4-Anilino-5-carboxamido-2-pyridone Derivatives as Noncompetitive Inhibitors of Mitogen-Activated Protein Kinase Kinase

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Received April 17, 2007

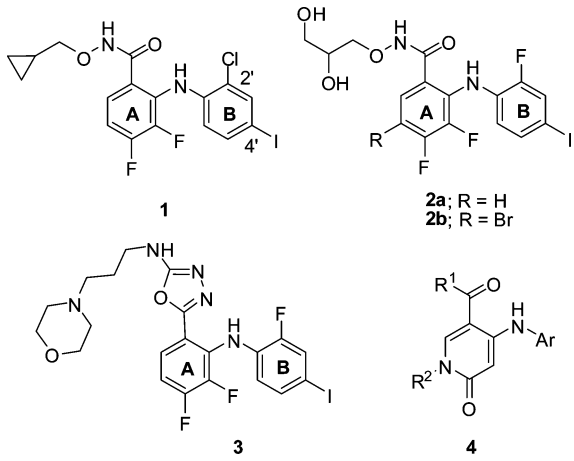
A new series of MEK1 inhibitors, the 4-anilino-5-carboxamido-2-pyridones, were designed and synthesized using a combination of medicinal chemistry, computational chemistry, and structural elucidation. The effect of variation in the carboxamide side chain, substitution on the pyridone nitrogen, and replacement of the 4′-iodide were all investigated. This study afforded several compounds which were either equipotent or more potent than the clinical candidate CI-1040 (**1**) in an isolated enzyme assay, as well as murine colon carcinoma (C26) cells, as measured by supression of phosphorylated ERK substrate. Most notably, pyridone **27** was found to be more potent than **1** *in vitro* and produced a 100% response rate at a lower dose than **1**, when tested for *in vivo* efficacy in animals bearing C26 tumors.

Introduction

The Ras/Raf/MEK/ERK mitogen-activated protein (MAP) kinase signaling pathway is responsible for the coordination and regulation of cell growth and differentiation in response to extracellular stimulation.[1−3] Numerous studies have shown that the MAP kinase pathway plays an integral part in the formation, progression, and survival of tumors, in addition to participating in many inflammatory processes. MAP kinase kinase 1 and 2 (MEK1, MEK2) are closely related, dual-specificity, tyrosine/threonine protein kinases that phosphorylate the downstream target ERK (a MAP kinase) on specific tyrosine and threonine residues.[4] Therefore, small-molecule inhibitors of MEK have the potential to be anticancer, antiviral, and antiinflammatory therapeutics. As a result, this target has been the focus of intense medicinal chemistry efforts by a number of research groups in recent years,[5−8] with several compounds advancing into clinical development, including Pfizer compounds CI-1040 (**1**, Chart 1, also known as PD184352)[5] and PD325901 (**2a**)[9] as well as the Array BioPharma candidate ARRY-142886 (AZD6244).[10]

In 1999 the diarylamine **1** was reported to be a highly selective, potent inhibitor of MEK1 and MEK2. It was found to utilize a unique noncompetitive mechanism of inhibition and had significant antitumor activity *in vivo*.[11−14] On the basis of its promising preclinical profile, compound **1** was advanced into clinical trials[15] where a number of pharmacological issues were identified, such as metabolic instability and low overall bioavailability.[16,17] These were addressed by the second-generation dihydroxyalkyl hydroxamate compound **2a** (PD325901), of the same class, which displayed more potency and improved bioavailability compared to **1**.

Chart 1. Compound **1** and Analogues



Throughout this development process we also sought to identify additional inhibitor templates that would retain the significant advantages of **2a** but provide an even better pharmacological profile.

A structural basis for the unique, noncompetitive nature of MEK inhibition possessed by compounds such as **1** was provided by the crystal structures of analogues **2b** (PD318088) and **3** (PD334581) bound to MEK1 and MEK2, respectively.[18,19] These structures revealed that the inhibitors bind in a novel allosteric binding pocket separate from, but adjacent to, the MgATP site. Several key interactions were observed between the inhibitors and amino acid residues within this binding site. First, the B ring of the inhibitor was bound within a deep hydrophobic pocket formed by Met143, Ile141, Leu118, and Phe209, creating numerous van der Waals interactions with the amino acid side chains involved, while Phe209 also formed a critical edge-to-face aromatic interaction with the B ring.

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[a] Abbreviations: MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; MgATP, magnesium adenosine triphosphate; CoMFA, comparative molecular field analysis; SAR, structure−activity relationship; pERK, phosphorylated extracellular signal-regulated kinase; DMT-MM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; PFP−TFA, pentafluorophenyl trifluoroacetate; DABCO, 1,4-diazabicyclo[2.2.2]octane; TFA, trifluoroacetic acid; PyBOP, benzotriazolyloxytris-(pyrrolidino)phosphonium hexafluorophosphate; BID, twice daily; TID, three times daily.

10.1021/jm0704548 CCC: $37.00
Published on Web 09/19/2007

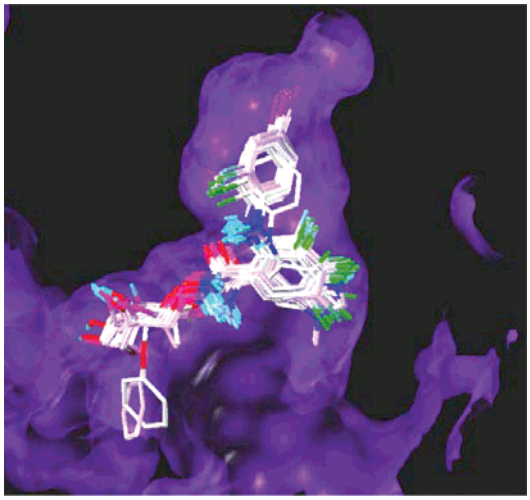


Figure 1. Result of GOLD docking of a set of compounds into the inhibitor binding site (Purple Connolly Surface) of the ternary complex of MEK1, MgATP, and **2a** (PDB Code: 1S9J). The GOLD docking model served as alignment rule for Comparative Molecular Field Analysis (CoMFA).

Second, an important electrostatic interaction between the backbone carbonyl oxygen of Val127 and the 4′-iodide was observed at the back of the pocket. Finally, a hydrogen bond interaction was shown to exist between the 4-fluoro atom of the inhibitor A ring and the backbone amide of Ser212.

This structural information was combined with Comparative Molecular Field Analysis (CoMFA) techniques to enable the structure-guided design of a new class of potent inhibitors of MEK1 and MEK2, the 4-(phenylamino)-5-carboxamido-2-pyridones **4**, where R^1, R^2, and Ar were each varied independently in order to establish an optimal arrangement of substituents.

Results and Discussion

The key protein−ligand interactions derived from the crystal structures of MEK1 and MEK2 provided the context for the structure-guided discovery of new MEK inhibitor templates. This information was used as a template for docking experiments to aid in the discovery of novel structures. The docking of compounds with known potencies was used as an alignment rule for a Comparative Molecular Field Analysis (CoMFA) model.[20] The docking mode of compounds within the allosteric inhibitor binding site of MEK1 was used to generate the CoMFA model which is illustrated in Figure 1.

By providing suggestions for increasing the biological activity of compounds using the CoMFA contours, the model was useful in prioritizing the synthesis of novel compounds. The CoMFA contours within the inhibitor-binding site are shown in Figure 2 and suggest that the bioactivity of novel compounds can be increased by placing more steric bulk into the green volumes and less bulk in the yellow volumes, more negative charge in the red volumes, and less negative charge in the blue. From the green contours, one can see the importance of having bulky groups within the iodophenyl binding pocket. In addition, there is a red region off the 4-fluoro position of the A-ring, signaling the importance of maintaining the hydrogen bond to the amide of Ser212.

The general protocol for submitting new compounds for structure-based drug design involved initially docking the compounds into the inhibitor-binding site using GOLD then CoMFA scoring all docking poses of each molecule.[20] The compounds were then prioritized based on consistency of binding mode, predicted IC_{50}, and ease of synthesis. On the basis of these observations, we chose to investigate replacement of the difluorophenyl group of the original class of inhibitors with a pyridone derivative to determine whether the pyridone carbonyl group would provide an improved hydrogen bond interaction with Ser212. We then explored a variety of carboxamide side chains as alternatives to the hydroxamate of **1**. In combination with both of the above modifications we also investigated the effect of substitution on the pyridone nitrogen. Finally, we looked at structural changes involving replacement of the 4′-iodo substituent with other groups designed to explore binding in the hydrophobic pocket while interacting with the carbonyl oxygen of Val127.

For SAR determination, all compounds were first evaluated in a coupled Raf-MEK-ERK cascade assay, measuring the inhibition of phosphorylated ERK (pERK) formation as the terminal endpoint. Specific details are provided in the Experimental Section. Selected compounds were also evaluated in a cellular assay that measured the inhibition of ERK phosphorylation in murine colon carcinoma cells.

Optimization of the Carboxamide Side Chain. The synthesis of the 4-(phenylamino)-5-carboxamido-2-pyridones **4**

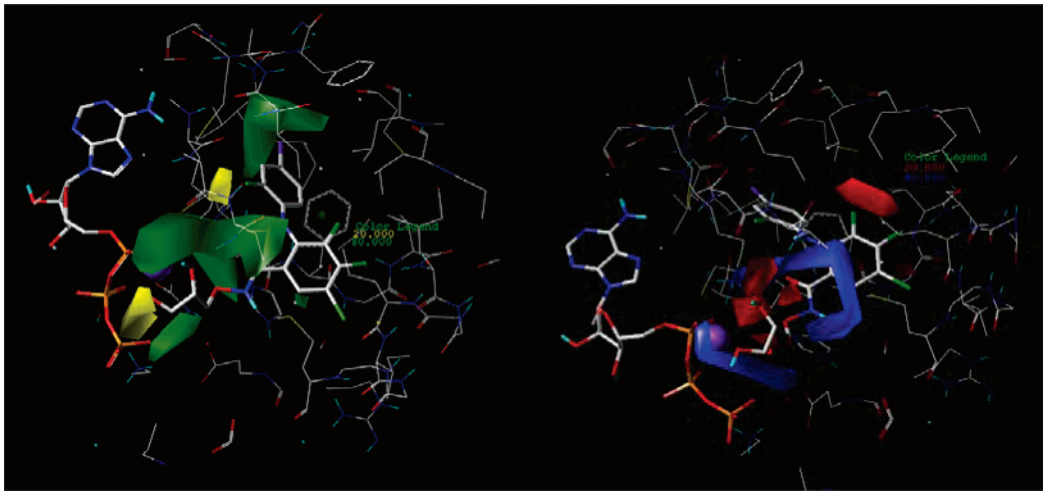
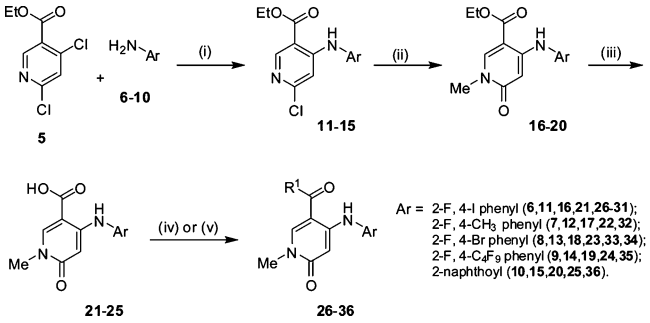


Figure 2. CoMFA Contours shown in the MEK1 ternary complex coordinate frame provides insights into the design of novel chemical entities. The contours suggest that the compounds' activity can be optimized by placing more negative charge density and steric bulk in the red and green volumes, respectively, and less charge density and steric bulk in the blue and yellow volumes.

Scheme 1[a]



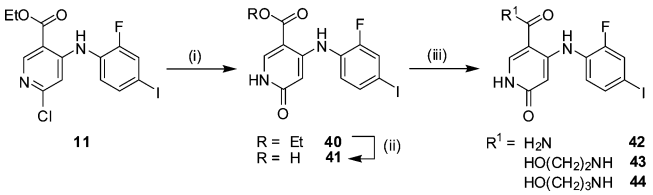
[a] Reagents and conditions: (i) EtOH, concd HCl (cat.), 90 °C, 15 h; (ii) a. Me_2SO_4, $CHCl_3$, 0 °C to reflux, b. TEA, AcOH, EtOH, reflux 2 h; (iii) 1 M NaOH, EtOH, RT, 15 h; (iv) amine R^1, DMT−MM, MeOH/THF, RT, 15 h; (v) a. PFP−TFA, pyridine, DMA, RT, 2 h, b. amine R^1, DIEA, THF, RT, 2 h.

involved the initial selective acid-catalyzed displacement of the 4-chlorine atom of ethyl 4,6-dichloronicotinate **5** with substituted anilines **6**−**10** to give intermediate 4-anilino-6-chloropyridine esters **11**−**15** (Scheme 1).

