** were then selected for further assessment on the basis of their potency on the enzyme, antiproliferative activity, and preliminary solubility in neutral buffer.

The three selected hits were subsequently screened against a panel of 55 serine-threonine and tyrosine kinases and results are reported in Table 4. Concerning the members of the CDK family that were part of the panel, compounds **50**, **52**, and **59** turned out to be potent inhibitors of CDK2/CyA, CDK2/CyE, CDK1/CyB, and CDK5/p25, and to inhibit also CDK4/CyD1, although with lower potency. Among all the other enzymes in the panel, only GSK-3 β (ratio vs CDK2/CyA of 14 \times , 16 \times , and 179 \times , respectively, for **50**, **52**, and **59**),

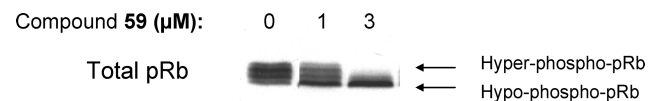


Figure 4. Decrease of pRb phosphorylation induced by compound **59** in cells after 24 h treatment.

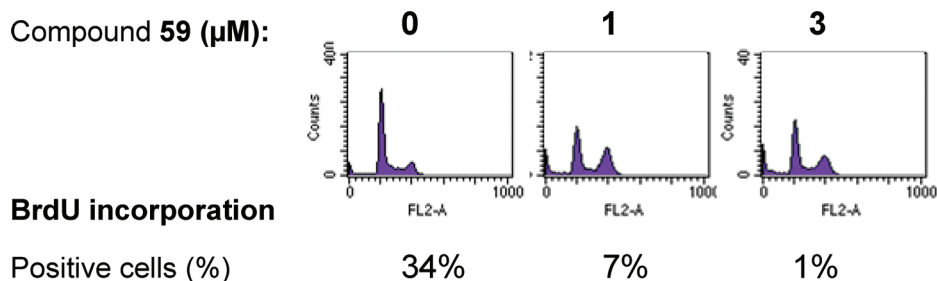


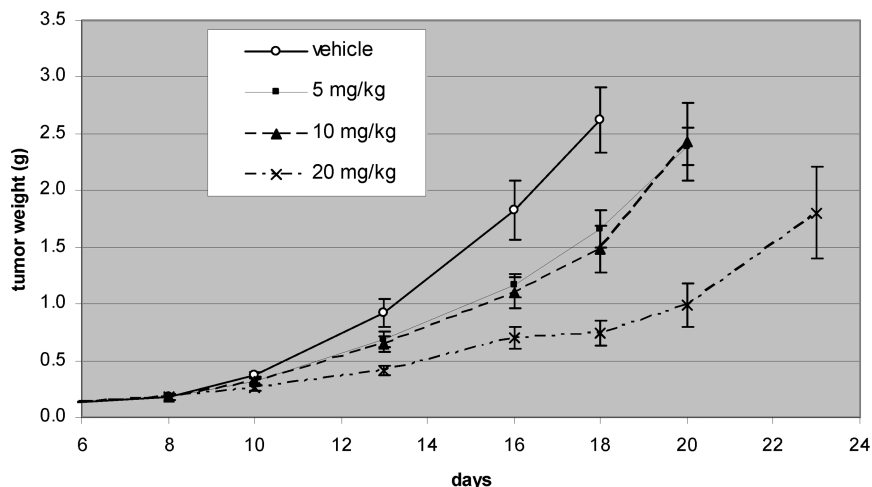
Figure 3. Effects of compound **59** on cell cycle progression and DNA synthesis after 24 h treatment.

Table 5. In Vitro ADME for Selected Compound **59**^a

preliminary in vitro ADME					
CL _{intr} hepatocytes mL/min/kg	PAMPA permeability 10 ⁻⁶ cm/sec	Caco2 cells permeability	solubility 5% dextrose (mg/mL)	CYP4503A4 (% remaining)	PPB (%)
5	11.9	high	4.0	88	69

^aDosed in 5% dextrose as hydrochloride salt.**Table 6.** In Vivo Pharmacokinetic Parameters for Selected Compound **59**^a

in vivo PK (mouse) 10 mg/kg iv			in vivo PK (mouse) 10 mg/kg os			
t _{1/2} (h)	CL (mL/h/kg)	V _{ss} (mL/kg)	t _{1/2} (h)	C _{max} (μM)	AUC (os; μM·h)	F (%)
1.7	814.2	1390.2	3.4	2.87	15.31	55.4

^aDosed in 5% dextrose as hydrochloride salt.**Figure 5.** Compound **59** shows 38, 46, and 75% tumor growth inhibition (TGI) against a human ovarian cancer model (A2780) transplanted into nude mice when orally administered respectively at doses of 5 (square), 10 (triangle), and 20 (cross) mg/kg bid for 10 consecutive days. Vehicle-treated (control) curve is reported with open circles.

and ERK2 (106×, 75×, and 311×, respectively) were inhibited in the high nanomolar range, while Aur-A was inhibited in a submicromolar range (ratio vs CDK2/CyA of 223×) only by **52**. All the other tested kinases were substantially not inhibited (IC₅₀ > 10 μM).

We focused our attention on compound **59** as the most potent and selective multi-CDK inhibitor in the series. Its distinctive selectivity profile was explained by obtaining the crystal structure of the compound in complex with CDK2/CyA (Figure 2). The amino-pyrimidine group is anchored at the hinge region by two hydrogen bonds to the nitrogen and oxygen backbone atoms of Leu83, while the carbonyl oxygen of the amide forms a hydrogen bond with the conserved Lys33. Interaction between the amide nitrogen and the carboxyl group of Asp145 that generally is present for compounds bearing a primary amide (data not shown) is lost for this compound because the secondary amide adopts a CIS conformation. This conformation can explain the experimental data indicating that amide steric hindrance tends to negatively affect activity on CDK2. The methansulfonyl group of the piperidine ring makes a hydrogen bond with backbone nitrogen of Asp86 and an additional weak interaction with Lys89, which is solvent exposed and very flexible. Interestingly, the piperidine ring or more generally a cycloalkyl ring is detrimental for activity against Aur-A and therefore crucial for compound selectivity. This moiety occupies the solvent accessible region, a part of the ATP pocket where Aur-A and CDK2 are structurally different for the presence of an

inserted glycine (Gly216) in Aur-A that is absent in CDK2.¹⁹ This additional residue in Aur-A induces a different protein conformation between the hinge region and the beginning of the C-terminal domain, which in combination with the presence of Leu139 (CDK2 Ile10) and Thr217 (CDK2 Asp86), tends to render the accommodation of nonplanar groups less favorable. In contrast, the CDK2 pocket, probably due to a better shape complementarity, can well tolerate a cycloalkyl ring.

The pharmacological profile of compound **59** was then characterized in depth.

The effects of compound **59** on the cell cycle progression and DNA synthesis were analyzed using flow cytometry analysis and BrdU incorporation, respectively, on A2780 ovarian carcinoma cells in exponential growth in the presence or absence of compound, for 24 h, at 1 and 3 μM. Compound **59** modulates cell cycle with an increase in G2/M phase and a reduction in S phase as expected for an inhibitor able to block both CDK2 and CDK1 activity. The reduction of S phase population was linked to a strong reduction of the percentage of BrdU incorporating cells, meaning that DNA synthesis in these cells was impaired (Figure 3). In addition, the effect on the phosphorylation status of a known CDK substrate such as pRb was analyzed in cells treated with compound **59** at 1 and 3 μM. A clear reduction of the hyperphosphorylated form of pRb was observed in the extracts of treated cells in comparison with the untreated cells, confirming the inhibitory effect exerted by the compound on the activity of CDK2 (Figure 4).

Analysis of the cell cycle profile, DNA synthesis, and CDK2 substrate phosphorylation status were consistent with an antiproliferative effect mediated by multi-CDK inhibition.

A preliminary in vitro ADME study (solubility in 5% dextrose, stability to human CYP4503A4, plasma protein binding, or PPB) was accomplished and in vivo pharmacokinetic data were determined as key-parameters. As reported in Table 5, compound **59** revealed high metabolic stability to human CYP4503A4 (88% remaining), good plasma protein binding (69% bound), good solubility (4.0 mg/mL in 5% dextrose as hydrochloric salt), and good permeability (PAMPA and Caco2 cells).

The compound, after intravenous administration, showed a low clearance (CL), which accounted for approximately 15% of the hepatic blood flow. The volume of distribution (V_{ss} 2-fold of the total body water) suggested a good tissue distribution. The terminal half-life was 1.7 h. After oral administration at a dose of 10 mg/kg, compound **59** showed high oral bioavailability (55%) with an AUC of 15.31 $\mu\text{M}\cdot\text{h}$, C_{max} of 2.87 μM , and half-life of 3.4 h (Table 6). Overall, compound **59** showed low clearance, suitable volume of distribution, and high oral bioavailability in the experimental conditions used in the study.

On the basis of the data presented above, compound **59** proceeded to further in vivo characterization in the human A2780 xenograft mouse model. The doses of 5, 10, and 20 mg/kg were selected and administered orally twice a day, for 10 consecutive days (on days 8–17 from tumor cell injection). A pharmacokinetic analysis was also performed in a satellite group of three mice after the last oral administration at 20 mg/kg twice a day, for 10 consecutive days, and confirmed the good and dose-proportional exposure obtained with the compound **59** (AUC = 56.21 $\mu\text{M}\cdot\text{h}$; C_{max} of 6.40 μM). Figure 5 illustrates the results from this study. Compound **59** caused a dose-dependent inhibition of A2780 tumor growth up to 75% at the dose of 20 mg/kg on day 18 ($P < 0.0001$ vs control group, Mann–Whitney U-test) and a corresponding tumor growth delay of 6.4 days.

Conclusions

Exploration of the pyrazoloquinazoline class focused on R^1 , R^2 , and R^3 modifications afforded several compounds that showed good potency as multi-CDK inhibitors, good selectivity when tested on a panel of serine-threonine and tyrosine kinases, and antiproliferative activity in A2780 human ovarian carcinoma cells. Optimization of the physical properties and pharmacokinetic profile led to the identification of the highly potent, orally available compound **59**. Analysis of the cell cycle profile, DNA synthesis, and CDK2 substrate phosphorylation status were consistent with an antiproliferative effect mediated by multi-CDK inhibition. The compound exhibited good efficacy in vivo on A2780 xenograft model when orally administered at dose of 20 mg/kg bid for 10 consecutive days. The complete pharmacological profile of compound **59** will be reported in a separate paper.

