Identification of 4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3*S*)-3-piperidinylmethyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-methyl-3-butyn-2-ol (GSK690693), a Novel Inhibitor of AKT Kinase

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Overexpression of AKT has an antiapoptotic effect in many cell types, and expression of dominant negative AKT blocks the ability of a variety of growth factors to promote survival. Therefore, inhibitors of AKT kinase activity might be useful as monotherapy for the treatment of tumors with activated AKT. Herein, we describe our lead optimization studies culminating in the discovery of compound **3g** (GSK690693). Compound **3g** is a novel ATP competitive, pan-AKT kinase inhibitor with IC_{50} values of 2, 13, and 9 nM against AKT1, 2, and 3, respectively. An X-ray cocrystal structure was solved with **3g** and the kinase domain of AKT2, confirming that **3g** bound in the ATP binding pocket. Compound **3g** potently inhibits intracellular AKT activity as measured by the inhibition of the phosphorylation levels of GSK3β. Intraperitoneal administration of **3g** in immunocompromised mice results in the inhibition of GSK3β phosphorylation and tumor growth in human breast carcinoma (BT474) xenografts.

Introduction

AKT kinases have been implicated as key mediators of proliferation and survival pathways that are critical for cancer growth. As such, aberrant activation of AKT pathway has been observed in a variety of malignancies including breast, ovarian, prostate, pancreatic, and skin cancers. For example, increased AKT1 activity has been observed in about 40% of breast and ovarian cancers and >50% of prostate carcinomas. In addition, activation of AKT2 kinase has been observed in 30−40% of ovarian and pancreatic cancers whereas increased AKT3 enzymatic activity was found in estrogen-receptor-deficient breast cancer and androgen-insensitive prostate cancer cell lines. Furthermore, overexpression of AKT has an antiapoptotic effect in many cell types and expression of dominant negative AKT blocks the ability of a variety of growth factors to promote survival.[1] Perturbations in both positive and negative regulators of AKT activity commonly occur in human cancers.[2−4] Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a critical negative regulator of AKT, is lost in many cancers, including breast and prostate carcinomas, glioblastomas, and several cancer syndromes including Bannayan−Zonana syndrome, Cowden disease, and Lhermitte−Duclos disease.[5,6]

The importance of AKT mediated signaling in tumor proliferation and survival makes AKT kinases promising targets for therapeutic intervention.[7−9] Therefore, inhibitors of AKT kinase activity could be useful for the treatment of tumors with activated AKT. In fact, several small molecule AKT inhibitors have recently been reported.[10−13] Herein, we describe our lead optimization studies, which starting from compounds **1** and **2**, culminated in the discovery of compound **3g** (GSK690693) (Figure 1). Compound **3g** is a novel ATP competitive, pan-AKT kinase inhibitor with IC_{50} values of 2, 13, and 9 nM against AKT1, 2 and 3, respectively. Compound **3g** inhibits proliferation and induces apoptosis in a subset of tumor cells with activity that is consistent with intracellular inhibition of AKT kinase activity. Compound **3g** also demonstrated pharmacodynamic activity in vivo and has been shown to inhibit BT474 tumor xenograft growth in mice.[16] Compound **3g** is currently in clinical trials as an iv agent to treat patients with solid tumors or hematological malignancies.

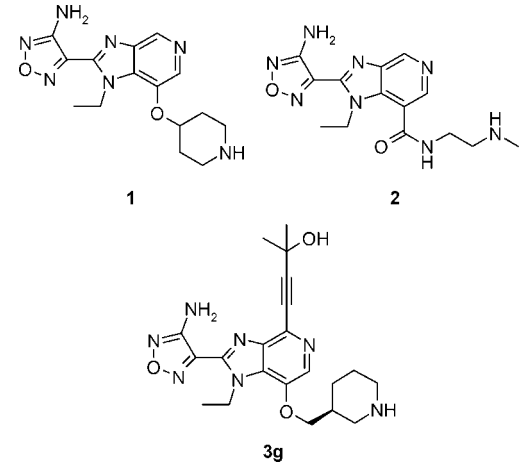

Figure 1. Lead structures.

Chemistry

Compounds **2** and **4**−**9** were prepared according to literature methods.[17,18] The remaining compounds were prepared according to Schemes 1−6. The synthesis of

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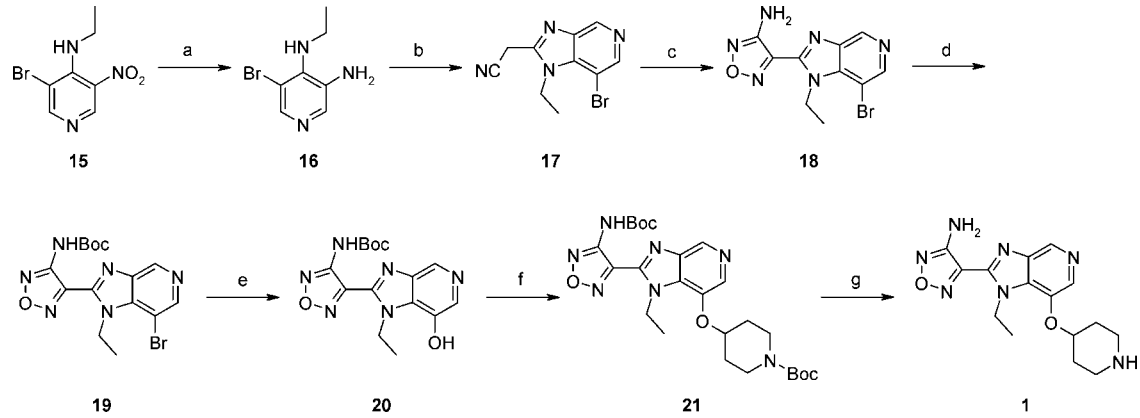
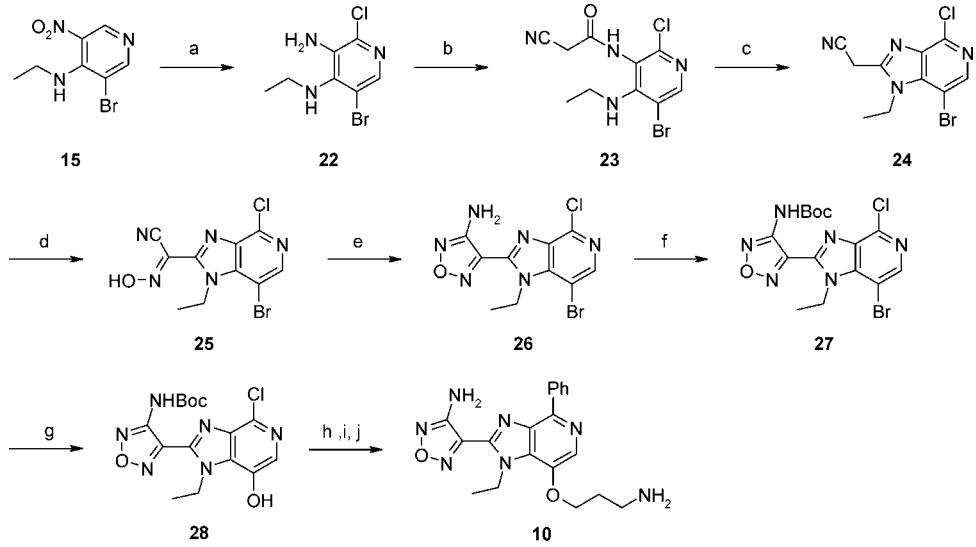
Scheme 1. Preparation of Compound **1**[a]



[a] Reagents and conditions: (a) Fe powder, AcOH, 75 °C, 1 h (93%); (b) ethyl cyanoacetate, 190 °C, 1 h (59%); (c) (i) $NaNO_2$, MeOH; (ii) NH_2OH/NaOH, 90 °C, 2 h (67%); (d) $(Boc)_2O$, DMAP, pyridine, dichloroethane, 90 °C, 18 h (58%); (e) (i) *n*-BuLi, THF, −78 °C; (ii) $B(OMe)_3$, THF, −78 °C to room temp, 1.5 h; (iii) H_2O_2/aq NaOH, room temp, 0.5 h (83%); (f) 1,1-dimethylethyl 4-hydroxy-1-piperidinecarboxylate, diethyl azadicarboxylate, polymer bound Ph_3P, CH_2Cl_2, 0 °C, 1.5 h (43%); (g) TFA, CH_2Cl_2, room temp, 40 min (97%).

Scheme 2. Preparation of Compound **10** and Key Intermediate **26**[a]



[a] Reagents and conditions: (a) $SnCl_2$, HCl, room temp, 1 h (80%); (b) cyanoacetic acid, EDC, NMM, DMF, 0 °C to room temp, 3 h (quant); (c) AcOH, 90 °C, 1 h (88%); (d) $NaNO_2$, HCl, room temp, 0.5 h (99%); (e) (i) aq NH_2OH, Et_3N, THF, 90 °C, 1.5 h; (ii) Et_3N, dioxane, 150 °C, 1.5 h (52%); (f) $(Boc)_2O$, pyridine, DMAP, dichloroethane, 85 °C, 1 h (78%); (g) (i) *n*-BuLi, THF, −100 °C; (ii) $B(OMe)_3$, THF, −100 °C to room temp, 1.5 h; (iii) H_2O_2/NaOH, room temp, 1 h (84%); (h) $PhB(OH)_2$, $Pd(Ph_3P)_4$, Na_2CO_3, dioxane, 90 °C, 18 h (75%); (i) 1,1-dimethylethyl (3-hydroxypropyl)carbamate, polymer-bound Ph_3P, DEAD, CH_2Cl_2, 0 °C, 1 h; (j) TFA, CH_2Cl_2, room temp, 2 h (27%, three steps).

compound **1** started from 3-bromo-*N*-ethyl-5-nitro-4-pyridinamine[2] (**15**) using established methods to append the 4-amino-1,2,5-oxadiazole.[19,20]

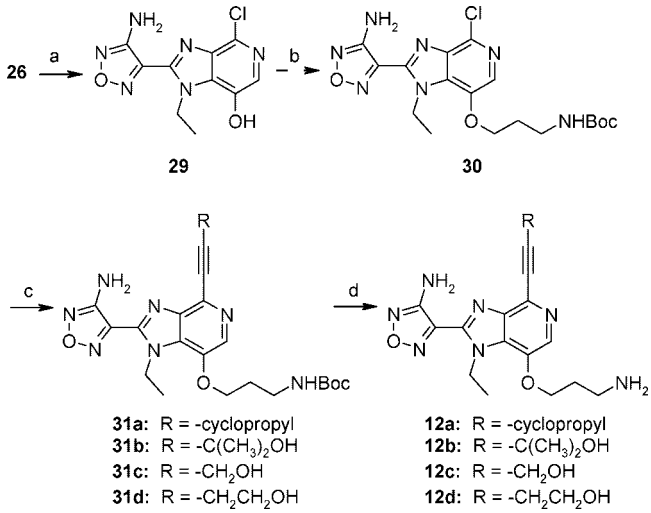
The synthesis of compound **10** and a key intermediate **26** was carried out as detailed in Scheme 2. Notable features include a tin(II) chloride mediated reduction of the nitro group in **15** with concomitant chlorination to give **22**.[21] The 4-amino-1,2,5-oxadiazole moiety was introduced as noted above to give **26**. The phenol moiety in **28** was introduced by a selective halogen−metal exchange followed by reaction with trimethoxy borane and subsequent oxidation. Reaction with phenylboronic acids under Suzuki[22] conditions followed by a Mitsunobu reaction with 1,1-dimethylethyl (3-hydroxypropyl)carbamate gave **10** after removal of the protecting groups.

The alkyne-containing analogues **12a**−**d** were synthesized in a similar manner starting from **26** (Scheme 3). Subsequent to the preparation of **28**, we discovered that it was not necessary to protect the amino group in the 4-amino-1,2,5-oxadiazole moiety during the halogen−metal exchange reaction. Therefore, **26** was converted directly to the corresponding phenolic compound **29**. A Cs_2CO_3-mediated alkylation with the corresponding alkyl halide followed by a reaction of the product with an appropriate terminal acetylene under Sonogashira conditions[23] gave the penultimate products. Trifluoroacetic acid mediated deprotection gave the final compounds **12a**−**d**.

The alkyne-containing analogues **3a**−**h** were also prepared from **29** (Scheme 4). Compound **29** was alkylated with an appropriate Boc-protected alkyl chloride or bromide. A Sonogashira reaction with 2-methyl-3-butyn-2-ol followed by removal of the protecting group gave compounds **3a**−**h**.

The heteroaryl containing analogues **11a**−**d** were prepared by reacting suitably protected arylboronic acids or esters with **30** (Scheme 5). Removal of the protecting groups then furnished the final compounds.

Scheme 3. Preparation of compounds **12a**−**d**[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, THF, −100 °C; (ii) $B(OMe)_3$, THF, −100 °C to room temp, 3 h; (iii) H_2O_2/NaOH, room temp, 45 min (59%); (b) 1,1-dimethylethyl (3-bromopropyl)carbamate, Cs_2CO_3, DMF, 35 °C, 15 h (77%); (c) H−C≡C−R, *i*-Pr_2NH, $Pd(PPh_3)_4$, dioxane, 110 °C, 2 h; (d) TFA, CH_2Cl_2.

Compounds **14a**,**b** were prepared by first alkylating **29** with 1,3-dibromopropane to give **35** (Scheme 6). Reaction with 4-methoxyphenethyl amine or (2*S*)-3-amino-1,2-propanediol followed by a Sonogashira reaction with 2-methyl-3-butyn-2-ol completed the syntheses.

Results and Discussion

Lead Identification. The oxadiazole-containing compounds **1** and **2** were identified from the GSK compound collection as starting points for lead optimization, as both compounds were submicromolar inhibitors of AKT1 (Table 1). Furthermore, compounds structurally related to **1** and **2** have previously been described as ATP competitive inhibitors that bind in the ATP binding pocket of ROCK1 and MSK1 kinase.[18−20] Not surprisingly, potent MSK1 and ROCK1 inhibition was also observed with **1** and **2** (Table 1).

Side Chain Modifications at Position-7 To Optimize AKT Potency. A model of **1** in the ATP binding pocket of AKT2 was generated on the basis of the published cocrystal structure of an activated form of the AKT kinase domain containing a S474D mutation with GSK3 peptide and AMP-PNP.[24] The amine side chain of **1** was in proximity to the side chain carboxylic acid of Glu236 and the backbone carbonyl of Glu279 (Figure 2A). Initially, the lead optimization effort focused on increasing AKT kinase potency by varying the presentation of the amine-containing side chain to further optimize this interaction.

A spacer consisting of four or five atoms between the imidazopyridine core and the amino group gave analogues with low micromolar to submicromolar IC_{50} values against the AKT isoforms (Table 2). There was no obvious preference for a primary versus secondary amine. There was a trend toward poorer AKT activity in the amide series when the side chain was constrained into a ring (**2** versus **4**, **5**, **6**, and **8**). An ether link between the amine containing side chain and the imidazopyridine core consistently gave better AKT potency over the corresponding amide or alkylamine linked analogues (**1** versus **5**; **7** versus **8** or **9**).

Back-Pocket Selectivity Element. While modifications at the 7-position of the imidazopyridine core influenced AKT activity, they had very little impact on kinase selectivity as measured by inhibition of MSK1 and ROCK1. Inspection of **1** docked into AKT2 revealed that the vector projecting from C4 of the imidazopyridine ring system was positioned to introduce a substituent into a pocket in the back cleft (Figure 2B).[25] In AKT, a leucine residue lines the back of this pocket, whereas the corresponding residue in ROCK1 is a methionine. We reasoned that we could take advantage of this residue difference and improve selectivity for AKT by introducing a steric element at C4 of the imidazopyridine core.