The *N*-methylpyridone esters **16**−**20** were prepared by quaternization of the chloropyridine esters **11**−**15** with dimethyl sulfate followed by *in situ* conversion to the pyridones **16**−**20** with AcOH/TEA.[21] Deprotection of ethyl esters **16**−**20** under basic conditions gave the acids **21**−**25** which were coupled with amines or hydroxylamines, using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT−MM) (Scheme 1). Alternatively, the acids were first converted to the corresponding pentafluorophenyl esters with pentafluorophenyl trifluoroacetate (PFP−TFA) and then reacted with amines.

Initial efforts focused on the preparation of 2-fluoro-4-iodoanilino-substituted pyridones, owing to the previously observed[17] potency enhancement vis-à-vis the 2-chloro-4-iodoanilines. Thus, ester **16** was prepared according to Scheme 1, further saponified and derivatized as amides. From our previous work, it was expected that introduction of a carboxamide would both increase the potency and improve the pharmacokinetic properties relative to the acid. Indeed, the simple carboxamide **27** was highly potent against the Raf-MEK-ERK cascade and was significantly active in the colon26 cellular assay. Mono- (**28**) or dimethylation (**29**), however, greatly diminished this potency.

Scheme 2[a]



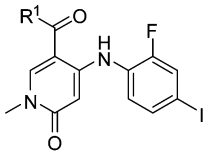
[a] Reagents and conditions: (i) AcOH, H_2O, reflux, 6 d; (ii) 1 M NaOH, EtOH, RT, 15 h; (iii) a. PFP−TFA, py, DMA, RT, 2 h, b. amine R^1, DIEA, THF, RT, 2 h.

Notably, the simple amide was nearly as potent *in vitro* as **1** and was considerably more potent than **1** in C26 cells. The latter fact might be attributable to its greater aqueous solubility and Caco permeability (Table 1). It was expected that the hydroxyethylhydroxamate would further improve the potency relative to the simple carboxamide. However, hydroxamate **26** and amide **27** were essentially equipotent. This is in marked contrast to **37**/**38** (IC_{50}s 0.0012 μM vs 0.0174 μM) and may indicate that a tighter H-bond of the pyridone carbonyl to Ser212 obviates the need to highly optimize the carboxamide substituent. The hydroxyamides **30** and **31** were slightly less effective, with **31**, the hydroxamate isostere, being 2−3-fold more potent than the hydroxyethylamide of **30**. Table 1 also lists the structure-guided CoMFA prediction for all of the molecules in this series. The molecules that were listed as unable to predict gave inconsistent binding modes and predicted values.

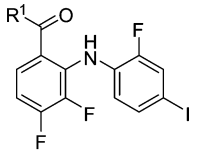
Substitution on the Pyridone Nitrogen. The *N*H-pyridone ester **40** was prepared by heating ethyl 6-chloro-4-(2-fluoro-4-iodoanilino)nicotinate **11** at reflux in a 3:1 mixture of AcOH/H_2O[22] (Scheme 2). Deprotection of ester **40** and coupling of the resulting acid **41** with amines to give the target amides (**42**−**44**) was carried out under the same conditions (using PFP−TFA as the coupling agent) as detailed in Scheme 1.

Primary amide **42** showed a loss of potency in both isolated enzyme and cellular assays (Table 2). The hydroxyamides **43** and **44** were also prepared, on the basis that the corresponding *N*-methylpyridones **30** and **31** showed appreciable activity (Table 1). Unfortunately, this was not the case for *N*H-pyridones, as **43** and **44** had IC_{50}s of 1.17 and 2.68 μM, respectively, against the isolated enzyme and both IC_{50}s were >5 μM in the

Table 1. Variation of the Carboxamide Side Chain



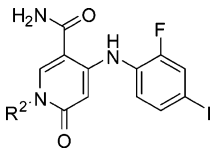
16, **21**, **26-31**



37-39

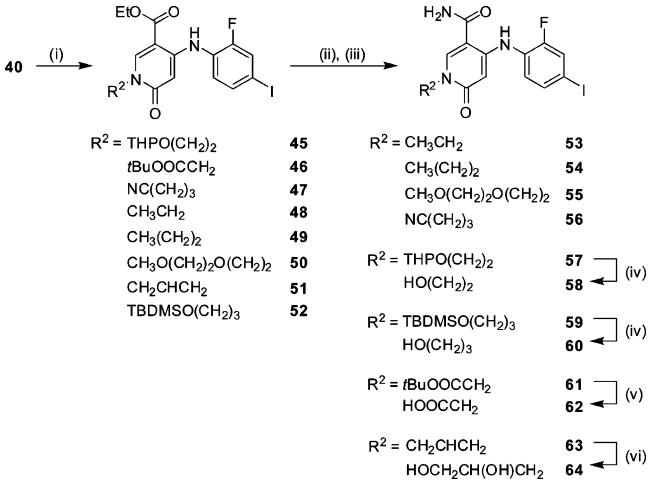
compd	R^1	cascade IC_{50} (μM)	cellular IC_{50} (μM)	aq solution (μg/mL)	Caco (cm/sec)	COMFA prediction
1	$cPrCH_2ONH$	0.016	0.046	<1	0	0.017
37	$HOCH_2CH_2ONH$	0.0012	0.002	7	43.4	0.0009
38	NH_2	0.0174	0.030	<1	0	0.0522
39	$HOCH_2CH_2NH$	0.0842	0.068	11	21.2	0.0036
16	CH_3CH_2O	0.669	0.063	−	−	0.0465
21	OH	0.425	>5	770	0	unable to predict
26	$HOCH_2CH_2ONH$	0.018	0.005	610	0	0.0044
27	NH_2	0.023	0.004	6	24.8	unable to predict
28	CH_3NH	0.195	−	31	−	0.0624
29	$(CH_3)_2N$	0.627	−	1200	−	0.0217
30	$HOCH_2CH_2NH$	0.206	0.180	29	0	0.0212
31	$HOCH_2CH_2CH_2NH$	0.061	0.095	68	0	0.0014

Table 2. Substitution on the Pyridone Nitrogen



compd	R^2	cascade IC_{50} (μM)	cellular IC_{50} (μM)
27	CH_3	0.023	0.004
42	H	0.100	0.63
53	CH_3CH_2	1.00	–
54	$CH_3CH_2CH_2$	2.54	0.43
55	$CH_3O(CH_2)_2O(CH_2)_2$	1.13	–
56	$NCCH_2CH_2CH_2$	4.89	–
58	$HOCH_2CH_2$	2.54	>5
60	$HOCH_2CH_2CH_2$	>3	–
62	HO_2CCH_2	>10	–
63	$H_2C{=}CHCH_2$	0.091	–
64	$HOCH_2CH(OH)CH_2$	>10	–

Scheme 3[a]



[a] Reagents and conditions: (i) a. NaH, DMF, 0 °C to RT, b. R^2X, DMF, RT; (ii) 1 M NaOH, EtOH, RT, 15 h; (iii) a. PFP−TFA, py, DMA, RT, 2 h, b. ammonia, THF, RT, 2 h; (iv) 1 M HCl, EtOH, RT, 2 h; (v) TFA, CH_2Cl_2, RT, 2 h; (vi) OsO_4, $K_3Fe(CN)_6$, K_2CO_3, DABCO, tBuOH/H_2O, RT, 15 h.

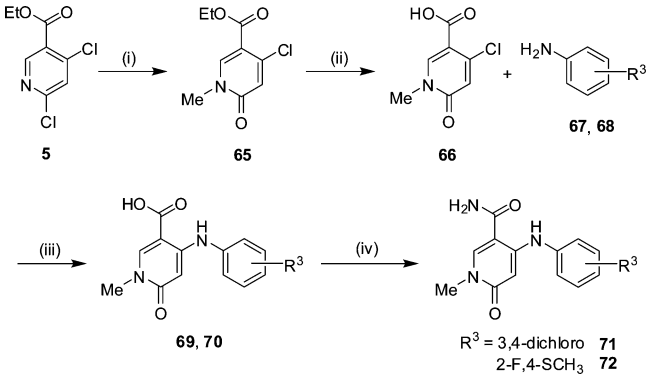
cellular assay. This drop in activity may be attributed to the loss of the key hydrogen bond to Ser212 due to partial tautomerization of the pyridone system, providing further evidence for the importance of this interaction for inhibition of the MEK1 and MEK2 protein kinases.

The crystal structures of MEK1 and MEK2 show a significant area of space adjacent to the 4- and 5-positions of the diphenylamine A-rings of **2b** and **3**, which corresponds to the position occupied by the pyridone series A-ring nitrogen atom. It was therefore decided to explore the impact of increased substitution on this nitrogen atom.

Hence, the *N*H-pyridone ester **40** was alkylated with various alkyl halides using NaH in DMF to give intermediates **45**−**52** (Scheme 3). Subsequent hydrolysis and amide coupling to give final products **53**−**56** and **63** was performed as described in Schemes 1 and 2.

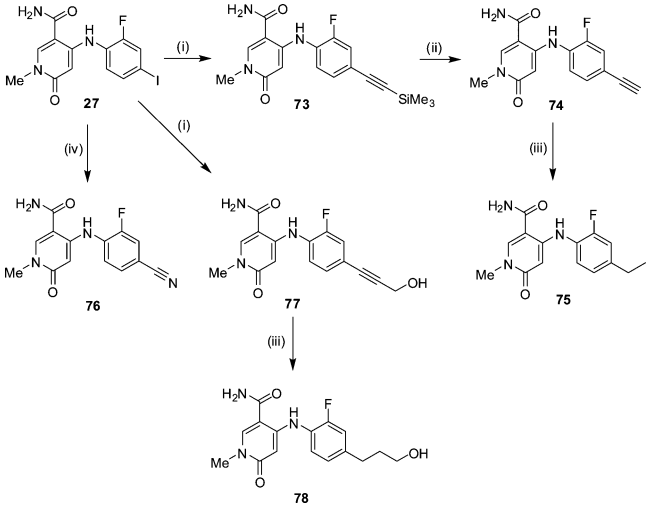
In the cases of compounds **57** and **59** the THP and TBDMS protecting groups were removed using 1 M HCl in EtOH at room temperature, giving the 4′-hydroxyethyl **58** and 4′-hydroxypropyl **60** derivatives, respectively. For compound **61**, deprotection of the *tert*-butyl ester was carried out in TFA/CH_2Cl_2 to give the desired *N*-acetic acid **62**. Compound **64** was obtained *via* dihydroxylation of the allyl compound **63** with OsO_4 in the presence of $K_3Fe(CN)_6$ and 1,4-diazabicyclo[2.2.2]-octane.[23]

Scheme 4[a]



[a] Reagents and conditions: (i) a. Me_2SO_4, 120 °C, b. sat. $NaHCO_3$, CH_3CN, RT, 18 h.; (ii) 1 M NaOH, EtOH, RT, 15 h; (iii) $Li[Si(CH_3)_2]$, THF, 0 °C, 1 h. (iv) amine R^1, PyBOP, CH_2Cl_2, RT, 15 h.

Scheme 5[a]

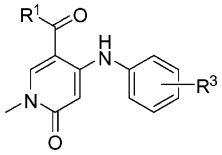


[a] Reagents and conditions: (i) Alkyne, $(Ph_3P)_2PdCl_2$, CuI, TEA, THF, RT, 15 h.; (ii) K_2CO_3, MeOH/THF, RT, 48 h.; (iii) 5% Pd/C, H_2, MeOH, RT, 15 h.; (iv) KCN, CuI, $(Ph_3P)_4Pd$, DMF, 110 °C, 4 h.

However, even with the presence of a pocket near the pyridone nitrogen, substitution other than methyl does not appear to be tolerated (Table 2). Extension of the *N*-methyl **27** to *N*-ethyl **53** or *N*-propyl **54** resulted in a progressive loss of activity (IC_{50}s = 0.023, 1.00, and 2.54 μM, respectively), although the *N*-allyl compound **63** was surprisingly potent with an IC_{50} of 0.091 μM against the isolated enzyme. Recognizing that Arg189 lined the potential binding pocket for this position, the pyridone nitrogen was alkylated with hydroxylated side chains (Scheme 3) to probe potential hydrogen bonding (direct or water-bridged) with the Arg residue. However all substitutions of this type afforded micromolar inhibitors (2.54 to >10 μM), well below the level of activity possessed by the *N*-methyl lead compound **27**.