Experimental Section

Chemistry. All solvents and reagents, unless otherwise stated, were commercially available, of the best grade, and were used without further purification. All experiments dealing with moisture-sensitive compounds were conducted under dry nitrogen or argon. 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 F₂₅₄ precoated plates. Column chromatography was conducted either under medium pressure on silica (Merck silica gel 40–63 μm) or on prepacked silica gel cartridges (Biotage). Components were visualized by UV light (λ : 254 nm) and by iodine vapor. ^1H NMR spectra were recorded at a constant temperature of 28 °C on a Varian INOVA 400 spectrometer operating at 400.45 MHz and equipped with a 5 mm Indirect Detection PFG Probe ($^1\text{H}\{^15\text{N}-^31\text{P}\}$). Chemical shifts were referenced with respect to the residual solvent signals (DMSO- d_6 : 2.50 ppm for ^1H). Data are reported as follows: chemical shift (δ), multiplicity (s = singlet, bs = broad signal, d = doublet, t = triplet, q = quartet, quin = quintet, sxt = sextet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, td = triplet of doublets, m = multiplet), coupling constants (Hz), and number of protons. Electrospray (ESI) mass spectra were obtained on a Finnigan LCQ ion trap. Unless otherwise specified, all final compounds were homogeneous (purity of not less than 95%), as determined by high-performance liquid chromatography (HPLC). 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 UV6000LP 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 flow rate, using a Waters X Terra RP 18 column (4.6 \times 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 re-equilibration. ESI(+) high resolution mass spectra (HRMS) were obtained on a Waters Q-ToF Ultima directly connected with micro-HPLC 1100 Agilent as previously described.²⁰

Elemental analyses were performed on a Carlo Erba 1110 instrument, and C, H, and N results were within $\pm 0.4\%$ of theoretical values unless specified. All reactants were commercially available or were prepared as previously described.¹⁸

Ethyl 1-Methyl-8-(methylsulfonyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (3). To a solution of ethyl (6E)-6-[(dimethylamino)methylidene]-1-methyl-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate **2** (9.0 g, 69 mmol, prepared according to ref 17) in dry DMF (350 mL), dry potassium acetate (13.4 g, 138 mmol), and *S*-methylisothiourea sulfate (19.18 g, 69 mmol) were added. The mixture was maintained at 90 °C under stirring for 8 h. The solvent was then evaporated, the residue dissolved with dichloromethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The crude was finally triturated with diethyl ether and collected by filtration to give **3** (15.5 g, 74%). ^1H NMR (400 MHz, DMSO- d_6) δ ppm 1.31 (t, $J = 7.1$ Hz, 3H), 2.56 (s, 3H), 2.91 (m, 2H), 3.00 (m, 2H), 4.29 (d, $J = 7.1$ Hz, 2H), 4.33 (s, 3H), 8.55 (s, 1H); LCMS (ESI) m/z 305 (M + H)⁺.

1-Methyl-8-(methylsulfonyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (4). A suspension of **3** (13.0 g, 0.043 mol) in a mixture of methanol (200 mL), DMF (200 mL), and 33% NH_4OH (200 mL) was stirred at 65 °C in a closed bottle for about 8 h. The solvent was then evaporated to dryness, the residue dissolved with dichloromethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by flash chromatography on a silica gel column (cyclohexane/ethyl acetate, 8/2), giving **4** (6.16 g, 52%). ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.56 (s, 3H), 2.88 (m, 2H), 3.01 (m, 2H), 4.30 (s, 3H), 7.27 (bs, 1H), 7.36–7.69 (m, 1H), 8.53 (s, 1H); LCMS (ESI) m/z 276 (M + H)⁺.

1-Methyl-8-(methylsulfonyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (5). A solution of **4** (6.0 g, 0.022 mol)

in DMF (1000 mL) and potassium monopersulfate triple salt (oxone, 40.57 g, 0.066 mol) was stirred at room temperature for 16 h. Water and ethyl acetate were then added and the layers separated. The organic phase was finally dried over Na_2SO_4 and evaporated. The residue was triturated with diethyl ether and **5** (5.40 g, 80%) was collected by filtration. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 3.08 (s, 4H), 3.45 (s, 3H), 4.31 (s, 3H), 7.33 (bs, 1H), 7.55 (bs, 1H), 8.93 (s, 1H); LCMS (ESI) m/z 308 ($\text{M} + \text{H}^+$).

8-(Benzylamino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (1). To a solution of **5** (400 mg, 1.30 mmol) in dry DMSO (20 mL), benzylamine (0.28 mL, 2.60 mmol) was added. After 6 h at 95 °C under nitrogen atmosphere, the solvent was evaporated. The residue was then dissolved with dichloromethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. By chromatography on a silica gel column (dichloromethane/acetone, 9/1) **1** was obtained (304 mg, 70%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.68–2.76 (m, 2H), 2.84–2.99 (m, 2H), 4.16 (bs, 3H), 4.53 (d, $J = 6.2$ Hz, 2H), 7.15–7.23 (m, 2H), 7.25–7.35 (m, 4H), 7.40 (bs, 1H), 7.68 (bs, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 335 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O} + \text{H}^+$, 335.1615; found, 335.1610; Anal. ($\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}$) C, H, N.

Compounds **14**, **19**–**26**, and **28**. By employment of the above-described procedure, starting from **5** and using the suitable amine, compounds **14**, **19**–**26**, and **28** were prepared.

8-[(3-Methoxybenzyl)amino]-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (14). Yield, 72%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.71 (t, $J = 7.6$ Hz, 2H), 2.92 (t, $J = 7.6$ Hz, 2H), 3.71 (s, 3H), 4.17 (s, 3H), 4.49 (d, $J = 6.3$ Hz, 2H), 6.73–6.80 (m, 1H), 6.89 (d, $J = 1.3$ Hz, 1H), 6.89–6.92 (m, 1H), 7.15–7.28 (m, 2H), 7.39 (s, 1H), 7.66 (t, $J = 6.6$ Hz, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 365 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{20}\text{N}_6\text{O}_2 + \text{H}^+$, 365.1721; found, 365.1725; Anal. ($\text{C}_{19}\text{H}_{20}\text{N}_6\text{O}_2$) C, H, N.

1-Methyl-8-[[3-(4-methylpiperazin-1-yl)benzyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (19). Yield, 66%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.21 (s, 3H), 2.43 (m, 4H), 2.67–2.75 (m, 2H), 2.86–2.97 (m, 2H), 3.05–3.12 (m, 4H), 4.19 (s, 3H), 4.46 (d, $J = 6.2$ Hz, 2H), 6.72–6.80 (m, 2H), 6.89–6.96 (m, 1H), 7.08–7.16 (m, 1H), 7.21 (s, 1H), 7.40 (s, 1H), 7.62 (s, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 433 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{28}\text{N}_8\text{O} + \text{H}^+$, 433.2459; found, 433.2454; Anal. ($\text{C}_{23}\text{H}_{28}\text{N}_8\text{O}$) C, H, N.

1-Methyl-8-[[4-(4-methylpiperazin-1-yl)benzyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (20). Yield, 68%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.26 (bs, 3H), 2.43–2.56 (m, 4H), 2.71 (m, 2H), 2.92 (m, 2H), 3.09 (s, 4H), 4.21 (s, 3H), 4.42 (d, $J = 6.2$ Hz, 2H), 6.86 (d, $J = 8.7$ Hz, 2H), 7.18 (d, $J = 8.7$ Hz, 2H), 7.21 (s, 1H), 7.40 (t, 1H), 7.56 (t, $J = 6.1$ Hz, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 433 ($\text{M} + \text{H}^+$); Anal. ($\text{C}_{23}\text{H}_{28}\text{N}_8\text{O}$) C, H, N.

1-Methyl-8-[(pyridin-4-ylmethyl)amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (21). Yield, 45%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.68–2.78 (m, 2H), 2.86–2.98 (m, 2H), 4.07 (bs, 3H), 4.54 (d, $J = 6.2$ Hz, 2H), 7.21 (bs, 1H), 7.27–7.34 (m, 2H), 7.40 (bs, 1H), 7.78 (t, $J = 6.8$ Hz, 1H), 8.22 (s, 1H), 8.38–8.54 (m, 2H); LCMS (ESI) m/z 336 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{17}\text{N}_7\text{O} + \text{H}^+$, 336.1568; found, 336.1572; Anal. ($\text{C}_{17}\text{H}_{17}\text{N}_7\text{O}$) C, H, N.

1-Methyl-8-[(pyridin-3-ylmethyl)amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (22). Yield, 40%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.68–2.97 (m, 4H), 4.16 (s, 3H), 4.55 (d, $J = 6.2$ Hz, 2H), 7.21 (s, 1H), 7.32 (dd, $J = 7.8, 4.7$ Hz, 1H), 7.40 (s, 1H), 7.63–7.81 (m, 1H), 7.72 (dt, $J = 7.7, 1.8$ Hz, 1H), 8.23 (s, 1H), 8.42 (dd, $J = 4.7, 1.5$ Hz, 1H), 8.55 (d, $J = 1.8$ Hz, 1H); LCMS (ESI) m/z 342 ($\text{M} + \text{H}^+$); Anal. ($\text{C}_{17}\text{H}_{17}\text{N}_7\text{O}$) C, H, N.

1-Methyl-8-[(pyridin-2-ylmethyl)amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (23). Yield, 42%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.68–2.76 (m, 2H), 2.92 (m, 2H), 4.04 (bs, 3H), 4.60 (d, $J = 6.1$ Hz, 2H), 7.10–7.28 (m, 2H), 7.31

(d, $J = 7.8$ Hz, 1H), 7.39 (bs, 1H), 7.71 (td, $J = 7.6, 1.83$ Hz, 2H), 8.22 (s, 1H), 8.47–8.50 (m, 1H); LCMS (ESI) m/z 336 ($\text{M} + \text{H}^+$); Anal. ($\text{C}_{17}\text{H}_{17}\text{N}_7\text{O}$) C, H, N.

1-Methyl-8-[[2-(morpholin-4-yl)ethyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (24). Yield, 46%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.41 (m, 4H), 2.67–2.77 (m, 2H), 2.88–3.02 (m, 2H), 3.43 (q, $J = 6.5$ Hz, 2H), 3.57 (m, 4H), 4.30 (s, 3H), 6.92 (t, $J = 5.9$ Hz, 1H), 7.22 (bs, 1H), 7.41 (bs, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 358 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{23}\text{N}_7\text{O}_2 + \text{H}^+$, 358.1986; found, 358.1971; Anal. ($\text{C}_{17}\text{H}_{23}\text{N}_7\text{O}_2$) C, H, N.

8-[(2-Hydroxyethyl)amino]-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (25). Yield, 48%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.67–2.98 (m, 4H), 3.34–3.60 (m, 4H), 4.29 (s, 3H), 4.65 (s, 1H), 6.95 (t, $J = 5.7$ Hz, 1H), 7.22 (s, 1H), 7.41 (s, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 289 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{16}\text{N}_6\text{O}_2 + \text{H}^+$, 289.1408; found, 289.1410; Anal. ($\text{C}_{13}\text{H}_{16}\text{N}_6\text{O}_2$) C, H, N.

1-Methyl-8-[[2-(piperidin-1-yl)ethyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (26). Yield, 40%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.38 (m, 2H), 1.44–1.58 (m, 4H), 2.38 (bs, 4H), 2.46 (bs, 2H), 2.72 (m, 2H), 2.93 (m, 2H), 3.41 (q, $J = 6.3$ Hz, 2H), 4.30 (s, 3H), 6.86 (bs, 1H), 7.22 (bs, 1H), 7.41 (bs, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 356 ($\text{M} + \text{H}^+$); Anal. ($\text{C}_{18}\text{H}_{25}\text{N}_7\text{O}$) C, H, N.

8-[(1-Ethylpiperidin-4-yl)amino]-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (28). Yield, 63%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.00 (t, $J = 7.2$ Hz, 3H), 1.42–1.57 (m, 2H), 1.86–1.93 (m, 2H), 1.93–2.02 (m, 2H), 2.33 (q, $J = 7.2$ Hz, 2H), 2.71 (t, $J = 7.6$ Hz, 2H), 2.87 (m, 2H), 2.93 (t, $J = 7.62$ Hz, 2H), 3.57–3.76 (m, 1H), 4.29 (s, 3H), 6.98 (d, $J = 7.9$ Hz, 1H), 7.21 (s, 1H), 7.42 (s, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 356 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{25}\text{N}_7\text{O} + \text{H}^+$, 356.2193; found, 356.2184.

Ethyl 8-Methoxy-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (6). To a solution of **2** (2.0 g, 7.2 mmol) in acetonitrile (200 mL), *O*-methylisourea sulfate (17.4 g, 70.6 mmol) and potassium carbonate (10.0 g, 72.4 mmol) were added. The reaction mixture was stirred at reflux for 16 h. The solvent was then evaporated, and the residue was dissolved with dichloromethane and washed with water. The organic layer was dried over dry Na_2SO_4 and concentrated. After flash chromatography on a silica gel column (dichloromethane) **6** was obtained (1.7 g, 80%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.31 (t, $J = 7.1$ Hz, 3H), 2.87–2.93 (m, 2H), 2.99 (m, 2H), 3.95 (s, 3H), 4.29 (q, $J = 7.1$ Hz, 2H), 4.33 (s, 3H), 8.52 (s, 1H); LCMS (ESI) m/z 289 ($\text{M} + \text{H}^+$).