The side chain at C7 was fixed as an aminopropyl ether, while various groups were introduced at C4 of the imidazopyridine core (Table 3). In terms of AKT activity, there was a clear preference for five-membered over six-membered aryl rings in the back pocket (**11a**−**c** compared to **10**). The nature and position of heteroatoms within the five-membered ring aryl groups impacted AKT activity. For example, pyrrole-containing analogues (**11a** and **11c**) were slightly better inhibitors than the corresponding furanyl analogue **11b**. In contrast, the pyrazole substituent **11d** was not tolerated. Although AKT activity comparable to **1** and **7** could be maintained by introducing certain five-membered ring heteroaromatic groups at C4 of the imidazopyridine core, only modest improvements in kinase selectivity were achieved. We speculate that the five-membered heteroaryl rings did not penetrate deep enough into the back pocket to significantly impact the kinase selectivity with respect to ROCK1.

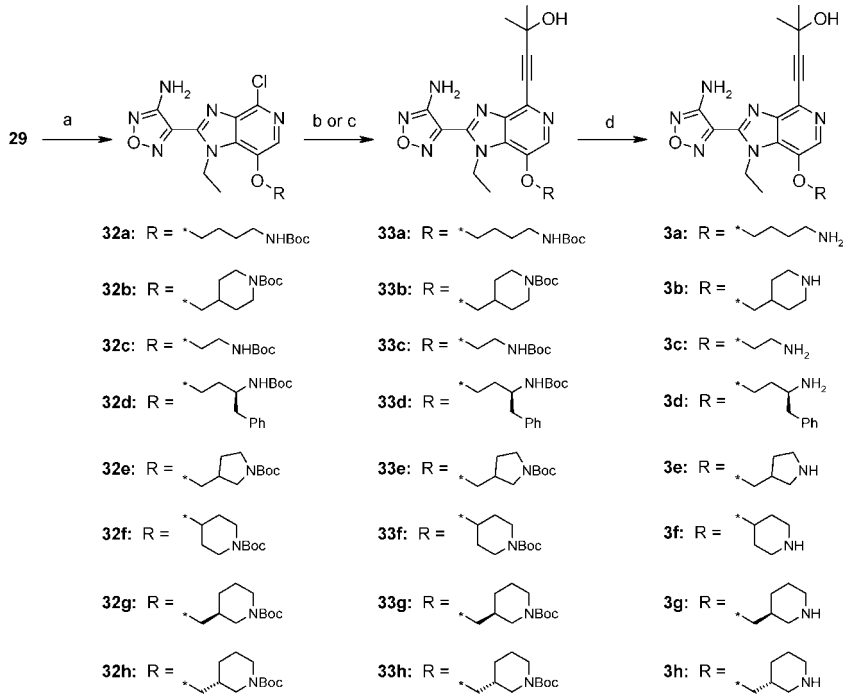
Closer inspection of the model of **1** in AKT2 revealed that the opening into the back pocket was narrow. We reasoned that an alkyne spacer would fit better through this opening and potentially allow a range of substituents to be introduced at the terminus of the triple bond to explore interactions within this pocket. Indeed, the cyclopropylalkyne **12a** retained AKT activity while significantly improving kinase selectivity against ROCK1 and RSK1. Introducing an alcohol at the terminus of the alkyne to give **12b** substantially boosted AKT activity over **12a** and further increased selectivity against ROCK and RSK1 by 83 and >200-fold, respectively. It was also clear that the geminal dimethyl groups contributed significantly to the selectivity (**12b** versus **12c**). Adding an extra methylene between the alkyne and the carbinol moiety (**12d**) was detrimental to AKT activity, as was the introduction of an amine (**13**).

Side Chain Modification To Optimize Cell Potency. The compounds listed in Table 3 were also evaluated for their ability to inhibit AKT-mediated phosphorylation of GSK3β in BT474 cells.[7] By use of this assay, some of the more potent AKT analogues demonstrated low micromolar inhibition of GSK3β phosphorylation (**11a**, **11c**, and **12a**−**c**). Having identified the 2-methyl-3-butyn-2-ol group as a key kinase selectivity element, efforts were redirected to further SAR exploration around the amine-containing side chain at position-7 of the imidazopyridine core to try to increase cellular potency.

In general, relatively minor changes in AKT activity and kinase selectivity were seen as a result of the structural modifications at R^7 (Table 4). However, these changes did impact cellular activity. For example, introducing a pendant aromatic ring (**3d** and **14a**) or including a cyclic constraint (**3f** and **3g**) led to an increase in the inhibition of GSK3β phosphorylation in BT474 cells over the simple linear side chain (**12b**). Incorporation of polar functionality into the amine-containing side chain, as exemplified by **14b**, was detrimental to cell activity overall.

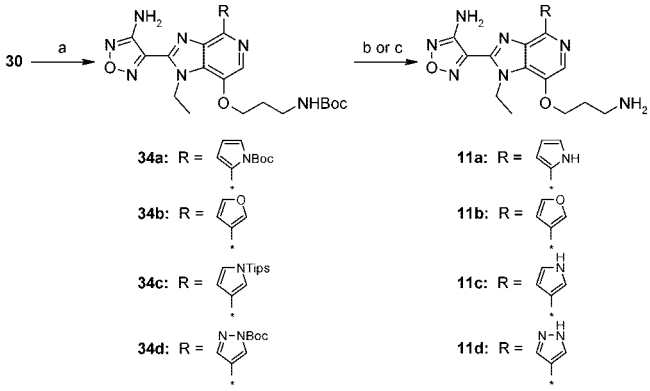
As these compounds were shown to inhibit AKT-mediated phosphorylation of GSK3β in BT474 cells, they were also tested

Scheme 4. Preparation of Compounds **3a**−**h**[a]



[a] Reagents and conditions: (a) R-Br or R-Cl, Cs_2CO_3, DMF, 35 °C, 15 h; (b) 2-methyl-3-butyn-2-ol, $Pd(Ph_3P)_4$, *i*-Pr_2NH, dioxane, 110 °C, 2 h; (c) 2-methyl-3-butyn-2-ol, $Pd(Ph_3P)_4$, Zn dust, NaI, DBU, Et_3N, DMSO, 80 °C, 3 h; (d) TFA, CH_2Cl_2.

Scheme 5. Preparation of Compounds **11a**−**d**[a]



[a] Reagents and conditions: (a) R-$B(OH)_2$ or R-(tetramethyldioxaborolane), $Pd(Ph_3P)_4$, K_2CO_3, dimethoxyethane, 90 °C, 5 h; (b) TFA, CH_2Cl_2; (c) (i) TFA, CH_2Cl_2; (ii) *n*-Bu_4NF, THF.

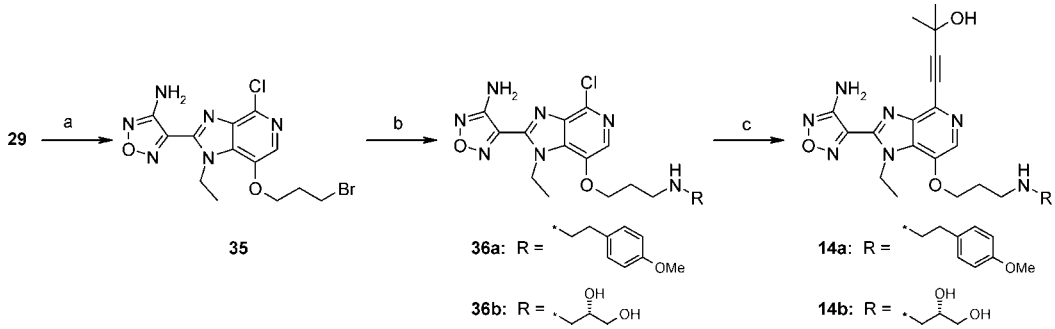
for their ability to inhibit cellular proliferation.[7] In fact, **3b**−**d**,**g**,**h** and **14a** inhibited proliferation of BT474[26] and LNCaP[27] cells with submicromolar IC_{50} values. The antiproliferative effects of these compounds against human foreskin fibroblast (HFF) cells were also measured and used as a surrogate for general cytotoxicity, as these are nontumor derived cells. Although the aminoethyl side chain analogue **3c** was potent in the BT474 and LNCaP proliferation assays, it exhibited a poorer window of selectivity against HFF cells. Therefore, compound **3g** was selected for further profiling, as it represented the best combination of cellular potency and selectivity within this set of analogues.

Biochemical Characterization of 3g. To estimate the selectivity of **3g**, we measured its ability to inhibit >250 in vitro expressed human protein kinases in either activity assays or binding assays.[16,28] IC_{50} values were generated against 95 kinases. Kinases with IC_{50} values less than 100 nM are shown in Table 5. Compound **3g** is very selective for the AKT isoforms versus the majority of kinases in other families. It is also selective against some members of the AGC kinase family such as MSK1, ROCK1, and RSK1 but less so for other AGC kinases including PKA, PrkX, and PKC isozymes. The poorer selectivity for PKA, PrkX, and the PKC isozymes was not entirely surprising on the basis of the high degree of homology in the ATP binding pocket within these kinases. Other kinases inhibited by **3g** are AMPK and DAPK3 from the CAMK family and PAK4, 5, and 6 from the STE family.

Some kinase inhibitors exhibit time-dependent or slow onset of inhibition of their target enzymes due to either slow on- or off-rate for binding.[14] The potency of such inhibitors can be grossly underestimated if the time-dependent inhibition is not properly accounted for.[15,32] To capture potential time-dependent inhibition, our normal IC_{50} assays included a 30 min incubation of enzyme and compound prior to the initiation of the reaction with substrate addition.[16] To understand whether **3g** inhibits the AKT enzymes in a time-dependent fashion, IC_{50} values were determined with and without a 30 min preincubation step and found to decrease by 12- and 20-fold upon incubation with AKT1 (Figure 3A) and AKT2,[33] respectively. Because of the time dependence of inhibition, **3g** was tested for reversibility by rapidly diluting the E−I complex into a high concentration of ATP. Full activity was recovered with half-life values for dissociation of 38 and 30 min for AKT1 (Figure 3B) and AKT2,[33] respectively. To probe the mechanism of inhibition with respect to ATP, steady state IC_{50} values were found to increase linearly with the ATP concentration; this pattern is consistent with an ATP competitive mechanism of inhibition for AKT1 (Figure 3C) and AKT2.[33]

As **3g** is a potent inhibitor of AKT1 and AKT2 and since a nominal concentration of 10 nM AKT1 or AKT2 was used in the IC_{50} assays, it was not clear whether the potency determined for **3g** reflected the fraction of the kinase that was capable of binding to the inhibitor or the true potency of the compound. By use of an assay format with lower concentration of the AKT

Scheme 6. Preparation of Compounds **14a** and **14b**[a]



[a] Reagents: (a) 1,3-dibromopropane, Cs_2CO_3, DMF, 60 °C, 3.5 h, (67%); (b) R-NH_2, Et_3N, DMF, 60 °C, 30 min; (c) 2-methyl-3-butyn-2-ol, $Pd(Ph_3P)_4$, Zn dust, NaI, DBU, Et_3N, DMSO, 80 °C, 3 h.

Table 1. Kinase Activity of Compounds **1** and **2** against AKT1, ROCK1, and MSK1

	IC_{50} (μM)[a]	
kinase	**1**	**2**
AKT1	0.120	0.355
MSK1	0.021	0.015
ROCK1	0.009	0.006

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

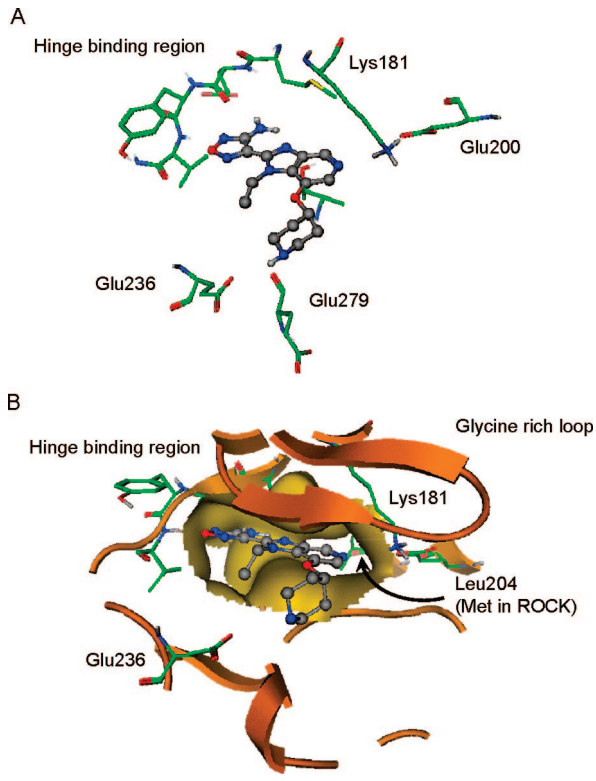
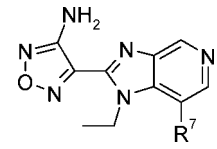


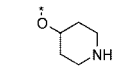
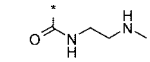
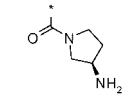
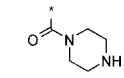
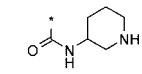
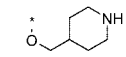
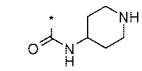
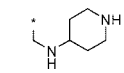
Figure 2. Compound **1** modeled into AKT2.

enzymes (0.33, 1, and 0.2 nM of AKT1, 2, and 3, respectively) and a 1 h incubation, the K_i* of **3g** was determined to be 1, 4, and 13 nM toward AKT1, 2, and 3, respectively. The K_i* values determined for AKT1 and AKT2 were within 3-fold of their IC_{50} values, indicating that the IC_{50} values represented the true potency of this compound rather than the active fraction of enzyme.

Compound **3g** was also tested against the mouse AKT1 and 2 enzymes and was found to have very similar potency as against the human enzymes (data not shown).

Table 2. Position-7 Modification To Improve Potency

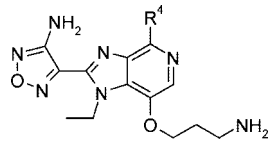


Compd	R^7	IC_{50} (μM)				
		AKT1	AKT2	AKT3	MSK1	ROCK1
1		0.079	1.00	0.398	0.021	0.008
2		0.126	3.98	1.41	0.015	0.005
4		0.331	2.34	1.29	0.003	0.005
5		0.501	2.51	1.549	0.047	0.020
6		0.631	10.0	3.02	0.011	0.040
7		0.056	0.204	0.380	0.001	0.003
8		0.562	2.57	2.29	0.013	0.006[a]
9		0.200	0.741	0.646	0.008	0.038[a]

[a] Rat ROCK1.

