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Identification of *N*,1,4,4-Tetramethyl-8-{[4-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (PHA-848125), a Potent, Orally Available Cyclin Dependent Kinase Inhibitor[#,†]

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The discovery of a novel class of inhibitors of cyclin dependent kinases (CDKs) is described. Starting from compound **1**, showing good potency as inhibitor of CDKs but being poorly selective against a panel of serine−threonine and tyrosine kinases, new analogues were synthesized. Enhancement in selectivity, antiproliferative activity against A2780 human ovarian carcinoma cells, and optimization of the physical properties and pharmacokinetic profile led to the identification of highly potent and orally available compounds. Compound **28** (PHA-848125), which in the preclinical xenograft A2780 human ovarian carcinoma model showed good efficacy and was well tolerated upon repeated daily treatments, was identified as a drug candidate for further development. Compound **28** is currently undergoing phase I and phase II clinical trials.

Introduction

Cyclin dependent kinases (CDKs[a]) are a family of serine/threonine kinases that, in concert with cyclins (positive regulators) and natural inhibitors (CDKI), play a crucial role in the cell cycle progression.[1] Deregulation of the activity of CDKs, due to alterations of expression and/or genetic mutations of cyclins, CDKs, CDKIs, and other components of the retinoblastoma protein (pRB) pathway, has been reported in more than 90% of human neoplasms. For example, cyclins E and A have been found overexpressed in 50% of breast and lung cancer whereas decreased levels of the inhibitor p27 indicate a poor prognosis in breast, prostate, colon, gastric, lung, and esophageal cancer.[2] The high frequency of alterations found in the core members of this pathway in human tumors led to the suggestion that its deregulation, leading to increased activity of CDK/cyclin complexes, is an obligatory event for the development of all human cancers. Despite recent genetic studies in mice indicating that normal cells are not dependent on interphase CDKs (CDK4 and CDK2) for their growth, certain tumor cells, depending on their origin and their pathogenic spectrum of mutation, may be sensitive to the inhibition of CDKs.[1] Several small molecules inhibitors of CDKs are in clinical development. Among the first generation of CDK inhibitors, flavopiridol has been granted orphan drug status for the treatment of chronic lymphocytic leukemia.[3] A second generation of inhibitors with greater selectivity for CDKs such as roscovitine/CYC-202,[4] BMS-387032/SNS-032[5] (both mainly CDK2 but also CDK7 and CDK9 inhibitors), PD0332991[6] (selective CDK4 and CDK6 inhibitor), and recently R547[7] (CDK1, -2, -4 inhibitor), SCH-727965[8] (CDK1, -2, -5, -9 inhibitor), AT7519[9] (CDK1, -2, -4, -5 inhibitor), and AZD5597[10] (CDK1, -2, and -9 inhibitor) suggest that agents that inhibit the function of multiple CDKs may be clinically more successful than very selective inhibitors of CDKs.

We recently reported on a series of benzodipyrazoles[11] as CDK2 inhibitors (Figure 1). As a further development of the fused pyrazole ring systems, we identified a novel series of pyrazolo[4,3-*h*]quinazoline-3-carboxamides as CDK inhibitors. Preliminary expansion of this class led to compound **1** that, notwithstanding being a potent inhibitor of CDKs, was poorly selective against a panel of serine−threonine and tyrosine kinases (see Experimental Session, Kinase Assays). The remarkable antiproliferative activity of this compound against A2780 human ovarian carcinoma cells and the potency on CDKs prompted us to start a medicinal chemistry program with the aim of optimizing its profile with respect to selectivity, physicochemical properties, and pharmacokinetic profile.

[#] Coordinates of the CDK2 complexes with compounds **2** and **28** have been deposited in the Protein Data Base under accession codes 2WIP and 2WIH, together with the corresponding structure factor files.

[†] This paper is dedicated to the memory of our colleague Valter Croci.

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[a] Abbreviations: CDK, cyclin dependent kinase; CDK2/A, (cyclin dependent kinase 2)/(cyclin A); Aur-A, Aurora A; TRKA, thropomyosin receptor kinase A, catalytic receptor for the neurothrophin nerve growth factor; NGF, nerve growth factor; pRB, retinoblastoma protein; PPB, plasma protein binding; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; HOBt·NH_3, 1-hydroxybenzotriazole ammonium salt; (±)-BINAP, (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene.

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Structural information was used to support the development of this class. Specifically, the crystal structure of CDK2/cyclin A complexed with carboxylic acid **2** was solved (Figure 2). This compound, which shows a similar potency but is more soluble than compound **1**, binds in the ATP pocket of the active conformation of the kinase: the pyrazoloquinazoline ring system occupies the adenine region of the ATP pocket, while the phenyl moiety points toward the solvent accessible region. Compound **2** makes two hydrogen bonds with the protein backbone of the hinge region: the N atom at position 7 of the pyrazoloquinazoline core interacts with the backbone NH of Leu83, while the adjacent amino group binds to the carbonyl oxygen of Leu83. In addition, the carboxylate group of the ligand is within hydrogen bonding distance of the conserved lysine (Lys33).

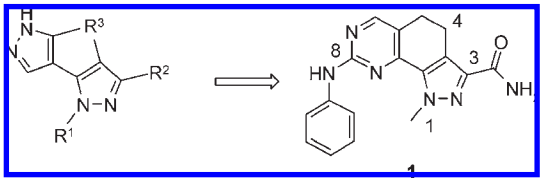


Figure 1. From benzodipyrazole series to hit compound **1**.

Chemistry

The synthetic approach to this class of compounds[12] (Scheme 1) started from commercially available 1,2-cyclohexanedione that, after conversion into enol ether **3**, was condensed with diethyl oxalate to provide diketoester **4** that further reacted with methylhydrazine to form regioselectively the pyrazole **5.** Condensation with *N*,*N*-dimethylformamide di-*tert*-butyl acetal gave rise to enaminone **6a**. The preparation of intermediate **6b** was accomplished by using dimedone as starting material. The formation of enol ether **7** was followed by a reaction sequence encompassing reduction of the conjugated double bond, elimination, and epoxidation to give intermediate **8**. Oxirane ring-opening by methanol led to enol ether **9**, regioisomer of **7**. Subsequent condensation with diethyl oxalate and reaction of resulting 1,3-diketone intermediate with methylhydrazine regioselectively gave rise to the pyrazole derivative **10**. Condensation with *N*,*N*-dimethylformamide di-*tert*-butyl acetal afforded the enaminone **6b**.

The two enaminones **6a** and **6b** were condensed with phenylguanidine carbonate in DMF to afford the arylamino derivatives **11a** and **11b** (Scheme 2). Hydrolysis of carboxylic esters gave the corresponding acids, which were submitted to the coupling with amines to provide amides **1**, **16**−**21**, and **26**.

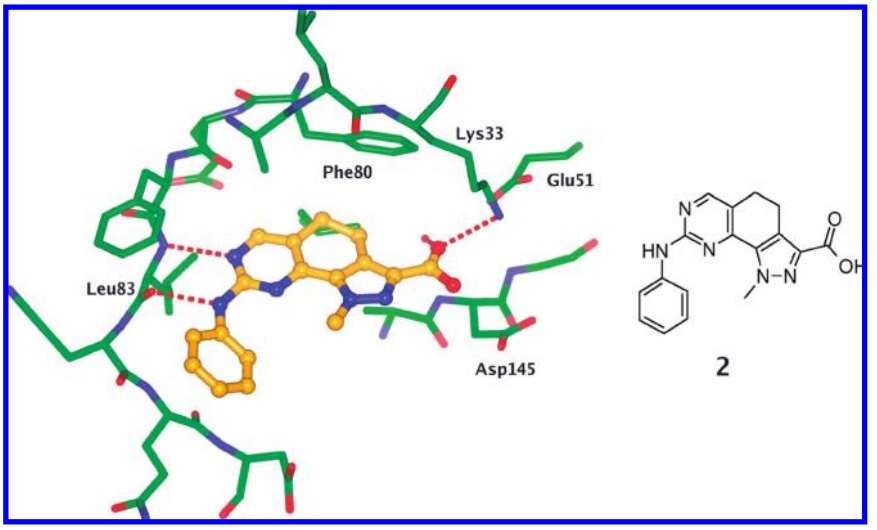
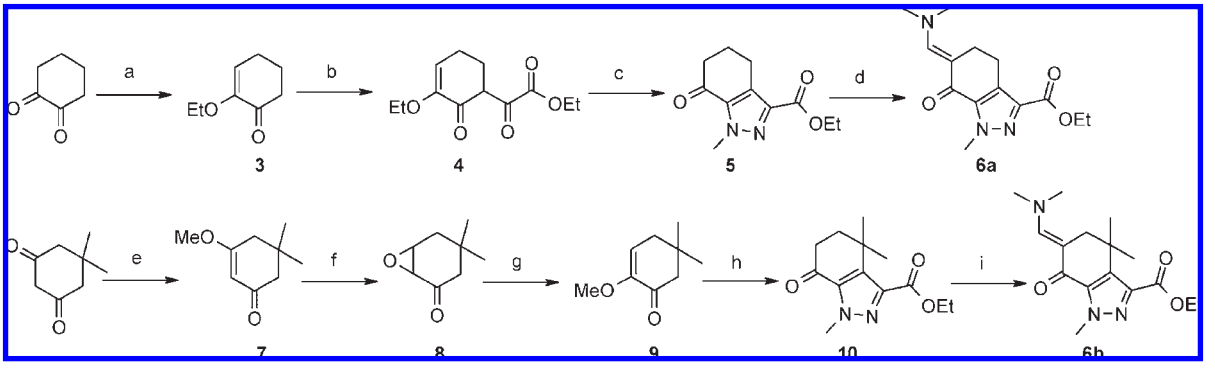