Replacement of the 4′-Iodide. Finally, we investigated the impact of the (presumably) stronger pyridone H-bond on the preference for iodine at the 4′-position. The primary amide (R_1 = NH_2) was set as the best amide side chain and a series of compounds synthesized with a selection of 4′-substituents (Scheme 1). An alternative procedure to the desired pyridone carboxamides **4** involved the quaternization and conversion to the pyridone being performed first, on ethyl 4,6-dichloronico-

Table 3. Replacement of the 4′-Iodide



compd	R^1	R^3	cascade IC_{50} (μM)	cellular IC_{50} (μM)
27	NH_2	2-F-4-I	0.023	0.004
32	NH_2	2-F-4-CH_3	2.97	–
33	NH_2	2-F-4-Br	1.91	0.093
34[a]	$HOCH_2CH_2ONH$	2-F-4-Br	3.63	0.12
35	NH_2	2-F-4-$CF_2CF_2CF_2CF_3$	>10	–
36	NH_2	3,4-benzo	3.00	–
71	NH_2	3,4-dichloro	5.72	3.9
72	NH_2	2-F-4-SCH_3	0.356	0.15
74	NH_2	2-F-4-CCH	0.100	0.021
75	NH_2	2-F-4-CH_2CH_3	2.62	0.110
76	NH_2	2-F-4-CN	>10	–
78	NH_2	2-F-4-$CH_2CH_2CH_2OH$	>10	–

[a] Compound **34** has been published previously in ref 7 (as compound **10**).

tinate **5**, to give the 4-chloropyridone ester **65** (Scheme 4). Deprotection of the ester **65** was carried out under basic conditions, followed by reaction of the resultant acid **66** with substituted anilines (**67**, **68**) in the presence of $LiN[Si(CH_3)_3]_2$ to give substituted *N*-methylpyridones **69** and **70** as obtained in Scheme 1. These acids were then reacted with ammonia to give the target amides **71** and **72**, using benzotriazolyloxytris-(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) as the coupling agent. Generally speaking, the overall yield of the target compounds **4** was superior for Scheme 1 as compared to Scheme 4.

In the case of amide **27** further elaboration of the iodide was carried out *via* Sonogashira coupling with either TMS-acetylene or propargyl alcohol to give silyl alkyne or hydroxymethyl alkyne derivatives (**73** and **77**, respectively) (Scheme 5).

Deprotection of the trimethylsilyl protecting group of **73** with K_2CO_3 in MeOH/THF gave the acetylene (**74**), followed by hydrogenation to give the corresponding 4′-ethyl compound (**75**). Similarly, hydrogenation of the hydroxymethyl alkyne **77** gave the target 4′-saturated alcohol (**78**). Palladium-catalyzed reaction of the 4′-iodide **27** with KCN also gave the corresponding 4′-nitrile (**76**).

From the results obtained, it was clear that the interaction which exists between the 4′-iodide and Val127 in both MEK1 and MEK2 crystal structures is critical to the activity of this series of MEK inhibitors. All compounds were significantly less potent than the corresponding 4′-iodide **27** (Table 3). For example, even the closely related bromides (**33**, **34**[7]) had IC_{50}s against the isolated enzyme in the micromolar range (1.91 μM for **33** and 3.63 μM for **34**) versus the nanomolar range for the corresponding iodides (0.023 μM and 0.018 μM for **27** and **26**, respectively). These bromides (**33**, **34**) and iodides (**27**, **26**) also provide an interesting comparison with a series of pyridone-based inhibitors of MEK prepared and published by workers at Array BioPharma.[7] After completion of this study, we became aware that both our group and the group at Array Biopharma had independently[24,25] established the utility of the pyridone template in the preparation of inhibitors of MEK. Array BioPharma prepared a series of pyridine-2(1*H*)-ones as MEK inhibitors, based on a 2′-fluoro-4′-bromo template such as **34**, with variation at either the carboxamide/hydroxamate side chain or the C5-position of the pyridone ring. From our own work, however, it appears that the 4′-iodide provides superior potency compared to the 4′-bromide of the Array BioPharma series. In the current study, the only sub-micromolar derivatives other than iodide were the 4′-SMe **72** and 4′-acetylene **74** with IC_{50}s of 0.356 and 0.100 μM, respectively. The acetylene **74** was also a potent inhibitor in the cellular assay (IC_{50} = 0.021 μM). Although the 4′-hydroxypropyl derivative was designed to provide an H-bond donor interaction to Val127, it was in fact inactive, illustrating the overriding preference for nontraditional donor−acceptor interactions in the context of the highly hydrophobic binding pocket. The presumed electrostatic interaction of Val127 with the iodine originates from the crystal structure of **2b**,[18] and the potent activity of the acetylenic variant was predicted based on modeling, because of the presence of a CH-hydrogen bond. Although it was not possible to cocrystallize a pyridone template with a MEK1 construct, we were able to obtain a crystal structure of an analogue of **1** with an acetylene substituent (**79**), and this is illustrated in Figure 3. The acetylene

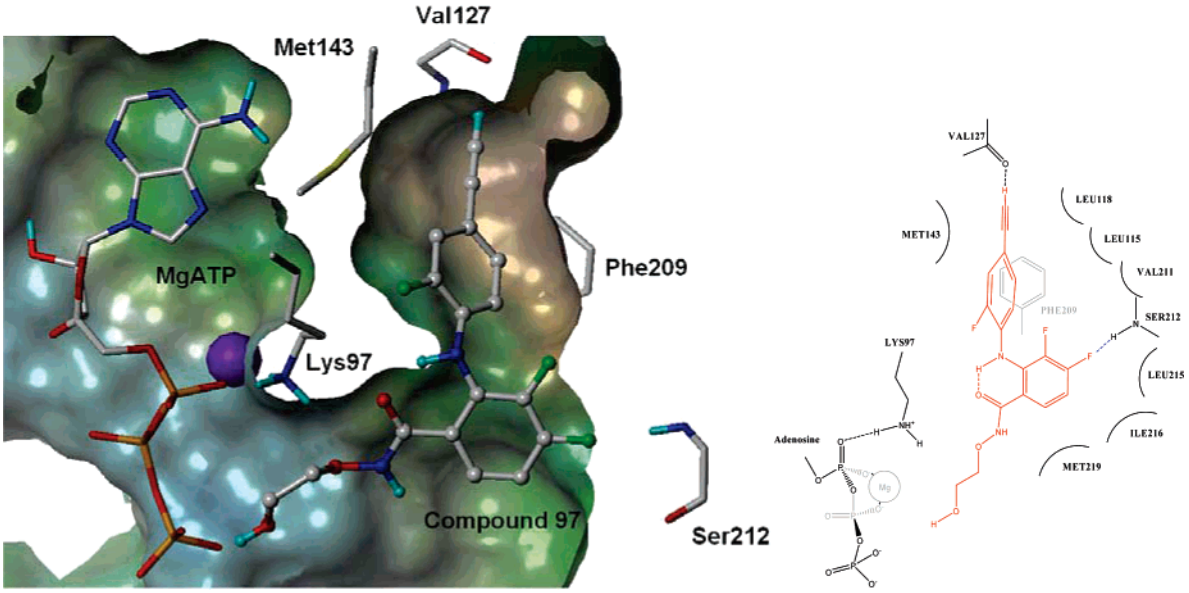


Figure 3. MEK1 with MgATP and compound **79** bound (PDB Code: 2P55). The acetylene of compound **79** forms a nontraditional hydrogen bond with the backbone carbonyl oxygen of Val127.

Table 4. Tumor Levels of pERK after Treatment with Pyridone **27**

	pyridone **27**: % inhibition of tumor pERK (hours postdose)		
dose	6 h	10 h	24 h
200 mpk	95	91	0
100 mpk	58	83	0
50 mpk	77	91	0

Table 5. Antitumor Efficacy of Pyridone **27**

	dose[a,b]	schedule	nonspecific deaths	CR, %[c]	PR, %[d]	T − C[e]
27	50	BID, D10-23	0/5	100	-	20.9
1	300	TID, D10-23	0	70	30	12.7

[a] Tumor doubling time was 4.1 days. [b] Dose is in mg/kg/injection. [c] Complete response is defined as a 100% reduction of initial tumor mass. [d] Partial response is defined as at least a 50% reduction of initial tumor mass. [e] T − C is the difference, in days, for the median treated and control tumors to reach a fixed evaluation size of 750 mg.

forms a nontraditional hydrogen bond between the terminal carbon of the acetylene and the carbonyl oxygen of Val127 with a nonbonded distance of 3.07 A.

Activity of the Optimized Pyridone 27 *in Vivo*. The *in vivo* activity of the best pyridone, amide **27**, was evaluated in an *ex vivo* pharmacodynamic assay in colon 26 tumor-bearing mice. The animals were administered a single dose of drug by oral gavage over a wide range of doses as summarized in Table 4. Tumors were excised at the indicated time points and tumor levels of pERK measured by immunoblotting methodology.

As can be seen in Table 4, treatment with pyridone **27** resulted in significant inhibition of ERK phosphorylation in tumors within 6 h of dosing, as reflected by 58% to 95% suppression over the range of doses tested. Furthermore, this high level of target suppression was maintained out to 10 h postdosing. By 24 h, control levels of ERK phosphorylation were observed at all doses of pyridone **27**. It is unclear why the degree of target inhibition did not correlate with dose of drug but may reflect tumor heterogeneity in this single experiment.

Pyridone **27** was also examined for *in vivo* efficacy in animals bearing C26 tumors treated on a BID dosing schedule. A dose of 50 mpk administered twice daily proved to be well tolerated with no lethality or weight loss, whereas the 100 and 200 mpk BID dosing regimens were not tolerated, as reflected by 40% and 100% lethality at these two doses, respectively. However, as summarized in Table 5, pyridone **27** proved to be more efficacious than **1**. Despite being administered at a significantly lower dose than **1** as well as being administered less frequently, this compound nonetheless resulted in a complete response rate of 100% compared to 70% for **1**. As is further shown in Table 5, tumor growth delay was also significantly greater for pyridone **27** compared to **1**.

Conclusions

Using information derived from both the crystal structures of MEK1 and MEK2[18] and molecular modeling, a new series of inhibitors based on a novel template, the 4-anilino-5-carboxamido-2-pyridones, was designed and prepared. Replacement of the difluorophenyl group (A-ring) of the diphenylamine series of inhibitors with a pyridone resulted in analogues (**26**, **27**) which were of similar potency to the corresponding diphenylamines (**37**, **38**) and more potent than clinical candidate **1**. One of these pyridones, **27**, was further shown to be more efficacious than **1** as measured by response rate as well as tumor growth delay. It was also found possible to replace the hydroxamate side chain of **1** with a more metabolically stable primary or secondary alkyl amide (**27**, **30**, **31**) with little or no loss of activity. Any substituent other than methyl on the pyridone nitrogen, however, was detrimental to activity although the crystal structure indicated a pocket in this area of the MEK1 protein. In the case of 4′-substituents, those compounds (**72**, **74**) which modeling predicted would interact with the protein in a manner similar to the 4′-iodide of a compound **1**-type template were found to have superior activity.

Experimental Section

Measurement of ERK Phosphorylation *in Vitro*. All compounds were first evaluated in a coupled Raf-MEK-ERK cascade assay, measuring the inhibition of pERK formation as the terminal endpoint. Each individual IC_{50} was determined from an 11-point dose−response curve which was carried out in duplicate. Each IC_{50} was determined twice using this method.

Exponentially growing murine colon 26 carcinoma cells cultured in the presence of 10% fetal bovine serum were treated with various concentrations of the test compounds (or vehicle control) for 1 h at 37 °C. After drug treatment, cells were harvested in a lysis solution containing 50 mM B-glycerophosphate, 10 mM HEPES, pH 7.4, 70 mM NaCl, 2 mM EDTA, and 1% SDS. The protein lysates were diluted 1:15 with supplied assay buffer prior to the execution of the assay. pERK was measured by ELISA assays employing phospho-specific antibodies to ERK1 and ERK2 (Assay Designs Inc, Ann Arbor, MI). Each IC_{50} was determined from a 7-point dose−response curve which was carried out in duplicate. Each IC_{50} was determined four times using this method. The highest concentration tested for any given compound was 5 μM. The estimated error is 30% based on historical variability of the control compound **1**.

Measurement of ERK Phosphorylation in Tumors. *Ex vivo* tissue samples were immediately frozen at −80 °C after dissection. Homogenates were subsequently prepared from frozen tissues by thawing the samples in lysis buffer (70 mM NaCl, 50 mM glycerol phosphate, 10 mM HEPES, pH 7.4, 1% Triton X-100, 1 mM Na_3VO_4, 100 μM PMSF, 10 μM leupeptin, and 10 μM pepstatin) followed by disruption with a Polytron homogenizer. After centrifugation, supernatants were assayed for protein concentration and evaluated for pERK levels by standard Western blotting methodology employing commercially available phospho-specific antibodies for ERK1 and ERK2.

Measurement of Xenograft Growth Delay. Colon 26 tumor fragments (approximately 3 mm^3 in size) were implanted subcutaneously into the right axillae of CD2F1 male mice at 4−6 weeks old. The tumors were grown to approximately 200 mg over 8 days, and then the drug (or vehicle) was administered from days 9 to 15. The vehicle for all compounds was 0.5% hydropropylmethylcellulose and 0.2% Tween 80 in water. All compounds were administered orally. Tumor size was evaluated periodically by calliper measurements, generally three times per week. The time required, in days, for the median-treated and nontreated tumors to reach a fixed evaluation size of 750 mg was then determined.