Ethyl 8-Hydroxy-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (7). To a solution of **6** (1.5 g, 5.2 mmol) in acetonitrile (90 mL), sodium iodide (1.6 g, 10.6 mmol), and trimethylsilylchloride (1.5 mL, 12 mmol) were added in sequence. After 24 h under stirring and nitrogen atmosphere at room temperature, the solvent was evaporated, and the residue was dissolved with a mixture dichloromethane/methanol 4/1 and washed with a saturated aqueous solution of sodium thiosulfate. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from methanol leading **7** (1.1 g, 78%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.30 (t, $J = 7.1$ Hz, 3H), 2.71 (m, 1H), 2.93 (m, 1H), 4.29 (q, $J = 7.1$ Hz, 2H), 4.27 (s, 3H), 7.85 (s, 1H), 11.69 (s, 1H); LCMS (ESI) m/z 275 ($\text{M} + \text{H}^+$); HPLC purity 80%.

Ethyl 1-Methyl-8-[[trifluoromethyl)sulfonyl]oxy]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (8). A solution of **7** (0.60 g, 2.19 mmol) and triethylamine (0.31 mL, 2.19 mmol) in dichloromethane (60 mL) was stirred at –78 °C for 5 h. After this time, trifluoromethanesulfonic anhydride (0.72 mL, 2.19 mmol) was added. The reaction was stirred overnight and allowed to come to room temperature, washed with aqueous NaHCO_3 , dried over Na_2SO_4 , and evaporated to dryness. The residue was triturated with diethyl ether/acetone and **8** was collected by filtration (0.60 g, 67%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm

1.31 (t, $J = 7.1$ Hz, 3H), 3.07 (s, 4H), 4.27 (s, 3H), 4.31 (q, $J = 7.1$ Hz, 2H), 8.84 (s, 1H); LCMS (ESI) m/z 407 (M + H)⁺.

1-Methyl-8-[[4-(methylsulfonyl)benzyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (18). A solution of **8** (1.5 g, 3.69 mmol) and 4-methylsulfonyl benzylamine hydrochloride (1.6 g, 7.38 mmol) in dry 1,4-dioxane (100 mL) was stirred at room temperature for 6 h, then the solvent was removed in vacuo. The residue was taken up with dichloromethane and washed with aqueous NaHCO₃. The organic layer was then dried over Na₂SO₄ and evaporated. The crude was triturated with diethyl ether and filtered, giving ethyl 1-methyl-8-[[4-(methylsulfonyl)benzyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (2.3 g, 70%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.29 (t, $J = 7.1$ Hz, 3H), 2.75 (t, $J = 7.6$ Hz, 2H), 2.91 (t, $J = 7.6$ Hz, 2H), 3.16 (s, 3H), 4.08 (s, 3H), 4.27 (q, $J = 7.1$ Hz, 2H), 4.62 (d, $J = 6.2$ Hz, 2H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.86 (m, 3H), 8.24 (s, 1H); LCMS (ESI) m/z 442 (M + H)⁺. HRMS (ESI) calcd for C₂₁H₂₃N₅O₄S + H⁺, 442.1543; found, 442.1543. A suspension of the above-described ethyl carboxylate (2.0 g, 4.54 mmol) in dry ethanol (40 mL) was treated with a 1.5 M solution of KOH in ethanol (9.10 mL, 13.62 mmol) at reflux temperature for 2 h. After cooling in an ice bath, the resulting precipitate was collected by filtration to give potassium 1-methyl-8-[[4-(methylsulfonyl)benzyl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (1.54 g, 75%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.63 (t, $J = 7.6$ Hz, 2H), 2.87 (t, $J = 7.6$ Hz, 2H), 3.16 (s, 3H), 3.97 (s, 3H), 4.60 (d, $J = 6.1$ Hz, 2H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.67 (t, $J = 6.3$ Hz, 1H), 7.85 (d, $J = 8.4$ Hz, 2H), 8.13 (s, 1H); LCMS (ESI) m/z 414 (M + H)⁺. HRMS (ESI): calcd for C₁₉H₁₉N₅O₄S + H⁺, 414.1231; found, 414.1234. A solution of the above-described carboxylic acid potassium salt (298 mg, 0.66 mmol) was dissolved in a mixture of dry DMF (5 mL) and dry THF (5 mL). After cooling to 0 °C, *N,N*-diisopropyl-*N'*-ethylamine (DIPEA; 0.46 mL, 2.64 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC; 380 mg, 1.98 mmol) were added in sequence to the solution. The reaction mixture was maintained at the same temperature for 30 min and then *N*-hydroxybenzotriazole ammonium salt (HOBT·NH₃; 300 mg, 1.98 mmol) was added under stirring. The mixture was warmed to room temperature and kept at this temperature overnight. The solvent was then evaporated and the residue taken up with dichloromethane and washed with aqueous NaHCO₃. The organic layer was dried over Na₂SO₄ and the solvent removed in vacuo. After trituration with diethyl ether, **18** (167 mg, 61%) was collected by filtration. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.72 (m, 2H), 2.92 (m, 2H), 3.16 (s, 3H), 4.07 (bs, 3H), 4.62 (d, $J = 6.2$ Hz, 2H), 7.21 (bs, 1H), 7.39 (bs, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.79–7.84 (m, 1H), 7.86 (d, $J = 8.5$ Hz, 2H), 8.22 (s, 1H); LCMS (ESI) m/z 413 (M + H)⁺; HPLC purity 90%.

8-(Cyclopentylamino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (29). By employment of the above-described procedure, starting from **8**, and using cyclopentylamine, compound **29** was prepared. Yield, 55%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.47–1.78 (m, 6H), 1.86–1.99 (m, 2H), 2.71 (m, 2H), 2.93 (t, $J = 7.6$ Hz, 2H), 4.08–4.20 (m, 1H), 4.31 (s, 3H), 7.06 (d, $J = 6.8$ Hz, 1H), 7.22 (s, 1H), 7.41 (s, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 313 (M + H)⁺; HRMS (ESI) calcd for C₁₆H₂₀N₆O + H⁺, 313.1772; found, 313.1774.

8-(Cyclohexylamino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (27). A solution of **8** (1.5 g, 3.69 mmol) and cyclohexylamine (0.84 mL, 7.33 mmol) in dry 1,4-dioxane (100 mL) was stirred at room temperature for 6 h, and then the solvent was removed in vacuo. The residue was taken up with dichloromethane and washed with aqueous NaHCO₃. The organic layer was then dried over Na₂SO₄ and evaporated. The crude was triturated with diethyl ether and filtered, giving ethyl 8-(cyclohexylamino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (1.07 g, 82%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.06–1.35 (m, 5H), 1.30 (t, $J = 7.1$ Hz, 3H), 1.53–1.64 (m, 1H), 1.68–1.78 (m, 2H), 1.85–2.01 (m, 2H), 2.74

(t, $J = 7.6$ Hz, 2H), 2.92 (t, $J = 7.6$ Hz, 2H), 3.60–3.75 (m, 1H), 4.28 (q, $J = 7.1$ Hz, 2H), 4.32 (s, 3H), 6.96 (d, $J = 5.4$ Hz, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 356 (M + H)⁺; HRMS (ESI) calcd for C₁₉H₂₅N₅O₂ + H⁺, 356.2081; found, 356.2081. A solution of the above-described ethyl carboxylate (250 mg, 0.70 mmol) in a mixture of methanol (10 mL), DMF (10 mL), and 33% NH₄OH (5 mL) was stirred at 70 °C in a closed bottle for 8 h. The solvent was removed under reduced pressure and the residue dissolved with dichloromethane and washed with water. The organic layer was dried over Na₂SO₄ and evaporated. The crude was purified by flash chromatography on a silica gel column (dichloromethane/methanol) affording **27** (137 mg, 60%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.05–1.42 (m, 5H), 1.54–1.99 (m, 5H), 2.71 (t, $J = 7.6$ Hz, 2H), 2.93 (t, $J = 7.6$ Hz, 2H), 3.61–3.78 (m, 1H), 4.29 (s, 3H), 6.93 (d, $J = 6.9$ Hz, 1H), 7.21 (s, 1H), 7.42 (s, 1H), 8.18 (s, 1H); LCMS (ESI) m/z 327 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₂₂N₆O + H⁺, 327.1928; found, 327.1928; Anal. (C₁₇H₂₂N₆O) C, H, N.

1-Methyl-8-[(1-methylpiperidin-4-yl)amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (48). By employment of the above-described procedure, starting from **8**, and using 1-methylpiperidin-4-yl-amine, compound **48** was prepared. Yield, 50%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.44–1.64 (m, 2H), 1.89 (m, 2H), 1.96–2.08 (m, 2H), 2.20 (s, 3H), 2.71 (t, $J = 7.6$ Hz, 2H), 2.79 (m, 2H), 2.93 (t, $J = 7.6$ Hz, 2H), 3.58–3.73 (m, 1H), 4.29 (s, 3H), 6.99 (d, $J = 6.3$ Hz, 1H), 7.21 (s, 1H), 7.42 (s, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 342 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₂₃N₇O₄ + H⁺, 342.2037; found, 342.2024.

8-(Cyclohexylamino)-*N*-1-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (47). A solution of ethyl 8-(cyclohexylamino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (200 mg, 0.56 mmol), prepared as described for the synthesis of compound **27** in a mixture of ethanol (30 mL) and 33 wt % methylamine (10 mL) in absolute ethanol, was stirred at 60 °C in a closed bottle for 8 h. The solvent was then removed in vacuo and the crude purified by flash chromatography on a silica gel column (dichloromethane/methanol/triethylamine 95/5/5) affording **47** (149 mg, 78%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.24 (m, 4H), 1.61 (m, 2H), 1.75 (m, 2H), 1.94 (m, 2H), 2.70–2.74 (m, 2H), 2.75 (d, $J = 4.7$ Hz, 3H), 2.95 (m, 2H), 3.69 (m, 1H), 4.31 (s, 3H), 6.94 (d, $J = 5.6$ Hz, 1H), 8.06 (q, $J = 4.7$ Hz, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 341 (M + H)⁺; HRMS (ESI) calcd for C₁₈H₂₄N₆O + H⁺, 341.2084; found, 341.2089.

By employment of the above-described procedure, starting from **8**, and using the suitable amine, compounds **30** and **49** were prepared.

8-(Cyclopentylamino)-*N*-1-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (30). Yield, 51%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.43–1.79 (m, 6H), 1.87–2.03 (m, 2H), 2.74 (d, $J = 4.7$ Hz, 3H), 2.77 (t, $J = 7.6$ Hz, 2H), 2.98 (t, $J = 7.6$ Hz, 2H), 4.19 (s, 1H), 4.30 (s, 3H), 7.67–7.90 (m, 1H), 8.10 (q, $J = 4.7$ Hz, 1H), 8.22 (s, 1H); LCMS (ESI) m/z 327 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₂₂N₆O + H⁺, 327.1928; found, 327.1933; HPLC purity 95%.

***N*-1-Dimethyl-8-[(1-methylpiperidin-4-yl)amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (49).** Yield, 50%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.46–1.60 (m, 2H), 1.88 (m, 2H), 1.98–2.07 (m, 2H), 2.20 (s, 3H), 2.68–2.75 (m, 2H), 2.73 (d, $J = 4.7$ Hz, 3H), 2.76–2.82 (m, 2H), 2.93 (t, $J = 7.6$ Hz, 2H), 3.61–3.73 (m, 1H), 4.29 (s, 3H), 6.99 (d, $J = 7.6$ Hz, 1H), 8.04 (q, $J = 4.7$ Hz, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 356 (M + H)⁺; HRMS (ESI) calcd for C₁₈H₂₅N₇O + H⁺, 356.2193; found, 356.2176; HPLC purity 95%.