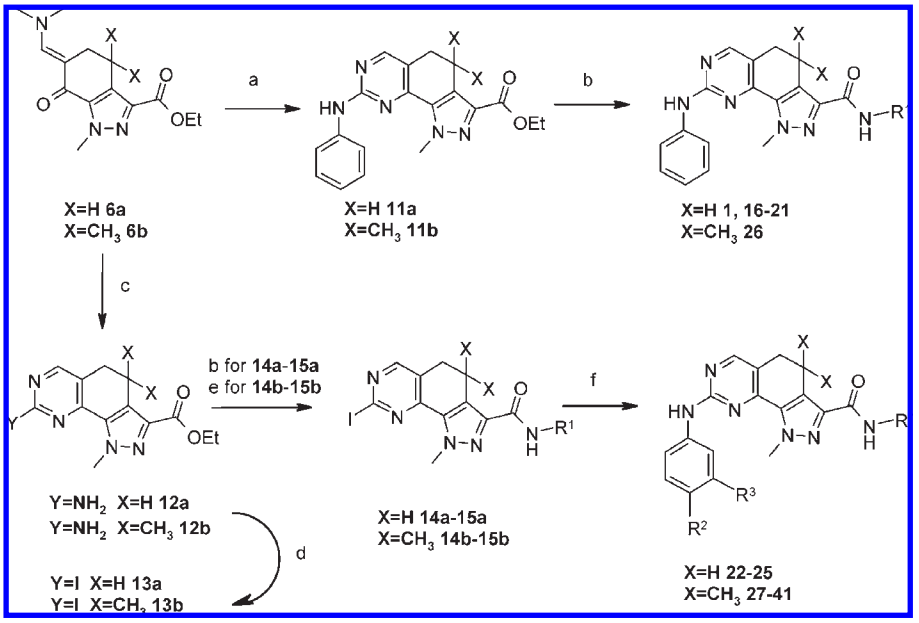
Figure 2. Crystal structure of pyrazolo[4,3-*h*]quinazoline-3-carboxylic acid derivative **2** in complex with CDK2/cyclin A (PDB code 2WIP).

Scheme 1. Synthesis of Adducts **6a** and **6b**[a]



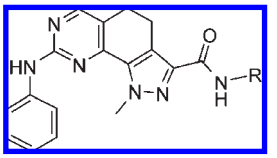
[a] Reagents and conditions: (a) *p*-TSA cat., toluene, EtOH, reflux, 48 h, 66%; (b) 1 M $LiN(TMS)_2$ in THF, diethyl oxalate, Et_2O, −50 °C to room temp, 76%; (c) methylhydrazine, CH_3COOH, room temp, 6 h, 63%; (d) *N*,*N*-dimethylformamide di-*tert*-butyl acetal, DMF, 60 °C, 8 h, 90%; (e) $TiCl_4$, MeOH, room temp, 1 h, 92%; (f) 1 M $LiAlH_4$ in THF, 0 °C to room temp, 4 h; 2 M H_2SO_4; H_2O_2, 2% NaOH, MeOH, 0−4 °C, 20 h, 78%; (g) KOH, MeOH, room temp, 20 h, 68%; (h) NaH, diethyl oxalate, THF, reflux, 1 h; methylhydrazine, CH_3COOH, room temp, 12 h, 48%; (i) *N*,*N*-dimethylformamide di-*tert*-butyl acetal, DMF, 60 °C, 2 h, 87%.

Scheme 2. Synthesis of Final Compounds **1** and **16**−**41**[a]



[a] Reagents and conditions: (a) phenylguanidine carbonate, DMF, 110 °C, 6 h, 72−90%; (b) 1.5 N KOH in 95% EtOH, room temp; HOBt·NH_3, EDC, DIPEA, DMF, room temp or R^1NH_2, EDC, HOBt, DIPEA, DMF, room temp, 46−83%; (c) guanidine hydrochloride, K_2CO_3, DMF, 110 °C, 92−93%; (d) isopentyl nitrite, CsI, I_2, CuI, DME, 65 °C, 40−46%; (e) K_2CO_3, H_2O, dioxane, reflux; HOBt·NH_3, EDC, DIPEA, DMF, room temp or R^1NH_2, EDC, HOBt, DIPEA, DMF, room temp, 56−65%; (f) substituted aniline, $Pd(OAc)_2$, (±)-BINAP, K_2CO_3, DMF, 80 °C, 30−71%.

Table 1. R^1 Modification[a]



compd	R^1	IC_{50}, μM CDK2/A	IC_{50}, μM Aur-A	IC_{50}, μM A2780	solubility at pH 7, μM
1	H	0.002	0.050	0.50	3
16	CH_3	0.019	0.312	2.63	1
17	cyclopropyl	0.081	0.213	3.82	1
18	CH_2CH_2OH	0.074	0.364	2.38	7
19	cyclopentyl	3.90	> 10	> 10	1
20	phenyl	0.574	> 10	> 10	1
21	benzyl	1.053	0.374	0.58	1

[a] Values are the mean of two or more experiments.

Alternatively, the two enaminones **6a** and **6b** were condensed with guanidine hydrochloride in DMF to afford the fused pyrimidine derivatives **12a** and **12b**, which were converted into the corresponding iodo derivatives **13a** and **13b**. Hydrolysis of carboxylic esters gave the corresponding acids, which were submitted to the coupling with amines to provide amides **14a**,**15a** and **14b**,**15b**. Palladium catalyzed coupling with substituted anilines furnished the arylamino derivatives **22**−**25** and **27**−**41**.

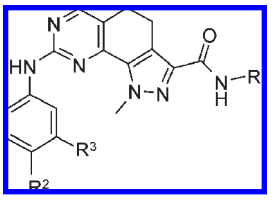
Results and Discussion

An initial set of amides **1** and **16**−**21** (Table 1) was synthesized in order to explore the phosphate region of the ATP binding site of the CDK2/cyclin A, which accommodates the carboxamide groups. The primary carboxamide **1** proved to be the most active compound. Analogously, secondary amides bearing small substituents such as methyl, cyclopropyl, and hydroxyethyl (**16**−**18**) displayed interesting enzymatic activity as CDK2/cyclin A inhibitors, whereas bulkier residues such as cyclopentyl, phenyl, and benzyl (**19**−**21**) caused a marked decrease in activity on CDK2/cyclin A. However, no particular improvement in terms of selectivity vs the serine−threonine kinase Aurora A (used as indicator of compound selectivity) and of cellular antiproliferative activity was achieved moving from the primary amide **1** to the most interesting secondary amides **16**−**18**.

Exploration of the solvent accessible region of the ATP binding site of CDK2/cyclin A was realized by introducing solubilizing moieties on the phenyl ring to give arylamines **22**−**25** (Table 2).

Early comparison of analogues **22** and **23** with parent compounds **1** and **16**, respectively, indicated that introduction of the 4-methylpiperazinyl moiety on the phenyl ring para position afforded potent enzyme inhibitors with an improvement of the antiproliferative activity in A2780 human ovarian carcinoma cells as well as solubility in neutral buffer. Introduction of the 4-methylpiperazinyl group in the meta position

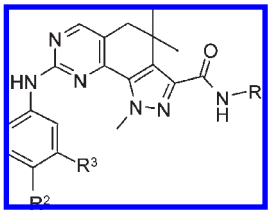
Table 2. R^1, R^2, R^3 Modifications[a]



compd	R^1	R^2	R^3	IC_{50}, μM CDK2/A	Aur-A	A2780	solubility at pH 7, μM
22	H	4-methylpiperazin-1-yl	H	0.002	0.053	0.03	156
23	CH_3	4-methylpiperazin-1-yl	H	0.045	0.175	0.19	211
24	H	H	4-methylpiperazin-1-yl	0.001	0.030	0.03	47
25	H	morpholin-4-yl	H	0.014	0.051	0.12	10

[a] Values are the mean of two or more experiments.

Table 3. R^1, R^2, R^3 Modifications[a]



compd	R^1	R^2	R^3	IC_{50}, μM CDK2/A	Aur-A	A2780	solubility at pH 7, μM
26	H	H	H	0.024	> 10	0.17	< 1
27	H	4-methylpiperazin-1-yl	H	0.017	0.582	0.05	98
28	CH_3	4-methylpiperazin-1-yl	H	0.045	1.051	0.20	191
29	CH_3	H	4-methylpiperazin-1-yl	0.031	1.00	0.53	182
30	CH_3	morpholin-4-yl	H	0.137	0.649	0.37	46
31	CH_3	H	morpholin-4-yl	0.248	0.625	0.70	51
32	CH_3	1-methylpiperidin-4-yloxy	H	0.029	1.343	0.17	183
33	CH_3	H	1-methylpiperidin-4-yloxy	0.047	1.584	0.39	160
34	CH_3	4-methylpiperazin-1-ylmethyl	H	0.055	1.615	0.24	170
35	CH_3	H	4-methylpiperazin-1-ylmethyl	0.059	1.184	0.57	162
36	CH_3	morpholin-4-ylmethyl	H	0.036	0.535	0.35	133
37	CH_3	H	morpholin-4-ylmethyl	0.160	0.638	0.87	121
38	CH_3	dimethylamino	H	0.059	0.855	0.50	56
39	CH_3	H	dimethylamino	0.220	1.437	3.01	41
40	CH_3	dimethylaminomethyl	H	0.035	> 5	0.11	206
41	CH_3	H	dimethylaminomethyl	0.083	2.940	0.5	193

[a] Values are the mean of two or more experiments.

