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Discovery of *N*-(2,4-Di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770, Ivacaftor), a Potent and Orally Bioavailable CFTR Potentiator

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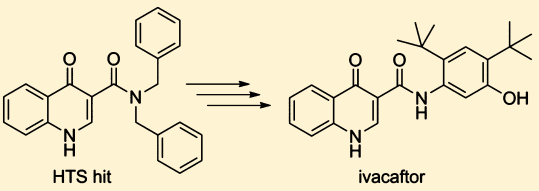
Supporting Information

ABSTRACT: Quinolinone-3-carboxamide **1**, a novel CFTR potentiator, was discovered using high-throughput screening in NIH-3T3 cells expressing the F508del-CFTR mutation. Extensive medicinal chemistry and iterative structure−activity relationship (SAR) studies to evaluate potency, selectivity, and pharmacokinetic properties resulted in the identification of *N*-(2,4-di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770, **48**, ivacaftor), an investigational drug candidate approved by the FDA for the treatment of CF patients 6 years of age and older carrying the G551D mutation.



INTRODUCTION

Cystic fibrosis (CF) is a lethal genetic disease that affects approximately 70 000 patients worldwide.[1] CF is caused by defective or deficient cystic fibrosis transmembrane conductance regulator (CFTR) protein resulting from mutations on both alleles of the CFTR gene. The CFTR protein is a PKA-regulated anion channel that is critical for the transport of chloride and bicarbonate ions in epithelial cells from multiple organs including the lung, pancreas, intestine, reproductive tract, and sweat duct. Over 1900 different mutations in the CFTR gene have been identified. Mutations of the CFTR gene can result in reduced amounts of CFTR at the cell surface, a decrease in the ability of CFTR to transport ions, or both.[2] The most common mutation is F508del, a deletion of phenylalanine at position 508, which is found in at least one allele in about 90% of CF patients and interferes with CFTR folding, trafficking, membrane stability, and channel gating. The resulting decrease of CFTR-dependent chloride secretion into the airway leads to an increase in fluid absorption across the surface epithelia causing the mucus to have abnormal composition and increased viscosity. This leads to airway obstruction and prevents clearance of harmful bacteria. There is still a large unmet medical need for CF patients, as the majority of the treatments such as antibiotics and mucus-clearing agents are only symptomatic.[3]

Our strategy was to discover small molecules that target the underlying mechanism of CF by restoring the function of the mutant CFTR channel. Assays were developed to monitor the activity of compounds that affect the function of CFTR by two complementary mechanisms, CFTR potentiation and CFTR correction. Correctors are agents that improve the cellular processing and delivery of F508del-CFTR to the cell surface to increase CFTR-mediated Cl^- secretion.[4−6] CFTR potentiators act on CFTR to increase the flow of ions through CFTR channels that are present at the cell surface, usually by increasing their open probability (P_o).[4] Initial in vitro data suggested that CFTR potentiators are therapeutically useful in CF patients who have CFTR at the cell surface, including patients with gating mutations such as G551D. Additionally, potentiators might augment the effect of other agents, such as correctors, that increase CFTR presence at the cell surface. Thus, a potentiator that acts on different mutations, including F508del and G551D-CFTR, would be desirable.

Several classes of CFTR potentiators have been reported[7] (Figure 1) such as flavones (genistein), chromen-4-ones (UC_{CF}-29), sulfonamides (SF-01), phenylglycines (PG-01), and our previously described pyrazoles (VRT-532).[4,8] It is speculated that these molecules interact directly with the nucleotide binding domain region of CFTR.[9] However, many of these compounds display low potency, limited selectivity, and poor pharmacokinetic properties in preclinical species, making them unsuitable for development as human therapeutics.[10] Herein, we describe the discovery and subsequent evaluation of a novel class of CFTR potentiators, the quinolinone-3-carboxamides. Extensive structure−activity relationship around compound **1**, the starting point of our medicinal chemistry program, led to the identification of compounds that potentiated a number of mutant CFTR forms in vitro, including F508del-CFTR, G551D-CFTR, and others, culminating in the discovery of VX-770 (**48**).[11] VX-770 (also known by its generic name ivacaftor and now marketed under the name Kalydeco) was subsequently evaluated in randomized, placebo-controlled clinical trials[12,13] and was approved by the FDA on January 31, 2012 for the treatment of CF in patients aged 6 years or older who have the G551D mutation.[14]