Ethyl 8-Amino-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (9). To a solution of **2** (16.0 g, 0.06 mol) in ethanol (600 mL), sodium ethylate (3.90 g, 0.06 mol) and guanidine hydrochloride (5.44 g, 0.06 mol) were added in sequence. The mixture was stirred under reflux for 12 h. The solvent was then evaporated, the residue taken up with dichloromethane

and washed with water. The organic layer was then dried over Na_2SO_4 and concentrated. The residue was triturated with diethyl ether and **9** was collected by filtration (13.9 g, 85%) as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.30 (t, $J = 7.1$ Hz, 3H), 2.74 (m, 2H), 2.92 (m, 2H), 4.28 (q, $J = 7.1$ Hz, 2H), 4.32 (s, 3H), 6.56 (s, 2H), 8.18 (s, 1H); LCMS (ESI) m/z 274 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{15}\text{N}_5\text{O}_2 + \text{H}^+$, 274.1298; found, 274.1299.

8-Amino-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (10a). A suspension of **9** (300 mg, 1.1 mmol) in a mixture of 33% NH_4OH (20 mL), methanol (20 mL), and DMF (10 mL) was stirred at 65 °C for 16 h in a closed bottle. The solvent was evaporated in vacuo, and the residue was taken up with dichloromethane and washed with aqueous NaHCO_3 . The organic phase was dried over Na_2SO_4 and evaporated, giving **10a** (135 mg, 50%) after trituration with diethyl ether. ^1H NMR (401 MHz, $\text{DMSO}-d_6$) δ ppm 2.71 (t, $J = 7.6$ Hz, 2H), 2.92 (m, 2H), 4.29 (s, 3H), 6.52 (s, 2H), 7.21 (bs, 1H), 7.41 (bs, 1H), 8.16 (s, 1H); LCMS (ESI) m/z 245 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{12}\text{N}_6\text{O} + \text{H}^+$, 245.1146; found, 245.1143.

8-[(4-Methoxybenzyl)amino]-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (15). To a solution of **10a** (244 mg, 1.0 mmol) in a mixture of glacial acetic acid/methanol/water 1/1/1 (30 mL) in a round-bottom flask, *p*-methoxybenzaldehyde (0.88 mL, 6.0 mmol) and then 85% sodium cyanoborohydride (420 mg, 4.0 mmol) were added. The solution was stirred at room temperature overnight. The reaction mixture was then poured into ice-water (200 mL), the pH was adjusted to 10 by addition of saturated aqueous Na_2CO_3 , and the solution was extracted with ethyl acetate. The collected organic extracts were washed with brine until neutral, washed with water, and dried over Na_2SO_4 . Evaporation of the solvent under reduced pressure left a yellow solid residue that was purified by flash chromatography on silica gel (dichloromethane/methanol, 95/5) to yield a yellow compound. Crystallization from methanol afforded crystalline **15** (225 mg, 62%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.71 (m, 2H), 2.92 (m, 2H), 3.71 (s, 3H), 4.20 (bs, 3H), 4.45 (s, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 7.21 (bs, 1H), 7.25 (d, $J = 8.8$ Hz, 2H), 7.40 (bs, 1H), 7.62 (bs, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 365 ($\text{M} + \text{H}^+$).

By employment of the above-described procedure, starting from **10a** and using the suitable aldehyde, compounds **16** and **17** were prepared.

8-[(4-Bromobenzyl)amino]-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (16). Yield, 60%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.68–3.00 (m, 4H), 4.15 (s, 3H), 4.49 (d, $J = 6.3$ Hz, 2H), 7.21 (s, 1H), 7.29 (d, $J = 8.5$ Hz, 2H), 7.40 (s, 1H), 7.49 (d, $J = 8.5$ Hz, 2H), 7.65–7.77 (m, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 413 ($\text{M} + \text{H}^+$).

8-[(4-Acetylamino)benzyl]amino]-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (17). Yield, 58%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.00 (s, 3H), 2.71 (t, $J = 7.7$ Hz, 2H), 2.92 (t, $J = 7.7$ Hz, 2H), 4.18 (s, 1H), 4.46 (d, $J = 6.2$ Hz, 1H), 7.21 (s, 1H), 7.24 (d, $J = 8.5$ Hz, 2H), 7.40 (s, 1H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.56–7.68 (m, 1H), 8.21 (s, 1H), 9.80 (none, 1H); LCMS (ESI) m/z 392 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{21}\text{N}_7\text{O}_2 + \text{H}^+$, 392.183; found, 392.1835; Anal. ($\text{C}_{20}\text{H}_{21}\text{N}_7\text{O}_2$) C, H, N.

8-Amino-*N*-1-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (10b). A solution of **9** (200 mg, 0.73 mmol) in a mixture of ethanol (10 mL) and 33 wt % methylamine in absolute ethanol (30 mL) was stirred at 60 °C in a closed bottle for 8 h. The solvent was then removed in vacuo and the crude was purified by flash chromatography on a silica gel column (dichloromethane/methanol/triethylamine, 95/5/5) affording **10b** (147 mg, 78%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 2.68–2.73 (m, 2H), 2.73 (d, $J = 4.8$ Hz, 3H), 2.93 (t, $J = 7.6$ Hz, 2H), 4.29 (s, 3H), 6.52 (s, 2H), 7.97–8.06 (m, 1H), 8.16 (s, 1H); LCMS (ESI) m/z 259 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{14}\text{N}_6\text{O} + \text{H}^+$, 259.1302; found, 259.1300.

8-[(1-Acetylpiperidin-4-yl)amino]-*N*,1-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (51). To a suspension of

10b (490 mg, 1.9 mmol) in dry DMF (12 mL), 1-acetyl-4-piperidone (470 μL , 3.8 mmol), trifluoroacetic acid (1 mL, 12.8 mmol), and sodium triacetoxyborohydride (886 mg, 4.2 mmol) were added. After 18 h, 0.33 N NaOH (80 mL, 26.4 mmol) was added dropwise to the mixture. The precipitate was filtered, washed with water, and dried in an oven to dryness to give **51** (509 mg, 70%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.84 (m, 4H), 2.03 (s, 3H), 2.76 (d, $J = 4.8$, 3H), 2.84 (m, 2H), 3.17 (m, 2H), 4.21 (m, 1H), 4.24 (s, 3H), 7.28 (s, 1H), 8.19 (s, 1H), 9.10 (q, $J = 4.8$ Hz, 1H); LCMS (ESI) m/z 384 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{25}\text{N}_7\text{O}_2 + \text{H}^+$, 384.2142; found, 384.2145.

By employment of the above-described procedure, starting from **10b** and using the suitable cycloalkylketone, compounds **53**, **55**, **59**, and **61** were prepared.

***N*-1-Dimethyl-8-[[1-(phenylcarbonyl)piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (53).** Yield, 70%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.50 (s, 2H), 1.97 (s, 2H), 2.73 (d, $J = 4.7$ Hz, 3H), 2.76 (m, 2H), 2.96 (t, $J = 7.7$ Hz, 2H), 3.05–3.77 (m, 3H), 4.03 (m, 1H), 4.29 (s, 3H), 4.39 (m, 1H), 7.35–7.41 (m, 2H), 7.43–7.47 (m, 3H), 7.58 (s, 1H), 8.08 (q, $J = 4.7$ Hz, 1H), 8.24 (s, 1H); LCMS (ESI) m/z 891 ($2\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{27}\text{N}_7\text{O}_2 + \text{H}^+$, 891.4525; found, 891.4491.

***N*-1-Dimethyl-8-[[1-[(4-methylpiperazin-1-yl)carbonyl]piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (55).** Yield, 50%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.41–1.50 (m, 2H), 1.90 (m, 2H), 2.19 (s, 3H), 2.31 (s, 4H), 2.69–2.75 (m, 2H), 2.73 (d, $J = 4.7$ Hz, 3H), 2.80–2.89 (m, 2H), 2.94 (t, $J = 7.6$ Hz, 2H), 3.10–3.17 (m, 4H), 3.58 (dt, $J = 13.3$, 3.3 Hz, 2H), 3.78–3.93 (m, 1H), 4.29 (s, 3H), 7.07 (s, 1H), 8.04 (q, $J = 4.7$ Hz, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 468 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{33}\text{N}_9\text{O}_2 + \text{H}^+$, 468.2830; found, 468.2831.

Ethyl 4-[[1-Methyl-3-(methylcarbamoyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazolin-8-yl]amino]piperidine-1-carboxylate (57). Yield, 60%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.18 (t, $J = 7.1$ Hz, 3H), 1.32–1.49 (m, 2H), 1.90 (m, 2H), 2.70–2.75 (m, 1H), 2.73 (d, $J = 4.7$ Hz, 1H), 2.94 (t, $J = 7.7$ Hz, 1H), 2.94 (t, $J = 7.7$ Hz, 1H), 3.84–3.93 (m, 1H), 3.92–3.98 (m, 1H), 4.04 (q, $J = 7.1$ Hz, 2H), 4.29 (s, 3H), 7.08 (d, $J = 5.9$ Hz, 1H), 8.04 (q, $J = 4.7$ Hz, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 414 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{27}\text{N}_7\text{O}_3 + \text{H}^+$, 414.2248; found, 414.2263.

***N*-1-Dimethyl-8-[[1-(methylsulfonyl)piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (59).** Yield, 72%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.59 (m, 2H), 2.02 (m, 2H), 2.73 (m, 2H), 2.75 (d, $J = 4.7$ Hz, 3H), 2.86 (m, 2H), 2.88 (s, 3H), 2.96 (t, $J = 7.6$ Hz, 2H), 3.56 (m, 2H), 3.87 (m, 1H), 4.29 (s, 3H), 7.43 (m, 1H), 8.08 (m, 1H), 8.23 (s, 1H); LCMS (ESI) m/z 420 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{25}\text{N}_7\text{O}_3\text{S} + \text{H}^+$, 420.1812; found, 420.1814; Anal. ($\text{C}_{18}\text{H}_{25}\text{N}_7\text{O}_3\text{S}$) C, H, N, S.

***N*-1-Dimethyl-8-[[1-(phenylsulfonyl)piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (61).** Yield, 68%; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.59 (m, 2H), 1.98 (m, 2H), 2.56 (m, 2H), 2.74 (m, 2H), 2.72 (d, $J = 4.8$ Hz, 3H), 2.94 (t, $J = 7.6$ Hz, 2H), 3.58 (m, 2H), 3.74 (m, 1H), 4.22 (s, 3H), 7.53 (s, 1H), 7.66 (m, 2H), 7.74 (m, 1H), 7.74–7.76 (m, 2H), 8.06 (q, $J = 4.8$ Hz, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 482 ($\text{M} + \text{H}^+$); HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{27}\text{N}_7\text{O}_3\text{S} + \text{H}^+$, 482.1969; found, 482.1959.