(**24**) or of the morpholin-4-yl group in the para position of the phenyl ring (**25**) was compatible with the maintenance of the CDK2 inhibitory activity and potency in the cellular antiproliferative assay, albeit solubility in neutral buffer was diminished. Unfortunately, improvement in the selectivity vs Aurora A, as well as vs many of the kinases in our panel, was not achieved with this series, and the exploration of the small CDK2 cavity at the back of the pocket (buried region) was considered. Size and shape of this cavity vary among the different kinases and are often exploited for improving selectivity. The buried region of CDK2, defined by Ala31, Val64, Phe80, and Ala144,[13] is small mainly because of the presence of a hindered and rigid gatekeeper residue such as Phe80. Structure−activity relationship (SAR) data generated by us[11,13,14] and others[15] on multiple chemical classes have shown that occupation of this buried area by small hydrophobic moieties is beneficial to CDK2 binding. By visual inspection and modeling experiments, position 4 of the pyrazoloquinazoline skeleton was identified as suitable for placing a moiety such as a dimethyl group that could increase the hydrophobic contact in the CDK2 buried region. Compound **26** (Table 3) showed interesting CDK2 inhibitory activity and potency in the A2780 cells assay. Moreover, incorporation of the dimethyl moiety at the 4-position resulted in a significant improvement of selectivity against Aurora A for compound **26** when compared to compound **1**, supporting the working hypothesis.

With the aim of improving solubility in neutral buffer, an expansion on the 4,4-dimethylsubstituted pyrazoloquinazoline skeleton was performed by keeping the 3-substituent fixed as primary or *N*-methylamide and varying the arylamines at position 8. Introduction of the 4-methylpiperazinyl moiety in the para position of the phenyl ring (**27** and **28**) provided compounds with good activity against CDK2/cyclin A and in A2780 cell proliferation assay as well as good solubility in neutral buffer. Selectivity toward Aurora A was reasonably improved by the presence of the 4,4-dimethyl moiety, in particular when comparing **28** with **23**. As far as buffer solubility is concerned, compound **28**, albeit less potent than compound **27**, displayed higher solubility. The binding mode

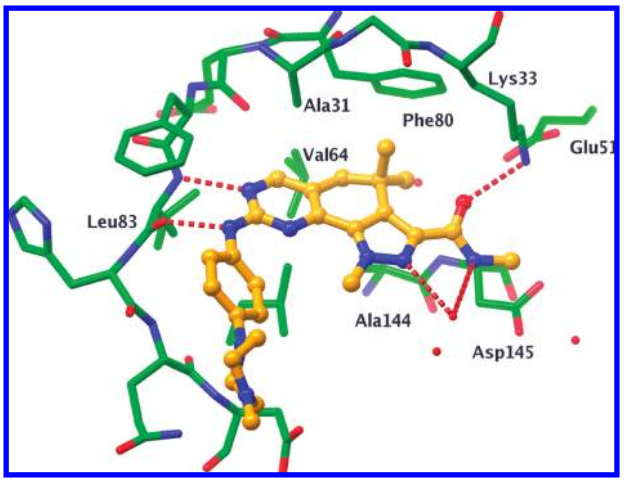


Figure 3. Crystal structure of compound **28** (PHA-848125) in complex with CDK2/cyclin A (PDB code 2WIH).

of the 4,4-dimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline skeleton was confirmed by the crystal structure of compound **28** in complex with CDK2/cyclin A (Figure 3).

Compound **28** is anchored, in a similar manner as compound **2**, to the ATP pocket of CDK2/cyclin A by three hydrogen bonds to the protein backbone of the hinge region (Leu83) and to the conserved Lys33. Selectivity enhancement of compound **28** with respect to **23** could be explained by the more favorable hydrophobic interactions of the 4,4-dimethyl group with the CDK2 gatekeeper residue Phe80 (compared with the Aurora A gatekeeper Leu210) and by the presence of the smaller side chain of CDK2-Val64 (Aurora A-Leu194).

CDK2/cyclin A inhibitory activity was retained by introduction of the 4-methylpiperazinyl group in the meta position, as in compound **29**, but a lower potency in the cellular antiproliferative assay was observed with respect to compound **28**. Introduction of the morpholin-4-yl group in the para and meta positions of the phenyl ring led to compounds **30** and **31**, respectively, that were less potent on the enzyme and in the cellular antiproliferative assays and less soluble in neutral buffer than compound **28**. The role of the 4-methylpiperazinyl moiety, as far as potency and solubility are concerned, was explored by replacement with different solubilizing groups attached to the phenyl ring in the para and meta positions (**32**−**41**). All these compounds, with the exception of **37** and **39**, displayed good activity on CDK2/cyclin A and from moderate to good selectivity vs Aurora A. In particular, the para substituted compounds were endowed with a higher potency in the antiproliferative assay than the corresponding meta derivatives (compare **32**, **34**, **36**, **38**, **40** to **33**, **35**, **37**, **39**, **41**). All compounds of this series showed good solubility except for the dimethylamino derivatives **38** and **39**.

Compounds **27**, **28**, **32**, **34**, **40** were selected for further assessment on the basis of their potency on the enzyme, antiproliferative activity, and preliminary solubility in neutral buffer. Further selection criteria were applied using preliminary in vitro physicochemical ADME properties (solubility in 5% dextrose, stability to human CYP4503A4, human plasma protein binding) and in vivo pharmacokinetic data as key parameters. As reported in Table 4, compound **28** emerged among these because of its high stability to human CYP4503A4 (85% remaining), acceptable plasma protein binding (98% bound), and good solubility (10 mg/mL in 5% dextrose as hydrochloride salt).

Table 4. In Vitro Physicochemical ADME Properties of Selected Compounds[a]

	in vitro ADME properties		
compd	solubility in 5% dextrose, mg/mL	CYP4503A4, % remaining	PPB, %
27	3.4	82	99
28	10	85	98
32	10	63	100
34	10	50	99
40	10	77	95

[a] Dosed in 5% dextrose as hydrochloride salt.

Table 5. In Vivo Pharmacokinetic Parameters of Selected Compounds[a]

	in vivo PK (mouse), 10 mg/kg iv			in vivo PK (mouse), 10 mg/kg os			
compd	$t_{1/2}$, h	CL, (mL/h)/kg	V_{ss}, mL/kg	$t_{1/2}$, h	C_{max}, μM	AUC, μM·h	F, %
28	2.6	1873	4822	2.9	2.35	8.29	85
32	1.9	2621	5273	3.9	0.60	2.51	38
34	1.8	1898	3227	4.2	0.77	3.37	42
40	1.4	3200	4040	3.4	0.57	1.97	28

[a] Dosed in 5% dextrose as hydrochloride salt.

Table 6. Selectivity Profile of Compound **28**[a]

IC_{50}, μM						
CDK2/A	CDK4/D1	CDK5/p35	CDK2/E	CDK1/B	CDK7/H	TRKA
0.045	0.160	0.265	0.363	0.398	0.150	0.053

[a] Values are the mean of two or more experiments.

In addition, **28** possessed better pharmacokinetic parameters than the other compounds tested so far (**32**, **34**, and **40** in Table 5). In vivo results in healthy nude mouse (10 mg/kg by iv administration) suggested a half-life of 2.6 h, with a moderate clearance (about 36% of the hepatic blood flow). The volume of distribution (V_{ss} about 7-fold of the total body water) pointed toward a high tissue distribution. Compound **28**, after oral administration at a dose of 10 mg/kg, showed the highest oral bioavailability (85%) with an AUC of 8.29 μM·h, a C_{max} of 2.35 μM, and a half-life of 2.9 h.

Compound **28** displayed a combination of cellular potency and physicochemical properties that made it suitable for further profiling as an orally active CDK inhibitor. It was subsequently screened against a panel of 43 serine−threonine and tyrosine kinases. As shown in Table 6, among the members of the CDK family that were assessed, **28** turned out to be a potent inhibitor of CDK2/cyclin A but also, albeit less potent, an inhibitor of CDK4/cyclin D1 (ratio vs CDK2/cyclin A of 3.5×), CDK5/p35 (6×), CDK2/cyclin E (8×), CDK1/cyclin B (8.5×), and CDK7/cyclin H (3×). Among all the other enzymes in the panel, only TRKA was inhibited in the same nanomolar range by compound **28**. It is worth noting that overexpression of TRKA correlates with an aggressive phenotype and poor clinical outcome in malignant solid tumors such as prostate,[16] pancreatic,[17] and breast cancer.[18]

The effects of compound **28** on the cell cycle progression and DNA synthesis (Table 7) 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 μM. At this concentration, the compound was able to show a clear reduction of S phase population, which was associated with an increase of G1 population as expected for a CDK2/cyclin A inhibitor. The reduction of S phase population was linked to a strong reduction of the percentage of BrdU incorporating cell, meaning that DNA synthesis in these cells was impaired.

In addition, the effect on the phosphorylation status of a known CDK substrate such as retinoblastoma protein (pRb) was analyzed in cells treated with compound **28** at 1 and 3 μM. A clear reduction of the hyperphosphorylated form of pRb and an accumulation of the hypophosphorylated form of pRb were observed in the extracts of treated cells in comparison with the untreated cells, indicative of an effect on the activity of CDK2 (Figure 4).

On the basis of the data presented above, compound **28** was prioritized for further in vivo characterization in the human ovarian A2780 xenograft mouse model. The doses of 20, 30, and 40 mg/kg were selected and administered orally twice a day for 10 consecutive days on the basis of the plasma levels reached in the preliminary in vivo PK study. Figure 5 illustrates the results from this study. Compound **28** caused a dose-dependent inhibition of A2780 tumor growth up to 91% at a dose of 40 mg/kg.

Table 7. Effects on Cell Cycle Progression and DNA Synthesis of Compound **28** at 1 μM[a]

G0/G1, %	S, %	G2/M, %	BrdU incorporation, %
+49	−68	−40	−75

[a] Versus control cells.

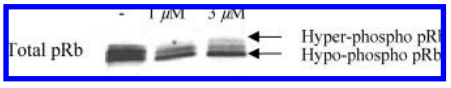


Figure 4. Compound **28** decreases the amount of pRB phosphorylation in treated cells in comparison with untreated cells.