Crystallography. Compound **3g** was successfully cocrystallized within the kinase domain of AKT2 (148−481).[34] The overall conformation of AKT2 in this structure is very similar to that of the kinase domain of activated AKT containing a S474D mutation (PDB code 1o6k).[24] As predicted from the docking studies, **3g** occupied the ATP binding pocket, making a key hydrogen bonding contact between N5 of the oxadiazole moiety and the backbone NH of Ala232 in the hinge binding region of AKT2 (Figure 4). Another key feature is an intramolecular hydrogen bond between N3 of the imidazopyridine ring and the exocyclic NH of the oxadiazole ring, keeping the two

Table 3. Back-Pocket Elements To Improve Selectivity[b]



Compd	R^4	IC_{50} (μM) Kinase AKT1	MSK1	ROCK1	RSK1	Cellular Activity GSK3β[a]
10		0.331	1.00	NT	0.044	>30
11a		0.032	NT	0.158	0.013	3.38 ± 0.88
11b		0.079	0.162	0.562	0.071	>30
11c		0.032	NT	0.126	NT	2.76 ± 0.38
11d		3.16	NT	0.224	NT	>30
12a		0.079	NT	1.995	0.251	3.90 ± 0.14
12b		0.006	15.85	0.501	1.259	1.05 ± 0.60
12c		0.002	NT	0.020	0.001	1.13 ± 0.80
12d		0.050	NT	0.126	0.006	6.20
13		0.794	NT	0.200	0.251	NT

[a] Inhibition of phosphorylation of GSK3β in BT474 cells. [b] NT: not tested.

rings coplanar with respect to each other. The glycine rich loop (residues 158−165) is tucked down over the plane of the imidazopyridine ring, maximizing hydrophobic interactions between the kinase and the inhibitor. The piperidinyl side chain of **3g** is engaged in a hydrogen bond with the carboxylic acid side chain of Glu236 found in the substrate binding area. Another important interaction was the hydrogen bonding network between the backbone NH of Phe294 and the side chain carboxylic acid of Glu200 with the alkynol −OH. Interestingly, this hydroxyl group appears to have displaced a water molecule present in published structures of AKT2 crystallized with the nonhydrolyzable ATP mimetic AMP-PNP.[24] We speculate that the ability of **3g** to displace this water molecule may contribute to the observed time-dependent inhibition.

In Vivo Characterization of 3g. In vivo characterization of **3g** showed that this compound effectively inhibited GSK3β phosphorylation in BT474 xenografts in a dose dependent fashion when analyzed 4 h following ip dosing in SCID mice (Figure 5A). Dose dependent increases in blood glucose and insulin levels were also observed. We believe that this was due, in part, to the potent inhibition of AKT2 by **3g**, as this AKT isoform has been implicated in glucose homeostasis.[29] In a separate experiment, **3g** was found to significantly decrease GSK3β phosphorylation in BT474 tumor for up to 8 h after single ip dose in SCID mice at 20 mg/kg (Figure 5B). Furthermore, daily dosing of **3g** (ip, 30 mg/kg) resulted in 64% inhibition of tumor growth compared to vehicle treated mice in BT474 xenografts. Details of the in vivo characterization of **3g** in xenograft models are described in a separate publication.[16]

Pharmacokinetic profiles of **3g** in rat, dog, and monkey were determined. Although **3g** had poor oral exposure in all three species, blood concentrations of approximately 3 μM could be achieved following iv administration of the compound (Table 6). We have previously reported that this concentration of **3g** correlated with a sustained decrease of GSK3β phosphorylation in BT474 tumor xenografts.[16] Compound **3g** is also soluble in aqueous buffer (>5 mg/mL, sodium acetate buffer in 5% mannitol, pH 4). Therefore, an iv formulation of **3g** was selected for evaluation in patients with solid tumors or hematological malignancies.

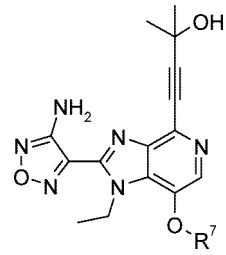
Conclusion

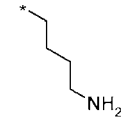
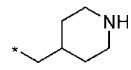
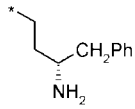
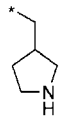
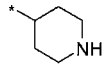
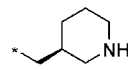
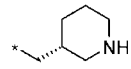
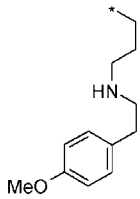
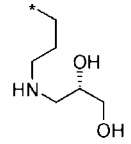
In summary, we have described novel pyridylimidazole-oxadiazolyl amines as potent inhibitors of AKT1, 2, and 3. AKT kinase potency and selectivity were optimized guided by insights gleaned from a docking model of the lead structure **1** in AKT2. The validity of this model was subsequently verified by solving a cocrystal structure of **3g** bound within the kinase domain of AKT2 (148−481). Inhibition of AKT activity by **3g** was manifested in cells by the inhibition of phosphorylation of a downstream substrate of AKT (GSK3β) and inhibition of proliferation in cells with activated AKT. In immune compromised mice implanted with human breast carcinoma (BT474) xenografts, a single intraperitoneal administration of **3g** inhibited GSK3β phosphorylation in the tumor in a dose- and time-dependent manner. In addition, GSK3β phosphorylation in BT474 tumor xenografts showed >60% decrease for up to 8 h following a single dose of 20 mg/kg. Repeated daily ip administration of **3g** produced significant delay in tumor growth in mice bearing established human BT474 breast carcinoma xenografts.[16] Compound **3g** is currently in clinical development as an iv agent for use in patients with solid tumors or hematological malignancies.

Experimental Section

All materials and reagents were used as is unless otherwise indicated. Air- or moisture-sensitive reactions were carried out under a nitrogen atmosphere. Microwave irradiation was carried out in a Personal Chemistry Emrys Optimizer microwave. Flash chromatography was performed using silica gel (EM Science, 230−400 mesh) under standard techniques or using silica gel cartridges (RediSep normal phase disposable flash columns) on an Isco CombiFlash. Preparative and analytical HPLC were carried out on Agilent 1100 series or Gilson 306 HPLC systems. Unless otherwise stated, the conditions for the preparative reverse phase HPLC purifications used YMC Combiscreen ODS-A 75 mm × 30 mm (30 mL/min; gradient, (A) acetonitrile−0.1%TFA, (B) water−0.1% TFA; 10−70% A during 10 min) with UV detection at 214. The ^{1}H NMR spectra were recorded at 400 MHz using a Bruker Avance 400 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield of tetramethylsilane (δ scale). Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (*J*) are reported in hertz and

Table 4. Side Chain Modification To Improve Cell Potency[c]



		IC50 (μM)							
		Kinase				Cellular Activity			
Compd	R^7	AKT1	AKT2	AKT3	Selectivity	GSK3β[a]	BT474[b]	LNCaP[b]	HFF[b]
3a		0.009	0.009	0.069	MSK1 15.8 ROCK1 0.50 RSK1 0.79	1.51 ± 1.37	0.70 ± 0.22	0.99 ± 0.47	>30
3b		0.010	0.008	0.040	ROCK1 2.51 RSK1 2.51	2.10 ± 1.01	0.38 ± 0.34	0.22 ± 0.11	>30
3c		0.002	0.020	NT	ROCK1 0.50 RSK1 0.50	0.23 ± 0.01	0.42 ± 0.03	0.30 ± 0.10	7.06 ± 0.90
3d		0.003	0.004	NT	ROCK1 1.26 RSK1 1.56	0.10 ± 0.02	0.36 ± 0.07	0.03 ± 0.02	4.17 ± 0.51
3e		0.008	0.013	NT	MSK1 10.0 ROCK1 0.79 RSK1 1.99	1.01 ± 0.05	1.03 ± 0.26	0.46 ± 0.15	20.1 ± 1.60
3f		0.008	0.008	NT	ROCK1 1.00 RSK1 3.98	0.56 ± 0.12	1.28	0.18 ± 0.06	18.1 ± 1.02
3g		0.002	0.013	0.009	MSK1 7.94 ROCK1 0.89 RSK1 1.26	0.14 ± 0.05	0.05 ± 0.02	0.02 ± 0.01	16.3 ± 4.50
3h		0.003	NT	NT	ROCK1 1.58 RSK1 1.58	0.40 ± 0.06	0.76 ± 0.21	0.19 ± 0.03	21.2
14a		0.005	0.006	0.016	MSK1 19.9 ROCK1 3.98 RSK1 2.51	0.14 ± 0.09	0.26 ± 0.14	0.05 ± 0.02	4.85 ± 2.52
14b		0.003	0.005	NT	ROCK1 0.25 RSK1 0.50	>30	14.2 ± 1.32	2.89 ± 0.76	>30

[a] Inhibition of phosphorylation of GSK3β in BT474 cells. [b] Inhbition of proliferation. [c] NT: not tested.

Table 5. Enzyme Activity of **3g** against Other Selected Protein Kinases

family	kinase	**3g**, IC_{50} (μM)[a]
AGC	AKT1	0.002
	AKT2	0.013
	AKT3	0.009
	PKA	0.024
	PKCη	0.002
	PKCθ	0.002
	PKCβ1	0.019
	PKCδ	0.014
	PKCε	0.021
	PKG1β	0.033
	PrkX	0.005
CAMK	AMPK[b]	0.050
	DAPK3[c]	0.081
STE	PAK4[c]	0.010
	PAK5[c]	0.052
	PAK6[c]	0.006

[a] All IC_{50} determinations were carried out with ATP concentration at K_m(app) ATP. [b] Rat enzyme. All others are human enzymes. [c] Data are from binding assays. All other data are from activity assays.

refer to apparent multiplicities and not true coupling constants. Mass spectra were recorded on an Applied Biosystems MSD Sciex API 150EX single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ.

5-Bromo-*N*^{4}-ethylpyridine-3,4-diamine (16). A mixture of ethyl (3-bromo-5-nitropyridin-4-yl)amine (7.0 g, 28 mmol) and iron powder (9.51 g, 170 mmol, <50 μm mesh) in acetic acid (100 mL) was stirred at 75 °C for 1 h. The reaction mixture was allowed to cool to room temperature, then diluted with EtOAc/CH_2Cl_2 (1:4) and filtered through Celite. The filtrate was concentrated under reduced pressure. Flash chromatography (silica gel, 96:4 EtOAc/MeOH) afforded 5.68 g (93%) of **16**. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.88 (s, 1 H), 7.77 (s, 1 H), 3.41 (q, J = 7.07 Hz, 2 H), 1.19 (t, J = 7.20 Hz, 3 H). MS m/z 216 $[M + H]^+$.

(7-Bromo-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)acetonitrile (17). A solution of **16** (5.68 g, 26.3 mmol) in ethyl cyanoacetate (5.6 mL, 53 mmol) was stirred at 190 °C for 1 h. A precipitate was observed following cooling and the addition of EtOAc (50 mL). The solid was collected by filtration, washed with EtOAc, and then air-dried to afford 4.15 g (59%) of **17**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1 H), 8.48 (s, 1 H), 4.69 (s, 2 H), 4.46 (q, J = 7.07 Hz, 2 H), 1.39 (t, J = 7.20 Hz, 3 H). MS m/z 265 $[M + H]^+$.

4-(7-Bromo-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)[1,2,5]oxadiazolidin-3-ylamine (18). Sodium nitrite (1.67 g, 24.2 mmol) was added portionwise to a solution of **17** (3.20 g, 12.1 mmol) in methanol (40 mL). After being stirred for 2 h, the suspension was adjusted to pH 12 with 50% aqueous NaOH. Aqueous 50% NH_2OH (33 mL) was added, and the mixture was stirred at 90 °C for 2 h. A precipitate was formed upon cooling to room temperature. The solid was collected by filtration to afford 2.50 g (67%) of **18**. ^{1}H NMR (400 MHz, MeOH-d_4) δ 9.05 (s, 1 H), 8.56 (s, 1 H), 5.15 (q, J = 7.07 Hz, 2 H), 1.59 (t, J = 7.07 Hz, 3 H). MS m/z 309 $[M + H]^+$.

[4-(7-Bromo-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)furazan-3-yl]carbamic Acid *tert*-Butyl Ester (19). A solution of **18** (2.14 g, 6.95 mmol), di-*tert*-butyl dicarbonate (2.27 g, 10.4 mmol) and DMAP (0.85 g, 6.95 mmol) in CH_2Cl_2 (10 mL) and pyridine (20 mL) was stirred at 90 °C in a sealed tube for 2.5 h. More di-*tert*-butyl dicarbonate (2.27 g, 10.4 mmol) was added, and the mixture was stirred at 90 °C for an additional 18 h. The reaction was quenched by partitioning between EtOAc and water. The organic layer was washed with water, then brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash chromatography (silica gel, 20% EtOAc/hexanes) to afford 1.60 g (58.4%) of **19** as an off-white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.96 (s, 1 H), 8.47 (s, 1 H), 4.98 (q, J = 7.07 Hz, 2 H), 1.50 (t, J = 7.07 Hz, 3 H), 1.45 (s, 9 H). MS m/z 409 $[M + H]^+$.

[4-(1-Ethyl-7- hydroxy-1*H*-imidazo[4,5-*c*]pyridin-2-yl)furazan-3-yl]carbamic Acid *tert*-Butyl Ester (20). To a solution of **19** (0.20 g, 0.50 mmol) in THF (4 mL) at −78 °C was added *n*-BuLi (0.5 mL, 2.5 M solution in hexane, 1.25 mmol). After 20 min at −78 °C, trimethyl borate (0.17 mL, 1.50 mmol) in THF (1 mL) was added. After the reaction was allowed to warm to room temperature over 1.5 h, a solution of 30% H_2O_2 (1.10 mL) in 3 N NaOH (0.35 mL) was added and stirring continued at room temperature for 30 min. The reaction mixture was diluted with EtOAc and then extracted with 1 N NaOH. The combined aqueous extracts were acidified with 6 N HCl and then extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford 144 mg (83%) of **20** as an orange solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.62 (s, 1 H), 7.90 (s, 1 H), 5.04 (q, J = 7.07 Hz, 2 H), 1.59 (s, 9 H), 1.54−1.58 (m, 3 H). MS m/z 347 $[M + H]^+$.

4-[2-(4-*tert*-Butoxycarbonylaminofurazan-3-yl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yloxy]piperidine-1-carboxylic Acid *tert*-Butyl Ester (21). To a stirred mixture of triphenylphosphine on polystyrene resin (2.40 g, 1.20 mmol/g, 2.88 mmol) in CH_2Cl_2 (25 mL) was added 4-hydroxypiperidine-1-carboxylic acid *tert*-butyl ester (1.15 g, 5.76 mmol) followed by diethyl azodicarboxylate (0.45 mL, 2.88 mmol). After 10 min at room temperature, the mixture was cooled to 0 °C and a solution of **20** (0.20 g, 0.58 mmol) in CH_2Cl_2 (15 mL) was added. After 1.5 h at 0 °C the resin was removed by filtration and washed with CH_2Cl_2. The combined organic extracts were washed with 1 N NaOH, then water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue subjected to preparative HPLC to afford 131 mg (43%) of **21** as an off white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1 H), 8.82 (s, 1 H), 8.07 (s, 1 H), 4.96 (q, J = 7.07 Hz, 2 H), 4.72−4.84 (m, 1 H), 3.61−3.79 (m, 2 H), 3.32−3.47 (m, 2 H), 2.02−2.16 (m, 2 H), 1.77−1.91 (m, 2 H), 1.55 (s, 9 H), 1.48 (t, J = 6.95 Hz, 3 H), 1.42 (s, 9 H). MS m/z 530 $[M + H]^+$.