Crystallography. The crystal structure of MEK1 in a complex with MgATP and compound **79** was determined essentially as previously described.[18] The data were collected to a resolution of 2.8 Å on beam-line 17-ID at the Advanced Photon Source in Argonne, IL.[26] The crystallographic data were indexed, scaled and error reduced using the program *HKL200*.[27] The structure was determined by Fourier difference methods using the MEK1 structure 1S9J and refined against the crystallographic data using the program *Refmac5* in the CCP4 suite of programs.[28,29] The statistics for the final crystallographic model are 24.0% for the R_{work} and 29.5% for the R_{free} value.

Medicinal Chemistry. Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or by Quantitative Technologies Inc., Whitehouse, NJ. Melting points were determined using an Electrothermal Model 9200 or Gallenkamp digital melting point apparatus and are as read. NMR spectra were measured on Bruker AM-400, Bruker Avance 400, or Varian

Unity 400 spectrometers and referenced to Me_4Si. Mass spectra were recorded either on a Varian VG 7070 spectrometer at nominal 5000 resolution or a Finnigan MAT 900Q spectrometer. All final compound purities were determined to be >95% by HPLC.

Procedure A: Ethyl 6-Chloro-4-(2-fluoro-4-iodoanilino)-nicotinate (11). Ethyl 4,6-dichloronicotinate **5** (prepared by reaction of ethyl 4,6-dihydroxynicotinate with $POCl_3$ according to a literature procedure[30]) (4.00 g, 18.2 mmol) and 2-fluoro-4-iodoaniline **6** (4.30 g, 18.2 mmol) were dissolved in EtOH (80 mL), to which was added concd HCl (6 drops). This mixture was heated at 90 °C for 15 h, and then the solution was cooled, whereupon the desired product crystallized out of solution as fine needles. The product was isolated by filtration and washed with 10% Et_2O/hexanes to give compound **11** as white needles (3.79 g, 50%): mp (EtOAc/hexanes) 162−164 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.62 (s, 1 H), 8.69 (s, 1 H), 7.82 (dd, J = 9.9, 1.9 Hz, 1 H), 7.61−7.66 (m, 1 H), 7.33 (t, J = 8.5 Hz, 1 H), 6.67 (d, J = 1.8 Hz), 4.37 (q, J = 7.1 Hz, 2 H), 1.35 (t, J = 7.1 Hz, 3 H). LCMS (APCI$^+$) calcd for $C_{14}H_{12}ClFIN_2O_2$ 421 (MH^+), found 421. Anal. ($C_{14}H_{11}ClFIN_2O_2$) C, H, N.

Procedure B: Ethyl 4-(2-Fluoro-4-iodoanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate (16). Compound **11** (200 mg, 0.48 mmol) was dissolved in $CHCl_3$ (5 mL) and the solution cooled (ice/water). Dimethyl sulfate (0.27 mL, 2.86 mmol) was added and the solution allowed to warm to RT and then heated at reflux for 20 h. Upon cooling, a mixture of triethylamine (1.41 mL), acetic acid (0.94 mL), and EtOH (0.94 mL) was added and the reaction heated at reflux for a further 2 h. After cooling, water (10 mL) was added and the mixture was partitioned between EtOAc (100 mL) and water (50 mL). The EtOAc layer was washed with further water (50 mL) and brine (50 mL) and then dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **16** as a white solid (143 mg, 72%), mp (EtOAc/n-hexane) 169−170 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.31 (s, 1 H), 8.54 (s, 1 H), 7.77 (dd, J = 10.1, 1.9 Hz, 1 H), 7.60 (br dd, J = 8.3, 1.0 Hz, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.46 (s, 1 H), 4.30 (q, J = 7.1 Hz, 2 H), 3.43 (s, 3 H), 1.32 (t, J = 7.1 Hz, 3 H). LCMS (APCI$^+$) calcd for $C_{15}H_{15}FIN_2O_3$ 417 (MH^+), found 417. Anal. ($C_{15}H_{14}FIN_2O_3$) C, H, N.

Procedure C: 4-(2-Fluoro-4-iodoanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (21). Compound **16** (140 mg, 0.34 mmol) was suspended in EtOH (10 mL), to which was added 1 M NaOH (10 mL). This mixture was stirred at RT for 15 h and then diluted with 1 M HCl (50 mL) and the resulting precipitate extracted into EtOAc (2 × 50 mL). The combined EtOAc fractions were dried (Na_2SO_4) and filtered, and the solvent was removed under reduced pressure to afford compound **21** as a white solid (132 mg, 100%), mp (acetone/MeOH) 254−257 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.30 (v br s, 1 H), 9.66 (s, 1 H), 8.52 (s, 1 H), 7.76 (dd, J = 10.1, 1.9 Hz, 1 H), 7.59 (ddd, J = 8.4, 1.7, 0.8 Hz, 1 H), 7.31 (t, J = 8.5 Hz, 1 H), 5.49 (d, J = 0.7 Hz, 1 H), 3.41 (s, 3 H). LCMS (APCI$^-$) calcd for $C_{13}H_9FIN_2O_3$ 387 (M − H), found 387. Anal. ($C_{13}H_{10}FIN_2O_3$) C, H, N.

Procedure D: 4-(2-Fluoro-4-iodoanilino)-*N*-(2-hydroxyethoxy)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (26). To a mixture of compound **21** (130 mg, 0.34 mmol) and 2-(aminooxy)-ethanol (52 mg, 0.67 mmol) in MeOH/THF (1:1, 20 mL) was added DMT−MM (187 mg, 0.67 mmol) and the reaction stirred at RT for 15 h. All solvent was removed under reduced pressure and the oily residue partitioned between water (100 mL) and EtOAc (100 mL). The EtOAc fraction was then washed with water (2 × 100 mL) and brine (100 mL), dried (Na_2SO_4), and filtered and the solvent removed under reduced pressure. Purification by recrystallization from EtOAc/MeOH gave compound **26** as a white, crystalline solid (83 mg, 55%): mp (EtOAc/MeOH) 148−151 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.65 (v br s, 1 H), 9.48 (br s, 1 H), 8.13 (s, 1 H), 7.74 (dd, J = 10.2, 1.8 Hz, 1 H), 7.58 (br d, J = 8.6 Hz, 1 H), 7.28 (t, J = 8.5 Hz, 1 H), 5.55 (s, 1 H), 4.76 (v br s, 1 H), 3.90 (t, J = 4.9 Hz, 2 H), 3.61 (t, J = 4.9 Hz, 2 H), 3.36 (s, 3 H). LCMS (APCI$^+$) calcd for $C_{15}H_{16}FIN_3O_4$ 448 (MH^+), found 448. Anal. ($C_{15}H_{15}FIN_3O_4$) C, H, N.

Procedure E: 2,3,4,5,6-Pentafluorophenyl 4-(2-Fluoro-4-iodoanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate (80). Compound **21** (894 mg, 2.30 mmol) and pyridine (909 mg, 11.5 mmol) were dissolved in DMA (15 mL). To this mixture was added PFP−TFA (3.22 g, 11.5 mmol), and then the solution was allowed to stir at RT for 2 h. The DMA solution was diluted with EtOAc (150 mL) and was washed sequentially with 1 M HCl (2 × 100 mL), water (100 mL), sat. $NaHCO_3$ (2 × 100 mL), and brine (100 mL). The EtOAc fraction was then dried (Na_2SO_4) and filtered and the solvent removed under reduced pressure to yield a viscous oil. Purification by flash column chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **80** as a cream foam (1.22 g, 96%) which was used directly in subsequent steps. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.03 (s, 1 H), 8.70 (s, 1 H), 7.79 (dd, J = 10.1, 1.9 Hz, 1 H), 7.62 (br dd, J = 8.4, 1.0 Hz, 1 H), 7.29 (t, J = 8.4 Hz, 1 H), 5.36 (d, J = 1.6 Hz, 1 H), 3.45 (s, 3 H). LCMS (APCI$^+$) calcd for $C_{19}H_9F_6N_2O_3$ 555 (MH^+), found 555.

Procedure F: 4-(2-Fluoro-4-iodoanilino)-*N*-(2-hydroxyethyl)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (30). Compound **80** (600 mg, 1.08 mmol) was dissolved in THF (15 mL) to which was added DIEA (697 mg, 5.40 mmol), followed by 2-aminoethanol (132 mg, 2.17 mmol). This mixture was stirred at RT for 2 h, then the solvent was removed under reduced pressure and the resulting white solid suspended in Et_2O. This solid was collected by filtration and recrystallized from EtOAc/MeOH to give compound **30** (394 mg, 85%) as a white solid: mp (EtOAc/MeOH) 205−208 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.10 (s, 1 H), 8.43 (br s, 1 H), 8.28 (s, 1 H), 7.72 (dd, J = 10.2, 1.8 Hz, 1 H), 7.56 (br d, J = 8.5 Hz, 1 H), 7.28 (t, J = 8.5 Hz, 1 H), 5.58 (s, 1 H), 4.76 (br t, J = 5.2 Hz, 1 H), 3.50 (q, J = 5.8 Hz, 2 H), 3.36 (s, 3 H), 3.32−3.26 (m, 2 H). LCMS (APCI$^+$) calcd for $C_{15}H_{16}FIN_3O_3$ 432 (MH^+), found 432. Anal. ($C_{15}H_{15}FIN_3O_3$) C, H, N.

4-(2-Fluoro-4-iodoanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (27). Compound **80** was reacted with concd NH_3 solution in THF according to procedure F. Recrystallization from EtOAc/MeOH afforded compound **27** as white crystals (84%): mp (EtOAc/MeOH) 283−285 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.40 (s, 1 H), 8.34 (s, 1 H), 7.88 (br s, 1 H), 7.74 (dd, J = 9.9, 1.9 Hz, 1 H), 7.57 (br d, J = 7.8 Hz, 1 H), 7.46 (br s, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.56 (d, J = 0.7 Hz, 1 H), 3.36 (s, 3 H). LCMS (APCI$^+$) calcd for $C_{13}H_{12}FIN_3O_2$ 388 (MH^+), found 388. Anal. ($C_{13}H_{11}FIN_3O_2$) C, H, N.

4-(2-Fluoro-4-iodoanilino)-*N*,1-dimethyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (28). Compound **80** was reacted with methylamine (40% aq solution) in THF according to procedure F. Trituration with hexane afforded compound **28** as a white solid (73%); mp 252−254 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.10 (br s, 1 H), 8.41 (br d, J = 4.4 Hz, 1 H), 8.22 (s, 1 H), 7.72 (dd, J = 10.2, 1.9 Hz, 1 H), 7.57 (dt, J = 8.4, 0.9 Hz, 1 H), 7.28 (t, J = 8.4 Hz, 1 H), 5.58 (d, J = 0.9 Hz, 1 H), 3.36 (s, 3 H), 2.75 (d, J = 4.4 Hz, 3 H). HRMS (FAB$^+$) calcd for $C_{14}H_{14}FIN_3O_2$ 402.0115 (MH^+), found 402.0119.

4-(2-Fluoro-4-iodoanilino)-*N*,*N*,1-trimethyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (29). Compound **80** was reacted with dimethylamine (40% aq solution) in THF according to procedure F. Trituration with hexane gave compound **29** as a pale yellow solid (58%); mp 106−111 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 8.21 (s, 1 H), 7.79 (s, 1 H), 7.72 (dd, J = 10.2, 1.9 Hz, 1 H), 7.56 (dt, J = 8.4, 1.2 Hz, 1 H), 7.16 (t, J = 8.4 Hz, 1 H), 5.41 (d, J = 1.2 Hz, 1 H), 3.34 (s, 3 H), 2.98 (s, 6 H). HRMS (FAB$^+$) calcd for $C_{15}H_{16}FIN_3O_2$ 416.0271 (MH^+), found 416.0270.

4-(2-Fluoro-4-iodoanilino)-*N*-(3-hydroxypropyl)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (31). Compound **80** was reacted with 3-aminopropanol in THF in the presence of DIEA according to procedure F. Purification by flash chromatography on silica gel (50% acetone/CH_2Cl_2) gave compound **31** as a white solid (95%), mp (Et_2O) 81−86 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.11 (s, 1 H), 8.40 (br s, 1 H), 8.24 (s, 1 H), 7.74 (d, J = 9.7 Hz, 1 H), 7.58 (d, J = 8.2 Hz, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.59 (s, 1 H), 4.48 (t, J = 5.1 Hz, 1 H), 3.48 (q, J = 5.7 Hz, 2 H), 3.37 (s, 3 H), 3.30−3.24 (m, 2 H), 1.67 (pentet, J = 6.7 Hz, 2 H). LCMS

(APCI$^+$) calcd for $C_{16}H_{18}FIN_3O_3$ 446 (MH^+), found 446. Anal. ($C_{16}H_{17}FIN_3O_3$·0.5H_2O) C, H, N.