1-Methyl-8-[[1-[(4-methylpiperazin-1-yl)carbonyl]piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (54). To a suspension of **9** (355 mg, 1.30 mmol) in dry DMF (10 mL), 1-[(4-methylpiperazin-1-yl)carbonyl]piperidin-4-one dihydrochloride (562 mg, 2.50 mmol, prepared as described in ref 17, trifluoroacetic acid (1.3 mL, 16.64 mmol), and sodium triacetoxyborohydride (528 mg, 2.50 mmol) were added. After 18 h, the mixture was poured into a solution of potassium carbonate (500 mg in 100 mL of water) and extracted with dichloromethane. The organic layer was concentrated and the residue purified by flash chromatography on a silica gel column (dichloromethane/ethanol 9/1) affording ethyl 1-methyl-8-[[1-[(4-methylpiperazin-

1-yl)carbonyl]piperidin-4-yl]amino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (315 mg, 50%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 1.45 (m, 2H), 1.89 (m, 2H), 2.18 (s, 3H), 2.37 (m, 4H), 2.75 (t, *J* = 7.7 Hz, 2H), 2.85 (m, 2H), 2.93 (t, *J* = 7.6 Hz, 2H), 3.13 (m, 4H), 3.57 (m, 2H), 3.87 (m, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.31 (s, 3H), 7.11 (s, 1H), 8.22 (s, 1H); LCMS (ESI) *m/z* 483 (M + H)⁺; HRMS (ESI) calcd for C₂₄H₃₄N₈O₃ + H⁺, 483.2827; found, 483.2820. A suspension of the above-described ethyl carboxylate (300 mg, 0.62 mmol) in a mixture of 33% NH₄OH (10 mL), methanol (5 mL), and DMF (5 mL) was stirred at 65 °C in a closed bottle for 16 h. After that time, the solvent was removed and the residue was partitioned between dichloromethane and aqueous NaHCO₃. The organic layer was then dried over Na₂SO₄ and evaporated affording **54** (170 mg, 61%), after purification by flash chromatography (dichloromethane/ethanol/33% NH₄OH, 90/9/1). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.87 (m, 4H), 2.44 (s, 3H), 2.49 (m, 4H), 2.86 (m, 2H), 3.13 (m, 2H), 3.39 (m, 2H), 3.50 (m, 2H), 3.80 (m, 2H), 4.05 (m, 1H), 4.24 (s, 3H), 7.28 (s, 1H), 7.27 (bs, 1H), 7.45 (bs, 1H), 8.18 (s, 1H); LCMS (ESI) *m/z* 454 (M + H)⁺; HRMS (ESI) calcd for C₂₂H₃₁N₉O₂ + H⁺, 454.2673; found, 454.2687.

1-Methyl-8-[[1-(methylsulfonyl)piperidin-4-yl]amino]-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (58). By employment of the above-described procedure, compound **58** was prepared. Yield, 52%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.50–1.66 (m, 2H), 2.02 (m, 2H), 2.74 (t, *J* = 7.7 Hz, 2H), 2.84–2.92 (m, 2H), 2.88 (s, 3H), 2.95 (t, *J* = 7.6 Hz, 2H), 3.52–3.61 (m, 2H), 3.80–3.92 (m, 1H), 4.29 (s, 3H), 7.24 (s, 1H), 7.34 (s, 1H), 7.44 (s, 1H), 8.23 (s, 1H); LCMS (ESI) *m/z* 406 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₂₃N₇O₃S + H⁺, 406.1656; found, 406.1653.

8-(Cyclopentylamino)-*N*-[1-(dimethylamino)propan-2-yl]-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (31). A suspension of **9** (546 mg, 2 mmol) in dry DMF (10 mL) was treated with cyclopentanone (212 μL, 2.4 mmol), trifluoroacetic acid (1 mL, 12.8 mmol), and sodium triacetoxyborohydride (632 mg, 3 mmol). After 16 h, 0.5 N NaOH (60 mL, 30 mmol) was added dropwise to the mixture. The precipitate was filtered, washed with water, and dried. The residue was then purified by flash chromatography on a silica gel column (dichloromethane/methanol, 99/1) affording ethyl 8-(cyclopentylamino)-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (500 mg, 73%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 1.44–1.61 (m, 4H), 1.63–1.73 (m, 2H), 1.92 (m, 2H), 2.75 (m, 2H), 2.92 (m, 2H), 4.15 (m, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.33 (s, 3H), 7.08 (d, *J* = 5.6 Hz, 1H), 8.20 (s, 1H); LCMS (ESI) *m/z* 342 (M + H)⁺; HRMS (ESI) calcd for C₁₈H₂₃N₅O₂ + H⁺, 342.1924; found, 342.1924. A suspension of the above-described ethyl carboxylate (230 mg, 0.67 mmol) was suspended in dry ethanol (5 mL) and treated with a 1.5 M solution of KOH in ethanol (1.33 mL, 2.01 mmol) at reflux temperature for 1.5 h. After cooling in an ice bath, the resulting precipitate was collected by filtration to give potassium 8-(cyclopentylamino)-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (210 mg, 89%) as a crystalline solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.39–1.76 (m, 6H), 1.80–1.99 (m, 2H), 2.63 (t, *J* = 7.7 Hz, 2H), 2.88 (t, *J* = 7.7 Hz, 2H), 4.09–4.17 (m, 1H), 4.18 (s, 3H), 6.87 (d, *J* = 6.8 Hz, 1H), 8.10 (s, 1H); LCMS (ESI) *m/z* 314 (M + H)⁺; HRMS (ESI) calcd for C₁₆H₁₉N₅O₂ + H⁺, 314.1611; found, 314.1615. To a suspension of the above-described carboxylic acid potassium salt (210 mg, 0.58 mmol) in DMF (4 mL), 1-hydroxybenzotriazole (HOBt, 101 mg, 0.75 mmol) and EDC (144 mg, 0.75 mmol) were added in sequence. The mixture was stirred at room temperature for 30 min, then *N*¹,*N*¹-dimethylpropane-1,2-diamine (130 μL, 1 mmol) was added. After 20 h the reaction mixture was poured into water and extracted with dichloromethane. The organic phase was dried over Na₂SO₄ and concentrated. The crude was then purified by flash chromatography on a silica gel column (dichloromethane/ethanol/33% NH₄OH, 94/5.5/0.5). The residue was tritu-

rated with diethyl ether and **31** was collected by filtration (81 mg, 35%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.10 (d, *J* = 6.6 Hz, 3H), 1.60 (m, 4H), 1.84 (m, 2H), 2.07 (m, 2H), 2.27 (s, 6H), 2.30 (m, 2H), 2.85 (m, 2H), 3.11 (m, 2H), 4.12 (m, 1H), 4.24 (s, 3H), 4.57 (quin, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 8.02 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H); LCMS (ESI) *m/z* 398 (M + H)⁺; HRMS (ESI) calcd for C₂₁H₃₁N₇O + H⁺, 398.2663; found, 398.2671.

By employment of the above-described procedure, starting from **9** and using the suitable amine, compounds **32** and **33** were prepared.

8-(Cyclopentylamino)-*N*-[2-(dimethylamino)ethyl]-*N*-1-dimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (32). Yield, 45%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.64 (m, 4H), 1.84 (m, 4H), 2.03 (m, 2H), 2.27 (s, 6H), 2.41 (t, *J* = 6.5 Hz, 2H), 2.87 (m, 2H), 2.92 (s, 3H), 2.98 (m, 2H), 3.70 (t, *J* = 6.5 Hz, 2H), 4.23 (s, 3H), 4.57 (quin, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 8.18 (s, 1H); LCMS (ESI) *m/z* 398 (M + H)⁺; HRMS (ESI) calcd for C₂₁H₃₁N₇O + H⁺, 398.2663; found, 398.2676.

[8-(Cyclopentylamino)-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazolin-3-yl](4-methylpiperazin-1-yl)methanone (33). Yield, 51%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.64 (m, 4H), 1.86 (m, 2H), 2.08 (m, 2H), 2.47 (s, 3H), 2.55 (m, 4H), 2.89 (m, 2H), 3.03 (m, 2H), 3.64 (m, 2H), 3.71 (m, 2H), 4.22 (s, 3H), 4.57 (quin, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 8.20 (s, 1H); LCMS (ESI) *m/z* 396 (M + H)⁺; HRMS (ESI) calcd for C₂₁H₂₉N₇O + H⁺, 396.2506; found, 396.2518.

8-[(1-Acetylpiperidin-4-yl)amino]-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (50). To a suspension of **9** (5.187 g, 19 mmol) in dry DMF (120 mL), 1-acetyl-4-piperidone (4.7 mL, 38 mmol), trifluoroacetic acid (10 mL, 128 mmol), and sodium triacetoxyborohydride (8.862 g, 42 mmol) were added. After 18 h, 0.33 N NaOH (800 mL, 264 mmol) was added dropwise to the mixture. The precipitate was filtered, washed with water, and dried in oven to dryness to give of ethyl 8-[(1-acetylpiperidin-4-yl)amino]-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (5.3 g, 70%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 1.43 (m, 2H), 1.94 (m, 2H), 2.01 (s, 3H), 2.76 (m, 3H), 2.93 (t, *J* = 7.8 Hz, 2H), 3.15 (m, 1H), 3.33 (m, 1H), 3.81 (m, 1H), 3.94 (m, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.32 (s, 3H), 7.13 (d, *J* = 7.2 Hz, 1H), 8.23 (s, 1H); LCMS (ESI) *m/z* 399 (M + H)⁺; HRMS (ESI) calcd for C₂₀H₂₆N₆O₃ + H⁺, 399.2139; found, 399.2140. A suspension of the above-described ethyl carboxylate (1.0 g, 2.50 mmol) in anhydrous ethanol (20 mL) was treated with a 1.5 M solution of KOH in ethanol (5 mL, 7.5 mmol) at reflux temperature for 1.5 h. After cooling in ice bath, the resulting precipitate was collected by filtration to give potassium 8-[(1-acetylpiperidin-4-yl)amino]-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (836 mg, 82%) as a crystalline solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.38 (m, 2H), 1.79–1.99 (m, 2H), 2.00 (s, 3H), 2.64 (m, 2H), 2.74 (td, *J* = 12.78, 3.1 Hz, 1H), 2.89 (m, 2H), 2.96 (m, 1H), 3.14 (m, 2H), 3.81 (m, 1H), 3.86–3.99 (m, 1H), 4.17 (s, 3H), 4.23–4.32 (m, 1H), 6.93 (d, *J* = 7.3 Hz, 1H), 8.12 (s, 1H); LCMS (ESI) *m/z* 371 (M + H)⁺; HRMS (ESI) calcd for C₁₈H₂₂N₆O₃ + H⁺, 371.1826; found, 371.1825. A suspension of the above-described carboxylic acid potassium salt (500 mg, 1.23 mmol) in a mixture of dry DMF (10 mL) and dry THF (10 mL) was cooled to 0 °C, then DIPEA (0.86 mL, 4.92 mmol) and EDC (708 mg, 3.69 mmol) were added in sequence. After stirring at the same temperature for 30 min, HOBt·NH₃ (560 mg, 3.69 mmol) was added. The mixture was then maintained at room temperature overnight and then the solvent was removed under reduced pressure. The residue was taken up with dichloromethane and washed with aqueous NaHCO₃. The organic layer was finally dried over Na₂SO₄ and the solvent was evaporated. After trituration with diethyl ether, **50** (272 mg, 60%) was collected by filtration. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.16–1.52 (m, 3H), 1.83–1.99 (m, 2H), 2.01 (s, 3H), 2.72 (m, 2H), 2.93 (m, 2H), 3.16 (m, 2H), 3.81 (m, 2H), 3.87–4.02 (m, 2H), 4.27 (bs, 2H), 4.29 (s, 3H), 7.10 (bs, 1H), 7.22 (bs, 1H), 7.42 (bs, 1H),

8.21 (s, 1H); LCMS (ESI) m/z 370 (M + H)⁺; HRMS (ESI) calcd for C₁₈H₂₃N₇O₂ + H⁺, 370.1986; found, 370.1982.

By employment of the above-described procedure, compounds **52**, **56**, and **60** were prepared.

1-Methyl-8-[[1-(phenylcarbonyl)piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (52). Yield, 45%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.91 (m, 4H), 2.86 (m, 4H), 3.08 (m, 2H), 3.61 (m, 4H), 4.02 (m, 1H), 4.24 (s, 3H), 7.26 (bs, 1H), 7.28 (bs, 1H), 7.47 (bs, 1H), 7.55 (m, 3H), 7.78 (m, 2H), 8.20 (s, 1H); LCMS (ESI) m/z 432 (M + H)⁺; HRMS (ESI) calcd for C₂₃H₂₅N₇O₂ + H⁺, 432.2142; found, 432.2135.