4-[1-Ethyl-7-(piperidin-4-yloxy)-1*H*-imidazo[4,5-*c*]pyridin-2-yl-]furazan-3-ylamine, Trifluoroacetate (1). A solution of **21** (130 mg, 0.25 mmol) and TFA (0.75 mL) in CH_2Cl_2 (1.5 mL) was stirred at room temperature for 40 min. The solvent was removed under reduced pressure and the residue subjected to preparative HPLC to afford 80 mg (97%) of **1**. ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 8.85 (s, 1 H), 8.30 (s, 1 H), 4.96−5.13 (m, 1 H), 4.84 (q, J = 6.82 Hz, 2 H), 3.22−3.35 (m, 2 H), 3.10−3.23 (m, 2 H), 2.19−2.32 (m, 2 H), 1.93−2.12 (m, 2 H), 1.47 (t, J = 7.07 Hz, 3 H). MS m/z 330 $[M + H]^+$. Anal. ($C_{15}H_{19}N_7O_2 \cdot 1.45C_2HF_3O_2$) calcd: C, 43.46; H, 4.17; N, 19.82. Found: C, 43.93; H, 4.13; N, 19.35.

5-Bromo-2-chloro-*N*^{4}-ethylpyridine-3,4-diamine (22). To a solution of ethyl (3-bromo-5-nitropyridin-4-yl)amine (22.0 g, 89.4 mmol) in concentrated HCl (250 mL) was added portionwise tin(II) chloride dihydrate (60.5 g, 270 mmol). The mixture was stirred at room temperature for 1 h and then poured into ice (300 g). EtOAc (500 mL) was added and the aqueous phase made basic with NaOH. The EtOAc extracts were sequentially washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was subjected to flash chromatography (silica gel, 25% EtOAc/hexanes) to afford 17.8 g (80%) of **22**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (s, 1 H), 5.20 (s, 2 H), 4.86 (t, J = 6.6 Hz, 1 H), 3.23−3.40 (m, 2 H), 1.07 (t, J = 7.1 Hz, 3 H). MS m/z 250 $[M + H]^+$.

***N*-[5-Bromo-2-chloro-4-(ethylamino)-3-pyridinyl]-2-cyanoacetamide (23).** To a solution of **22** (17.7 g, 70.9 mmol) in DMF (100 mL) at 0 °C was added cyanoacetic acid (9.06 g, 106 mmol), *N*-methylmorpholine (39 mL, 350 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (20.3 g, 106 mmol). The cooling bath was removed, and the mixture was stirred for 3 h. EtOAc (300 mL) was added, and the organic extracts were washed with water and then brine. The solvent was removed under reduced pressure to give a solid. Recrystallization from EtOAc/hexanes afforded 22.5 g (quantitative) of **23** as a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-

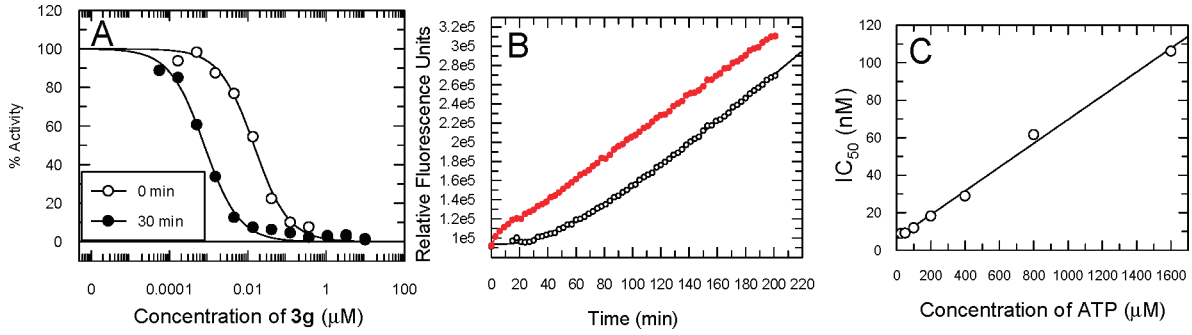


Figure 3. Kinetic characterization of **3g** against AKT1. (A) IC_{50} of compound **3g** with (filled circles) and without (open circles) a preincubation with AKT1. (B) Recovery of enzymatic activity following rapidly diluting the preformed AKT1−**3g** complex into substrate mix. Filled circles represent the control reaction in the absence of **3g**, and open circles represent the dissociation of **3g** from AKT1. (C) IC_{50} values of **3g** as a function of ATP concentration against AKT1.

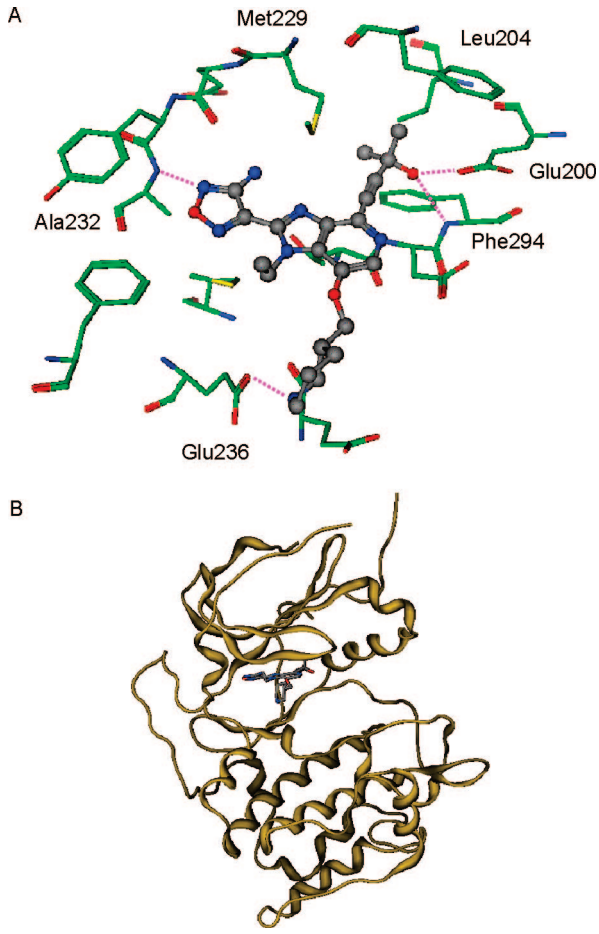


Figure 4. Cocrystal structure of **3g** in AKT2(148−481). (A) Compound **3g** cocrystal structure in AKT2(148−481) with the glycine rich loop omitted for clarity. Hydrogen bonds are indicated as dotted lines. (B) Ribbon diagram representation of AKT2(148−481) cocrystallized with **3g**. Graphics were prepared using MOE 2006.08, Chemical Computing Group, Inc.

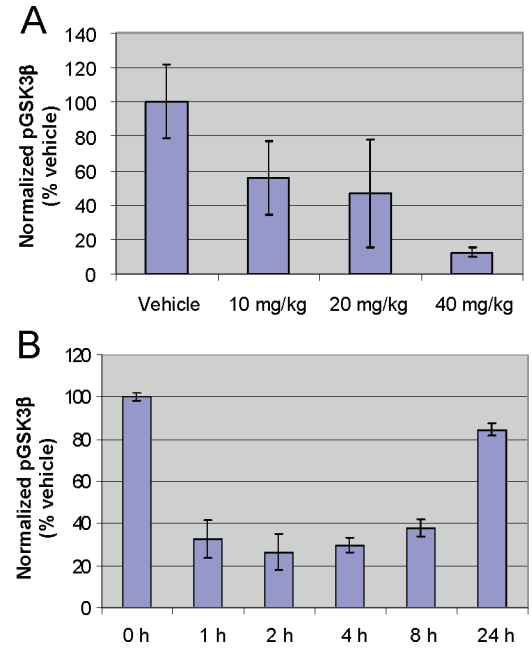


Figure 5. Effect of **3g** on GSK3β phosphorylation in BT474 xenografts. (A) Dose response effect of **3g** on GSK3β phosphorylation in BT474 tumors in female SCID mice analyzed 4 h following administration of **3g**. (B) Time course of inhibition on GSK3β phosphorylation in BT474 tumors in female SCID mice by **3g** dosed at 20 mg/kg (ip).

d_6) δ 10.02 (s, 1 H), 8.12 (s, 1 H), 6.04 (t, J = 6.2 Hz, 1 H), 3.93 (s, 2 H), 3.38−3.52 (m, 2 H), 1.12 (t, J = 7.1 Hz, 3 H). MS m/z 317 $[M + H]^+$.

(7-Bromo-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)acetonitrile (24). A solution of **23** (35.6 g, 112 mmol) in glacial acetic acid (300 mL) was heated to 90 °C. After 1 h, LC/MS analysis indicated that the reaction was complete. The solvent was removed under reduced pressure to give 29.5 g (87.8%) of **24** that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1 H), 4.72 (s, 2 H), 4.49 (q, J = 7.3 Hz, 2 H), 1.40 (t, J = 7.2 Hz, 3 H). MS m/z 299 $[M + H]^+$.

(7-Bromo-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)hydroxyiminoacetonitrile (25). To a mixture of **24** (29.5 g, 98.0 mmol) in 2 N HCl (400 mL) at room temperature was added, portionwise over 20 min, sodium nitrite (14.0 g, 203 mmol). After the mixture was stirred for an additional 30 min, the resulting precipitate was isolated by filtration, washed with water, and dried to afford 32 g (99%) of **25** that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.00 (s, 1 H), 8.41 (s, 1 H), 4.77 (q, J = 7.1 Hz, 2 H), 1.44 (t, J = 7.1 Hz, 3 H). MS m/z 328 $[M + H]^+$.

4-(7-Bromo-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine (26). A solution of the compound of **25** (32 g, 97 mmol), Et_3N (40 mL), and 50% aqueous hydroxylamine (16 mL) in THF (250 mL) was heated to 90 °C in a sealed pressure vessel for 1.5 h. After cooling to room temperature, the mixture was poured into water and extracted with EtOAc. The combined organic extracts were washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure. The residue was dissolved in dioxane (200 mL) and Et_3N (35 mL) and then heated to 150 °C in a sealed pressure vessel for 1.5 h. After the mixture was allowed to cool to room temperature, the solvent was removed under reduced pressure to give a solid. Recrystallization from

Table 6. Single Dose, iv, Pharmacokinetic Summary for **3g**

species	iv dose (mg/kg)	C_{max} (μg/mL)	$AUC_{(0-inf)}$ (μg·h)/mL)	CL ((mL/min)/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)
rat	10.6[a]	2.13 ± 0.19	3.15 ± 0.32	56.7 ± 5.5	15.2 ± 1.3	8.92 ± 1.03
dog	4.2[b]	1.30 ± 0.11	2.01 ± 0.11	34.6 ± 2.0	5.29 ± 0.24	3.50 ± 0.33
monkey	4.3[b]	1.50 ± 0.10	3.25 ± 0.67	22.5 ± 4.3	6.54 ± 0.89	5.52 ± 0.43

[a] Vehicle: 0.9% saline. [b] Vehicle: 1% DMSO and 20% Encapsin in saline.

CH_2Cl_2 afforded 17.3 g (51.7%) of **26**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1 H), 6.94 (s, 2 H), 4.93 (q, J = 7.1 Hz, 2 H), 1.51 (t, J = 7.1, 3 H). MS m/z 343 $[M + H]^+$.

1,1-Dimethylethyl [4-(7-Bromo-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-yl]carbamate (27). A solution of the compound of **26** (8.50 g, 24.7 mmol), pyridine (70 mL), di-*tert*-butyl dicarbonate (21.5 g, 98.8 mmol), and DMAP (3.01 g, 24.7 mmol) in 1,2-dichloroethane (140 mL) was stirred at 85 °C in a sealed flask for 1 h. The product mixture was partitioned between EtOAc and 1 N HCl. The layers were separated, and the organic extract was washed with 1 N HCl, brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the resulting solid triturated with EtOAc to afford **27** as a beige solid (5.06 g). The mother liquor was evaporated to dryness and treated with 2% trifluoroacetic acid in CH_2Cl_2 (100 mL) for 20 h. The reaction mixture was neutralized with saturated $NaHCO_3$, washed with brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was subjected to flash chromatagraphy (20% EtOAc/hexane, silica gel), affording an additional crop and giving a combined yield of 8.55 g (78%) of **27**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1 H,) 8.49 (s, 1 H), 4.80 (q, J = 7.07 Hz, 2 H), 1.49 (t, J = 7.07 Hz, 3 H), 1.37 (s, 9 H). MS m/z 443 $[M + H]^+$.

1,1-Dimethylethyl [4-(4-Chloro-1-ethyl-7-hydroxy-1*H*-imidazo-[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-yl]carbamate (28). To a solution of the compound of **27** (2.00 g, 4.51 mmol) in THF (60 mL), at −100 °C was added dropwise *n*-BuLi (4.50 mL, 2.5 M in hexane, 11.3 mmol). After 5 min, a solution of $B(OMe)_3$ (1.50 mL, 13.5 mmol) in THF (2 mL) was added. The cooling bath was removed after a further 10 min. After 1.5 h, 3 M NaOH (3 mL) and 30% w/w H_2O_2 (9 mL) were added to the reaction. After an additioal 1 h, the reaction was quenched by adding EtOAc and washing sequentially with 1 N HCl, H_2O, and brine and then drying over Na_2SO_4. The solvent was removed under reduced pressure and the residue triturated with EtOAc to afford 1.45 g (84.5%) of **28**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1 H,) 10.36 (s, 1 H), 7.80 (s, 1 H), 4.73 (q, J = 6.91 Hz, 2 H), 1.45 (t, J = 7.07 Hz, 3 H), 1.41 (s, 9 H). MS m/z 381 $[M + H]^+$.

4-{7-[(3-Aminopropyl)oxy]-1-ethyl-4-phenyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl}-1,2,5-oxadiazol-3-amine, Bis-trifluoroacetate (10). Compound **28** (1.40 g, 3.67 mmol), phenylboronic acid (0.90 g, 7.34 mmol), and $Pd(PPh_3)_4$ (0.24 g) were added to 1,4-dioxane (40 mL) and 2 M Na_2CO_3 (4.04 mL, 8.1 mmol). The reaction vessel was purged with nitrogen, sealed, and heated to 90 °C for 18 h. After the mixture was allowed to cool to room temperature, the solids were removed by filtration. The filtrate was concentrated under reduced pressure and the residue subjected to flash chromatography (75% EtOAc/hexanes, silica gel) to give 1.16 g (74.7%) of 1,1-dimethylethyl [4-(1-ethyl-7-hydroxy-4-phenyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-yl]carbamate. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.16 (d, J = 7.07 Hz, 2 H), 7.98 (s, 1 H), 7.54 (t, J = 7.58 Hz, 2 H), 7.44 (t, J = 7.33 Hz, 1 H), 5.05 (q, J = 6.91 Hz, 2 H), 1.54−1.59 (m, 12H). MS m/z 423 $[M + H]^+$.

To a suspension of polymer-bound PPh_3 (0.87 g, 1.2 mmol/g loading, 1.05 mmol) in CH_2Cl_2 (10 mL) was added 1,1-dimethylethyl (4-hydroxypropyl)carbamate (0.37 g, 2.10 mmol) and DEAD (0.16 mL, 1.05 mmol) dropwise. After 30 min, the suspension was cooled to 0 °C. A solution of 1,1-dimethylethyl [4-(1-ethyl-7-hydroxy-4-phenyl-1 *H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-yl]carbamate (0.09 g, 0.21 mmol) in CH_2Cl_2 (5 mL) was added. After 1 h at 0 °C, solids were removed by filtration and exhaustively washed with CH_2Cl_2. The combined filtrates were concentrated under reduced pressure and the resulting residue was subjected to flash chromatography (35% EtOAc/hexane, silica gel) to give 1,1-dimethylethyl (4-{7-[(3-aminopropyl)oxy]-1-ethyl-4-phenyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl}-1,2,5-oxadiazol-3-yl)carbamate. This was used without further purification in the step below. MS m/z 580 $[M + H]^+$.