Ethyl 6-Chloro-4-(2-fluoro-4-methylanilino)nicotinate (12). 2-Fluoro-4-methylaniline **7** and **5** were reacted according to procedure A. Trituration with 10% Et_2O/hexane gave compound **12** (44%); mp (EtOH/water) 107−109 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (br s, 1 H), 8.77 (s, 1 H), 7.20 (t, J = 8.2 Hz, 1 H), 7.05−6.99 (m, 2 H), 6.65 (d, J = 1.6 Hz, 1 H), 4.41 (q, J = 7.1 Hz, 2 H), 1.43 (t, J = 7.1 Hz, 3 H). LRMS (FAB$^+$) calcd for $C_{15}H_{15}N_2O_2FCl$, 309 (MH^+), found 309.

Ethyl 4-(2-Fluoro-4-methylanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate (17). Compound **12** was reacted according to procedure B. Purification by recrystallization (EtOAc/hexane) afforded compound **17** (84%); mp (EtOAc/hexane) 148−150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (br s, 1 H), 8.20 (s, 1 H), 7.27−7.21 (m, 1 H), 6.99−6.92 (m, 2 H), 5.76 (s, 1 H), 4.35 (q, J = 7.1 Hz, 2 H), 3.53 (s, 3 H), 2.35 (s, 3 H), 1.39 (t, J = 7.1 Hz, 3 H). LCMS (ACPI$^-$) calcd for $C_{16}H_{16}N_2O_3$ 303 (M − H), found 303. Anal. ($C_{16}H_{17}FN_2O_3$) C, H, N.

4-(2-Fluoro-4-methylanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (22). Hydrolysis of compound **17** was carried out according to procedure C. Compound **22** was isolated as a white solid (91%) and used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.20 (v br s, 1 H), 9.47 (br s, 1 H), 8.49 (s, 1 H), 7.34 (t, J = 8.3 Hz, 1 H), 7.18 (d, J = 11.7 Hz, 1 H), 7.07 (d, J = 7.8 Hz, 1 H), 5.33 (d, J = 1.1 Hz, 1 H), 3.39 (s, 3 H), 2.33 (s, 3 H).

4-(2-Fluoro-4-methylanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (32). Compound **22** was reacted according to procedure E. Purification by flash chromatography on silica gel (60% EtOAc/hexane) gave the intermediate pentafluorophenyl ester (90%) which was reacted directly with concd NH_3 in THF according to procedure F. Recrystallization from EtOAc/hexane gave compound **32** (84%); mp (EtOAc/hexane) 285−288 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.14 (s, 1 H), 8.32 (s, 1 H), 7.86 (br s, 1 H), 7.43 (br s, 1 H), 7.31 (t, J = 8.4 Hz, 1 H), 7.16 (dd, J = 11.8, 1.1 Hz, 1 H), 7.05 (d, J = 8.2 Hz, 1 H), 5.38 (d, J = 1.1 Hz, 1 H), 3.34 (s, 3 H), 2.32 (s, 3 H). LCMS (ACPI$^-$) calcd for $C_{14}H_{13}FN_3O_2$ 274 (M − H), found 274. Anal. ($C_{14}H_{14}FN_3O_2$) C, H, N.

Ethyl 4-(4-Bromo-2-fluoroanilino)-6-chloronicotinate (13). 4-Bromo-2-fluoroaniline **8** and **5** were reacted according to procedure A. The product was isolated by filtration and washed with 10% Et_2O/hexane, affording compound **13** (58%); mp (EtOH/water) 150−152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (br s, 1 H), 8.81 (s, 1 H), 7.41 (dd, J = 9.6, 2.2 Hz, 1 H), 7.38−7.35 (m, 1 H), 7.25 (t, J = 8.3 Hz, 1 H), 6.71 (d, J = 1.5 Hz, 1 H), 4.42 (q, J = 7.1 Hz, 2 H), 1.43 (t, J = 7.1 Hz, 3 H). LRMS (FAB$^+$) calcd for $C_{14}H_{12}N_2O_2FBrCl$ 375 (MH^+), found 375.

Ethyl 4-(4-Bromo-2-fluoroanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate (18). Compound **13** was reacted according to procedure B. Recrystallization from EtOAc/hexane gave compound **18** (77%); mp (EtOAc/hexane) 159−162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (br s, 1 H), 8.22 (s, 1 H), 7.36−7.25 (m, 3 H), 5.85 (s, 1 H), 4.35 (q, J = 7.1 Hz, 2 H), 3.54 (s, 3 H), 1.39 (t, J = 7.1 Hz, 3 H). LCMS (ACPI$^-$) calcd for $C_{15}H_{13}BrFN_2O_3$, 367, 369 (M − H), found 367, 369. Anal. ($C_{15}H_{14}BrFN_2O_3$) C, H, N.

4-(4-Bromo-2-fluoroanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (23). Hydrolysis of compound **18** was carried out according to procedure C. Compound **23** was isolated as a white solid (89%) and used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.30 (v br s, 1 H), 9.65 (br s, 1 H), 8.52 (s, 1 H), 7.69 (dd, J = 10.3, 1.6 Hz, 1 H), 7.51−7.43 (m, 2 H), 5.47 (d, J = 1.2 Hz, 1 H), 3.41 (s, 3 H).

4-(4-Bromo-2-fluoroanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (33). Compound **23** was reacted according to procedure E. Purification by flash column chromatography on silica gel (60% EtOAc/hexane) gave the intermediate pentafluorophenyl ester (97%) which was reacted directly with concd NH_3 in THF according to procedure F. Recrystallization from EtOAc/hexane gave compound **33** (100%); mp (EtOAc/hexane) 282−284 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.39 (s, 1 H), 8.35 (s, 1 H), 7.89 (br s, 1 H), 7.66 (dd, J = 10.4, 1.7 Hz, 1 H), 7.51−7.44 (br m, 2 H), 7.42 (dd, J = 8.5, 1.9 Hz, 1 H), 5.55 (s, 1 H), 3.38 (s, 3 H). LCMS (ACPI$^-$) calcd for $C_{13}H_{10}BrFN_3O_2$ 338, 340 (M − H), found 338, 340. Anal. ($C_{13}H_{11}BrFN_3O_2$) C, H, N.

4-(4-Bromo-2-fluoroanilino)-*N*-(2-hydroxyethoxy)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (34). Compound **23** and 2-(aminooxy)ethanol were reacted the presence of DMT−MM in MeOH according to procedure D. Purification by flash column chromatography on silica gel (EtOAc followed by 10% MeOH/CH_2Cl_2) gave compound **34** (38%); mp (EtOAc/hexane) 124−128 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.64 (br s, 1 H), 9.50 (br s, 1 H), 8.13 (s, 1 H), 7.69−7.65 (m, 1 H), 7.47−7.41 (m, 2 H), 5.53 (d, J = 0.7 Hz, 1 H), 4.78 (s, 1 H), 3.91 (t, J = 5.0 Hz, 2 H), 3.61 (t, J = 5.0 Hz, 2 H), 3.36 (s, 3 H). LCMS (ACPI$^-$) calcd for $C_{15}H_{14}BrFN_3O_4$ 398, 400 (M − H), found 398, 400. Anal. ($C_{15}H_{15}BrFN_3O_4$·0.25 H_2O) C, H, N.

2-Fluoro-4-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)aniline (9). A dispersion of 1,1,1,2,2,3,3,4,4-nonafluoro-4-iodobutane (3.50 g, 10.1 mmol), 2-fluoro-4-iodoaniline **6** (2.00 g, 8.4 mmol), and copper bronze (1.93 g, 30.4 mmol) in DMSO (10 mL) was stirred at 120 °C for 15 h. Copper(I) iodide was removed by filtration through celite which was washed with Et_2O (100 mL). Water (100 mL) was then added to the filtrate, and the mixture was stirred at RT for 5 min. The organic layer was separated, washed with water (5 × 100 mL) to remove DMSO, dried (Na_2SO_4), and concentrated. Flash column chromatography on silica gel (10% EtOAc/hexane) afforded compound **9** (1.69 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25−7.14 (m, 2 H), 6.86−6.74 (m, 1 H), 4.07 (br s, 1 H). LCMS (ACPI$^-$) calcd for $C_{10}H_4NF_{10}$ 328 (M − H), found 328.

Ethyl 6-Chloro-4-[2-fluoro-4-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)anilino]nicotinate (14). Compounds **9** and **5** were reacted according to procedure A. Purification by flash column chromatography on silica gel (10% EtOAc/hexane as eluant) gave compound **14** (30%) which was used directly in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11 (br s, 1 H), 8.86 (s, 1 H), 7.57 (t, J = 8.0 Hz, 1 H), 7.46 (d, J = 9.7 Hz, 2 H), 6.98 (d, J = 0.7 Hz, 1 H), 4.44 (q, J = 7.1 Hz, 2 H), 1.44 (t, J = 7.1 Hz, 3 H).

Ethyl 4-[2-Fluoro-4-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)anilino]-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate (19). Compound **14** was reacted according to procedure B. Recrystallization from EtOAc/hexane gave compound **19** (47%). ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.50 (s, 1 H), 8.35 (s, 1 H), 7.58 (t, J = 8.3 Hz, 1 H), 7.52 (dd, J = 10.9, 1.8 Hz, 1 H), 7.34 (d, J = 8.1 Hz, 1 H), 5.59 (s, 1 H), 4.08 (q, J = 7.1 Hz, 2 H), 3.22 (s, 3 H), 1.10 (t, J = 7.1 Hz, 3 H).

4-[2-Fluoro-4-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)anilino]-1-methyl-6-oxo-1,6 -dihydro-3-pyridinecarboxylic Acid (24). Hydrolysis of compound **19** was carried out according to procedure C. Compound **24** was isolated as a white solid (75%) and used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.20 (v br s, 1 H), 10.21 (br s, 1 H), 8.56 (s, 1 H), 7.83 (t, J = 8.3 Hz, 1 H), 7.74 (d, J = 10.4 Hz, 1 H), 7.57 (d, J = 8.2 Hz, 1 H), 5.86 (s, 1 H), 3.44 (s, 3 H).

4-[2-Fluoro-4-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)anilino]-1-methyl-6-oxo-1,6 -dihydro-3-pyridinecarboxamide (35). Compound **24** was reacted according to procedure E, then the intermediate pentafluorophenyl ester reacted directly with c. NH_3 solution in THF according to procedure F. Purification by recrystallization (EtOAc/hexane) gave compound **35** (65%); mp (EtOAc/hexane) 112−118 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.92 (s, 1 H), 8.40 (s, 1 H), 7.93 (br s, 1 H), 7.79 (t, J = 8.3 Hz, 1 H), 7.70 (dd, J = 11.2, 1.7 Hz, 1 H), 7.53 (d, J = 7.8 Hz, 2 H), 5.92 (s, 1 H), 3.40 (s, 3 H). HRMS (FAB$^+$) calcd for $C_{17}H_{12}F_{10}N_3O_2$ 480.0770 (M^+), found 480.0762.

Ethyl 6-Chloro-4-(2-naphthylamino)nicotinate (15). Compounds **10** and **5** were reacted according to procedure A. Trituration with 10% Et_2O/hexane yielded compound **15** (68%) which was used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.91 (br s, 1 H), 8.71 (s, 1 H), 8.02 (d, J = 8.7 Hz, 1 H), 7.97−7.88 (m,

3 H), 7.59−7.48 (m, 3 H), 6.91 (s, 1 H), 4.39 (q, J = 7.1 Hz, 2 H), 1.37 (t, J = 7.1 Hz, 3 H).

Ethyl 1-Methyl-4-(2-naphthylamino)-6-oxo-1,6-dihydro-3-pyridinecarboxylate (20). Compound **20** was prepared from compound **15** according to procedure B. Purification by recrystallization (EtOAc/hexane) gave compound **20** as a white solid (57%); mp (EtOAc/hexane) 160−163 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.55 (s, 1 H), 8.57 (s, 1 H), 7.97 (d, J = 8.8 Hz, 1 H), 7.91 (dd, J = 7.7, 4.7 Hz, 2 H), 7.81 (d, J = 1.8 Hz, 1 H), 7.55−7.43 (m, 3 H), 5.79 (s, 1 H), 4.32 (q, J = 7.1 Hz, 2 H), 3.43 (s, 3 H), 1.35 (t, J = 7.1 Hz, 3 H). Anal. ($C_{19}H_{18}N_2O_3$) C, H, N.

1-Methyl-4-(2-naphthylamino)-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (25). Hydrolysis of compound **20** was carried out according to procedure C. Compound **25** (88%) was isolated as a white solid and used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.27 (v br s, 1 H), 9.95 (br s, 1 H), 8.53 (s, 1 H), 7.96 (d, J = 8.8 Hz, 1 H), 7.91 (d, J = 8.8 Hz, 2 H), 7.80 (d, J = 1.9 Hz, 1 H), 7.54−7.42 (m, 3 H), 5.81 (s, 1 H), 3.42 (s, 3 H).