Conclusions

Starting from compound **1**, showing good potency as CDK inhibitor but being poorly selective against a panel of serine−threonine and tyrosine kinases, a series of new analogues was synthesized. A remarkable enhancement in selectivity toward Aurora A was achieved through the incorporation of the dimethyl group at the 4-position of the skeleton. Several of the prepared compounds showed antiproliferative activity against A2780 human ovarian carcinoma cells mediated by CDK2 inhibition. Optimization of the physical properties and pharmacokinetic profile led to the identification of the highly potent, orally available compound **28** (PHA-848125). The inhibition of TRKA highlighted the value of **28** due to the involvement of this kinase in the development of solid tumors such as prostate, pancreatic, and breast cancer. The evaluation of this aspect will be reported elsewhere. Compound **28** is currently undergoing phase I and phase II clinical trials.

Experimental Section

Chemistry. All solvents and reagents, unless otherwise stated, were commercially available, were 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 F_{254} 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. ^{1}H NMR spectra were recorded at 28 °C on a Varian Inova 400 spectrometer in DMSO-d_6 or $CDCl_3$ using the residual solvent signal as reference. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) in Hz. The following abbreviations are used for multiplicities: s = singlet; bs = broad signal; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets. 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

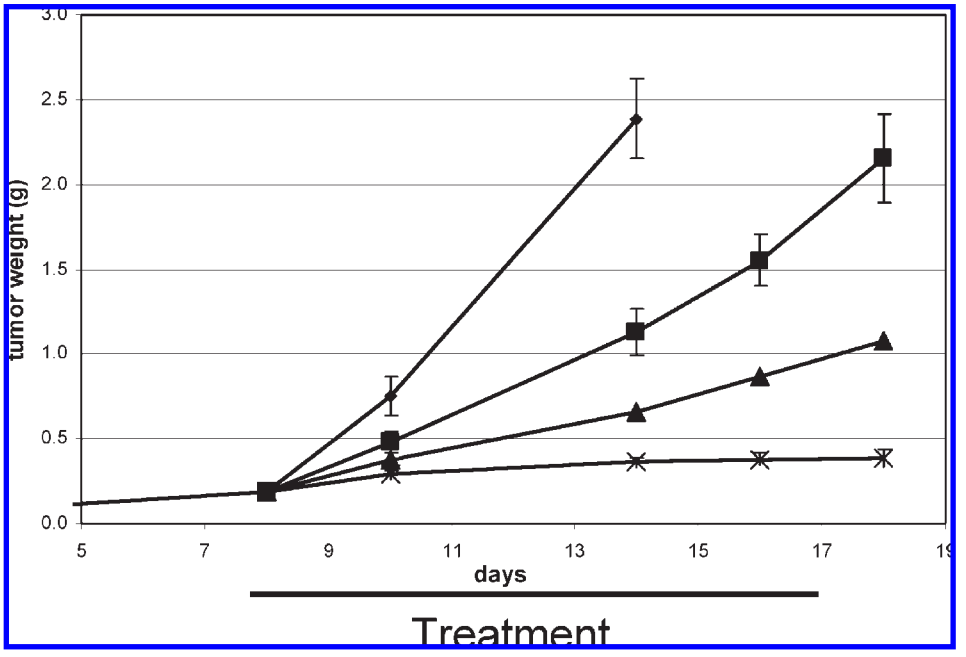


Figure 5. Compound **28** shows 53%, 76%, and 91% tumor growth inhibition (TGI) against a human ovarian cancer model (A2780) transplanted into nude mice when orally administered respectively at doses of 20 (■), 30 (▲), and 40 (×) mg/kg b.i.d. for 10 consecutive days.

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 at 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 (4.6mm×50 mm, 3.5 μm). Mobile phase A was 5 mM ammonium acetate buffer (pH 5.5 with acetic acid)/acetonitrile, 90:10, and mobile phase B was 5 mM ammonium acetate buffer (pH 5.5 with acetic acid)/acetonitrile, 10:90. The gradient was from 0 to 100% B in 7 min and then held at 100% B for 2 min before requilibration. ESI(+) high resolution mass spectra (HRMS) were obtained on a Waters Q-Tof Ultima directly connected to a micro-HPLC 1100 Agilent as previously described.[19] Elemental analyses were performed on a Carlo Erba 1110 instrument, and C, H, and N results were within ±0.4% of theoretical values unless otherwise specified. Melting points are uncorrected.

2-Ethoxycyclohex-2-en-1-one (3). 1,2-Cyclohexandione (50.0 g, 0.445 mol) was dissolved in a mixture of toluene (1.0 L) and ethanol (0.5 L). *p*-Toluenesulfonic acid (10.0 g, 52 mmol) was added and the solution heated under reflux for 2 days. The solvent was then evaporated and the residue dissolved with dichloromethane and washed with a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated. The crude was purified by chromatography on a silica gel column (cyclohexane/ethyl acetate, 98/2) to give **3** as an oil (41.41 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.99 (t, J = 4.5 Hz, 1H), 3.67 (q, J = 6.8 Hz, 2H), 2.33−2.39 (m, 4H), 1.82−1.88 (m, 2H) 1.20 (t, J = 6.8 Hz, 3H); LCMS (ESI) m/z 141 $(M + H)^+$.

Ethyl (3-Ethoxy-2-oxocyclohex-3-en-1-yl)(oxo)acetate (4). $LiN(TMS)_2$ (1 M in THF, 325 mL) was added dropwise at −50 °C to a solution of **3** (41.40 g, 0.29 mol) in diethyl ether (310 mL). After 30 min at the same temperature, diethyl oxalate (44.2 mL, 0.325 mol) was added. The solution was kept at room temperature overnight. Water (300 mL) was added, the pH adjusted to 4−5 by adding 1 N HCl, and the resulting solution extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude was purified by chromatography on a silica gel column (dichloromethane) to yield **4** as an oil (52.9 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.93 (m, 1H), 4.23 (q, J = 7.1 Hz, 2H), 3.70 (q, J = 6.9 Hz, 2H), 3.27 (m, 1H), 2.06−2.58 (m, 4H), 1.25 (t, J = 7.1 Hz, 3H), 1.20 (t, J = 6.9 Hz, 3H); LCMS (ESI) m/z 241 $(M + H)^+$; HRMS (ESI) calcd for $C_{12}H_{16}O_5 + H^+$ 241.1070, found 241.1072.

Ethyl 1-Methyl-7-oxo-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxylate (5). Compound **4** (30.0 g, 0.125 mol) was dissolved in glacial acetic acid (150 mL), and methylhydrazine (6.6 mL, 0.125 mol) was added. The mixture was stirred at room temperature for 6 h. The solvent was evaporated, the crude dissolved with water, the solution made basic with 30% NH_4OH and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography on a silica gel column (dichloromethane) and crystallized from a mixture *n*-hexane/diethyl ether to give **5** as a white solid (17.5 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (q, J = 6.9 Hz, 2H), 4.15 (s, 3H), 2.90 (t, J = 6.2 Hz, 2H), 2.51−2.53 (m, 2H), 2.03−2.07 (m, 2H), 1.33 (t, J = 6.9 Hz, 3H); LCMS (ESI) m/z 223 $(M + H)^+$; HRMS (ESI) calcd for $C_{11}H_{14}N_2O_3 + H^+$ 223.1077, found 223.1077.

Ethyl (6*E*)-6-[(Dimethylamino)methylidene]-1-methyl-7-oxo-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxylate (6a). Compound **5** (16.0 g, 0.072 mol) was dissolved in DMF (100 mL), and *N*,*N*-dimethylformamide di-*tert*-butyl acetal (32 mL, 0.13 mol) was added. The mixture was stirred at 60 °C for 8 h. The solvent was evaporated and the solid crystallized from ethanol to give **6a** as a white solid (17.9 g, 90%). ^{1}H NMR (400 MHz), DMSO-d_6) δ 7.48 (s, 1H), 4.26 (q, J = 6.9 Hz, 2H), 4.12 (s, 3H), 3.12 (s, 6H), 2.88−2.92 (m, 2H), 2.79−2.83 (m, 2H), 1.29 (t, J = 6.9 Hz, 3H); LCMS (ESI) m/z 278 $(M + H)^+$.

3-Methoxy-5,5-dimethyl-cyclohex-2-en-1-one (7). A solution of 5,5-dimethyl-1,3-cyclohexanedione (80.0 g, 0.57 mol) in anhydrous methanol (600 mL) was treated with 1 M solution of titanium chloride ($TiCl_4$) in dichloromethane (17.2 mL). After being stirred for 1 h at room temperature, the mixture was slowly poured into a cold 5% $NaHCO_3$ solution and extracted with diethyl ether (450 mL × 6). The organic layers were collected, washed with brine, dried over Na_2SO_4, and evaporated to dryness, affording **7** (80.7 g, 92%) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.68 (s, 3H), 2.26 (s, 2H), 2.19 (s, 2H), 1.05 (s, 6H).

4,4-Dimethyl-7-oxa-bicyclo[4.1.0]heptan-2-one (8). A solution of **7** (80.0 g, 0.519 mol) in anhydrous THF (270 mL) was treated dropwise with a 1 M solution of $LiAlH_4$ in THF (182 mL) under argon atmosphere and keeping the temperature of the reaction between 0 and 5 °C. The temperature was allowed to rise to 25 °C, and the mixture was stirred for 4 h. The resulting slurry was cooled in an ice bath, quenched with ethyl acetate (30 mL), and poured with caution into a cooled 2 M H_2SO_4 solution. The aqueous solution was then extracted with diethyl ether (300 mL×3), dried over Na_2SO_4, and evaporated under reduced pressure to remove most of the solvent. The crude material was dissolved in methanol (500 mL), cooled to 0 °C, and treated with 30% hydrogen peroxide (265 mL, 2.6 mol). The resulting solution was treated dropwise with a 2% NaOH solution (142 mL, 0.067 mol), keeping the reaction temperature around 0 °C. The mixture was allowed to stay at 4 °C for 20 h and was then diluted with water (900 mL) and extracted with ethyl ether (450 mL × 4). The extracts were collected, washed with 5% sodium metabisulfite solution and with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by distillation under vacuum to obtain **8** (56.7 g, 78%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.59 (t, J = 4.6 Hz, 1H), 3.20 (d, J = 3.8 Hz, 1H), 2.54 (d, J = 13.1 Hz, 1H), 1.91 (part of AB system, J = 14.6 Hz, 1H), 1.87 (part of AB system, J = 14.6, 4.3, 2.1, 1H), 1.74 (d, J = 13.1, 2.1, 1.1 Hz, 1H), 0.97 (s, 3H), 0.85 (s, 3H).