The 1,1-dimethylethyl (4-{7-[(3-aminopropyl)oxy]-1-ethyl-4-phenyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl}-1,2,5-oxadiazol-3-yl)carbamate was dissolved in TFA (1 mL) and CH_2Cl_2 (2 mL). After 2 h, the solvent was removed under reduced pressure and the residue subjected to preparative reverse phase HPLC to give 37 mg (27%) of **10** as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.30−8.36 (m, 1 H), 8.18−8.27 (m, 2 H), 7.67−7.79 (m, 3 H), 5.14 (q, J = 7.07 Hz, 2 H), 4.59 (t, J = 5.94 Hz, 2 H), 3.25−3.32 (m, 2 H), 2.35−2.48 (m, 2 H), 1.65 (t, J = 7.07 Hz, 3 H). MS m/z 380 $[M + H]^+$. Anal. ($C_{19}H_{21}N_7O_2 \cdot 2C_2HF_3O_2$) calcd: C, 45.48; H, 3.82; N, 16.14. Found: C, 45.77; H, 3.80; N, 16.22.

2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-ol (29). A solution of **26** (2.00 g, 5.80 mmol) in THF (270 mL) was cooled to −100 °C under an atmosphere of nitrogen. *n*-Butyllithium (7.20 mL, 18.0 mmol, 2.5 M in hexanes) at −78 °C was added over 4 min using a syringe pump. After an additional 3 min at −100 °C, trimethyl borate (2.10 mL, 19.0 mmol) was added. The cooling bath was removed, and the mixture was allowed to warm to room temperature. After 3 h, a solution of 30% aqueous hydrogen peroxide (13 mL) in 3 M NaOH (4.3 mL) was added. After an additional 45 min, the reaction was quenched by partitioning between EtOAc and 1 N HCl. The aqueous layer was extracted with additional EtOAc, and the combined organic extracts were washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was triturated with 3% MeOH/CH_2Cl_2 to give 0.96 g (59%) of **29** as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1 H), 7.79 (s, 1 H), 6.94 (br s, 2 H), 4.84 (q, J = 7.1 Hz, 2 H), 1.45 (t, J = 7.1 Hz, 3 H). MS m/z 281.2 $[M + H]^+$.

1,1-Dimethylethyl (3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}propyl)carbamate (30). To a stirred solution of **29** (250 mg, 0.89 mmol) in DMF (10 mL) at ambient temperature was added cesium carbonate (726 mg, 2.23 mmol) followed by 1,1-dimethylethyl (3-bromopropyl)carbamate (318 mg, 1.34 mmol). The reaction mixture was stirred under argon at 33 °C for 15 h, at which time it was diluted with ethyl acetate and washed with water. The combined aqueous washings were extracted with fresh ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a pale-yellow solid. The solid was suspended in a small volume of CH_2Cl_2/hexanes (2:1) and then isolated by filtration to afford 317 mg (77%) of pure **30** as an off-white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 1 H), 7.00 (t, J = 5.68 Hz, 1 H), 6.94 (s, 2 H), 4.83 (q, J = 6.99 Hz, 2 H), 4.28 (t, J = 5.81 Hz, 2 H), 3.18 (q, J = 6.48 Hz, 2 H), 1.90−2.00 (m, 2 H), 1.46 (t, J = 7.07 Hz, 3 H), 1.36 (s, 9 H).

1,1-Dimethylethyl {4-[4-(Cyclopropylethynyl)-1-ethyl-7-hydroxy-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-yl}carbamate (31a). Ethynylcyclopropane (70% in toluene) (0.55 mL, 4.07 mmol) and diisopropylamine (0.60 mL, 4.24 mmol) were added to a solution of **30** (150 mg, 0.34 mmol) and $Pd(PPh_3)_4$ (60 mg, 0.052 mmol) in dioxane (6.0 mL). The reaction vessel was sealed and heated to 110 °C for 2 h. After the mixture was cooled to room

temperature, the solvent was removed under reduced pressure and the residue was subjected to flash chromatography (silica gel, 0.5% to 10% methanol/chloroform) to afford 160 mg of **31a** (>80% pure by HPLC analysis). This material was used without further purification. MS *m/z* 468.4 $[M + H]^+$.

4-[7-[(3-Aminopropyl)oxy]-4-(cyclopropylethynyl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine, Bis-trifluoroacetate (12a). Trifluoracetic acid (1.6 mL) was added to a solution of **31a** (160 mg, 0.343 mmol) in CH_2Cl_2 (2.5 mL). After 1 h, the solvent was removed under reduced pressure and the residue was subjected to reverse phase HPLC to afford 60 mg of **12a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1 H), 7.81 (br s, 3 H), 7.01 (br s, 2 H), 4.82 (q, J = 6.8 Hz, 2 H), 4.40 (t, J = 5.8 Hz, 2 H), 3.04 (m, 2 H), 2.14 (m, 2 H), 1.70 (m, 1 H), 1.45 (t, J = 7.1 Hz, 3 H), 1.00 (m, 2 H), 0.82 (m, 2 H). MS *m/z* 368.0 $[M + H]^+$. Anal. ($C_{18}H_{21}N_7O_2 \cdot 2C_2HF_3O_2 \cdot 0.5H_2O$) calcd: C, 43.72; H, 4.00; N, 16.22. Found: C, 43.69; H, 3.75; N, 16.05.

4-{2-(4-Amino-1,2,5-oxadiazol-3-yl)-7-[(3-aminopropyl)oxy]-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl}-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (12b). Compound **12b** was prepared in a manner analogous to the preparation of **12a** by substituting 2-methyl-3-butyn-2-ol (0.35 mL, 3.60 mmol) for ethynylcyclopropane. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1 H), 7.82 (br s, 3 H), 7.06 (s, 2 H), 4.84 (q, J = 7.0 Hz, 2 H), 4.41 (t, J = 5.8 Hz, 2 H), 3.05 (m, 2 H), 2.15 (m, 2 H), 1.53 (s, 6 H), 1.45 (t, J = 7.1 Hz, 3 H). MS *m/z* 385.9 $[M + H]^+$. Anal. ($C_{18}H_{23}N_7O_3 \cdot 2C_2HF_3O_2$) calcd: C, 43.07; H, 4.11; N, 15.98. Found: C, 42.74; H, 4.31; N, 16.06.

3-{2-(4-Amino-1,2,5-oxadiazol-3-yl)-7-[(3-aminopropyl)oxy]-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl}-2-propyn-1-ol, Bis-trifluoroacetate (12c). Compound **12c** was prepared in a manner analogous to the preparation of **12a** by substituting (1,1-dimethylethyl)(dimethyl)(2-propyn-1-yloxy)silane (1.90 mL, 9.00 mmol) for ethynylcyclopropane and using dimethylformamide (30%v/v) as a cosolvent. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1 H), 7.83 (br s, 3 H), 6.98 (br s, 2 H), 4.83 (q, J = 7.0 Hz, 2 H), 4.42 (m, 4 H), 3.05 (m, 2 H), 2.15 (m, 2 H), 1.46 (t, J = 7.1 Hz, 3 H). MS *m/z* 357.8 $[M + H]^+$. Anal. ($C_{16}H_{19}N_7O_3 \cdot 2C_2HF_3O_2$) calcd: C, 41.03; H, 3.62; N, 16.75. Found: C, 41.10; H, 3.59; N, 16.97.

4-{2-(4-Amino-1,2,5-oxadiazol-3-yl)-7-[(3-aminopropyl)oxy]-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl}-3-butyn-1-ol, Bis-trifluoroacetate (12d). Compound **12d** was prepared in a manner analogous to the preparation of **12a** by substituting 3-butyn-1-ol (0.35 mL, 4.60 mmol) for ethynylcyclopropane. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1 H), 7.83 (br s, 3 H), 6.98 (br s, 2 H), 4.83 (q, J = 7.1 Hz, 2 H), 4.40 (t, J = 5.9 Hz, 2 H), 3.66 (t, J = 6.8 Hz, 2 H), 3.05 (m, 2 H), 2.69 (t, J = 6.8 Hz, 2 H), 2.15 (m, 2 H), 1.45 (t, J = 7.1 Hz, 3 H). MS *m/z* 371.7 $[M + H]^+$. Anal. ($C_{17}H_{21}N_7O_3 \cdot 2C_2HF_3O_2 \cdot 0.5H_2O$) calcd: C, 41.45; H, 3.98; N, 16.11. Found: C, 41.56; H, 3.72; N, 15.86.

4-[7-[(3-Aminopropyl)oxy]-4-(3-amino-1-propyn-1-yl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine, Tris-trifluoroacetate (13). A reaction vessel was charged with **30** (0.22 g, 0.50 mmol), 1,1-dimethylethyl 2-propyn-1-ylcarbamate (0.39 g, 2.51 mmol), zinc powder (9.9 mg, 0.15 mmol), NaI (23.0 mg, 0.15 mmol), $Pd(PPh_3)_4$ (58.0 mg, 0.05 mmol), Et_3N (0.21 mL, 1.51 mmol), DBU (0.23 mL, 1.51 mmol), and DMSO (5 mL). The reaction vessel was purged with nitrogen, sealed, and heated in an oil bath at 85 °C for 2 h. The reaction was quenched by pouring into saturated NH_4Cl, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a dark oil. This was subjected to flash chromatography (1% NH_4OH/9% MeOH/90% $CHCl_3$ to 100% $CHCl_3$, silica gel) to give 0.24 g of 1,1-dimethylethyl [3-(2-(4-amino-1,2,5-oxadiazol-3-yl)-7-{[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)propyl]oxy}-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-propyn-1-yl]carbamate that was used without further purification. MS *m/z* 556.9 $[M + H]^+$.

To the (1,1-dimethylethyl [3-(2-(4-amino-1,2,5-oxadiazol-3-yl)-7-{[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)propyl]oxy}-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-propyn-1-yl]carbamate) obtained above were added dichloromethane (4 mL) and trifluoroacetic acid (1.60 mL). After the mixture was stirred at room temperature for 3 h, the solvent was removed under reduced pressure. The residue was subjected to reverse phase HPLC purification to give 0.12 g of **13**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (br s, 3 H), 8.22 (s, 1 H), 7.91 (br s, 3 H), 6.96 (br s, 2 H), 4.84 (q, J = 6.8 Hz, 2 H), 4.43 (t, J = 5.8 Hz, 2 H), 4.18 (d, J = 5.3 Hz, 2 H), 3.05 (m, 2 H), 2.15 (m, 2 H), 1.46 (t, J = 6.9 Hz, 3 H). MS *m/z* 357.1 $[M + H]^+$. Anal. ($C_{16}H_{20}N_8O_2 \cdot 3C_2H_1F_3O_2 \cdot 0.75H_2O$) calcd: C, 37.11; H, 3.47; N, 15.74. Found: C, 37.26; H, 3.19; N, 15.41.

4-[7-[(4-Aminobutyl)oxy]-2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl]-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (3a). Compound **3a** was prepared in a manner analogous to the preparation of **12a** by substituting 2-methyl-3-butyn-2-ol (1.00 mL, 11.0 mmol) for ethynylcyclopropane and 1,1-dimethylethyl (4-iodobutyl)carbamate (640 mg. 2.14 mmol) for 1,1-dimethylethyl (3-bromopropyl)carbamate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1 H), 7.72 (br s, 3 H), 7.05 (s, 2 H), 4.84 (q, J = 7.0 Hz, 2 H), 4.35 (t, J = 6.2 Hz, 2 H), 2.92 (m, 2 H), 1.92 (m, 2 H), 1.76 (m, 2 H), 1.54 (s, 6 H), 1.46 (t, J = 7.1 Hz, 3 H). MS *m/z* 399.9 $[M + H]^+$. Anal. ($C_{19}H_{25}N_7O_3 \cdot 2C_2HF_3O_2 \cdot H_2O$) calcd: C, 42.80; H, 4.53; N, 15.19. Found: C, 42.79; H, 4.41; N, 15.10.

4-{2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-[(4-piperidinylmethyl)oxy]-1*H*-imidazo[4,5-*c*]pyridin-4-yl}-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (3b). Compound **3b** was prepared in a manner analogous to the preparation of **12a** by substituting 2-methyl-3-butyn-2-ol (211 mg, 2.51 mmol) for ethynylcyclopropane and 1,1-dimethylethyl 4-(bromomethyl)-1-piperidinecarboxylate (690 mg. 2.49 mmol) for 1,1-dimethylethyl (3-iodopropyl)carbamate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (br s, 1 H), 8.40 (br s, 1 H), 8.18 (s, 1 H), 7.06 (br s, 2 H), 4.85 (q, J = 7.0 Hz, 2 H), 4.25 (d, J = 5.8 Hz, 2 H), 3.37 (m, 2 H), 2.98 (m, 2 H), 2.21 (m, 1 H), 1.98 (m, 2 H), 1.59 (m, 8 H), 1.46 (t, J = 7.1 Hz, 3 H). MS *m/z* 426.1 $[M + H]^+$. Anal. ($C_{21}H_{27}N_7O_3 \cdot 2C_2HF_3O_2 \cdot 0.5H_2O$) calcd: C, 45.32; H, 4.56; N, 14.80. Found: C, 45.32; H, 4.47; N, 14.71.

1,1-Dimethylethyl (2-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}ethyl)carbamate (32c). A mixture of **29** (130 mg, 0.46 mmol) with Cs_2CO_3 (377 mg, 1.16 mmol) in DMF (4 mL) was stirred at room temperature for 20 min. To this was added 1,1-dimethylethyl (2-bromoethyl)carbamate (531 mg, 2.37 mmol), and stirring continued at room temperature for 18 h. The product mixture was partitioned between water (15 mL) and EtOAc (25 mL). The organic extract was washed with brine and dried over Na_2SO_4, and the solvent was removed. The residue was triturated in cyclohexane (15 mL) and the solid collected to afford 163 mg (83%) of **32c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (s, 1 H), 7.16 (t, J = 5.7 Hz, 1H), 6.94 (s, 2 H), 4.84 (q, J = 6.8 Hz, 2 H), 4.28 (t, J = 4.9 Hz, 2 H), 3.45 (q, J = 5.0 Hz, 2 H), 1.43 (t, J = 7.0 Hz, 3 H), 1.38 (s, 9 H). MS *m/z* 424 $[M + H]^+$.

1,1-Dimethylethyl (2-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-hydroxy-3-methyl-1-butyn-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}ethyl)carbamate (33c). In a thick wall pressure tube, a mixture consisting of **32c** (0.150 g, 0.35 mmol), 2-methyl-3-butyn-2-ol (0.089 g, 1.06 mmol), zinc (4.05 mg, 0.062 mmol), sodium iodide (9.28 mg, 0.062 mmol), DBU (0.080 mL, 0.53 mmol), TEA (0.074 mL, 0.53 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.016 g, 0.014 mmol) in DMSO (2 mL) was purged with AR, sealed, then heated with stirring at 80 °C for 3 h. Water (5 mL) and EtOAc (7 mL) were added, and the mixture was filtered. The residue was triturated in EtOAc, and the solid was collected to afford 92 mg (55%) of **33c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1 H), 7.15 (t, J = 5.7 Hz, 1 H), 7.05 (s, 2 H), 5.67 (s, 1 H), 4.85 (q, J = 7.0 Hz, 2 H), 4.31 (t, J = 5.2 Hz, 2 H), 3.46 (q, J = 5.2 Hz, 2 H), 1.54 (s, 6 H), 1.42 (t, J = 7.1 Hz, 3 H), 1.38 (s, 9 H). MS *m/z* 472 $[M + H]^+$.