1-Methyl-4-(2-naphthylamino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (36). Compound **25** was reacted according to procedure E. Purification by flash column chromatography on silica gel (60% EtOAc/hexane) gave the intermediate pentafluorophenyl ester (97%), used directly in the next step by reaction with c. NH_3 in THF according to procedure F. Recrystallization (MeOH/EtOAc) gave compound **36** as a white solid (87%); mp (MeOH/EtOAc) 254−257 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.56 (s, 1 H), 8.35 (s, 1 H), 7.95−7.86 (m, 4 H), 7.74 (d, J = 1.9 Hz, 1 H), 7.53−7.42 (m, 3 H), 7.39 (dd, J = 8.7, 2.1 Hz, 1 H), 5.86 (s, 1 H), 3.37 (s, 3 H). LCMS (APCI$^-$) calcd for $C_{17}H_{14}N_3O_2$ 292 (M − H), found 292. Anal. ($C_{17}H_{15}N_3O_2$) C, H, N.

Ethyl 4-(2-Fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxylate (40). Compound **11** (2.03 g, 4.83 mmol) was dissolved in acetic acid (75 mL), to which was added water (25 mL). This solution was heated at reflux for 144 h. The mixture was cooled, and a cream solid crystallized out. This material was isolated by filtration, washed well with water and hexanes, and then dried to afford compound **40** as a white solid (1.14 g, 59%), mp (acetone/MeOH) 262−264 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.59 (br s, 1 H), 9.32 (s, 1 H), 8.10 (s, 1 H), 7.77 (dd, J = 10.1, 1.9 Hz, 1 H), 7.61 (ddd, J = 8.4, 2.0, 0.8 Hz, 1 H), 5.38 (d, J = 1.3 Hz, 1 H), 4.27 (q, J = 7.1 Hz, 2 H), 1.30 (t, J = 7.1 Hz, 3 H). LCMS (APCI$^+$) calcd for $C_{14}H_{13}FIN_2O_3$ 403 (MH^+), found 403. Anal. ($C_{14}H_{12}FIN_2O_3$) C, H, N.

4-(2-Fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (41). Hydrolysis of compound **40** was carried out according to procedure C, giving compound **41** as a white solid (99%), used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.30 (v br s, 1 H), 11.44 (br s, 1 H), 9.75 (br s, 1 H), 8.06 (s, 1 H), 7.76 (dd, J = 10.2, 1.9 Hz, 1 H), 7.59 (ddd, J = 8.3, 1.9, 0.9 Hz, 1 H), 7.32 (t, J = 8.4 Hz, 1 H), 5.40 (d, J = 1.1 Hz, 1 H). LCMS (APCI$^+$) calcd for $C_{12}H_9FIN_2O_3$ 375 (MH^+), found 375.

4-(2-Fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (42). Compound **41** was reacted according to procedure E, and then the intermediate pentafluorophenyl ester reacted directly with c. NH_3 in THF according to procedure F. Recrystallization from EtOAc/MeOH gave compound **42** as cream needles (87%), mp (CH_2Cl_2/MeOH) 320−325 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.42 (s, 1 H), 10.53 (s, 1 H), 8.01 (s, 1 H), 7.96 (br s, 1 H), 7.73 (dd, J = 10.1 Hz, 1 H), 7.57 (br d, J = 8.4 Hz, 1 H), 7.41 (br s, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.47 (s, 1 H). Anal. ($C_{12}H_9FIN_3O_2$) C, H, N.

4-(2-Fluoro-4-iodoanilino)-*N*-(2-hydroxyethyl)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (43). Compound **41** was reacted according to procedure E, and then the intermediate pentafluorophenyl ester reacted directly with 2-aminoethanol according to procedure F. Purification by recrystallization from acetone/MeOH gave compound **43** as a white solid (74%), mp (acetone/MeOH) 254−256 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.38 (br s, 1 H), 10.24 (br s, 1 H), 8.51 (br s, 1 H), 7.96 (s, 1 H), 7.73 (dd, J = 10.1, 1.8 Hz, 1 H), 7.57 (dd, J = 8.4, 0.9 Hz, 1 H), 7.28 (t, J = 8.5 Hz, 1 H), 5.48 (s, 1 H), 4.75 (br s, 1 H), 3.48 (br s, 2 H), 3.26 (br q, J = 5.7 Hz, 2 H). LCMS (APCI$^+$) calcd for $C_{14}H_{14}FIN_3O_3$ 418 (MH^+), found 418. Anal. ($C_{14}H_{13}FIN_3O_3 \cdot 0.5H_2O$) C, H, N.

4-(2-Fluoro-4-iodoanilino)-*N*-(3-hydroxypropyl)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (44). Compound **41** was reacted according to procedure E, and then the intermediate pentafluorophenyl ester reacted directly with 3-aminoethanol according to procedure F. Purification by flash chromatography on silica gel (10% MeOH/CH_2Cl_2) gave compound **44** as a crystalline white solid (89%), mp (EtOAc) 253−255 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.39 (br s, 1 H), 10.26 (br s, 1 H), 8.47 (br s, 1 H), 7.92 (s, 1 H), 7.74 (dd, J = 10.2, 1.7 Hz, 1 H), 7.57 (dd, J = 8.5, 0.8 Hz, 1 H), 7.28 (t, J = 8.5 Hz, 1 H), 5.49 (s, 1 H), 4.46 (br s, 1 H), 3.48−3.41 (m, 2 H), 3.24 (q, J = 6.4 Hz, 2 H), 1.64 (pentet, J = 6.7 Hz, 2 H). LCMS (APCI$^+$) calcd for $C_{15}H_{16}FIN_3O_3$ 432 (MH^+), found 432. Anal. ($C_{15}H_{15}FIN_3O_3$) C, H, N.

Procedure G: Ethyl 4-(2-Fluoro-4-iodoanilino)-6-oxo-1-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]-1,6-dihydro-3-pyridinecarboxylate (45). 2-Iodoethanol was protected as the tetrahydropyranyl ether according to a literature procedure.[31] Compound **40** (383 mg, 0.95 mmol) was dissolved/suspended in dry DMF (15 mL), and the solution cooled (ice/water). NaH (42 mg, 1.05 mmol) was added, the flask placed under nitrogen, and the resulting mixture allowed to warm and then stir at RT for 2 h. A solution of the above-protected iodide (1.22 g, 4.77 mmol) in dry DMF (5 mL) was then added as a single portion and the entire mixture stirred at RT for 15 h. Water (100 mL) was added and the resulting aqueous suspension extracted with EtOAc (3 × 50 mL). The combined EtOAc fractions were then washed with water (2 × 50 mL) and brine (50 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure to afford an oil which was purified by flash column chromatography on silica gel (50% EtOAc/hexanes as eluant) to give compound **45** as a transparent oil (249 mg, 49%) which was used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.31 (s, 1 H), 8.49 (s, 1 H), 7.78 (dd, J = 10.2, 1.9 Hz, 1 H), 7.61 (br d, J = 8.6 Hz, 1 H), 7.31 (t, J = 7.5 Hz, 1 H), 5.45 (s, 1 H), 4.59 (br s, 1 H), 4.30 (q, J = 7.0 Hz, 2 H), 4.11 (t, J = 5.0 Hz, 2 H), 3.75 (pentet, J = 5.5 Hz, 1 H), 3.61−3.49 (m, 2 H), 3.40−3.32 (m, 1 H), 1.74−1.33 (m, 6 H), 1.30 (t, J = 7.1 Hz, 3 H). HRMS (EI$^+$) calcd $C_{21}H_{24}FIN_2O_5$ (M^+) 530.0714, found 530.0704.

4-(2-Fluoro-4-iodoanilino)-6-oxo-1-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]-1,6-dihydro-3-pyridinecarboxamide (57). Hydrolysis of compound **45** was carried out according to procedure C, giving the intermediate acid (88%) which was reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by flash chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **57** as an oily cream solid (76%), used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.49 (s, 1 H), 8.34 (s, 1 H), 7.93 (br s, 1 H), 7.74 (dd, J = 10.2, 1.8 Hz, 1 H), 7.57 (dd, J = 8.7, 1.0 Hz, 1 H), 7.48 (br s, 1 H), 7.30 (t, J = 8.6 Hz, 1 H), 5.55 (s, 1 H), 4.58−4.52 (m, 1 H), 4.04−3.91 (m, 2 H), 3.82−3.75 (m, 1 H), 3.63−3.35 (m, 3 H), 1.73−1.26 (m, 6 H). HRMS (EI$^+$) calcd $C_{19}H_{21}FIN_3O_4$ (M^+) 501.0561, found 501.0564.

Procedure H: 4-(2-Fluoro-4-iodoanilino)-1-(2-hydroxyethyl)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (58). Compound **57** (118 mg, 0.24 mmol) was dissolved in EtOH (8 mL), to which was added 1 M HCl (2 mL). This mixture was stirred at RT for 2 h and then diluted with water (80 mL). The resulting solution was extracted with EtOAc (3 × 40 mL), and then the combined EtOAc fractions were washed with water (2 × 50 mL) and brine (50 mL) and dried (Na_2SO_4). Filtration and removal of the solvent under reduced pressure afforded compound **58** as a white solid (95 mg, 97%), mp (EtOAc/MeOH) 212−215 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.45 (s, 1 H), 8.27 (s, 1 H), 7.94 (br s, 1 H), 7.74 (dd, J = 10.1, 1.7 Hz, 1 H), 7.57 (br d, J = 8.6 Hz, 1 H), 7.48 (br s, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.55 (s, 1 H), 4.93 (t, J = 5.3 Hz, 1 H), 3.85 (t, J = 5.5 Hz, 2 H), 3.60 (q, J = 5.4 Hz, 2 H). Anal. ($C_{14}H_{13}FIN_3O_3$) C, H, N.

Ethyl 1-Ethyl-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxylate (48). Compound **40** was reacted with NaH and iodoethane in DMF according to procedure G. Purification by flash column chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **48** as white needles (61%), mp (EtOAc/hexanes) 138−142 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.29 (s, 1 H), 8.52 (s, 1 H), 7.77 (dd, J = 10.1, 1.9 Hz, 1 H), 7.60 (ddd, J = 8.4, 2.0, 0.8 Hz, 1 H), 7.31 (t, J = 8.5 Hz, 1 H), 5.45 (s, 1 H), 4.30 (q, J = 7.1 Hz, 2 H), 3.93 (q, J = 7.1 Hz, 2 H), 1.33 (t, J = 7.1 Hz, 3 H), 1.20 (t, J = 7.1 Hz, 3 H). Anal. ($C_{16}H_{16}FIN_2O_3$) C, H, N.

1-Ethyl-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (53). Hydrolysis of compound **48** was carried out according to procedure C, giving the intermediate acid (100%) which was reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concentrated NH_3 solution in THF according to procedure F. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **53** as a white solid (84%), mp (EtOAc) 260−262 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.38 (s, 1 H), 8.31 (s, 1 H), 7.90 (br s, 1 H), 7.73 (dd, J = 10.2, 1.9 Hz, 1 H), 7.57 (ddd, J = 8.4, 1.9, 0.9 Hz, 1 H), 7.46 (br s, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.55 (d, J = 0.9 Hz, 1 H), 3.83 (q, J = 7.1 Hz, 2 H), 1.23 (t, J = 7.1 Hz, 3 H). Anal. ($C_{14}H_{13}FIN_3O_2$) C, H, N.

Ethyl 4-(2-Fluoro-4-iodoanilino)-6-oxo-1-propyl-1,6-dihydro-3-pyridinecarboxylate (49). Compound **40** was reacted with NaH and bromopropane in DMF according to procedure G. Purification by flash column chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **49** as a white solid (54%), mp (EtOAc/hexanes) 147−150 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.29 (s, 1 H), 8.50 (s, 1 H), 7.76 (dd, J = 10.1, 1.9 Hz, 1 H), 7.60 (ddd, J = 8.4, 2.0, 0.8 Hz, 1 H), 7.31 (t, J = 8.5 Hz, 1 H), 5.46 (d, J = 1.3 Hz, 1 H), 4.30 (q, J = 7.1 Hz, 2 H), 3.85 (t, J = 7.3 Hz, 2 H), 1.62 (sextet, J = 7.3 Hz, 2 H), 1.33 (t, J = 7.1 Hz, 3 H), 0.85 (t, J = 7.4 Hz, 3 H). Anal. ($C_{17}H_{18}FIN_2O_3$) C, H, N.