Ethyl 4-[[3-(Carbamoyl-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazolin-8-yl)amino]piperidine-1-carboxylate (56). Yield, 50%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.18 (t, *J* = 7.1 Hz, 3H), 1.39 (m, 2H), 1.91 (m, 2H), 2.69–2.76 (m, 2H), 2.94 (m, 4H), 3.88 (m, 1H), 3.91–3.98 (m, 2H), 4.04 (q, *J* = 7.1 Hz, 2H), 4.28 (s, 3H), 7.07 (bs, 1H), 7.22 (bs, 1H), 7.42 (bs, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 400 (M + H)⁺; HRMS (ESI) calcd for C₁₉H₂₅N₇O₃ + H⁺, 400.2092; found, 400.2095; Anal. (C₁₉H₂₅N₇O₃) C, H, N.

1-Methyl-8-[[1-(phenylsulfonyl)piperidin-4-yl]amino]-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (60). Yield, 43%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.60 (m, 2H), 1.99 (m, 2H), 2.55 (m, 2H), 2.71 (t, *J* = 7.6 Hz, 2H), 2.93 (m, 2H), 3.59 (m, 2H), 3.72 (m, 1H), 4.24 (s, 3H), 7.10 (d, *J* = 7.3 Hz, 1H), 7.23 (s, 1H), 7.41 (s, 1H), 7.62 (m, 2H), 7.75 (m, 1H), 7.77 (m, 2H), 8.19 (s, 1H); LCMS (ESI) m/z 468 (M + H)⁺; HRMS (ESI) calcd for C₂₂H₂₅N₇O₃S + H⁺, 468.1813; found, 468.1808.

8-(Cyclopentylamino)-*N*-hydroxy-*N*,1-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (34). To a suspension of potassium 8-(cyclopentylamino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (300 mg, 0.85 mmol, prepared as described for the preparation of compound **31**) in dry dichloromethane (60 mL) and a few drops of dry DMF, oxalyl chloride (85 μL, 1 mmol) was added at 0 °C. The mixture was stirred at room temperature for 6 h and then evaporated, dissolved in dry dichloromethane, and dropped into a solution of methylhydroxylamine hydrochloride (141 mg, 1.70 mmol) and triethylamine (490 μL, 3.40 mmol) in the same solvent (20 mL), cooled to 0 °C. After 4 h the mixture was washed with a saturated solution of NaHCO₃, dried over Na₂SO₄, and evaporated to dryness. The residue was triturated with diethyl ether and filtered to give **34** (175 mg, 60%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.53 (m, 4H), 1.66 (m, 2H), 1.93 (m, 2H), 2.65–2.87 (m, 4H), 3.33 (s, 3H), 4.15 (m, 1H), 4.29 (s, 3H), 7.04 (d, *J* = 6.6 Hz, 1H), 8.19 (s, 1H), 9.87 (s, 1H); LCMS (ESI) m/z 343 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₂₂N₆O₂ + H⁺, 343.1877; found, 343.1884; Anal. (C₁₇H₂₂N₆O₂) C, H, N.

By employment of the above-mentioned procedure and using the suitable substituted hydroxylamine, compounds **35** and **36** were prepared.

***N*-Cyclohexyl-8-(cyclopentylamino)-*N*-hydroxy-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (35).** Yield, 55%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.16–1.36 (m, 2H), 1.46–1.80 (m, 12H), 1.84–1.99 (m, 2H), 2.65–2.79 (m, 4H), 4.16 (m, 1H), 4.29 (s, 3H), 4.33 (m, 1H), 7.04 (d, *J* = 6.8 Hz, 1H), 8.18 (s, 1H), 9.44 (s, 1H); LCMS (ESI) m/z 411 (M + H)⁺; Anal. (C₂₂H₃₀N₆O₂) C, H, N.

***N*-Benzyl-8-(cyclopentylamino)-*N*-hydroxy-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (36).** Yield, 62%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.52 (m, 4H), 1.69 (m, 2H), 1.89 (m, 2H), 2.76 (m, 4H), 4.16 (m, 1H), 4.29 (s, 3H), 4.99 (s, 2H), 7.05 (d, *J* = 6.6 Hz, 1H), 7.20–7.41 (m, 5H), 8.19 (s, 1H), 9.87 (s, 1H); LCMS (ESI) m/z 419 (M + H)⁺; HRMS (ESI) calcd for C₂₃H₂₆N₆O₂ + H⁺, 419.2190; found, 419.2201.

Ethyl 1-Benzyl-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12a). Ethyl (3-ethoxy-2-oxocyclohex-3-en-1-yl)(oxo)acetate **11** (3.0 g, 12.5 mmol, prepared as described in ref 17) was dissolved in glacial acetic acid (15 mL) and benzylhydrazine dihydrochloride (2.44 g, 12.5 mmol) was added. The mixture

was stirred at room temperature for 6 h. The solvent was evaporated and the crude dissolved with water, the solution made basic with 33% NH₄OH and extracted with dichloromethane. The organic layer was dried over Na₂SO₄ and concentrated. The residue was chromatographed on a silica gel column (*n*-hexane/ethylacetate, 7/3) and triturated with a mixture *n*-hexane/diethyl ether to give **12a** (2.34 g, 63%). ¹H NMR (401 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 2.06 (quin, *J* = 6.3 Hz, 1H), 2.56 (dd, *J* = 7.3, 5.7 Hz, 2H), 2.95 (t, *J* = 6.1 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 5.72 (s, 2H), 7.19 (dd, *J* = 7.8, 1.6 Hz, 2H); LCMS (ESI) m/z 299 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₁₈N₂O₃ + H⁺, 299.1390; found, 299.1404.

By employment of the above-described procedure, starting from **11** and using the suitable substituted hydrazine, compounds **12b–i** were prepared.

Ethyl 7-Oxo-1-phenyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12b). Yield, 80%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.32 (t, *J* = 7.1 Hz, 3H), 2.08–2.17 (m, 2H), 2.56 (dd, *J* = 7.4, 5.5 Hz, 2H), 3.03 (t, *J* = 6.1 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 7.51 (s, 5H); LCMS (ESI) m/z 285 (M + H)⁺; HRMS (ESI) calcd for C₁₆H₁₆N₂O₃ + H⁺, 285.1234; found, 285.1236.

Ethyl 1-(4-Methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12c). Yield, 72%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.32 (t, *J* = 7.1 Hz, 3H), 2.03–2.15 (m, 2H), 2.54 (dd, *J* = 7.3, 5.5 Hz, 2H), 3.01 (t, *J* = 6.1 Hz, 2H), 3.83 (s, 3H), 4.33 (q, *J* = 7.1 Hz, 2H), 7.03 (d, *J* = 9.0 Hz, 2H), 7.42 (d, *J* = 9.0 Hz, 2H); LCMS (ESI) m/z 315 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₁₈N₂O₄ + H⁺, 315.1339; found, 315.1345.

Ethyl 7-Oxo-1-(4-sulfamoylphenyl)-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12d). Yield, 70%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.32 (t, *J* = 7.1 Hz, 3H), 2.08–2.17 (m, 2H), 2.55–2.61 (m, 2H), 3.03 (t, *J* = 6.1 Hz, 2H), 4.35 (q, *J* = 7.1 Hz, 2H), 7.53 (s, 2H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H); LCMS (ESI) m/z 364 (M + H)⁺; HRMS (ESI) calcd for C₁₆H₁₇N₃O₅S + H⁺, 364.0962; found, 364.0961.

Ethyl 7-Oxo-1-(pyridin-2-yl)-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12e). Yield, 75%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.32 (t, *J* = 7.1 Hz, 3H), 2.08–2.19 (m, 2H), 2.57 (dd, *J* = 7.3, 5.6 Hz, 2H), 3.03 (t, *J* = 6.2 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 7.60 (ddd, *J* = 7.4, 4.7, 1.0 Hz, 1H), 7.62 (dt, *J* = 7.9, 0.9 Hz, 1H), 8.06 (td, *J* = 7.7, 1.8 Hz, 1H), 8.56 (ddd, *J* = 4.7, 1.8, 0.8 Hz, 1H); LCMS (ESI) m/z 286 (M + H)⁺.

Ethyl 1-(2-Hydroxyethyl)-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12f). Yield, 72%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 1.99–2.09 (m, 2H), 2.51–2.56 (m, 2H), 2.93 (t, *J* = 6.1 Hz, 2H), 3.72 (t, *J* = 5.8 Hz, 2H), 4.25–4.34 (m, 2H), 4.54 (t, *J* = 5.8 Hz, 2H), 4.85 (bs, 1H); LCMS (ESI) m/z 253 (M + H)⁺; HRMS (ESI) calcd for C₁₂H₁₆N₂O₄ + H⁺, 253.1183; found, 253.1187.

Ethyl 7-Oxo-1-(2,2,2-trifluoroethyl)-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12g). Yield, 68%; ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 1.31 (t, *J* = 7.2 Hz, 3H), 2.07 (dt, *J* = 12.5, 6.26 Hz, 2H), 2.59 (dd, *J* = 7.2, 5.9 Hz, 2H), 2.96 (t, *J* = 6.1 Hz, 2H), 4.32 (q, *J* = 7.2 Hz, 2H), 5.48 (q, *J* = 8.7 Hz, 2H); LCMS (ESI) m/z 291 (M + H)⁺.

Ethyl 1-(1-Methylpiperidin-4-yl)-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12h). Yield, 79%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 1.82–2.10 (m, 6H), 2.26 (s, 3H), 2.46–2.51 (m, 2H), 2.50–2.58 (m, 2H), 2.89–2.98 (m, 2H), 2.93 (t, *J* = 6.2 Hz, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 4.92–5.10 (m, 1H); LCMS (ESI) m/z 306 (M + H)⁺; HRMS (ESI) calcd for C₁₆H₂₃N₃O₃ + H⁺, 306.1812; found, 306.1801.

Ethyl 1-(1-Acetylpiperidin-4-yl)-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (12i). Yield, 76% yield; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 1.73 (m, 1H), 1.95 (m, 3H), 2.00–2.08 (m, 2H), 2.04 (s, 3H), 2.56 (dd, *J* = 7.2, 5.6 Hz, 2H), 2.64–2.76 (m, 1H), 4.29 (t, *J* = 6.1 Hz, 2H), 3.20 (m, 2H), 3.87–3.99 (m, 1H), 4.99 (q, *J* = 7.1 Hz, 2H), 4.48 (m, 1H), 5.27 (m, 1H); LCMS (ESI) m/z 334 (M + H)⁺;

HRMS (ESI) calcd for $C_{17}H_{23}N_3O_4 + H^+$, 334.1762; found, 334.1757.

Ethyl 8-Amino-1-benzyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13a). To a solution of **12a** (500 mg, 1.67 mmol) in dry DMF (12 mL), *N,N*-dimethylformamide di-*tert*-butylacetate (4 mL, 16.3 mmol) was added. The mixture was stirred at 60 °C for 8 h. The solvent was then evaporated and the residue triturated with diethyl ether to give ethyl (6*E*)-1-benzyl-6-[(dimethylamino)methylidene]-7-oxo-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxylate (501 mg, 85%). 1H NMR (401 MHz, DMSO- d_6) δ ppm 1.29 (t, $J = 7.1$ Hz, 3H), 2.80–2.87 (m, 2H), 2.87–2.96 (m, 2H), 3.12 (s, 6H), 4.26 (q, $J = 7.1$ Hz, 2H), 5.80 (s, 2H), 7.18–7.22 (m, 2H), 7.23–7.34 (m, 2H), 7.51 (s, 1H); LCMS (ESI) m/z 354 (M + H) $^+$; HRMS (ESI) calcd for $C_{20}H_{23}N_3O_3 + H^+$, 354.1812; found, 354.1804. To a solution of the above-described ethyl carboxylate (450 mg, 1.27 mmol) in ethanol (20 mL), sodium ethylate (86 mg, 1.27 mmol) and guanidine hydrochloride (121 mg, 1.27 mmol) were added in sequence. The solution was stirred at reflux for 12 h. The solvent was then evaporated, the residue taken up with dichloromethane and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with diethyl ether and collected by filtration, giving **13a** (377 mg, 85%). 1H NMR (401 MHz, DMSO- d_6) δ ppm 1.30 (t, $J = 7.1$ Hz, 3H), 2.75 (t, $J = 7.7$ Hz, 2H), 2.94 (t, $J = 7.7$ Hz, 2H), 4.28 (q, $J = 7.1$ Hz, 2H), 6.07 (s, 2H), 6.63 (bs, 2H), 7.22–7.38 (m, 5H), 8.17 (s, 1H); LCMS (ESI) m/z 350 (M + H) $^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_5O_2 + H^+$, 350.1612; found, 350.1618.