4-[7-[(2-Aminoethyl)oxy]-2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl]-2-methyl-3-butyn-2-ol, Trifluoroacetate (3c). A solution of **33c** (75 mg, 0.16 mmol) in CH_2Cl_2 (2.7 mL) with TFA (0.3 mL, 3.89 mmol) was stirred at room temperature for 1 h. Toluene (2 mL) was added, and all volatiles

were removed at reduced pressure. The residue was subjected to reverse phase HPLC, and the product-containing fractions were collected and evaporated to dryness. The residue was sequentially triturated in EtOAc and water to give 41 mg (53%) of **3c** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1 H), 8.05 (s, 3 H), 7.06 (s, 2 H), 5.68 (s, 1 H), 4.90 (q, J = 7.1 Hz, 2 H), 4.54 (t, J = 4.9 Hz, 2 H), 3.35 (t, J = 4.8 Hz, 2 H), 1.54 (s, 6 H), 1.44 (t, J = 7.0 Hz, 3 H). MS m/z 372 $[M + H]^+$. Anal. ($C_{17}H_{21}N_7O_3 \cdot C_2HF_3O_2 \cdot 0.5H_2O$) calcd: C, 46.16; H, 4.69; N, 19.83. Found: C, 46.49; H, 4.37; N, 19.65.

1,1-Dimethylethyl [(1*S*)-3-Hydroxy-1-(phenylmethyl)propyl]carbamate. To a 0 °C solution of (3*S*)-3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-4-phenylbutanoic acid (1.35 g, 4.83 mmol) in THF (27 mL) was added $BH_3 \cdot$THF complex (1.0 M in tetrahydrofuran, 24.2 mL, 24.2 mmol) via syringe over a period of 1−2 min. The reaction mixture was stirred between 0 and 5 °C for 5 h. The reaction was quenched by careful addition of 9:1 methanol/acetic acid (25 mL) followed by stirring at ambient temperature for 15 min. Solvents were removed in vacuo, and the crude residue was redissolved in ethyl acetate (50 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was subjected to flash chromatography (silica gel, 30%−50% ethyl acetate/hexanes) to provide 700 mg (52%) of the desired material as a clear, viscous oil that crystallized on standing to a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27−7.35 (m, 2 H), 7.14−7.26 (m, 3 H), 4.46 (br s, 1 H), 3.98−4.19 (m, 1 H), 3.53−3.78 (m, 2 H), 2.82 (d, J = 6.57 Hz, 2 H), 1.77−1.93 (m, 1 H), 1.42 (s, 9 H).

1,1-Dimethylethyl [(1*S*)-3-Bromo-1-(phenylmethyl)propyl]carbamate. A solution of 1,1-dimethylethyl [(1*S*)-3-hydroxy-1-(phenylmethyl)propyl]carbamate (700 mg, 2.64 mmol) and carbon tetrabromide (1.14 g, 3.43 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 °C and treated with triphenylphosphine (969 mg, 3.69 mmol) in one portion. After the mixture was stirred at 0 °C for 2 h, the solvent was removed under reduced pressure. Purification by flash chromatography (silica gel, 10%−30% ethyl acetate/hexanes) gave 559 mg (61%) of the desired material as a white fluffy powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.43 (m, 2 H), 7.13−7.27 (m, 3 H), 3.87−4.03 (m, 1 H), 3.33−3.54 (m, 2 H), 2.82−2.96 (m, 1 H), 2.73−2.82 (m, 1 H), 2.01−2.17 (m, 1 H), 1.81−2.01 (m, 1 H), 1.41 (s, 9 H).

1,1-Dimethylethyl [(1*S*)-3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}-1-(phenylmethyl)propyl]carbamate (32d). To a stirred solution of **29** (214 mg, 0.76 mmol) in DMF (5 mL) at ambient temperature was added cesium carbonate (621 mg, 1.91 mmol) followed by 1,1-dimethylethyl [(1*S*)-3-bromo-1-(phenylmethyl)propyl]carbamate (250 mg, 0.76 mmol). The reaction mixture was stirred under argon at 33 °C for 19 h, at which time it was diluted with ethyl acetate and washed with water. The combined aqueous washings were extracted with fresh ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, 25%−50% ethyl acetate/hexanes) to afford 350 mg (83%) of **32d** as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1 H), 7.28−7.35 (m, 2 H), 7.17−7.27 (m, 3 H), 5.97 (s, 2 H), 4.76−4.92 (m, 2 H), 4.67 (d, J = 8.34 Hz, 1 H), 4.17−4.33 (m, 2 H), 2.89−3.06 (m, 1 H), 2.80 (dd, J = 13.26, 7.71 Hz, 1 H), 2.10−2.23 (m, 1 H), 1.81−1.94 (m, 1 H), 1.31−1.43 (m, 12 H).

1,1-Dimethylethyl [(1*S*)-3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-hydroxy-3-methyl-1-butyn-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}-1-(phenylmethyl)propyl]carbamate (33d). A mixture of **32d** (0.19 mL, 1.99 mmol), Et_3N (0.55 mL, 3.98 mmol), DBU (0.20 mL, 1.33 mmol), zinc dust (43 mg, 0.66 mmol), sodium iodide (99 mg, 0.66 mmol), and tetrakis(triphenylphosphine)palladium(0) (77 mg, 0.066 mmol) in DMSO (7 mL) was stirred under argon in a sealed flask at 80 °C for 3.5 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, 50%−100% ethyl acetate/hexanes) to give 271 mg (67%) of **33d** as a flaky yellow solid. ^{1}H NMR (400 MHz, CH_3OH-d_4) δ 7.95 (s, 1 H), 7.23−7.34 (m, 4 H), 7.16−7.22 (m, 1 H), 4.27−4.46 (m, 2 H), 3.99−4.10 (m, 1 H), 2.84−2.94 (m, 1 H), 2.73−2.82 (m, 1 H), 2.10−2.22 (m, 1 H), 1.82−1.95 (m, 1 H), 1.64 (s, 6 H), 1.42 (t, J = 7.07 Hz, 3 H), 1.32 (s, 9 H).

4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-7-{[(3*S*)-3-amino-4-phenylbutyl]oxy}-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (3d). A solution of **33d** (266 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) and trifluoroacetic acid (1 mL) was stirred at 30 °C for 1 h. The solvent was removed under reduced pressure to give a yellow solid. The solid was suspended in CH_2Cl_2 (3 mL), sonicated, and isolated by filtration. The solid was further washed with CH_2Cl_2 and air-dried to give 255 mg (77%) of **3d** as a white powder. $[\alpha]_D^{23}$ −13.2° (c 0.50, methanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1 H), 8.01−8.08 (m, 3 H), 7.25−7.41 (m, 5 H), 7.04 (br s, 2 H), 4.63−4.74 (m, 2 H), 4.34−4.48 (m, 2 H), 3.55−3.68 (m, 1 H), 3.07 (dd, J = 13.52, 5.18 Hz, 1 H), 2.88 (dd, J = 13.64, 8.84 Hz, 1 H), 2.01−2.15 (m, 2 H), 1.53 (s, 6 H), 1.28 (t, J = 7.07 Hz, 3 H). Anal. ($C_{25}H_{29}N_7O_3 \cdot 2C_2HF_3O_2$) calcd: C, 49.51; H, 4.44; N, 13.94. Found: C, 49.58; H, 4.69; N, 14.03.

1,1-Dimethylethyl 3-(Bromomethyl)-1-pyrrolidinecarboxylate. To a solution of 1,1-dimethylethyl 3-(hydroxymethyl)-1-pyrrolidinecarboxylate (0.56 g, 2.8 mmol) with carbon tetrabromide (1.39 g, 4.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise a solution of triphenylphosphine (0.73 g, 2.8 mmol in 5 mL of CH_2Cl_2). Upon completion, the mixture was stirred for 18 h at room temperature. The solvent was removed at reduced pressure and the residue stirred in 10% ethyl acetate and 90% hexane. The mixture was filtered and the resulting solution chromatographed on silica eluting with a gradient of 0−25% EtOAc in hexane to afford 0.41 g (55%) of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.51−3.63 (m, 2 H), 3.31−3.49 (m, 2 H), 3.13−3.28 (m, 1 H), 2.90−3.03 (m, 1 H), 2.49−2.65 (m, 1 H), 1.90−2.07 (m, 1 H), 1.54−1.72 (m, 1 H), 1.40 (s, 9 H). MS m/z 264 $[M + H]^+$.

1,1-Dimethylethyl 3-({[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}methyl)-1-pyrrolidinecarboxylate (32e). A mixture consisting of **29** (100 mg, 0.35mmol) in DMF (2 mL) with cesium carbonate (290 mg, 0.9 mmol) and 1,1-dimethylethyl 3-(bromomethyl)-1-pyrrolidinecarboxylate (290 mg, 1.1 mmol) was stirred at room temperature for 44 h. The mixture was poured into rapidly stirring ice−water (7 mL), and stirring continued for 10 min. To this was added cyclohexane (7 mL), and stirring continued for an additional 20 min. The solid was collected by filtration, then washed with cyclohexane and dried in vacuo to afford 111 mg (69%) of **32e**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (s, 1 H), 6.94 (s, 2 H), 4.82 (q, J = 7.1 Hz, 2 H), 4.20−4.38 (m, 2 H), 3.51−3.61 (m, 1 H), 3.39−3.48 (m, 1 H), 3.23−3.32 (m, 1 H), 3.12−3.23 (m, 1 H), 2.71−2.88 (m, 1 H), 2.02−2.15 (m, 1 H), 1.70−1.86 (m, 1 H), 1.46 (t, J = 7.1 Hz, 3 H), 1.40 (s, 9 H). MS m/z 464 $[M + H]^+$.

1,1-Dimethylethyl 3-({[2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-hydroxy-3-methyl-1-butyn-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}methyl)-1-pyrrolidinecarboxylate (33e). A thick walled pressure vessel was charged with **32e** (100 mg, 0.22 mmol), 2-methyl-3-butyn-2-ol (0.25 mL, 2.6 mmol), $(Ph_3P)_4Pd$ (30 mg), diisopropylamine (0.4 mL), and dioxane (4 mL). The vessel was purged with argon, sealed, and stirred 100 °C for 6 h. The mixture was concentrated at reduced pressure and then triturated with EtOAc (4 mL) to afford 82 mg (75%) of **33e**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1 H), 705 (s, 2 H), 5.68 (s, 1 H), 4.83 (q, J = 6.8 Hz, 2 H), 4.23−4.42 (m, 2 H), 3.50−3.63 (m, 1 H), 3.39−3.49 (m, 1 H), 3.23−3.33 (m, 1 H), 3.13−3.23 (m, 1 H), 2.71−2.86 (m, 1 H), 2.00−2.16 (m, 1 H), 1.72−1.88 (m, 1 H), 1.54 (s, 6 H), 1.45 (t, J = 7.1 Hz, 3 H), 1.40 (s, 9 H). MS m/z 512 $[M + H]^+$.

4-{2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-[(3-pyrrolidinylmethyl)oxy]-1*H*-imidazo[4,5-*c*]pyridin-4-yl}-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (3e). Compound **33e** (75 mg, 0.15 mmol) was stirred in a 20% solution of TFA in CH_2Cl_2 (3 mL) at room temperature for 20 min. Toluene (3 mL) was added, and all volatiles were removed at reduced pressure. The residue was purified by

preparative reversed phase HPLC to afford 40 mg (43%) of **3e**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69−8.85 (m, 2 H), 8.18 (s, 1H), 7.06 (s, 2 H), 4.83 (q, J = 7.0 Hz, 2 H), 4.29−4.45 (m, 2 H), 3.40−3.53 (m, 1 H), 3.19−3.38 (m, 2 H), 3.02−3.16 (m, 1 H), 2.79−2.95 (m, 1 H), 2.12−2.27 (m, 1 H), 1.77−1.90 (m, 1 H), 1.54 (s, 6 H), 1.46 (t, J = 7.1 Hz, 3 H). MS m/z 412 $[M + H]^+$. Anal. ($C_{20}H_{25}N_7O_3 \cdot 2C_2HF_3O_2 \cdot 2H_2O$) calcd: C, 42.67; H, 4.63; N, 14.51. Found: C, 42.48; H, 4.35; N, 14.47.

1,1-Dimethylethyl 4-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}-1-piperidinecarboxylate (32f). A mixture of **29** (0.25 g, 0.89 mmol), 1,1-dimethylethyl 4-bromo-1-piperidinecarboxylate (0.71 g, 2.67 mmol), and cesium carbonate (0.87 g, 2.67 mmol) in DMF (5 mL) was heated to 60 °C for 5 h. The mixture was diluted with water and extracted with ethyl acetate. The combined organic extract was washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue subjected to flash chromatography (10−50% EtOAc/hexane, silica gel) to give 95 mg (23%) of **32f** as a light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1 H), 5.89 (s, 2 H), 5.01 (q, J = 6.99 Hz, 2 H), 4.77−4.86 (m, 1 H), 3.71−3.82 (m, 2 H), 3.42−3.51 (m, 2 H), 2.09−2.20 (m, 2 H), 1.86−1.98 (m, 2 H), 1.56 (t, J = 7.20 Hz, 3 H), 1.51 (s, 9 H). MS m/z 464 $[M + H]^+$.

1,1-Dimethylethyl 4-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-hydroxy-3-methyl-1-butyn-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}-1-piperidinecarboxylate (33f). A pressure tube was charged with **32f** (93 mg, 0.20 mmol), 2-methyl-3-buty-2-ol (0.1 mL, 1.03 mmol), $(Ph_3P)_4Pd$ (10 mg, 0.009 mmol), Zn dust (10 mg, 0.15 mmol), NaI (10 mg, 0.067 mmol), DBU (0.10 mL, 0.66 mmol), Et_3N (0.1 mL, 0.71 mmol), and DMSO (3 mL). The mixture was purged with argon for 10 min, and then the pressure vessel was sealed and heated at 80 °C for 4 h. The mixture was diluted with water and extracted with ethyl acetate. The combined organic extract was washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue subjected to preparative reverse phase HPLC to give 121 mg (89%) of **33f** as a light-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (s, 1 H), 7.05 (s, 2 H), 4.96−5.11 (m, 1 H), 4.84 (q, J = 6.99 Hz, 2 H), 3.57−3.72 (m, 2 H), 3.24−3.42 (m, 2 H), 1.98−2.12 (m, 2 H), 1.66−1.81 (m, 2 H), 1.54 (s, 6 H), 1.38−1.49 (m, 12 H). MS m/z 512 $[M + H]^+$.

4-[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-(4-piperidinyloxy)-1*H*-imidazo[4,5-*c*]pyridin-4-yl]-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (3f). Compound **33f** (121 mg, 0.19 mmol) was dissolved in TFA (2 mL) and CH_2Cl_2 (3 mL). After 1 h, the solvent was removed under reduced pressure and the residue subjected to preparative reverse phase HPLC to give 54 mg (44%) of **3f** as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.23 (s, 1 H), 5.11−5.20 (m, 1 H), 5.05 (q, J = 7.07 Hz, 2 H), 3.43−3.54 (m, 2 H), 3.35−3.40 (m, 2 H), 2.37−2.51 (m, 2 H), 2.14−2.30 (m, 2 H), 1.68 (s, 6 H), 1.60 (t, J = 7.07 Hz, 3 H). MS m/z 412 $[M + H]^+$. Anal. ($C_{20}H_{25}N_7O_3 \cdot 2C_2HF_3O_2$) calcd: C, 45.08; H, 4.26; N, 15.33. Found: C, 45.03; H, 4.18; N, 15.16.