4-(2-Fluoro-4-iodoanilino)-6-oxo-1-propyl-1,6-dihydro-3-pyridinecarboxamide (54). Hydrolysis of compound **49** was carried out according to procedure C, giving the intermediate acid (100%) which was reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **54** as a white solid (97%), mp (EtOAc) 218−220 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.43 (s, 1 H), 8.30 (s, 1 H), 7.80 (br s, 1 H), 7.73 (dd, J = 10.2, 1.9 Hz, 1 H), 7.56 (dd, J = 8.4, 0.9 Hz, 1 H), 7.46 (br s, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.55 (d, J = 0.7 Hz, 1 H), 3.75 (t, J = 7.3 Hz, 2 H), 1.65 (sextet, J = 7.4 Hz, 2 H), 0.87 (t, J = 7.4 Hz, 3 H). Anal. ($C_{15}H_{15}FIN_3O_2$) C, H, N.

Ethyl 4-(2-Fluoro-4-iodoanilino)-1-[2-(2-methoxyethoxy)ethyl]-6-oxo-1,6-dihydro-3-pyridinecarboxylate (50). Compound **40** was reacted with NaH and 1-bromo-2-(2-methoxyethoxy)ethane in DMF according to procedure G. Purification by flash chromatography on silica gel (1% MeOH/CH_2Cl_2 as eluant) gave compound **50** as a pale yellow oil (41%). ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.30 (s, 1 H), 8.43 (s, 1 H), 7.77 (dd, J = 10.1, 1.9 Hz, 1 H), 7.60 (ddd, J = 8.3, 1.9, 0.8 Hz, 1 H), 7.31 (t, J = 8.5 Hz, 1 H), 5.44 (d, J = 1.3 Hz, 1 H), 4.31 (q, J = 7.1 Hz, 2 H), 4.06 (t, J = 5.1 Hz, 2 H), 3.61 (t, J = 5.2 Hz, 2 H), 3.54−3.50 (m, 2 H), 3.43−3.39 (m, 2 H), 3.22 (s, 3 H), 1.32 (t, J = 7.1 Hz, 3 H). HRMS (EI$^+$) calcd $C_{19}H_{22}FIN_2O_5$ (M^+) 504.0558, found 504.0552.

4-(2-Fluoro-4-iodoanilino)-1-[2-(2-methoxyethoxy)ethyl]-6-oxo-1,6-dihydro-3-pyridinecarboxamide (55). Hydrolysis of compound **47** was carried out according to procedure C, to give the intermediate acid (99%) which was reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by flash column chromatography on silica gel (50% acetone/CH_2Cl_2 as eluant) gave compound **55** as a white solid (58%), mp (EtOAc/n-pentane) 114−116 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.43 (s, 1 H), 8.28 (s, 1 H), 7.81 (br s, 1 H), 7.73 (dd, J = 10.2, 1.9 Hz, 1 H), 7.57 (dd, J = 8.4, 0.9 Hz, 1 H), 7.48 (br s, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.55 (s, 1 H), 3.95 (t, J = 5.5 Hz, 2 H), 3.63 (t, J = 5.5 Hz, 2 H), 3.54−3.51 (m, 2 H), 3.43−3.39 (m, 2 H), 3.22 (s, 3 H). Anal. ($C_{17}H_{19}FIN_3O_4$) C, H, N.

Ethyl 1-(3-Cyanopropyl)-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxylate (47). Compound **40** was reacted with NaH and 4-bromobutyronitrile in DMF according to procedure G. Purification by flash column chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **47** as a white solid (67%), mp (EtOAc) 157−159 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.30 (s, 1 H), 8.51 (s, 1 H), 7.77 (dd, J = 10.1, 1.9 Hz, 1 H), 7.61 (br d, J = 8.4 Hz, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.46 (s, 1 H), 4.31 (q, J = 7.1 Hz, 2 H), 3.96 (t, J = 7.2 Hz, 2 H), 2.53 (t, J = 7.2 Hz, 2 H), 1.93 (pentet, J = 7.2 Hz, 2 H), 1.33 (t, J = 7.1 Hz, 3 H). Anal. ($C_{18}H_{17}FIN_3O_3$) C, H, N.

1-(3-Cyanopropyl)-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (56). Hydrolysis of compound **47** was carried out according to procedure C, to give the intermediate acid (100%) which was reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **56** as a white solid (70%), mp (EtOAc) 146−150 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.36 (s, 1 H), 8.28 (s, 1 H), 7.91 (br s, 1 H), 7.73 (dd, J = 10.2, 1.9 Hz, 1 H), 7.57 (ddd, J = 8.4, 1.9, 0.9 Hz, 1 H), 7.48 (br s, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.56 (d, J = 0.9 Hz, 1 H), 3.87 (t, J = 7.2 Hz, 2 H), 2.55 (t, J = 7.2 Hz, 2 H), 1.96 (pentet, J = 7.2 Hz, 2 H). Anal. ($C_{16}H_{14}FIN_4O_2$) H, N. C: calcd, 43.7; found, 44.2.

1-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}propyl)-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (59). 3-Bromopropanol was protected as the *tert*-butyldimethylsilyl ether according to a literature procedure.[32] Compound **40** was reacted with NaH and the silyl ether-protected bromide in DMF according to procedure G, giving a crude product which was purified by column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant). Rather than isolating the desired compound **52**, hydrolysis to the intermediate acid occurred *in situ*. This acid (34%) was therefore reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **59** as a white solid (68%), used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.36 (s, 1 H), 8.24 (s, 1 H), 7.92 (br s, 1 H), 7.72 (dd, J = 10.2, 1.8 Hz, 1 H), 7.56 (br d, J = 8.9 Hz, 1 H), 7.46 (br s, 1 H), 7.28 (t, J = 8.5 Hz, 1 H), 5.55 (s, 1 H), 3.85 (t, J = 7.1 Hz, 2 H), 3.62 (t, J = 6.1 Hz, 2 H), 1.85 (pentet, J = 6.6 Hz, 2 H), 0.86 (s, 9 H), 0.03 (s, 6 H). HRMS (EI$^+$) calcd $C_{21}H_{29}FIN_3O_3Si$ (M^+) 545.1007, found 545.1019.

4-(2-Fluoro-4-iodoanilino)-1-(3-hydroxypropyl)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (60). Deprotection of compound **59** was carried out according to procedure H, giving compound **60** as a white solid which was recrystallized from EtOAc/MeOH (86%), mp (EtOAc/MeOH) 220−223 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.40 (s, 1 H), 8.29 (s, 1 H), 7.91 (br s, 1 H), 7.72 (dd, J = 10.2, 1.8 Hz, 1 H), 7.56 (br d, J = 8.4 Hz, 1 H), 7.46 (br s, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.56 (s, 1 H), 4.58 (t, J = 5.1 Hz, 1 H), 3.86 (t, J = 7.2 Hz, 2 H), 3.41 (q, J = 5.7 Hz, 2 H), 1.79 (pentet, J = 6.6 Hz, 2 H). Anal. ($C_{15}H_{15}FIN_3O_3$) C, H, N.

Ethyl 1-(2-*tert*-Butoxy-2-oxoethyl)-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxylate (46). Compound **40** was reacted with NaH and *tert*-butylbromoacetate in DMF according to procedure G. Purification by flash chromatography on silica gel (1% MeOH/CH_2Cl_2 as eluant) gave compound **46** as a white solid (72%), mp (EtOAc/hexanes) 149−151 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.34 (s, 1 H), 8.57 (s, 1 H), 7.78 (dd, J = 10.0, 1.9 Hz, 1 H), 7.61 (dd, J = 8.4, 1.0 Hz, 1 H), 7.31 (t, J = 8.5 Hz, 1 H), 5.43 (d, J = 1.2 Hz, 1 H), 4.62 (s, 2 H), 4.31 (q, J = 7.1 Hz, 2 H), 1.42 (s, 9 H), 1.33 (t, J = 7.1 Hz, 3 H). Anal. ($C_{20}H_{22}FIN_2O_5$) C, H, N.

***tert*-Butyl [5-(Aminocarbonyl)-4-(2-fluoro-4-iodoanilino)-2-oxo-1(2*H*)-pyridinyl)]acetate (61).** Hydrolysis of compound **46** was carried out according to procedure C but using 1 M K_2CO_3 instead of 1 M NaOH. The intermediate acid (64%) was reacted according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by flash column chromatography on silica gel (50% acetone/CH_2Cl_2 as eluant) gave compound **61** as a white solid (93%), used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.40 (s, 1 H), 8.30 (s, 1 H), 7.80 (br s, 1 H), 7.74 (dd, J = 10.2, 1.9 Hz, 1 H), 7.58 (br d, J = 8.4 Hz, 1 H), 7.54 (br s, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.53 (d, J = 0.8 Hz, 1 H), 4.46 (s, 2 H), 1.43 (s, 9 H). HRMS (FAB^+) calcd $C_{18}H_{20}FIN_3O_4$ (MH^+) 488.0483, found 488.0471.

[5-(Aminocarbonyl)-4-(2-fluoro-4-iodoanilino)-2-oxo-1(2*H*)-pyridinyl]acetic Acid (62). Compound **61** was dissolved in a mixture of CH_2Cl_2 (10 mL) and trifluoroacetic acid (10 mL) and stirred at RT for 2 h. All solvent was evaporated under a stream of nitrogen, and the resulting oil was redissolved in MeOH (10 mL) to which was added sat. $NaHCO_3$ (10 mL). This mixture was stirred at RT for 1 h, 1 M HCl (50 mL) added, and the resulting white precipitate collected by filtration. Purification was carried out by recrystallization from EtOAc/MeOH to afford compound **62** as a white solid (38%), mp (EtOAc/MeOH) 296−300 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.10 (v br s, 1 H), 10.42 (s, 1 H), 8.32 (s, 1 H), 7.86 (br s, 1 H), 7.75 (dd, J = 10.2, 1.9 Hz, 1 H), 7.58 (dd, J = 8.4, 0.8 Hz, 1 H), 7.52 (br s, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 5.53 (d, J = 1.0 Hz, 1 H), 4.49 (s, 2 H). Anal. ($C_{14}H_{11}FIN_3O_4 \cdot 0.5H_2O$) C, H, N.

Ethyl 1-Allyl-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxylate (51). Compound **40** was reacted with NaH and allyl bromide in DMF according to procedure G. Purification by column chromatography on silica gel (50% EtOAc/hexanes as eluant) gave compound **51** as a white solid (78%), mp (EtOAc) 138−141 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 9.30 (s, 1 H), 8.45 (s, 1 H), 7.77 (dd, J = 10.1, 1.9 Hz, 1 H), 7.61 (ddd, J = 8.4, 2.0, 0.8 Hz, 1 H), 7.31 (t, J = 8.5 Hz, 1 H), 5.98−5.87 (m, 1 H), 5.47 (d, J = 1.3 Hz, 1 H), 5.19 (ddd, J = 10.4, 2.7, 1.3 Hz, 1 H), 5.12 (ddd, J = 17.2, 3.0, 1.5 Hz, 1 H), 4.54 (br d, J = 5.5 Hz, 2 H), 4.31 (q, J = 7.1 Hz, 2 H), 1.32 (t, J = 7.1 Hz, 3 H). Anal. ($C_{17}H_{16}FIN_2O_3$) C, H, N.

1-Allyl-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (63). Hydrolysis of compound **51** was carried out according to procedure C, to give the intermediate acid (99%) which was reacted directly according to procedure E. This intermediate pentafluorophenyl ester was then reacted directly with concd NH_3 solution in THF according to procedure F. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **63** as a white solid (85%), mp (EtOAc) 215−217 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.43 (s, 1 H), 8.27 (s, 1 H), 7.90 (br s, 1 H), 7.73 (dd, J = 10.2, 1.9 Hz, 1 H), 7.57 (dd, J = 8.3, 0.8 Hz, 1 H), 7.49 (br s, 1 H), 7.30 (t, J = 8.5 Hz, 1 H), 6.00−5.88 (m, 1 H), 5.55 (s, 1 H), 5.19 (dd, J = 10.3, 1.3 Hz, 1 H), 5.11 (ddd, J = 17.2, 2.9, 1.5 Hz, 1 H), 4.41 (d, J = 5.5 Hz, 2 H). Anal. ($C_{15}H_{13}FIN_3O_2$) C, H, N.

1-(2,3-Dihydroxypropyl)-4-(2-fluoro-4-iodoanilino)-6-oxo-1,6-dihydro-3-pyridinecarboxamide (64). Compound **63** (400 mg, 0.97 mmol) was dissolved in a mixture of *tert*-butyl alcohol (60 mL) and water (60 mL), and to the resulting solution were added $K_3Fe(CN)_6$ (956 mg, 2.91 mmol), K_2CO_3 (400 mg, 2.91 mmol), OsO_4 (0.62 mL of a 4% w/w solution in water), and diazabicyclooctane (108 mg, 0.97 mmol). The reaction mixture was stirred at RT for 15 h, poured into 1 M $Na_2S_2O_4$ (200 mL), and extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with water (100 mL) and brine (100 mL) and dried (Na_2SO_4). The solution was filtered and then solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **64** as a white solid (312 mg, 72%), mp (EtOAc) 210−213 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.44 (s, 1 H), 8.26 (s, 1 H), 7.87 (br s, 1 H), 7.73 (dd, J = 10.2, 1.9 Hz, 1 H), 7.57 (dd, J = 8.4, 0.9 Hz, 1 H), 7.46 (br s, 1 H), 7.29 (t, J = 8.5 Hz, 1 H), 5.56 (d, J = 0.9 Hz, 1 H), 4.96 (d, J = 5.5 Hz, 1 H), 4.70 (t, J = 4.7 Hz, 1 H), 4.14 (dd, J = 13.1, 3.5 Hz, 1 H), 3.79−3.70 (m, 1 H), 3.51 (dd, J = 13.1, 8.3 Hz, 1 H), 3.40−3.30 (m, 2 H). Anal. ($C_{15}H_{15}FIN_3O_4$) C, H, N.