By employment of the above-described procedure, starting from intermediates **12b–i**, compounds **13b–i** were prepared.

Ethyl 8-Amino-1-phenyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13b). Yield, 56%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.32 (t, $J = 7.1$ Hz, 3H), 2.80 (m, 2H), 2.99 (m, 2H), 4.33 (q, $J = 7.1$ Hz, 2H), 6.04 (bs, 2H), 7.50 (m, 3H), 7.56 (m, 2H), 8.20 (s, 1H); LCMS (ESI) m/z 336 (M + H) $^+$; HRMS (ESI) calcd for $C_{18}H_{17}N_5O_2 + H^+$, 336.1455; found, 336.1449.

Ethyl 8-Amino-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13c). Yield, 52%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.32 (t, $J = 7.1$ Hz, 3H), 2.79 (t, $J = 7.5$ Hz, 2H), 2.98 (t, $J = 7.4$ Hz, 2H), 3.84 (s, 3H), 4.32 (q, $J = 7.1$ Hz, 2H), 6.08 (s, 2H), 7.0 (d, $J = 9.0$ Hz, 2H), 7.48 (d, $J = 9.0$ Hz, 2H), 8.19 (s, 1H); LCMS (ESI) m/z 366 (M + H) $^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_5O_3 + H^+$, 366.1561; found, 366.1564; HPLC purity 85%.

Ethyl 8-Amino-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13d). Yield, 60%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.33 (t, $J = 7.1$ Hz, 3H), 2.82 (t, $J = 7.5$ Hz, 2H), 3.00 (t, $J = 7.4$ Hz, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 6.12 (s, 2H), 7.43 (s, 2H), 7.81 (d, $J = 8.8$ Hz, 2H), 7.90–7.97 (m, 2H), 8.23 (s, 1H); LCMS (ESI) m/z 415 (M + H) $^+$; HRMS (ESI) calcd for $C_{18}H_{18}N_6O_4S + H^+$, 415.1183; found, 415.1174.

Ethyl 8-Amino-1-(pyridin-2-yl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13e). Yield, 58%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.33 (t, $J = 7.1$ Hz, 3H), 2.81 (t, $J = 7.5$ Hz, 2H), 2.99 (t, $J = 7.5$ Hz, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 6.00 (s, 2H), 7.56 (ddd, $J = 7.5, 4.8, 1.0$ Hz, 1H), 7.67 (dt, $J = 7.9, 0.9$ Hz, 1H), 8.04 (td, $J = 7.7, 1.9$ Hz, 1H), 8.20 (s, 1H), 8.51 (ddd, $J = 4.8, 1.9, 0.9$ Hz, 1H); LCMS (ESI) m/z 337 (M + H) $^+$; HRMS (ESI) calcd for $C_{17}H_{16}N_6O_2 + H^+$, 337.1407; found, 337.1394.

Ethyl 8-Amino-1-(2-hydroxyethyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13f). Yield, 70%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.31 (t, $J = 7.1$ Hz, 3H), 2.74 (t, $J = 7.6$ Hz, 2H), 2.92 (t, $J = 7.5$ Hz, 2H), 3.77–3.86 (m, 2H), 4.29 (q, $J = 7.1$ Hz, 2H), 4.78 (t, $J = 5.8$ Hz, 1H), 4.82 (t, $J = 5.9$ Hz, 2H), 6.53 (s, 2H), 8.17 (s, 1H); LCMS (ESI) m/z 304 (M + H) $^+$; HRMS (ESI) calcd for $C_{14}H_{17}N_5O_3 + H^+$, 304.1404; found, 304.1412; HPLC purity 80%.

Ethyl 8-Amino-1-(2,2,2-trifluoroethyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13g). Yield, 45%; 1H NMR (400

MHz, DMSO- d_6) δ ppm 1.33 (t, $J = 7.1$ Hz, 3H), 2.77 (t, $J = 7.8$ Hz, 2H), 2.97 (t, $J = 7.8$ Hz, 2H), 4.32 (q, $J = 7.1$ Hz, 2H), 5.82 (q, $J = 8.9$ Hz, 2H), 6.65 (s, 2H), 8.22 (s, 1H); LCMS (ESI) m/z 342 (M + H) $^+$.

Ethyl 8-Amino-1-(1-methylpiperidin-4-yl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13h). Yield, 68%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.31 (t, $J = 7.1$ Hz, 3H), 1.93 (m, 2H), 2.04 (m, 2H), 2.14–2.24 (m, 4H), 2.25 (s, 3H), 2.72 (m, 2H), 2.87 (bs, 2H), 2.91 (m, 2H), 4.29 (q, $J = 7.1$ Hz, 2H), 5.55 (tt, $J = 11.3, 4.2$ Hz, 1H), 6.53 (s, 2H), 6.73–6.74 (m, 1H), 6.77–6.79 (m, 1H), 8.18 (s, 1H); LCMS (ESI) m/z 357 (M + H) $^+$; HRMS (ESI) calcd for $C_{18}H_{24}N_6O_2 + H^+$, 357.2033; found, 357.2033.

Ethyl 1-(1-Acetylpiperidin-4-yl)-8-amino-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13i). Yield, 79%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.30 (t, $J = 7.1$ Hz, 3H), 1.72–2.05 (m, 4H), 2.07 (s, 3H), 2.71–2.76 (m, 2H), 2.73–2.85 (m, 1H), 2.92 (t, $J = 7.6$ Hz, 2H), 3.26–3.35 (m, 1H), 3.89–4.03 (m, 1H), 4.28 (q, $J = 7.1$ Hz, 2H), 4.46–4.57 (m, 1H), 5.77–5.95 (m, 1H), 6.60 (s, 2H), 8.19 (s, 1H); LCMS (ESI) m/z 385 (M + H) $^+$; HRMS (ESI) calcd for $C_{19}H_{24}N_6O_3 + H^+$, 385.1983; found, 385.1983.

1-Benzyl-8-(cyclopentylamino)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (37). To a suspension of **13a** (450 mg, 1.29 mmol) in dry DMF (10 mL), cyclopentanone (0.23 mL, 2.58 mmol), sodium triacetoxycoborohydride (600 mg, 2.84 mmol), and trifluoroacetic acid (0.7 mL, 9 mmol) were added in sequence. The mixture was stirred at room temperature overnight. After this time, 0.33 N NaOH (54 mL, 18 mmol) was added dropwise to the mixture. The precipitate was filtered, washed with water, and dried in an oven to dryness to give ethyl 1-benzyl-8-(cyclopentylamino)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (430 mg, 80%). 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.29 (t, $J = 7.1$ Hz, 3H), 1.42 (bs, 4H), 1.53–1.65 (m, 2H), 1.75 (bs, 2H), 2.77 (m, 2H), 2.97 (m, 2H), 4.00 (bs, 1H), 4.28 (q, $J = 7.1$ Hz, 2H), 6.10 (s, 2H), 7.09 (d, $J = 7.2$ Hz, 1H), 7.17 (m, 2H), 7.25 (m, 1H), 7.31 (m, 2H), 8.21 (s, 1H); LCMS (ESI) m/z 418 (M + H) $^+$; HRMS (ESI) calcd for $C_{24}H_{27}N_5O_2 + H^+$, 418.2238; found, 418.2236. A solution of the above-described ethyl carboxylate (300 mg, 0.72 mmol) in a mixture of methanol (10 mL), DMF (10 mL), and 33% NH_4OH (5 mL) was stirred at 70 °C in a close bottle for 8 h. The solvent was then removed under reduced pressure and the residue dissolved with dichloromethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by flash chromatography on a silica gel column (dichloromethane/methanol, 98/2) affording **37** (223 mg, 80%). 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.36–1.52 (m, 4H), 1.58–1.83 (m, 4H), 2.79 (t, $J = 7.6$ Hz, 2H), 3.02 (t, $J = 7.6$ Hz, 2H), 3.98 (s, 1H), 6.04 (s, 2H), 7.09–7.34 (m, 7H), 7.49 (s, 1H), 7.70 (s, 1H), 8.22 (s, 1H); LCMS (ESI) m/z 389 (M + H) $^+$; HRMS (ESI) calcd for $C_{22}H_{24}N_6O + H^+$, 389.2085; found, 389.2090.

By employment of the above-described procedure, starting from intermediates **13b–i**, compounds **38–44** and **46** were prepared.

8-(Cyclopentylamino)-1-phenyl-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (38). Yield, 55%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.01–1.62 (m, 8H), 2.79 (t, $J = 7.62$ Hz, 2H), 3.02 (t, $J = 7.62$ Hz, 2H), 3.25–3.31 (m, 1H), 6.75 (s, 1H), 7.32 (s, 1H), 7.43–7.56 (m, 5H), 7.59 (s, 1H), 8.16 (s, 1H); LCMS (ESI) m/z 375 (M + H) $^+$; HRMS (ESI) calcd for $C_{21}H_{22}N_6O + H^+$, 375.1928; found, 375.1933.

8-(Cyclopentylamino)-1-(pyridin-2-yl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (39). Yield, 50%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 1.04–1.63 (m, 8H), 2.80 (t, $J = 7.7$ Hz, 2H), 3.02 (t, $J = 7.7$ Hz, 2H), 3.11–3.61 (m, 1H), 6.77 (s, 1H), 7.35 (s, 1H), 7.56 (ddd, $J = 7.5, 4.8, 1.1$ Hz, 1H), 7.63 (s, 1H), 7.66 (dt, $J = 7.9, 0.9$ Hz, 1H), 8.02–8.08 (m, 1H), 8.17 (s, 1H), 8.56 (ddd, $J = 4.8, 1.8, 0.9$ Hz, 1H); LCMS (ESI) m/z 376 (M + H) $^+$.

8-(Cyclopentylamino)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (40). Yield, 52%; 1H NMR (400 MHz, DMSO- d_6) δ ppm 0.98–1.66 (m, 8H), 2.77 (t,

$J = 7.6$ Hz, 2H), 3.01 (t, $J = 7.6$ Hz, 2H), 3.20–3.42 (m, 1H), 3.80 (s, 3H), 6.77 (d, $J = 5.8$ Hz, 1H), 7.02 (d, $J = 9.0$ Hz, 2H), 7.29 (s, 1H), 7.44 (d, $J = 8.9$ Hz, 2H), 7.56 (s, 1H), 8.14 (s, 1H); LCMS (ESI) m/z 405 (M + H)⁺; Anal. (C₂₂H₂₄N₆O₂) C, H, N.