1,1-Dimethylethyl (3*S*)-3-(Bromomethyl)-1-piperidinecarboxylate. To a solution of 1,1-dimethylethyl (3*S*)-3-(hydroxymethyl)-1-piperidinecarboxylate (30.0 g, 139 mmol) and carbon tetrabromide (72.0 g, 217 mmol) in CH_2Cl_2 (150 mL) was added dropwise a solution of triphenyl phosphine (42.4 g, 162 mmol) in CH_2Cl_2 (150 mL). An ice bath was used to maintain an internal temperature between 20 and 25 °C during the addition. After the mixture was stirred at ambient temperature for 1 h, cyclohexane (500 mL) was added. Approximately one-half of the solvent was removed under reduced pressure. The remaining solution was cooled in an ice bath, and the resulting precipitate was removed by filtration. The filtrate was concentrated under reduced pressure and the residue subjected to flash chromatography (0−25% ethyl acetate/hexanes, silica gel) to give 35.1 g (91%) of the desired product as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99−4.12 (m, 1 H), 3.82−3.92 (m, 1 H), 3.26−3.36 (m, 2 H), 2.81−2.92 (m, 1 H), 2.65−2.79 (m, 1 H), 1.77−1.99 (m, 2H), 1.61−1.72 (m, 2 H), 1.41−1.55 (m, 9 H), 1.25−1.39 (m, 1 H). MS m/z 278 $[M + H]^+$.

1,1-Dimethylethyl (3*S*)-3-({[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}methyl)-1-piperidinecarboxylate (32g). A mixture consisting of **29** (25.0 g, 89.1 mmol), cesium carbonate (41.0 g, 126 mmol), and 1,1-dimethylethyl (3 *S*)-3-(bromomethyl)-1-piperidinecarboxylate (35.0 g, 126 mmol) in DMF (200 mL) was stirred at 40 °C for 8 h and then at 35 °C for 18 h. The mixture was poured into rapidly stirring ice−water (800 mL). After 10 min, ethyl acetate (300 mL) was added and the stirring continued for an additional 20 min. The solid was collected by filtration, washed with ethyl acetate (50 mL), and dried to give 36 g (85%) of **32g**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (s, 1 H), 6.94 (s, 2 H), 4.83 (q, J = 7.3 Hz, 2 H), 3.92−4.26 (m, 3 H), 3.74−3.88 (m, 1 H), 2.67−3.01 (m, 2 H), 1.95−2.11 (m, 1 H), 1.81−1.94 (m, 1 H), 1.62−1.76 (m, 1 H), 1.48 (t, J = 7.1 Hz, 3H), 1.29−1.43 (m, 11 H). MS m/z 478 $[M + H]^+$.

1,1-Dimethylethyl (3*S*)-3-({[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-hydroxy-3-methyl-1-butyn-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}methyl)-1-piperidinecarboxylate (33g). Two thick-walled pressure vessels were each charged with **32g** (18.0 g, 37.7 mmol), 2-methyl-3-buty-2-ol (8.0 mL, 82.5 mmol), $(Ph_3P)_4Pd$ (0.5 g, 0.43 mmol), Zn dust (0.5 g., 7.4 mmol), NaI (1.10 g, 7.4 mmol), DBU (8.0 mL, 53.5 mmol), triethylamine (7.5 mL, 54.5 mmol), and DMSO (150 mL). Both vessels were purged with argon for 10 min, then sealed and heated at 80 °C for 4 h. The mixture from both vessels were poured into a single vessel containing rapidly stirring ice−water (1000 mL). After 10 min, ethyl acetate (300 mL) was added and stirring continued for an additional 20 min. The solid was collected by filtration, washed with ethyl acetate (50 mL), and dried to give 35.5 g (90%) of **33g**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1 H), 7.05 (s, 2 H), 5.68 (s, 1 H), 4.75−4.95 (m, 2 H), 4.13−4.29 (m, 2 H), 3.91−4.12 (m, 1 H), 3.71−3.89 (m, 1 H), 2.65−3.05 (m, 2 H), 1.98−2.09 (m, 1 H), 1.81−1.94 (m, J = 8.8 Hz, 1 H), 1.66−1.74 (m, 1 H), 1.54 (s, 6 H), 1.48 (t, J = 7.0 Hz, 3 H), 1.30−1.45 (m, 11 H). MS m/z 526 $[M + H]^+$.

4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3*S*)-3-piperidinylmethyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-methyl-3-butyn-2-ol (3g). Compound **33g** (35.0 g, 66.6 mmol) and TFA (350 mL of a 20% solution in CH_2Cl_2, 808 mmol) was stirred at ambient temperature for 2.5 h. The solution was poured slowly into a rapidly stirring mixture of water, NaOH (36 g, 900 mmol), ethyl acetate (200 mL), and THF (1000 mL). The organic layer was separated, and the aqueous layer was extracted with additional ethyl acetate/THF (1:5 v/v, 150 mL). The combined organic extract was washed with saturated NaCl and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting solid was recrystallized from hot ethanol (1200 mL) to give 26.3 g (93%) of **3g** as a white crystalline solid. $[\alpha]_D^{20}$ −21.7° (c 1.00, water). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1 H), 7.06 (s, 2 H), 5.71 (s, 1 H), 4.83 (q, J = 7.1 Hz, 2 H), 4.17 (d, J = 6.3 Hz, 2 H), 3.10 (dd, J = 11.8, 2.9 Hz, 1 H), 3.34 (s, 1H), 2.81−2.93 (m, 1 H), 2.40−2.49 (m, 2 H), 1.94−2.05 (m, 1 H), 1.87 (dd, J = 12.1, 3.0 Hz, 1 H), 1.59−1.67 (m, 1 H), 1.54 (s, 6 H), 1.45 (t, J = 7.1 Hz, 3 H), 1.21−1.42 (m, 2 H). MS m/z 426 $[M + H]^+$. Anal. ($C_{21}H_{27}N_7O_3 \cdot 0.5H_2O$) calcd: C, 58.05; H, 6.50; N, 22.57. Found: C, 58.32; H, 6.28; N, 22.32.

1,1-Dimethylethyl (3*R*)-3-(Bromomethyl)-1-piperidinecarboxylate. The title compound was prepared in a manner analogous to the preparation of 1,1-dimethylethyl (3*S*)-3-(bromomethyl)-1-piperidinecarboxylate by substituting dimethylethyl (3*R*)-3-(hydroxymethyl)-1-piperidinecarboxylate (1.00 g, 4.60 mmol) for dimethylethyl (3*S*)-3-(hydroxymethyl)-1-piperidinecarboxylate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93−4.21 (m, 1 H), 3.80−3.91 (m, 1 H), 3.24−3.36 (m, 2 H), 2.75−2.93 (m, 1 H), 2.47−2.72 (m, 1 H), 1.80−1.97 (m, 2 H), 1.62−1.74 (m, 2 H), 1.48 (s, 9 H), 1.24−1.40 (m, 1 H). MS m/z 278 $[M + H]^+$.

1,1-Dimethylethyl (3*R*)-3-({[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}methyl)-1-piperidinecarboxylate (32h). Compound **32h** was prepared in a manner analogous to the preparation of **32g** by substituting 1,1-dimethylethyl (3*R*)-3-(bromomethyl)-1-piperidinecarboxylate (600 mg, 2.16 mmol) for 1,1-dimethylethyl (3*S*)-3-(bromomethyl)-1-

piperidinecarboxylate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, 1 H), 6.94 (s, 2 H), 4.85 (q, J = 7.2 Hz, 2 H), 3.93−4.26 (m, 3 H), 3.72−3.89 (m, 1 H), 2.66−3.02 (m, 2 H), 1.95−2.13 (m, 1 H), 1.82−1.95 (m, 1 H), 1.64−1.76 (m, 1 H), 1.49 (t, J = 7.1 Hz, 3 H), 1.27−1.45 (m, 11 H). MS m/z 478 $[M + H]^+$.

4-(4-Chloro-1-ethyl-7-{[(3*R*)-3-piperidinylmethyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine. A solution of **32h** (200 mg, 0.42 mmol) in CH_2Cl_2 (3.2 mL) with TFA (0.8 mL, 10.4 mmol) was stirred at room temperature for 45 min. The solvent was removed at reduced pressure to give 220 mg (87%) the title compound as a TFA salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1 H), 6.95 (s, 2 H), 4.81 (q, J = 7.2 Hz, 2 H), 4.13−4.27 (m, 2 H), 3.41 (dd, J = 12.0, 3.4 Hz, 1 H), 3.15−3.31 (m, 2 H), 2.77−2.89 (m, 2 H), 2.27−2.39 (m, 1 H), 1.83−1.95 (m, 2 H), 1.63−1.72 (m, 1 H), 1.39−1.49 (m, 4 H). MS m/z 378 $[M + H]^+$.

4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3*R*)-3-piperidinylmethyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (3h). In a thick wall pressure tube, a mixture consisting of 4-(4-chloro-1-ethyl-7-{[(3*R*)-3-piperidinylmethyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine (125 mg, 0.33 mmol), 2-methyl-3-butyn-2-ol (83 mg, 0.99 mmol), tetrakis(triphenylphosphine)palladium(0) (15.3 mg, 0.013 mmol), zinc (3.8 mg, 0.058 mmol), sodium iodide (8.7 mg, 0.058 mmol), DBU (0.075 mL, 0.495 mmol), and TEA (0.069 mL, 0.495 mmol) in DMSO (3 mL) was purged with argon, sealed, and then heated with stirring at 80 °C for 3 h. The product mixture was subjected to reverse phase HPLC to afford 79 mg (36%) of **3h**. $[\alpha]_D^{23}$ +6.7° (*c* 0.75, methanol). ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 8.15 (s, 1 H), 4.83 (q, J = 7.2 Hz, 2 H), 4.19−4.33 (m, 2 H), 3.38−3.47 (m, 1 H), 3.29 (d, J = 12.9 Hz, 1 H), 2.84 (t, J = 12.1 Hz, 2 H), 2.27−2.40 (m, 1 H), 1.85−1.97 (m, 2 H), 1.60−1.75 (m, 1 H), 1.53 (s, 6 H), 1.45 (t, J = 7.1 Hz, 3 H), 1.41−1.50 (m, 1 H). MS m/z 426 $[M + H]^+$. Anal. ($C_{21}H_{27}N_7O_3 \cdot 2TFA \cdot H_2O$) calcd: C, 44.71; H, 4.65; N, 14.60. Found: C, 44.87; H, 4.48; N, 14.23.

1,1-Dimethylethyl 2-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-7-{[3-({[(1,1-dimethylethyl)oxy]-carbonyl}amino)propyl]oxy}-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-1*H*-pyrrole-1-carboxylate (34a). A mixture of **30** (317 mg, 0.720 mmol), (1-{[(1,1-dimethylethyl)oxy]-carbonyl}-1*H*-pyrrol-2-yl)boronic acid (382 mg, 1.81 mmol), tetrakis(triphenylphosphine)palladium(0) (125 mg, 0.110 mmol), and 2 M aqueous potassium carbonate (1.09 mL, 2.17 mmol) in 1,2-dimethoxyethane (11 mL) was heated in a sealed tube at 90 °C for 5 h. The mixture was cooled to ambient temperature, diluted with ethyl acetate (15 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, 50% ethyl acetate in hexanes) to furnish 334 mg (69%) of **34a** as a pale-yellow foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1 H), 7.43 (dd, J = 3.16, 1.64 Hz, 1 H), 6.61−6.71 (m, 1 H), 6.35 (t, J = 3.28 Hz, 1 H), 5.74 (br s, 2 H), 5.03 (q, J = 6.91 Hz, 2 H), 4.64−4.77 (m, 1 H), 4.35 (t, J = 6.06 Hz, 2 H), 3.42 (q, J = 5.98 Hz, 2 H), 2.09−2.21 (m, 2 H), 1.54 (t, J = 6.95 Hz, 3 H), 1.46 (s, 9 H), 1.22 (s, 9 H).

4-[7-[(3-Aminopropyl)oxy]-1-ethyl-4-(1*H*-pyrrol-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine, Bis-trifluoroacetate (11a). A solution of **34a** (334 mg, 0.59 mmol) in CH_2Cl_2 (3 mL) was treated with trifluoroacetic acid (0.75 mL). The mixture was stirred at 30 °C for 2 h and then concentrated under reduced pressure. The crude residue was redissolved in a mixture of methanol (5 mL) and dimethyl sulfoxide (1.5 mL) and subjected to purification by reverse phase HPLC to give 209 mg (57%) of **11a** as a flaky light-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (br s, 1 H), 8.09 (s, 1 H), 7.90 (br s, 3 H), 7.20−7.29 (m, 1 H), 7.07 (d, J = 1.01 Hz, 1 H), 6.98 (br s, 2 H), 6.26−6.34 (m, 1 H), 4.86 (q, J = 6.99 Hz, 2 H), 4.40 (t, J = 5.94 Hz, 2 H), 3.00−3.14 (m, 2 H), 2.10−2.24 (m, 2 H), 1.49 (t, J = 7.07 Hz, 3 H). Anal. ($C_{17}H_{20}N_8O_2 \cdot 2C_2HF_3O_2 \cdot 0.5H_2O$) calcd: C, 41.66; H, 3.83; N, 18.51. Found: C, 41.85; H, 3.51; N, 18.49.

1,1-Dimethylethyl (3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-furanyl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}propyl)-carbamate (34b). Tetrakis(triphenylphosphine)palladium(0) (20 mg, 0.017 mmol) was added to a mixture of **30** (50 mg, 0.11 mol), 2 N Na_2CO_3 (0.16 mL, 0.32 mmol), 3-furylboronic acid (25 mg, 0.23 mmol), and 1,4-dioxane (3 mL). The flask was flushed with argon, sealed, and heated with stirring for 10 min at 160 °C in a microwave apparatus. The resulting mixture was then diluted with 1,4-dioxane, filtered, and concentrated under reduced pressure to afford **34b** as a brownish-red residue. This material was used in the next step without further purification. MS m/z 470.4 $[M + H]^+$.

4-[7-[(3-Aminopropyl)oxy]-1-ethyl-4-(3-furanyl)-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine, Bis-trifluoroacetate (11b). Trifluoroacetic acid (2 mL) was added to a solution of crude **34b** in CH_2Cl_2 (6 mL) at ambient temperature. After 30 min, the solvent was removed under reduced pressure and the residue was subjected to reverse phase preparative HPLC to give 35 mg (49% over two steps) of **11b**. ^{1}H NMR (400 MHz, CH_3OH-d_4) δ 8.82 (s, 1 H), 8.17 (s, 1 H), 7.84 (t, J = 1.77 Hz, 1 H), 7.35 (dd, J = 1.77, 0.76 Hz, 1 H), 5.09 (q, J = 7.07 Hz, 2 H), 4.53 (t, J = 5.94 Hz, 2 H), 3.28 (t, J = 7.71 Hz, 2 H), 2.23−2.51 (m, 2 H), 1.62 (t, J = 7.07 Hz, 3 H). Anal. ($C_{17}H_{19}N_7O_3 \cdot 2C_2HF_3O_2 \cdot H_2O$) calcd: C, 40.98; H, 3.77; N, 15.93. Found: C, 40.89; H, 3.48; N, 15.68.