2-Methoxy-5,5-dimethyl-cyclohex-2-en-1-one (9). A solution of **8** (44.0 g, 0.31 mol) in methanol (150 mL) was added to a solution of 85% potassium hydroxide (20.7 g, 0.31 mol) in methanol (450 mL) at room temperature. The mixture was kept at this temperature for 20 h. After cooling, the solution was diluted with water (1.2 L) and extracted with diethyl ether (350 mL × 5). The organic extracts were collected, washed with brine, dried over Na_2SO_4, and evaporated under vacuum to remove most of the solvent. The crude material was purified by distillation to obtain **9** (32.8 g, 68%) as an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.83 (t, J = 4.4 Hz, 1H), 3.49 (s, 3H), 2.29 (s, 2H), 2.28 (d, J = 4.4 Hz, 2H), 0.98 (s, 6H).

Ethyl 1,4,4-Trimethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxylate (10). A sample of 60% sodium hydride in mineral oil (2.41 g, 60.3 mmol) was suspended in anhydrous THF (60 mL) under argon atmosphere and treated with a solution of **9** (6.2 g, 40.2 mmol) in anhydrous THF (50 mL). After 15 min, a solution of diethyl oxalate (8.17 mL, 60.3 mmol) in anhydrous THF (50 mL) was added and the mixture was refluxed for 1 h. The slurry was diluted with water (800 mL), acidified with 1 N HCl (50 mL), and extracted with ethyl acetate (500 mL × 2). The organic layers were collected, washed with brine, dried over Na_2SO_4, and evaporated to dryness to obtain an orange oil, which was dissolved in acetic acid (65 mL) and treated dropwise with a solution of methylhydrazine (2.14 mL, 40.2 mmol) in acetic acid (20 mL). The solution was stirred at room temperature overnight. The mixture was then diluted with water (800 mL) and extracted with ethyl acetate (500 mL × 2). The organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The crude material was

chromatographed on silica gel (dichloromethane/ethyl acetate, 100/5) to obtain **10** (4.82 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.43 (q, J = 7.0 Hz, 2H), 4.19 (s, 3H), 2.61 (t, J = 6.4 Hz, 2H), 1.98 (t, J = 6.4 Hz, 2H), 1.49 (s, 6H), 1.42 (t, J = 7.0 Hz, 3H).

Ethyl (6*E*)-6-[(Dimethylamino)methylidene]-1,4,4-trimethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxylate (6b). A solution of **10** (4.8 g, 19.2 mmol) in anhydrous DMF (30 mL) was treated with *N*,*N*-dimethylformamide di-*tert*-butyl acetal (9.19 mL, 38.35 mmol) at 60 °C for 2 h. The mixture was evaporated to dryness, and the crude material was crystallized from *n*-hexane to give **6b** (5.1 g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (s, 1H), 4.27 (q, J = 7.1 Hz, 1H), 4.09 (s, 3H), 3.11 (s, 6H), 2.76 (s, 2H), 1.32 (s, 6H), 1.29 (t, J = 7.1 Hz, 3H).

Ethyl 1-Methyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (11a). Phenylguanidine carbonate (2.13 g, 13.31 mmol) was added to a suspension of compound **6a** (3.0 g, 10.81 mmol) in DMF (30 mL), and the mixture was stirred at 110 °C for 6 h. After the mixture was cooled, the solvent was evaporated and the residue was dissolved in 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 filtered to give **11a** as a white solid (2.72 g, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.42 (s, 1H), 7.69−7.71 (m, 2H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.36 (s, 3H), 4.30 (q, J = 7.2 Hz, 2H), 2.98 (t, J = 7.7 Hz, 2H), 2.84 (t, J = 7.7 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H); LCMS (ESI) m/z 350 (M + H)$^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_5O_2 + H^+$ 350.1611, found 350.1614.

Ethyl 1,4,4-Trimethyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (11b). By employment of the above-described procedure, starting from **6b**, compound **11b** was prepared. Yield, 90%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.41 (s, 1H), 7.68−7.70 (m, 2H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.34 (s, 3 H), 4.29 (q, J = 7.1 Hz, 2H), 2.73 (s, 2H), 1.32 (s, 6H), 1.31 (t, J = 7.15 Hz, 3H); LCMS (ESI) m/z 378 (M + H)$^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_5O_2 + H^+$ 378.1925, found 378.1919.

1-Methyl-8-phenylamino-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylic Acid (2). To a suspension of **11a** (0.50 g, 1.43 mmol) in anhydrous ethanol (25 mL), 1.5 M KOH in 95% ethanol (3.7 mL, 5.55 mmol) was added under good stirring, and the mixture was left at room temperature overnight. After the mixture was cooled in an ice bath, 1 N HCl was added until neutral pH was attained. Water was added and the resulting precipitate collected by filtration to give **2** (390 mg, 85%) as a white solid: mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (bs, 1H), 9.51 (s, 1H), 8.41 (s, 1H), 7.79−7.71 (m, 2H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.34 (s, 3H), 2.96 (t, J = 7.6 Hz, 2H), 2.83 (t, J = 7.6 Hz, 2H); LCMS (ESI) m/z 322 (M + H)$^+$. Anal. ($C_{17}H_{15}N_5O_2$) C, H, N.

1-Methyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (1). A solution of **2** (150 mg, 0.46 mmol) in anhydrous DMF (5 mL) was treated with *N*,*N*-diisopropylethylamine (0.12 mL, 0.69 mmol) and EDC (131 mg, 0.69 mmol). The mixture was treated with 1-hydroxybenzotriazole ammonium salt[20] (HOBt·NH_3) (105 mg, 0.69 mmol). The mixture was kept at room temperature overnight and then was diluted with water, and the resulting precipitate was collected by filtration to afford **1** (132 mg, 90%): mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.40 (s, 1H), 7.68−7.71 (m, 2H), 7.45 (bs, 1H), 7.29 (m, 2H), 7.24 (bs, 1H), 6.93−6.97 (m, 1H), 4.32 (s, 3H), 2.98 (t, J = 7.8 Hz, 2H), 2.81 (t, J = 7.8 Hz, 2H); LCMS (ESI) m/z 321 (M + H)$^+$; HRMS (ESI) calcd for $C_{17}H_{16}N_6O + H^+$ 321.1458, found 321.1458. Anal. ($C_{17}H_{16}N_6O$) C, H, N.

***N*,1-Dimethyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (16).** To a solution of **2** (150 mg, 0.46 mmol) in anhydrous DMF (5 mL) were added *N*,*N*-diisopropylethylamine (0.12 mL, 0.69 mmol), HOBT (93 mg, 0.69 mmol), EDC (131 mg, 0.69 mmol), 2 M methylamine in THF (0.345 mL, 0.69 mmol), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into water (25 mL) and extracted with dichloromethane (4 × 25 mL). The combined organic extracts were washed with water and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash chromatography on silica gel (dichloromethane/methanol, 98/2) to afford **16** (141 mg, 92%): mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.40 (s, 1H), 8.07 (q, J = 4.9 Hz, 1H), 7.69−7.71 (m, 2H), 7.30 (m, 2H), 6.96 (m, 1H), 4.33 (s, 3H), 2.99 (t, J = 7.9 Hz, 2H), 2.81 (t, J = 7.9 Hz, 2H), 2.73 (d, J = 4.9 Hz, 3H); LCMS (ESI) m/z 335 (M + H)$^+$; HRMS (ESI) calcd for $C_{18}H_{18}N_6O + H^+$ 335.1615, found 335.1627.

Compounds 17−21. By employment of the above-described procedure, starting from **2** and using suitable amines, compounds **17−21** were prepared.

***N*-Cyclopropyl-1-methyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (17).** Yield, 55%; mp 186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.40 (s, 1H), 8.13 (d, J = 4.5 Hz, 1H), 7.68−7.70 (m, 2H), 7.27−7.31 (m, 2H), 6.93−6.98 (m, 1H), 4.32 (s, 3H), 2.98 (t, J = 7.8 Hz, 2H), 2.81 (t, J = 7.8 Hz, 2H), 2.78−2.84 (m, 1H), 0.57−0.71 (m, 4H); LCMS (ESI) m/z 361 (M + H)$^+$; HRMS (ESI) calcd for $C_{20}H_{20}N_6O$ + H$^+$ 361.1772, found 361.1779. Anal. ($C_{20}H_{20}N_6O$) C, H, N.

***N*-(2-Hydroxyethyl)-1-methyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (18).** Yield, 68%; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.40 (s, 1H), 7.93 (t, J = 5.7 Hz, 1H), 7.69−7.71 (m, 2H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.73 (t, J = 5.4 Hz, 1H), 4.34 (s, 3H), 3.49 (m, 2H), 3.30 (m obscured by water signal, 2H), 2.99 (t, J = 7.9 Hz, 2H), 2.81 (t, J = 7.9 Hz, 2H); LCMS (ESI) m/z 365 (M + H)$^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_6O_2 + H^+$ 365.1721, found 365.1727; HPLC purity 90%.