4-Chloro-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (66). Compound **5** (3.19 g, 14.5 mmol) and dimethyl sulfate (6.0 mL, 63 mmol) were combined in a thick-walled glass tube with a Teflon cap. The tube was sealed and heated in a 120 °C sand bath. After 5 h, the reaction was cooled to RT and diluted with acetonitrile (100 mL) and saturated aq $NaHCO_3$ solution (100 mL). The reaction mixture was stirred vigorously for 18 h. This mixture was further diluted with water and was extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were washed with brine (200 mL) and dried ($MgSO_4$). The solution was filtered and then solvent removed under reduced pressure to afford ethyl 4-chloro-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate **65** (2.65 g, 85% yield) as an oil that solidified upon standing. This material was then hydrolyzed directly according to procedure C, followed by crystallization from methanol/ethyl acetate to give compound **66** (37%) as an off-white solid: ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.02 (br s, 1 H), 8.58 (s, 1 H), 6.58 (s, 1 H), 3.48 (s, 3 H).

Procedure I: Alternative Preparation of 4-(2-fluoro-4-iodoanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylic Acid (21). Compound **66** (133 mg, 0.709 mmol) and 2-fluoro-4-iodoaniline **6** (172 mg, 0.726 mmol) were combined in a round-bottom flask equipped with magnetic stir bar. The flask was immersed in an ice bath, and lithium bis(trimethylsilyl)amide (1.0 M in THF, 5.0 mL, 5.0 mmol) was added slowly (5 min) with vigorous stirring under an atmosphere of nitrogen. The reaction mixture was further stirred for 1 h at 0 °C and 1 h at RT. The reaction mixture was diluted with 1 M HCl and water and was extracted with EtOAc (3 × 100 mL). The extracts were dried ($MgSO_4$) and filtered, and upon concentration compound **21** (114 mg, 41% yield) was isolated directly from the EtOAc by filtration and drying *in vacuo*. Spectral data for this compound was in exact agreement with that obtained for compound **21** described in procedure C above.

Procedure J: 4-(3,4-Dichlorophenylamino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (71). Compound **66** and 3,4-dichloroaniline **67** were reacted in the presence of lithium diisopropylamide in THF according to procedure I. The resultant compound **69** was used without further purification in the following step. Compound **69** (234 mg) was dissolved in CH_2Cl_2 (5 mL) and ethanolic ammonia (2 M, 2.0 mL, 4.0 mmol). PyBOP (579 mg, 1.10 mmol) was added in one portion, and the reaction mixture was stirred overnight at RT. The reaction mixture was quenched with AcOH (ca. 0.5 mL), diluted with EtOAc (40 mL), and washed with water (2 × 10 mL) and brine (10 mL). The organics were dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure. Purification by flash column chromatography on silica gel (4:1 CH_2Cl_2/MeOH as eluant) gave compound **71** as a dark yellow solid (28 mg, 12%); mp (MeOH) > 250 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.38 (s, 1 H), 8.30 (s, 1 H), 7.85 (br s, 1 H), 7.57 (d, J = 8.8 Hz, 1 H), 7.48 (d, J = 2.4 Hz, 1 H), 7.45 (br s, 1 H), 7.23 (dd, J = 8.8, 2.4 Hz, 1 H), 5.70 (s, 1 H), 3.33 (s, 3 H). Anal. ($C_{13}H_{11}Cl_2N_3O_2$) C, H, N.

4-(2-Fluoro-4-methylsulfanylphenylamino)-1-methyl-6-oxo-1,6-dihydro-3 -pyridinecarboxylic Acid (70). Compound **66** and 2-fluoro-3-thiomethylaniline **68** were reacted in the presence of lithium diisopropylamide in THF according to procedure I. Compound **70** was isolated as a light yellow solid (85%). ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 13.22 (v br s, 1 H), 9.43 (s, 1 H), 8.47 (s, 1 H), 7.39 (t, 1 H), 7.26 (dd, 1 H), 7.13 (dd, 1 H), 5.33 (s, 1 H), 3.38 (s, 3 H), 2.48 (s, 3 H). MS ($APCI^+$) calcd for $C_{14}H_{14}FN_2O_3S$ 309 (MH^+), found 309.

4-(2-Fluoro-4-methylsulfanylphenylamino)-1-methyl-6-oxo-1,6-dihydro-3 -pyridinecarboxamide (72). Compound **70** was reacted according to procedure J. Compound **72** was isolated as a white solid (47%). ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.16 (s, 1 H), 8.36 (s, 1 H), 7.80 (v br s, 1 H), 7.40 (v br s, 1 H), 7.37 (t, 1

H), 7.25 (dd, 1 H), 7.10 (dd, 1 H), 5.38 (s, 1 H), 3.33 (s, 3 H), 2.46 (s, 3 H). MS (APCI$^+$) calcd for $C_{14}H_{15}FN_3O_2S$ 308 (MH^+), found 308.

Procedure K: 4-{2-Fluoro-4-[(trimethylsilyl)ethynyl]anilino}-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (73). Compound **27** (200 mg, 0.52 mmol), CuI (2 mg, 0.01 mmol), and $(Ph_3P)_2PdCl_2$ (8 mg, 0.01 mmol) were dissolved in THF (10 mL), and the flask was flushed with nitrogen. A solution of TMS-acetylene (56 mg, 0.06 mmol) in TEA (2 mL) was added dropwise over 5 min, and then the reaction was allowed to stir at RT for 15 h. This mixture was diluted with EtOAc (80 mL) which was subsequently washed with water (3 × 50 mL) and brine (50 mL) and dried (Na_2SO_4). Purification by flash column chromatography on silica gel (10% MeOH/CH_2Cl_2 as eluant) gave compound **73** as a pale yellow solid (185 mg, 100%), used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.66 (s, 1 H), 8.38 (s, 1 H), 7.92 (br s, 1 H), 7.50 (t, J = 8.5 Hz, 1 H), 7.48 (br s, 1 H), 7.42 (dd, J = 11.5, 1.8 Hz, 1 H), 7.30 (dd, J = 8.3, 1.4 Hz, 1 H), 5.73 (s, 1 H), 3.38 (s, 3 H), 0.23 (s, 9 H). HRMS (EI$^+$) calcd $C_{18}H_{20}FN_3O_2Si$ (M^+) 357.1309, found 357.1308.

4-(4-Ethynyl-2-fluoroanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (74). Compound **73** (185 mg, 0.52 mmol) was dissolved in a mixture of MeOH (9 mL) and THF (1 mL), to which was added solid K_2CO_3 (143 mg, 1.03 mmol). This mixture was allowed to stir for 15 h at RT and then diluted with EtOAc (50 mL). The EtOAc solution was washed with water (3 × 50 mL) and brine (50 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (50% acetone/CH_2Cl_2 as eluant) gave compound **74** as a pale yellow-orange solid (108 mg, 73%); mp (CH_2Cl_2/MeOH) 269−272 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.60 (s, 1 H), 8.37 (s, 1 H), 7.91 (br s, 1 H), 7.51 (t, J = 8.4 Hz, 1 H), 7.48 (br s, 1 H), 7.46 (dd, J = 11.4, 1.7 Hz, 1 H), 7.33 (dd, J = 8.3, 0.9 Hz, 1 H), 5.71 (s, 1 H), 4.25 (s, 1 H). Anal. ($C_{15}H_{12}FN_3O_2$•0.25H_2O) C, H, N.

Procedure L: 4-(4-Ethyl-2-fluoroanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (75). Compound **74** (77 mg, 0.27 mmol) was dissolved in a mixture of THF (10 mL) and EtOH (10 mL) to which was added 5% Pd/C (10 mg). This mixture was stirred under an atmosphere of H_2 at 60 psi for 18 h at RT, the Pd/C removed over celite, and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (50% acetone/CH_2Cl_2 as eluant) gave compound **75** as a cream solid (64 mg, 82%), mp (EtOAc) 268−272 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.14 (s, 1 H), 8.32 (s, 1 H), 7.84 (br s, 1 H), 7.43 (br s, 1 H), 7.33 (t, J = 8.4 Hz, 1 H), 7.19 (dd, J = 11.1, 1.7 Hz, 1 H), 7.08 (dd, J = 8.2, 1.5 Hz, 1 H), 5.39 (d, J = 1.1 Hz, 1 H), 3.36 (s, 3 H), 2.62 (q, J = 7.6 Hz, 2 H), 1.19 (t, J = 7.6 Hz, 3 H). Anal. ($C_{15}H_{16}FN_3O_2$) C, H, N.

4-(4-Cyano-2-fluoroanilino)-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (76). Compound **27** (0.20 g, 0.5 mmol) was added to potassium cyanide (0.23 g, 3.50 mmol), copper(I) iodide (667 mg, 3.50 mmol), and tetrakis(triphenylphosphine)palladium-(0) (20 mg) in DMF (30 mL) and heated at 110 °C for 4 h. The reaction mixture was allowed to cool to RT and filtered through celite which was washed well with 5% MeOH/EtOAc. The filtrate and washings were combined and concentrated, washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4), and filtered. Removal of the solvent under reduced pressure followed by trituration with EtOAc gave an approximately 1:1 mixture of unreacted compound **27** and the desired product **76**. Further purification by preparative HPLC [90% (H_2O/TFA)/(acetonitrile/TFA)−1% (H_2O/TFA)/(acetonitrile/TFA) gradient elution 0.8 mL/min, pH 2.5−2.6] yielded compound **76** (0.01 g, 7%); mp 261−266 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 11.01 (s, 1 H), 8.39 (s, 1 H), 7.91 (dd and br s, J = 11.2, 1.7 Hz, 2 H), 7.72 (t, J = 8.2 Hz, 1 H), 7.66 (dd, J = 8.6, 1.7 Hz, 1 H), 7.52 (br s, 1 H), 5.97 (s, 1 H), 3.40 (s, 3 H). MS (APCI$^+$) calcd for $C_{14}H_{12}N_4O_2F$ 287 (MH^+), found 387.

4-[2-Fluoro-4-(3-hydroxy-1-propynyl)anilino]-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (77). Compound **27** was reacted with propargyl alcohol according to procedure K. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **77** as an off-white solid (89%), used directly in the next step. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.57 (s, 1 H), 8.35 (s, 1 H), 7.90 (br s, 1 H), 7.50 (t, J = 8.5 Hz, 1 H), 7.48 (br s, 1 H), 7.39 (dd, J = 11.4, 1.8 Hz, 1 H), 7.28 (dd, J = 8.3, 1.5 Hz, 1 H), 5.69 (s, 1 H), 5.36 (t, J = 6.0 Hz, 1 H), 4.30 (d, J = 5.9 Hz, 2 H), 3.37 (s, 3 H). LCMS (APCI$^+$) calcd for $C_{16}H_{15}FN_3O_3$ 316 (MH^+), found 316.

4-[2-Fluoro-4-(3-hydroxypropyl)anilino]-1-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxamide (78). Compound **77** was hydrogenated according to procedure L. Purification by flash column chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) gave compound **78** as a crystalline cream solid (90%), mp (EtOAc/MeOH) 214−216 °C. ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 10.15 (s, 1 H), 8.32 (s, 1 H), 7.85 (br s, 1 H), 7.43 (br s, 1 H), 7.33 (t, J = 8.3 Hz, 1 H), 7.17 (dd, J = 11.8, 1.7 Hz, 1 H), 7.07 (dd, J = 8.2, 1.6 Hz, 1 H), 5.40 (s, 1 H), 4.50 (t, J = 5.1 Hz, 1 H), 3.41 (q, J = 6.0 Hz, 2 H), 3.36 (s, 3 H), 2.63 (t, J = 7.7 Hz, 2 H), 1.76−1.69 (m, 2 H). LCMS (APCI$^-$) calcd for $C_{16}H_{17}FN_3O_3$ 318 (MH^+), found 318. Anal. ($C_{16}H_{18}FN_3O_3$) C, H, N.

Acknowledgment. This work was supported by Pfizer Global Research and Development (USA) and the Auckland Division of the Cancer Society of New Zealand.

Supporting Information Available: Elemental analysis, HRMS, and HPLC data on final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0704548