8-(Cyclopentylamino)-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (41). Yield, 48%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.07–1.66 (m, 8H), 2.85 (t, $J = 7.4$ Hz, 2H), 3.06 (t, $J = 7.4$ Hz, 2H), 3.27–3.55 (m, 1H), 7.45 (s, 4H), 7.69 (s, 1H), 7.81 (d, $J = 8.6$ Hz, 2H), 7.93 (d, $J = 8.6$ Hz, 2H), 8.23 (s, 1H); LCMS (ESI) m/z 454 (M + H)⁺; HRMS (ESI) calcd for C₂₁H₂₃N₇O₃S + H⁺, 454.1656; found, 454.1651; Anal. (C₂₁H₂₃N₇O₃S) C, H, N, S.

8-(Cyclopentylamino)-1-(2-hydroxyethyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (42). Yield, 51%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.39–1.76 (m, 6H), 1.83–1.99 (m, 2H), 2.71 (t, $J = 7.7$ Hz, 2H), 2.93 (t, $J = 7.6$ Hz, 2H), 3.84 (t, $J = 6.1$ Hz, 2H), 4.07–4.24 (m, 1H), 4.71–4.88 (m, 1H), 4.80 (t, $J = 6.2$ Hz, 2H), 7.03 (d, $J = 6.0$ Hz, 1H), 7.22 (s, 1H), 7.41 (s, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 343 (M + H)⁺; HRMS (ESI) calcd for C₁₇H₂₂N₆O₂ + H⁺, 343.1877; found, 343.1876.

8-(Cyclopentylamino)-1-(2,2,2-trifluoroethyl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (43). Yield, 53%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.40–1.76 (m, 6H), 1.82–1.98 (m, 2H), 2.76 (t, $J = 7.7$ Hz, 2H), 2.97 (t, $J = 7.7$ Hz, 2H), 4.00–4.18 (m, 1H), 5.79 (q, $J = 8.8$ Hz, 2H), 7.20 (d, $J = 6.9$ Hz, 1H), 7.39 (s, 1H), 7.44 (s, 1H), 8.24 (s, 1H); LCMS (ESI) m/z 381 (M + H)⁺; Anal. (C₁₇H₁₉F₃N₆O) C, H, N.

8-(Cyclopentylamino)-1-(1-methylpiperidin-4-yl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (44). Yield, 58%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.4–1.63 (m, 4H), 1.68–1.79 (m, 2H), 1.83–2.11 (m, 4H), 2.09–2.32 (m, 6H), 2.67–2.73 (m, 2H), 2.92 (t, $J = 7.7$ Hz, 2H), 2.98 (bs, 2H), 4.17 (sxt, $J = 7.0$ Hz, 1H), 5.58 (bs, 1H), 7.08 (bs, 1H), 7.24 (bs, 1H), 7.33 (bs, 1H), 8.19 (s, 1H); LCMS (ESI) m/z 396 (M + H)⁺; HRMS (ESI) calcd for C₂₁H₂₉N₇O + H⁺, 396.2507; found, 396.2507.

1-(1-Acetylpiperidin-4-yl)-8-(cyclopentylamino)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (46). Yield, 49%; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.43–1.60 (m, 4H), 1.71 (m, 2H), 1.89–2.04 (m, 6H), 2.06 (s, 3H), 2.65 (bs, 1H), 2.71 (t, $J = 7.7$ Hz, 2H), 2.85–2.99 (m, 2H), 3.07–3.21 (m, 1H), 4.02 (m, 1H), 4.16 (m, 1H), 4.55 (m, 1H), 5.81 (m, 1H), 7.07 (bs, 1H), 7.21 (s, 1H), 7.41 (s, 1H), 8.20 (s, 1H); LCMS (ESI) m/z 424 (M + H)⁺; HRMS (ESI) calcd for C₂₂H₂₉N₇O₂ + H⁺, 424.2456; found, 424.2457.

8-(Cyclopentylamino)-*N*-methyl-1-(1-methylpiperidin-4-yl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (45). To a suspension of **13h** (1.0 g, 2.80 mmol) in dry DMF (20 mL), cyclopentanone (0.5 mL, 5.6 mmol), trifluoroacetic acid (1.53 mL, 19.6 mmol), and sodium triacetoxyborohydride (1.3 g, 6.16 mmol) were added. After 18 h, 0.33 N NaOH (119 mL, 39.2 mmol) was added dropwise to the mixture. The precipitate was filtered and dried in oven to give ethyl 8-(cyclopentylamino)-1-(1-methylpiperidin-4-yl)-4,5-dihydro-1H-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (949 mg, 80%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.31 (t, $J = 7.1$ Hz, 3H), 1.44–1.64 (m, 4H), 1.73 (m, 2H), 1.90–2.02 (m, 6H), 2.13 (m, 2H), 2.25 (bs, 3H), 2.73 (m, 2H), 2.92 (m, 2H), 2.99 (bs, 2H), 4.17 (m, 1H), 4.29 (q, $J = 7.1$ Hz, 2H), 5.63 (m, 1H), 7.12 (bs, 1H), 8.21 (s, 1H); LCMS (ESI) m/z 425 (M + H)⁺; HRMS (ESI) calcd for C₂₃H₃₂N₆O₂ + H⁺, 425.2660; found, 425.2658. To a solution of the above-described ethyl carboxylate (800 mg, 1.89 mmol) in ethanol (50 mL) was added 33 wt % methylamine in absolute ethanol (15 mL). The solution was stirred at 60 °C in a closed bottle for 8 h. The solvent was then removed under reduced pressure and the crude purified by flash chromatography on a silica gel column (dichloromethane/methanol/triethylamine, 95/5/5) affording **45** (603 mg, 78%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.43–1.62 (m, 4H), 1.74 (m, 2H), 1.87–2.06 (m, 6H), 2.13–2.20 (m, 2H), 2.21 (s, 3H), 2.67–2.73 (m, 2H), 2.75 (d, $J = 4.8$ Hz, 3H), 2.92 (t, $J = 7.7$ Hz, 2H), 2.96 (bs, 2H), 4.17 (sxt, $J = 7.2$ Hz, 1H), 5.57 (tt, $J = 11.4$, 3.9 Hz, 1H), 7.09 (bs, 1H), 7.93 (m, 1H), 8.19 (s, 1H); LCMS (ESI) m/z

410 (M + H)⁺; HRMS (ESI) calcd for C₂₂H₃₁N₇O + H⁺, 410.2663; found, 410.2668.

Crystallographic Methods. Expression, purification, crystallization, and soaking procedures of the CDK2/cyclin A complexes were carried out as previously described.²¹

Kinase Assays. Kinase assays were performed as previously described.²¹ The panel includes the following: c-ABL, ACK1, ALK, AKT1, Aur-A, Aur-B, BRK, BUB1, CDC7/DBF4, CDK2/CyA, CDK2/CyE, CDK1/CyB, CDK5/p25, CDK4/CyD1, GSK-3 β , CHK1, CK2, EEF2K, EGFR, ERK2, FAK, FGFR1, FLT3, IGF1R, IKK1, IKK2, IR, JAK1, JAK3, C-KIT, LCK, LYN, MAPKAPK2, MET, MNK2, MST4, NEK6, NIM, PAK4, PDGFR, PDK1, PERK, PIM1, PIM2, PKA α , PKC β , PLK1, RET, SYK, SULU1, TYK, TRKA, VEGFR2, VEGFR3, and ZAP70.

In Vitro Pharmacology. A2780 Cells Proliferation Assay. This cell line was selected for the screening of compounds active against CDKs because it has a high proliferation rate (doubling time of 21 h). Cells were seeded into 96 or 384 wells plates at final concentration ranging from 10000 to 30000 cells per cm² in appropriate medium plus 10% FCS. After 24 h, cells were treated with scalar doses of test compounds in two replicates. Compound dilutions were prepared in 100% DMSO at 1000-fold concentration, and then diluted in the culture medium to have in the wells the desired compound dose with a final DMSO concentration of 0.1% that does not interfere with cell proliferation. A total of 72 h after the treatment, the cell number was evaluated using the Cell Titer_Glo Assay (Promega). IC₅₀s were calculated using a Sygmoidal fitting (Symyx Assay Explorer). Experiments were replicated at least two times.

Flow Cytometry Analysis, Immunoblot, and BrdU Incorporation. A2780 cells (human ovary adenocarcinoma, from ECACC), were seeded in T-75 tissue culture flasks, 25000 cells/cm² in RPMI 1640, pH 7.4, 10% FBS, 2 mM L-glutamine, 1 \times penicillin-streptomycin, and maintained in 5% CO₂ at 37 °C with 96% relative humidity. After 24 h, cells were treated with the compound at 1 and 3 μ M for 24 h, approximately 3- and 10-fold the IC₅₀ measured in 72 h proliferation study. Both detached and adherent cells were collected using 0.25% trypsin, 0.02% EDTA, washed in PBS, and divided into three samples for flow cytometry analysis, immunoblot, and BrdU incorporation as previously described.²¹ In particular, the sample for cell cycle analysis was fixed in ethanol 70% in PBS for at least 12 h. DNA staining was performed using a solution of propidium iodide 25 μ g/mL in sodium citrate 1% containing RNase A (2 μ g/mL) and 0.002% NP-40 (Sigma). After 1 h at room temperature, samples were acquired and analyzed using a FACS-Calibur flow cytometer (Beckton Dickinson). The DNA histograms were analyzed by Modfit 3.0 (Verity Software House).

In Vivo Pharmacology. All animal studies were carried out in compliance with Italian Legislative Decree No. 116, dated January 27, 1992, and the European Communities Council Directive No. 86/609/EEC concerning the protection of animals for experimental purposes and according to institutional policy regarding the care and use of laboratory animals. Female nu/nu mice (Harlan), 5–6 weeks age (average weight 20–22 g) were used. A2780 human ovarian carcinoma cells were transplanted s.c. in athymic mice. Mice bearing a palpable tumor were selected and randomized into vehicle and treated groups (Mean: 180 mg). Treatments started the day after randomization. Compound **59** was dissolved in 5% dextrose. Treatments were administered twice a day for 10 consecutive days. Dimensions of the tumors were measured regularly using Vernier calipers for all the duration of the experiment and tumor masses and tumor growth inhibition were calculated as described.²¹ The effectiveness of the anticancer treatment was also determined as the delay in the onset of the exponential growth of the tumor according to the criteria of Bissery et al.²² Tumor growth delay

was defined as the difference between the treatment and the control group in the median time (in days) to reach the predetermined size of 1 g.

High-Throughput Solubility. Solubility at pH 7 was evaluated as previously described.²¹

Metabolic Stability. Compound was dissolved in DMSO at 10 μ M concentration. Human cDNA expressed cytochrome P450 isoforms (supersomes) were purchased from Gentest (Woburn, MA). All chemicals were of analytical grade and commercially available. The potential inhibitory effect was investigated against cDNA expressed human CYP4503A4 supersome using typical substrates incubated at their respective K_m concentration. The reference inhibitor ketoconazole was included to check the inhibition response. Analysis of both substrate and metabolite was done by LC/MS/MS.

Plasma Protein Binding. Plasma protein binding was evaluated as previously described.²¹

In Vivo Pharmacokinetics. The pharmacokinetic profile of the compounds was investigated in overnight fasted male Nu/Nu mice following a single dose given intravenously (iv) or orally (po). The vehicle used was 5% dextrose solution. A total of six mice were treated (three for each leg). Pharmacokinetic analysis was also repeated in three A2780 tumor bearing female Balb nu/nu mice the last day of treatment with 20 mg/kg twice a day orally for 10 consecutive days. Blood samples of each mouse were collected from the saphenous vein at predose, 0.083, 0.5, 1, 6, and 24 h postdosing following iv dosing, and at predose, 0.25, 0.5, 1, 6, and 24 h following oral dosing. Samples were centrifuged at 10000 g for 3 min at 4 °C and the plasma was stored at -80 °C until analysis. Samples were analyzed by LC/MS/MS technique.

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Supporting Information Available: X-ray crystallographic studies of compound **59**; Elemental analysis results of compounds **1**, **14**, **17**, **19–27**, **34**, **35**, **40**, **41**, **43**, **56**, and **59**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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