***N*-Cyclopentyl-1-methyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (19).** Yield, 72%; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.40 (s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.69−7.71 (m, 2H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.33 (s, 3H), 4.17−4.25 (m, 1H), 2.98 (t, J = 7.8 Hz, 2H), 2.81 (t, J = 7.8 Hz, 2H), 1.50−1.89 (m, 8H); LCMS (ESI) m/z 389 (M + H)$^+$.

1-Methyl-*N*-phenyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (20). Yield, 60%; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 9.53 (s, 1H), 8.43 (s, 1H), 7.80−7.83 (m, 2H), 7.70−7.73 (m, 2H), 7.30−7.35 (m, 4H), 7.06−7.10 (m, 1H), 6.95−6.99 (m, 1H), 4.41 (s, 3H), 3.05 (t, J = 7.9 Hz, 2H), 2.86 (t, J = 7.9 Hz, 2H); LCMS (ESI) m/z 397 (M + H)$^+$.

***N*-Benzyl-1-methyl-8-(phenylamino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (21).** Yield, 68%; mp 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.69 (t, J = 6.3 Hz, 1H), 8.40 (s, 1H), 7.69−7.71 (m, 2H), 7.22−7.32 (m, 5H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.42 (d, J = 6.3 Hz, 2H), 4.35 (s, 3H), 3.00 (t, J = 7.9 Hz, 2H), 2.82 (t, J = 7.9 Hz, 2H); LCMS (ESI) m/z 411 (M + H)$^+$.

1,4,4-Trimethyl-8-phenylamino-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (26). To a suspension of **11b** (0.50 g, 1.32 mmol) in anhydrous ethanol (25 mL), 1.5 M KOH in 95% ethanol (2.64 mL, 3.96 mmol) was added under good stirring, and the mixture was left at room temperature overnight. After the mixture was cooled in an ice bath, 1 N HCl was added until neutral pH was attained. Water was added, and the resulting precipitate was collected by filtration (420 mg, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 1H), 9.51 (s, 1H), 8.40 (s, 1H), 7.68−7.71 (m, 2H), 7.28−7.32 (m, 2H), 6.94−6.98 (m, 1H), 4.34 (s, 3H), 2.71 (s, 2H), 1.33 (s, 6H). A solution of the above carboxylic acid (349 mg, 1.0 mmol) in anhydrous DMF (10 mL) was treated with *N*,*N*-diisopropylethylamine (0.349 mL, 2.0 mmol) and EDC (269 mg, 1.4 mmol). The mixture was treated with HOBt·NH_3 (213 mg, 1.4 mmol). The mixture was kept at room temperature for 5 h. The mixture was diluted with water and the resulting precipitate was

collected by filtration to afford **26** (320 mg, 92%) as a yellow solid: mp 158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.39 (s, 1H), 7.68−7.71 (m, 2H), 7.55 (bs, 1H), 7.28−7.31 (m, 2H), 7.29 (bs, 1H), 6.94−6.98 (m, 1H), 4.31 (s, 3H), 2.69 (s, 2H), 1.33 (s, 6H); LCMS (ESI) m/z 349 (M + H)$^+$. Anal. ($C_{19}H_{20}N_6O$) C, H, N.

Ethyl 8-Amino-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (12a). Potassium carbonate (7.87 g, 57 mmol) and guanidine hydrochloride (5.44 g, 57 mmol) were added to a solution of compound **6a** (16.0 g, 57 mmol) in DMF (60 mL), and the mixture was stirred at 110 °C for 6 h. After cooling, the mixture was poured into water (300 mL) and the resulting precipitate was collected by filtration and dried to give **12a** (14.33 g, 92% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 6.55 (bs, 2H), 4.32 (s, 3H), 4.30 (q, J = 7.1 Hz, 2H), 2.91 (t, J = 7.7 Hz, 2H), 2.74 (t, J = 7.7 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H); LCMS (ESI) m/z 274 (M + H)$^+$; HRMS (ESI) calcd for $C_{13}H_{15}N_5O_2$ + H$^+$ 274.1298, found 274.1299.

Ethyl 8-Amino-1,4,4-trimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (12b). By employment of the above-described procedure, starting from **6b**, the compound **12b** was prepared. Yield, 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 6.56 (bs, 2H), 4.31 (s, 3H), 4.29 (q, J = 7.1 Hz, 2H), 2.62 (s, 2H), 1.30 (t, J = 7.1 Hz, 3H), 1.29 (s, 6 H); LCMS (ESI) m/z 302 (M + H)$^+$; HRMS (ESI) calcd for $C_{15}H_{19}N_5O_2$ + H$^+$ 302.1611, found 302.1615.

Ethyl 8-Iodo-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxylate (13a). To a well stirred suspension of **12a** (8.8 g, 32 mmol) in dimethoxyethane (1.2 L) maintained in an inert atmosphere of argon, cesium iodide (9.13 g, 35 mmol), iodine (4.45 g, 18 mmol), copper(I) iodide (2.01 g, 10 mmol), and isopentyl nitrite (7.00 mL, 52 mmol) were added in sequence. The reaction mixture was stirred vigorously at 65 °C for 18 h. After the mixture was cooled in an ice−water bath, the solid was filtered off and the filtrate was diluted with dichloromethane (2.0 L), washed with 30% ammonium hydroxide (150 mL), sodium thiosulphate (300 mL), brine, and dried over anhydrous Na_2SO_4. Concentrating to a volume of about 100 mL of dimethoxyethane, the crude **13a** precipitated. It was then filtered and washed with dimethoxyethane. Flash chromatography on silica gel (eluant, dichloromethane/methanol, 98/2) yielded the title compound (5.65 g, 46%). ^{1}H NMR (400 MHz), DMSO-d_6), δ 8.48 (s, 1H), 4.29 (q, J = 7.1 Hz, 2H), 4.26 (s, 3H), 3.01 (m, 2H), 2.93 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H); LCMS (ESI) m/z 385 (M + H)$^+$; HRMS (ESI) calcd for $C_{13}H_{13}IN_4O_2$ + H$^+$ 385.0156, found 385.0154.

Ethyl 8-Iodo-1,4,4-trimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]-quinazoline-3-carboxylate (13b). By employment of the above-described procedure, starting from **12b**, compound **13b** was prepared. Yield, 40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 4.30 (q, J = 7.2 Hz, 2H), 4.26 (s, 3H), 2.81 (s, 2H), 1.32 (s, 6H), 1.31 (t, J = 7.2 Hz, 3H); LCMS (ESI) m/z 413 (M + H)$^+$; HRMS (ESI) calcd for $C_{15}H_{17}IN_4O_2$ + H$^+$ 413.0469, found 413.0472.

8-Iodo-1-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (14a). To a suspension of **13a** (384 mg, 1.0 mmol) in anhydrous ethanol (10 mL), 1.5 M KOH in 95% ethanol (2.0 mL, 3.0 mmol) was added under good stirring, and the mixture was left at room temperature overnight. After the mixture was cooled in an ice bath, the resulting precipitate was collected by filtration and used without further purification. A suspension of the above carboxylic acid potassium salt in anhydrous DMF (10 mL) was treated with EDC (269 mg, 1.4 mmol). HOBt·NH$_3$ (213 mg, 1.4 mmol) was added, and the mixture was kept at room temperature overnight and then diluted with water. The resulting precipitate was collected by filtration to afford **14a** (220 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.51 (bs, 1H), 7.29 (bs, 1H), 4.23 (s, 3H), 3.01 (t, J = 8.0 Hz, 2H), 2.89 (t, J = 8.0 Hz, 2H); LCMS (ESI) m/z 356 (M + H)$^+$; HRMS (ESI) calcd for $C_{11}H_{10}IN_5O$ + H$^+$ 356.0003, found 356.0005.

8-Iodo-*N*,1-dimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (15a). By employment of the above-described procedure, starting from **13a** and using methylamine, compound **15a** was prepared. Yield, 75%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 8.12 (q, J = 4.8 Hz, 1H), 4.23 (s, 3H), 3.02 (dd, J = 7.6, 6.6 Hz, 2H), 2.89 (dd J = 7.6, 6.6 Hz, 2H), 2.73 (d, J = 4.8 Hz, 3H); LCMS (ESI) m/z 370 (M + H)$^+$; HRMS (ESI) calcd for $C_{21}H_{12}IN_5O$ + H$^+$ 370.0160, found 370.0170.

8-Iodo-1,4,4-trimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (14b). Potassium carbonate (1.38 g, 10 mmol) in water (10 mL) was added to a solution of compound **13b** (1.03 g, 2.5 mmol) in dioxane (20 mL), and the mixture was stirred under reflux for 30 h. After cooling, the mixture was poured into water (100 mL) and extracted with ethyl acetate (2 × 100 mL). The aqueous phase was acidified with 2 N HCl (12 mL), and the resulting precipitate was collected by filtration, washed with water, dried, and used without further purification. To a solution of the above carboxylic acid in anhydrous DMF (10 mL) were added *N*,*N*-diisopropylethylamine (0.51 mL, 3.0 mmol), EDC (572 mg, 3.0 mmol), and HOBt·NH$_3$ (456 mg, 3.0 mmol). The mixture was kept at room temperature overnight and then diluted with water. The resulting precipitate was collected by filtration and washed with water to afford **14b** (536 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 7.51 (bs, 1H), 7.30 (bs, 1H), 4.25 (s, 3H), 2.78 (s, 2H), 1.33 (s, 6H); LCMS (ESI) m/z 384 (M + H)$^+$.

8-Iodo-*N*,1,4,4-tetramethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (15b). By employment of the above-described procedure, starting from **13b** and using methylamine, compound **15b** was prepared. Yield, 65%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 8.20 (q, J = 4.6 Hz, 1H), 4.22 (s, 3H), 2.78 (s, 2H), 2.75 (d, J = 4.6 Hz, 3H), 1.32 (s, 6H); LCMS (ESI) m/z 398 (M + H)$^+$; HRMS (ESI) calcd for $C_{14}H_{16}IN_5O$ + H$^+$ 398.0473, found 398.0476.