1,1-Dimethylethyl {3-[(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-{1-[tris(1-methylethyl)silyl]-1*H*-pyrrol-3-yl}-1*H*-imidazo[4,5-*c*]pyridin-7-yl)oxy]propyl}carbamate (34c). A mixture of **30** (135 mg, 0.31 mmol), {1-[tris(1-methylethyl)silyl]-1*H*-pyrrol-3-yl}boronic acid (165 mg, 0.62 mmol), tetrakis(triphenylphosphine)palladium(0) (71 mg, 0.062 mmol), and 2 N Na_2CO_3 (0.48 mL, 0.95 mmol) in 1,4-dioxane (5 mL) was heated in a sealed tube at 105 °C for 2 h. The mixture was cooled to ambient temperature, diluted with ethyl acetate, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide **34c**. This material was used in the next step without further purification. MS m/z 625.4 $[M + H]^+$.

4-[7-[(3-Aminopropyl)oxy]-1-ethyl-4-(1*H*-pyrrol-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine, Bis-trifluoroacetate (11c). A solution of crude **34c** in CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (1 mL), and the resultant mixture was stirred at 30 °C for 1 h. The reaction mixture was diluted with methanol (5 mL) and concentrated under reduced pressure to yield 4-(7-[(3-aminopropyl)oxy]-1-ethyl-4-{1-[tris(1-methylethyl)silyl]-1*H*-pyrrol-3-yl}-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine. This material was used in the next step without further purification. MS m/z 525.4 $[M + H]^+$.

A solution of crude 4-(7-[(3-aminopropyl)oxy]-1-ethyl-4-{1-[tris(1-methylethyl)silyl]-1*H*-pyrrol-3-yl}-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine in THF (5 mL) was treated with tetrabutylammonium fluoride (1.0 M solution in tetrahydrofuran, 1 mL, 1.0 mmol), and the resultant mixture was stirred for 15 min. Concentration under reduced pressure followed by reverse phase HPLC afforded 127 mg (61%) of **11c** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (br s, 1 H), 8.18 (br s, 1 H), 8.07 (s, 1 H), 7.99−8.06 (m, 3 H), 7.10−7.16 (m, 1 H), 7.05−7.10 (m, 1 H), 6.87 (br s, 2 H), 4.87 (q, J = 6.74 Hz, 2 H), 4.41 (t, J = 5.56 Hz, 2 H), 3.01−3.14 (m, 2 H), 2.12−2.26 (m, 2 H), 1.50 (t, J = 7.07 Hz, 3 H). Anal. ($C_{17}H_{20}N_8O_2 \cdot 2C_2HF_3O_2$) calcd: C, 42.29; H, 3.72; N, 18.79. Found: C, 42.07; H, 3.55; N, 18.48.

1,1-Dimethylethyl (3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(1*H*-pyrazol-4-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}propyl)-carbamate (34d). A mixture of **30** (163 mg, 0.37 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (145 mg, 0.74 mmol), tetrakis(triphenylphosphine)palladium(0) (79 mg, 0.074 mmol), and 2 N Na_2CO_3 (0.58 mL, 1.16 mmol) in 1,4-dioxane (5 mL) was heated in a sealed tube at 105 °C for 3 h. The mixture was cooled to ambient temperature, diluted with ethyl acetate, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to furnish **34d** that was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (br s, 2 H), 8.11 (s, 1 H), 7.01 (t, J = 5.56 Hz, 1 H), 6.91 (br s, 2 H), 4.85 (q, J = 6.82 Hz, 2 H), 4.28 (t, J = 5.81 Hz, 2 H), 3.19 (q, J = 6.57 Hz, 2 H), 1.91−2.02 (m, 2 H), 1.47 (t, J = 6.95 Hz, 3 H), 1.36 (s, 9 H).

4-[7-[(3-Aminopropyl)oxy]-1-ethyl-4-(1*H*-pyrazol-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine, Bis-trifluoroacetate (11d). A solution of crude **34d** in CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (2 mL), and the resultant mixture was

stirred at 30 °C for 16 h. The reaction mixture was diluted with methanol (5 mL) and concentrated under reduced pressure. The residue was dissolved in a mixture of methanol (2.5 mL) and dimethyl sulfoxide (1 mL) and purified by reverse phase HPLC to give 180 mg (81%) of **11d** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (br s, 2 H), 8.17 (s, 1 H), 7.83−7.99 (m, 3 H), 6.89 (br s, 2 H), 4.86 (q, J = 6.91 Hz, 2 H), 4.41 (t, J = 5.81 Hz, 2 H), 3.01−3.12 (m, 2 H), 2.12−2.23 (m, 2 H), 1.49 (t, J = 7.07 Hz, 3 H). Anal. ($C_{16}H_{19}N_9O_2 \cdot 2C_2HF_3O_2 \cdot 0.5H_2O$) calcd: C, 39.61; H, 3.66; N, 20.79. Found: C, 39.77; H, 3.34; N, 20.54.

4-{7-[(3-Bromopropyl)oxy]-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl}-1,2,5-oxadiazol-3-amine (35). Anhydrous Cs_2CO_3 (7.00 g, 21.7 mmol) was added to a solution of **29** (4.70 g, 16.7 mmol) in DMF (50 mL) at room temperature. After 5 min, 1,3-dibromopropane (16.9 g, 84.0 mmol) was added and the mixture was heated to 60 °C for 3.5 h. The mixture was cooled to room temperature and quenched by partitioning between EtOAc and saturated NH_4Cl. The aqueous layer was extracted with additional EtOAc, and the combined extracts were washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated to a residue which was triturated with 0.5% methanol in CH_2Cl_2 to give 4.46 g (67% yield) of **35** as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1 H), 6.93 (s, 2 H), 4.82 (q, J = 6.9 Hz, 2 H), 4.41 (t, J = 5.8 Hz, 2 H), 3.75 (t, J = 6.6 Hz, 2 H), 2.40 (m, 2 H), 1.45 (t, J = 7.1 Hz, 3 H). MS m/z 401.2 $[M + H]^+$.

4-(4-Chloro-1-ethyl-7-{[3-({2-[4-(methyloxy)phenyl]ethyl}amino)propyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine (36a). Compound **35** (4.30 g, 10.7 mmol) was dissolved in DMF (40 mL). To this was added Et_3N (4.5 mL, 32.0 mmol) and 2-[4-(methyloxy)phenyl]ethanamine (3.10 mL, 21.0 mmol), and the resulting mixture was heated to 60 °C. After 30 min, the mixture was cooled to room temperature and quenched by partitioning between EtOAc and water. The aqueous layer was extracted with additional EtOAc, and the combined extracts were washed with water and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a brown solid. Trituration with cold EtOAc/hexane (3:1) gave 4.20 g (84% yield) of **36a** as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1 H), 7.10 (m, 2 H), 6.93 (s, 2 H), 6.78 (m, 2 H), 4.80 (m, 2 H), 4.32 (t, J = 6.1 Hz, 2 H), 3.68 (s, 3 H), 2.73 (m, 4 H), 2.64 (m, 2 H), 1.97 (m, 2 H), 1.76 (br s, 1 H), 1.43 (t, J = 7.1 Hz, 3 H). MS m/z 472.4 $[M + H]^+$.

4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[3-({2-[4-(methyloxy)phenyl]ethyl}amino)propyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-methyl-3-butyn-2-ol, Bis-trifluoroacetate (14a). Compound **14a** was prepared in a manner analogous to the preparation of **3g** by substituting **36a** (2.50 g, 5.30 mmol) for **32g**. The reaction was quenched by partitioning between EtOAc and water. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 5−100% MeOH/$CHCl_3$ + 1%NH_4OH). The isolated product was washed with a small amount of $CHCl_3$ and recrystallized from CH_3CN. The solid was suspended in acetonitrile/water (1:1), and trifluoracetic acid (0.44 mL, 5.76mmol) was added. After the mixture was stirred for 15 min, the solvent was removed under reduced pressure and the resulting solid was dried under vaccum at 40 °C to give 1.82 g of **14** as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (br s, 2 H), 8.17 (s, 0 H), 7.20 (d, J = 8.6 Hz, 2 H), 7.05 (s, 2 H), 6.91 (d, J = 8.8 Hz, 2 H), 4.84 (d, J = 7.1 Hz, 2 H), 4.42 (t, J = 5.8 Hz, 2 H), 3.73 (s, 3 H), 3.19 (br s, 4 H), 2.86 (m, 2 H), 2.21 (m, 2 H), 1.54 (s, 6 H), 1.45 (t, J = 7.1 Hz, 3 H). MS m/z 519.9 $[M + H]^+$. Anal. ($C_{27}H_{33}N_7O_4 \cdot 2C_2HF_3O_2 \cdot 0.5CH_3CN$) calcd: C, 50.03; H, 4.79; N, 13.68. Found: C, 50.31; H, 5.07; N, 13.62.

(2*S*)-3-[(3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-4-chloro-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}propyl)amino]-1,2-propanediol (36b). A solution of **35** (250 mg, 0.622 mmol) and (2*S*)-3-amino-1,2-propanediol (300 mg, 3.29 mmol) in CH_3CN (15 mL) was stirred at 60 °C for 18 h. The solvent was removed under reduced pressure, and the residue was triturated with water. The solid was collected and dried to give 251 mg (98%) of **36b** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (s, 1 H), 6.94 (s, 2 H), 4.83 (q, J = 7.0 Hz, 2 H), 4.49−4.64 (m, 1 H), 4.35 (t, J = 6.1 Hz, 2 H), 3.49−3.59 (m, 1 H), 3.24−3.34 (m, 1 H), 2.75 (t, J = 6.8 Hz, 2 H), 2.63 (dd, J = 11.8, 4.4 Hz, 1 H), 2.44−2.49 (m, 1 H), 1.93−2.05 (m, 2 H), 1.45 (t, J = 7.1 Hz, 3 H). MS m/z 412 $[M + H]^+$.

(2*S*)-3-[(3-{[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-4-(3-hydroxy-3-methyl-1-butyn-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-7-yl]oxy}propyl)-amino]-1,2-propanediol (14b). In a thick wall pressure flask, a mixture consisting of **36b** (250 mg, 0.61 mmol), 2-methyl-3-butyn-2-ol (153 mg, 1.82 mmol), tetrakis(triphenylphosphine)palladium(0) (28.1 mg, 0.024 mmol), zinc (7.0 mg, 0.106 mmol), sodium iodide (15.9 mg, 0.106 mmol), DBU (0.137 mL, 0.911 mmol), and TEA (0.127 mL, 0.911 mmol) in DMSO (3 mL) was purged with Ar, then heated with stirring at 80 °C for 3 h. The mixture was subjected to reverse phase HPLC, and the product-containing fractions were made slightly basic with 1 N NaOH. After concentrating to approximately $^1/_3$ volume, the resulting precipitate was collected and dried to afford 164 mg (60%) of **14b** as a white solid. $[\alpha]_D^{23}$ −3.5° (c 0.40, methanol). ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 8.12 (s, 1 H), 4.82 (q, J = 6.7 Hz, 2 H), 4.35 (t, J = 5.9 Hz, 2 H), 3.49−3.58 (m, 1 H), 3.25−3.37 (m, 2 H), 2.72 (t, J = 6.8 Hz, 2 H), 2.60 (dd, J = 11.9, 4.3 Hz, 1 H), 2.44 (dd, J = 11.9, 7.3 Hz, 1 H), 1.93−2.04 (m, 2 H), 1.52 (s, 6 H), 1.42 (t, J = 7.0 Hz, 3 H). MS m/z 460 $[M + H]^+$. Anal. ($C_{21}H_{29}N_7O_5 \cdot H_2O$) calcd: C, 52.82; H, 6.54; N, 20.53. Found: C, 53.21; H, 6.55; N, 20.64.

Biological Assays and in Vivo Studies. Details of the in vitro kinase inhibition assays, assays to measure inhibition of phosphorylation of GSK3β in BT474 cells, and studies involving tumor xenografts are described in a separate publication.[14] Cell proliferation assays were performed as previously described.[30]

Biochemical Characterization of 3g. Determination of Time-Dependent Inhibition of 3g against Human AKT1 and 2. To determine whether **3g** exhibits time-dependent inhibition, a filter-binding assay was used to measure IC_{50} values with and without a 30 min preincubation step of inhibitor with AKT1 and 2 prior to the start of the reaction (preincubation). The final reaction conditions were 15 nM AKT1 or 10 nM AKT2, 1 μM AKT-tide peptide (sequence, biotin-ARKRERAYSFGHHA-NH_2), 15 μM ATP, and 0.015 mCi/mL γ-^{33}P-ATP in buffer A (25 mM MOPS, pH 7.5, 0.5 mM CHAPS, 10 mM $MgCl_2$, 50 mM KCl, 1 mM DTT) and various concentrations of **3g** in 5% DMSO. The reactions were allowed to proceed for 10 min for AKT1 and 5 min for AKT2 and then stopped with the addition of an equal volume of 1% H_3PO_4. The stopped reaction was transferred to phosphocellulose filter plates and washed 5 times with 100 μL of 0.5% H_3PO_4. Microscint20 cocktail was added to the dried filter plates, and the plates were read using a Perkin-Elmer Microbeta liquid scintillation counter.

Determination of Off-Rates of 3g from Human AKT1 and 2. Compound **3g** and AKT1 were incubated at 50 and 125 nM each in buffer A for 50 min at room temperature. Formation of the AKT2−**3g** complex was similarly carried out. This amount of **3g** was found to be sufficient to achieve complete inhibition. The reaction was initiated by adding the complex of **3g** and AKT1 or AKT2 to the substrate solution containing 10 μM Sox-AKT-tide peptide substrate and 1 mM ATP in buffer A. The recovery of enzymatic activity was measured in a continuous fluorescence detection assay.[31] The phosphorylation of Sox-labeled AKT-tide peptide was quantified in an Envision 2102 instrument (PerkinElmer) using an excitation wavelength of 380 nm and an emission wavelength of 486 nm. The time course of the recovery of enzymatic activity following rapid dilution of a preformed enzyme and inhibitor complex was fitted to the following equation:[32]

$$y = v_s t + \frac{v_i - v_s}{k_{obs}}(1 - e^{-k_{obs}t}) + \text{background}$$

where v_i and v_s are the initial and steady state velocities of the reaction in the presence of the inhibitor, k_{obs} is the apparent first-order rate constant for the conversion from v_i to v_s, and t is time.

Under the experimental conditions, k_{obs} represents the dissociation rate constant (k_{off}) of the EI complex. The dissociation half-life ($t_{1/2}$) is related to k_{obs} by $t_{1/2} = 0.693/k_{off}$.

Mode of Inhibition of 3g toward ATP. The IC_{50} values for **3g** were determined against AKT1 and AKT2 in a continuous fluorescence detection assay. Various concentration of ATP were tested with 2.5 nM AKT1 or 2 nM AKT2 in buffer A containing 10 μM Sox-AKT-tide peptide. Rates of catalysis were derived from the linear portion of the progress curves.

Potency of Compound 3g (K_i*) Determined against the Human and Mouse Enzymes. Either AKT1 (final concentration 0.33 nM) or AKT2 (final concentration 1 nM) in buffer A was incubated with **3g** at various concentrations for 60 min at room temperature. The reaction was initiated by adding substrate mix in buffer A containing GSK3α peptide (Ac-KKGGRARTSSFAEPG-NH_2, final concentration 10 μM), ATP (final concentration 50 μM), and γ-^{33}P-ATP (final concentration 0.03 mCi/mL). After 120 min, the reaction was stopped with an equal volume of 1% H_3PO_4 and detected in the filter binding format as described above. K_i* values were calculated using the following equation:

$$IC_{50} = \frac{[E]}{2} + K_i^*\left(1 + \frac{[S]}{K_m}\right)$$

Supporting Information Available: Results from the combustion analysis of test compounds and from the kinetic characterization of **3g** against AKT2 kinase. This material is available free of charge via the Internet at http://pubs.acs.org.

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