1-Methyl-8-{[4-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (22). Pd(OAc)$_2$ (20 mg, 0.09 mmol), (±)-BINAP (55 mg, 0.09 mmol), and DMF (5 mL) were charged in a round-bottom flask flushed with argon. The flask was evacuated and backfilled with argon. The mixture was stirred under argon for 30 min and added to a mixture of **14a** (319 mg, 0.9 mmol), 4-(4-methylpiperazin-1-yl)phenylamine (515 mg, 2.7 mmol), and potassium carbonate (1.24 g, 9.0 mmol) in DMF (10 mL). The resulting mixture was stirred at 80 °C for 3 h under argon. After cooling to room temperature, the reaction mixture was filtered on a pad of Celite. The solvent was concentrated, and the crude solid was purified by flash chromatography on silica gel (eluant, dichloromethane/methanol, 95/5) to afford **22** (230 mg, 61%): mp 211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (bs, 1H), 8.33 (s, 1H), 7.52 (m, 2H), 7.46 (bs, 1H), 7.25 (bs, 1H), 6.90 (m, 2H), 4.31 (s, 3H), 3.05−3.07 (m, 4H), 2.97 (t, J = 7.9 Hz, 2H), 2.78 (t, J = 7.9 Hz, 2H), 2.46−2.48 (m, 4H), 2.23 (s, 3H); LCMS (ESI) m/z 419 (M + H)$^+$; HRMS (ESI) calcd for $C_{22}H_{26}N_8O$ + H$^+$ 419.2303, found 419.2297.

***N*,1-dimethyl-8-{[4-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (23).** By employment of the above-described procedure, starting from **15a**, compound **23** was prepared. Yield, 65%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.34 (s, 1H), 8.08 (q, J = 4.7 Hz, 1H), 7.55 (m, 9.2 Hz, 2H), 6.93 (d, J = 9.2 Hz, 2H), 4.33 (s, 3H), 3.04−3.08 (m, 4H), 2.97 (t, J = 7.9 Hz, 2H), 2.78 (t, J = 7.9 Hz, 2H), 2.75 (d, J = 4.7 Hz, 3H), 2.45−2.50 (m, 4H), 2.24 (s, 3H); LCMS (ESI) m/z 433 (M + H)$^+$; HRMS (ESI) calcd for $C_{23}H_{28}N_8O$ + H$^+$ 433.2459, found 433.2459.

Compounds 24 and 25. By employment of the above-described procedure, starting from **14a** and using the suitable substituted aniline, compounds **24** and **25** were prepared.

1-Methyl-8-{[3-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (24). Yield, 52%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34

(bs, 1H), 8.39 (s, 1H), 7.43 (bs, 1H), 7.26−7.28 (m, 2H), 7.26 (bs, 1H), 7.15 (t, J = 8.0 Hz, 1H), 6.60 (d, J = 8.4 Hz, 1H), 4.33 (s, 3H), 3.0−3.5 (bs obscured by water signal, 4H), 2.98 (t, J = 7.8 Hz, 2H), 2.85−2.95 (bs, 4H), 2.80 (t, J = 7.8 Hz, 2H), 2.52−2.59 (bs, 3H); LCMS (ESI) m/z 419 $(M + H)^+$; HRMS (ESI) calcd for $C_{22}H_{26}N_8O + H^+$ 419.2303, found 419.2306.

1-Methyl-8-[(4-morpholin-4-ylphenyl)amino]-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (25). Yield, 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (bs, 1H), 8.34 (s, 1H), 7.52−7.56 (m, 2H), 7.44 (bs, 1H), 7.23 (bs, 1H), 6.89−6.93 (m, 2H), 4.31 (s, 3H), 3.73−3.77 (m, 4H), 3.03−3.05 (m, 4H), 2.97 (t, J = 7.8 Hz, 2H), 2.78 (t, J = 7.8 Hz, 2H); LCMS (ESI) m/z 406 $(M + H)^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_7O_2 + H^+$ 406.1986, found 406.1991; HPLC purity 90%.

1,4,4-Trimethyl-8-{[4-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (27). By employment of the above-described procedure, starting from **14b** and using the suitable substituted aniline, tcompound **27** was prepared. Yield, 54%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (bs, 1H), 8.32 (s, 1H), 7.53 (bs, 1H), 7.49−7.53 (m, 2H), 7.27 (bs, 1H), 6.87−6.91 (m, 2H), 4.29 (s, 3H), 3.06−3.08 (m, 4H), 2.66 (s, 2H), 2.48−2.55 (m partially obscured by DMSO, 4H), 2.24 (s, 3H), 1.32 (s, 6H); LCMS (ESI) m/z 447 $(M + H)^+$; HRMS (ESI) calcd for $C_{24}H_{30}N_8O + H^+$ 447.2616, found 447.2616.

Compounds 28−41. By employment of the above-described procedure, starting from **15b** and using the suitable substituted aniline, the compounds **28**−**41** were prepared.

***N*,1,4,4-Tetramethyl-8-{[4-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (28).** Yield, 65%; mp 190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (bs, 1H), 8.32 (s, 1H), 8.13 (q, J = 4.8 Hz, 1H), 7.49−7.54 (m, 2H), 6.88−6.92 (m, 2H), 4.29 (s, 3H), 3.05−3.10 (m, 4H), 2.74 (d, J = 4.8 Hz, 3H), 2.66 (bs, 2H), 2.44−2.54 (m obscured by DMSO, 4H), 2.26 (bs, 3H), 1.32 (s, 6H); LCMS (ESI) m/z 461 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{32}N_8O + H^+$ 461.2772, found 461.2772. Anal. ($C_{25}H_{32}N_8O$) C, H, N.

***N*,1,4,4-Tetramethyl-8-{[3-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (29).** Yield, 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H), 8.37 (s, 1H), 8.14 (q, J = 4.9 Hz, 1H), 7.24 (dd, J = 8.0, 2.2 Hz, 1H), 7.21 (t, J = 2.2 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 6.56 (dd, J = 8.0, 2,2 Hz, 1H), 4.31 (s, 3H) 3.09−3.13 (m, 4H), 2.74 (d, J = 4.9 Hz, 3H), 2.67 (bs, 2H) 2.50−2.54 (m obscured by DMSO, 4H), 2.25 (bs, 3H), 1.32 (s, 6H); LCMS (ESI) m/z 461 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{32}N_8O + H^+$ 461.2772, found 461.2780.

***N*,1,4,4-Tetramethyl-8-[(4-morpholin-4-ylphenyl)amino]-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (30).** Yield, 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.33 (s, 1H), 8.13 (q, J = 4.9 Hz, 1H), 7.52−7.56 (m, 2H), 6.89−6.93 (m, 2H), 4.29 (s, 3H), 3.73−3.75 (m, 4H), 3.03−3.05 (m, 4H), 2.74 (d, J = 4.9 Hz, 3H), 2.66 (s, 2H), 1.32 (s, 6H); LCMS (ESI) m/z 448 $(M + H)^+$; HRMS (ESI) calcd for $C_{24}H_{29}N_7O_2 + H^+$ 448.2456, found 448.2455.

***N*,1,4,4-Tetramethyl-8-[(3-morpholin-4-ylphenyl)amino]-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (31).** Yield, 71%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.38 (s, 1H), 8.14 (q, J = 4.6 Hz, 1H), 7.28 (dd, J = 8.0, 2.0 Hz, 1H), 7.21 (t, J = 2.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 6.57 (dd, J = 8.0, 2.0 Hz, 1H), 4.31 (s, 3H), 3.73−3.75 (m, 4H), 3.07−3.09 (m, 4H), 2.74 (d, J = 4.6 Hz, 3H), 2.68 (s, 2H), 1.32 (s, 6H); LCMS (ESI) m/z 448 $(M + H)^+$; HRMS (ESI) calcd for $C_{24}H_{29}N_7O_2 + H^+$ 448.2456, found 448.2454.

***N*,1,4,4-Tetramethyl-8-({4-[(1-methylpiperidin-4-yl)oxy]phenyl}amino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (32).** Yield, 36%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H), 8.34 (s, 1H), 8.13 (q, J = 4.8 Hz, 1H), 7.54−7.58 (m, 2H), 6.90−6.94 (m, 2H), 4.34−4.41 (m, 1H), 4.28 (s, 3H), 2.82−2.93 (bs, 2H), 2.74 (d, J = 4.8 Hz, 3H), 2.67 (s, 2H), 2.37−2.47 (bs, 5H), 1.93−2.02 (bs, 2H), 1.66−1.81 (bs, 2H), 1.32 (s, 6H); LCMS (ESI) m/z 476 $(M + H)^+$; HRMS (ESI) calcd for $C_{26}H_{33}N_7O_2 + H^+$ 476.2768, found 476.2767; HPLC purity 95%.

***N*,1,4,4-Tetramethyl-8-({3-[(1-methylpiperidin-4-yl)oxy]phenyl}amino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (33).** Yield, 30%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.40 (s, 1H), 8.14 (q, J = 4.80 Hz, 1H), 7.40 (t, J = 2.1 Hz, 1H), 7.26 (ddd, J = 8.2, 2.1, 0.7 Hz, 1 H), 7.17 (t, J = 8.2 Hz, 1H), 6.55 (ddd, J = 8.2, 2.1, 0.7 Hz, 1 H), 4.33 (s, 3H), 4.28−4.34 (m, 1H), 2.74 (d, J = 4.8 Hz, 3H), 2.69 (s, 2H), 2.58−2.66 (m, 2H), 2.18−2.25 (bs, 2H), 2.20 (s, 3H), 1.91−1.99 (m, 2H), 1.60−1.70 (m, 2H), 1.33 (s, 6H); LCMS (ESI) m/z 476 $(M + H)^+$; HRMS (ESI) calcd for $C_{26}H_{33}N_7O_2 + H^+$ 476.2768, found 476.2776.

***N*,1,4,4-Tetramethyl-8-({4-[(4-methylpiperazin-1-yl)methyl]phenyl}amino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (34).** Yield, 55%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.38 (s, 1H), 8.15 (q, J = 4.8 Hz, 1H), 7.62−7.65 (m, 2H), 7.19−7.22 (m, 2H), 4.31 (s, 3H), 3.40 (s, 2H), 2.75 (d, J = 4.8 Hz, 3H), 2.69 (s, 2H), 2.26−2.45 (bs, 8H), 2.18 (bs, 3H), 1.33 (s, 6H); LCMS (ESI) m/z 475 $(M + H)^+$; HRMS (ESI) calcd for $C_{26}H_{34}N_8O + H^+$ 475.2928, found 475.2932.

***N*,1,4,4-Tetramethyl-8-({3-[(4-methylpiperazin-1-yl)methyl]phenyl}amino)-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (35).** Yield, 62%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.39 (s, 1H), 8.14 (q, J = 4.8 Hz, 1H), 7.73 (bs, 1H), 7.55 (d, J = 7.8, Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 4.33 (s, 3H), 3.41 (s, 2H), 2.75 (d, J = 4.8 Hz, 3H), 2.69 (s, 2H), 2.26−2.47 (bs, 8H), 2.17 (bs, 3H), 1.33 (s, 6H); LCMS (ESI) m/z 475 $(M + H)^+$; HRMS (ESI) calcd for $C_{26}H_{34}N_8O + H^+$ 475.2928, found 475.2931.

***N*,1,4,4-Tetramethyl-8-{[4-(morpholin-4-ylmethyl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (36).** Yield, 64%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.38 (s, 1H), 8.14 (q, J = 4.9 Hz, 1H), 7.63−7.68 (m, 2H), 7.19−7.25 (m, 2H), 4.31 (s, 3H), 3.56−3.61 (m, 4H), 3.40 (s, 2H), 2.74 (d, J = 4.9 Hz, 3H), 2.69 (s, 2H), 2.30−2.37 (bs, 4H), 1.32 (s, 6H); LCMS (ESI) m/z 462 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{31}N_7O_2 + H^+$ 462.2612, found 462.2621.

***N*,1,4,4-Tetramethyl-8-{[3-(morpholin-4-ylmethyl)phenyl]amino}-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (37).** Yield, 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.40 (s, 1H), 8.14 (q, J = 4.8 Hz, 1H), 7.74 (bs, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 4.34 (s, 3H), 3.54−3.60 (bs, 4H), 3.42 (bs, 2H), 2.74 (d, J = 4.8 Hz, 3H), 2.69 (s,2H), 2.33−2.40 (bs, 4H), 1.33 (s, 6H); LCMS (ESI) m/z 462 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{31}N_7O_2 + H^+$ 462.2612, found 462.2618; HPLC purity 95%.

8-{[4-(Dimethylamino)phenyl]amino}-*N*,1,4,4-tetramethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (38). Yield, 44%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.40 (s, 1H), 8.16 (q, J = 4.8 Hz, 1H), 7.55 (d, J = 9.2 Hz, 2H), 6.93 (d, J = 9.2 Hz, 2H), 4.33 (s, 3H), 2.88 (s, 6H), 2.76 (d, J = 4.8 Hz, 3H), 2.67 (s, 2H), 1.32 (s, 6H); LCMS (ESI) m/z 406 $(M + H)^+$; HRMS (ESI) calcd for $C_{22}H_{27}N_7O + H^+$ 406.2350, found 406.2349.

8-{[3-(Dimethylamino)phenyl]amino}-*N*,1,4,4-tetramethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (39). Yield, 52%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.37 (s, 1H), 8.14 (q, J = 4.8 Hz, 1H), 7.18 (dd, J = 8.2, 2.1 Hz, 1H), 7.09 (t, J = 8.2 Hz, 1H), 6.97 (t, J = 2.1 Hz, 1H), 6.37 (dd, J = 8.2, 2.1 Hz, 1H), 4.32 (s, 3H), 2.88 (s, 6H), 2.74 (d, J = 4.8 Hz, 3H), 2.68 (s, 2H), 1.32 (s, 6H); LCMS (ESI) m/z 406 $(M + H)^+$; HRMS (ESI) calcd for $C_{22}H_{27}N_7O + H^+$ 406.2350, found 406.2346; HPLC purity 90%.

8-({4-[(Dimethylamino)methyl]phenyl}amino)-*N*,1,4,4-tetramethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (40). Yield, 54%; mp 161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (bs, 1H), 8.39 (s, 1H), 8.15 (q, J = 4.8 Hz, 1H),

7.64−7.68 (m, 2H), 7.20−7.25 (m, 2H), 4.32 (s, 3H), 3.43 (bs, 2H), 2.74 (d, $J = 4.8$ Hz, 3H), 2.69 (s, 2H), 2.21 (bs, 6H), 1.32 (s, 6H); LCMS (ESI) m/z 420 $(M + H)^+$; HRMS (ESI) calcd for $C_{23}H_{29}N_7O + H^+$ 420.2506, found 420.2509.

8-({3-[(Dimethylamino)methyl]phenyl}amino)-*N*,1,4,4-tetramethyl-4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline-3-carboxamide (41). Yield, 62%; mp 181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.39 (s, 1H), 8.14 (q, $J = 4.8$ Hz, 1H), 7.76 (s, 1H), 7.53 (d, $J = 7.8$ Hz, 1H), 7.23 (t, $J = 7.8$ Hz, 1H), 6.88 (d, $J = 7.8$ Hz, 1H), 4.33 (s, 3H), 3.34−3.38 (s obscured by water, 2H), 2.75 (d, $J = 4.8$ Hz, 3H), 2.69 (s, 2H), 2.17 (s, 6H), 1.33 (s, 6H); LCMS (ESI) m/z 420 $(M + H)^+$; HRMS (ESI) calcd for $C_{23}H_{29}N_7O + H^+$ 420.2506, found 420.2498.

Registry Numbers (RN). 1,2-Cyclohexanedione (RN, 765-87-7), 5,5-dimethyl-1,3-cyclohexanedione (RN, 126-81-8), phenylguanidine carbonate (RN, 14018-90-7), guanidine hydrochloride (RN, 50-01-1), 4-(4-methylpiperazin-1-yl)aniline (RN, 16153-81-4), 3-(4-methylpiperazin-1-yl)aniline (RN, 148546-99-0), 4-morpholin-4-ylaniline (RN, 2524-67-6), 3-morpholin-4-ylaniline (RN, 159724-40-0), 4-[(1-methylpiperidin-4-yl)oxy]aniline (RN, 358789-72-7), 3-[(1-methylpiperidin-4-yl)oxy]aniline (RN, 790667-66-2), 4-[(4-methylpiperazin-1-yl)methyl]aniline (RN, 70261-82-4), 3-[(4-methylpiperazin-1-yl)methyl]aniline (RN, 198281-55-9), 4-(morpholin-4-ylmethyl)aniline (RN, 51013-67-3), 3-(morpholin-4-ylmethyl)-aniline (RN, 123207-48-7), *N*,*N*-dimethylbenzene-1,4-diamine (RN, 536-46-9), *N*,*N*-dimethylbenzene-1,3-diamine (RN, 3575-32-4), 4-[(dimethylamino)methyl]aniline (RN, 6406-74-2), 3-[(dimethylamino)methyl]aniline (RN, 27958-77-6).

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 c-ABL, AKT1, ALK, Aur-A, Aur-B, CDC7, CDK2/A, CDK1/B, CDK2/E, CDK4/D1, CDK5/p25, CDK7/H, CHK1, CK2, EGFR, ERK2, FGFR1, GSK3β, IGF1R, IKKi, IKK2, IR, C-KIT, LCK, LYN, MAP-KAPK2, MET, NEK-6, NIM, PAK4, PDGFR, PDK1, PKAα, PKCβ, PLK1, P38α, P38β, RET, STLK2, SULU1, TRKA, VEGFR2, VEGFR3, and ZAP-70.

In Vitro Pharmacology. A2780 Cells Proliferation Assay. Cells were seeded into 96- or 384-well plates at final concentration ranging from 10 000 to 30 000 cells per cm^2 in appropriate medium plus 10% FCS. After 24 h cells were treated using serial dilution of compounds in two replicates. At 72 h after treatment the amount of cells were evaluated using the Cell Titer_Glo assay (Promega). IC_{50} values were calculated using a sigmoidal fitting (Assay Explorer MDL). Experiments were replicated at least two times.

Flow Cytometry Analysis and BrdU Incorporation. A2780 cells (human adenocarcinoma ovary, from ECACC) were seeded in T-75 tissue culture flasks, 25 000 cells/cm^2 in RPMI 1640, pH 7.4, 10% FBS, 2 mM L-glutamine, 1× penicillin−streptomycin, and maintained in 5% CO_2 at 37 °C with 96% relative humidity. After 24 h, cells were treated with compounds at 1 μM for 24 h. Cells in the supernatant and adherent cells were collected using 0.25% trypsin and 0.02% EDTA. Cells were washed with PBS and were divided into three samples for flow cytometry analysis and for immunoblot and BrdU incorporation, as previously described.[21]

In Vivo Pharmacology. Evaluation of antitumor efficacy was performed as previously described.[21]

High-Throughput Solubility. Solubility at pH 7 was performed as previously described.[21]

Metabolic Stability. Compounds were dissolved in DMSO at 10 μM. Human cDNA expressed cytochrome P450 isoforms (supersomes) were purchased from Gentest (Woburn, MA). All chemicals used 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 known 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 performed as previously described.[21]

In Vivo Pharmacokinetics. The pharmacokinetic profiles of the compounds were 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). 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 compounds **2** and **28**; elemental analyses results of compounds **1**, **2**, **17**, **26**, **28**; kinase selective profile of compound **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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