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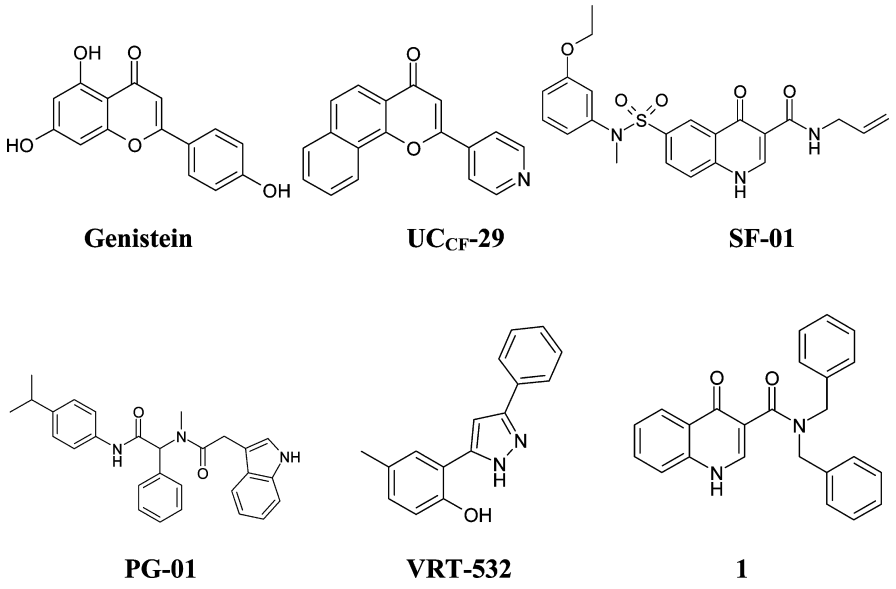

Figure 1. Examples of CFTR potentiators.

Ivacaftor exhibits excellent activity and pharmacokinetic properties, providing the opportunity to treat the underlying cause of CF either as monotherapy or in combination with CFTR correctors, depending on the CFTR mutation.

High throughput screening in NIH-3T3 cells expressing the F508del-CFTR mutation was developed using a fluorescence-based assay that measured changes in membrane potential due to CFTR-mediated chloride efflux in response to stimulation by forskolin, an adenylcyclase activator. To correct the defective processing and trafficking of F508del-CFTR to the cell surface, the cells were incubated at 27 °C for 16 h prior to monitoring compounds' activity.[11] Compound **1** displayed an EC_{50} of 2.1 ± 1.4 μM in this assay, a 4-fold improvement over genistein (EC_{50} = 8.0 ± 2.0 μM). The response to **1** was observed only after forskolin addition, indicating that **1** was a potentiator and not an activator of CFTR function. To confirm the activity of **1**, human bronchial epithelial cells (HBE) isolated from bronchi of CF subjects carrying the F508del mutation on both alleles were used. When cultured as monolayers, these cells show impaired salt and fluid transport, defective cilia beating, and the formation of a thick mucus layer characteristic of CF airway epithelia in vivo.[11] CFTR potentiation was measured as an increase in transepithelial current in the presence of forskolin, and the EC_{50} in F508del/F508del-HBE for **1** was 1.5 μM. In addition, compound **1** increased the chloride secretion in G551D/F508del-HBE cells with an EC_{50} of 12.2 μM, showing the potential for potentiation of multiple CFTR mutations. These findings, in combination with a relatively low molecular weight of 368 and cLogP of 2.9,[15] made **1** an attractive, validated starting point for the CFTR potentiator project. In the remainder of this study, unless noted otherwise, all potentiator EC_{50} data were obtained in the fluorescence-based assay in NIH-3T3 cells expressing the F508del-CFTR mutation. Key compounds were also profiled in F508del-HBE and G551D/F508del-HBE cells where we observed no more than 10-fold potency differences between NIH-3T3, F508del-HBE, and G551D/F508del-HBE cells.

RESULTS AND DISCUSSION

To begin the investigation, information around compound **1** was generated by using the central amide bond to synthesize analogs and probe independently the role of both the quinolinone and the amine moieties. To simplify the structure−activity (SAR) interpretation in this work, we hypothesized that the molecular target is a single protein, most likely F508del-CFTR. A range of distinct analogs were synthesized to examine the requirements for the quinolinone moiety which could exist in the keto or enol form, either of which could be differentially replicated using different isosteric replacements. Removal of the quinolinone 4-oxo group as shown in compound **2**, truncation of the fused phenyl with pyridone **3**, alkylation of the quinolinone nitrogen **4**, or replacement of the quinolinone ring with pyridopyrimidone **5** all led to reduced potencies, whereas quinolinone replacement with naphthol derivative **6** retained activity (Table 1). Unexpectedly, the data in Table 1 suggested that the quinolinone ring has two chemical features important for retaining activity. These are the hydrophobic phenyl ring and the quinolinol tautomer which is stabilized by intramolecular hydrogen bonding with the lone pair of electrons on the carbonyl oxygen of the amide (Figure 2).

To determine if changes in the amine portion of the molecule would lead to increased potency, a diverse set of ∼70 amines including primary, secondary, aliphatic, aromatic, and heterocyclic amines were selected for synthesis. As a result, 6-indolyl derivative **16** (Table 2) was identified, with an EC_{50} of 0.1 μM, a 20-fold improvement in potency over compound **1**.

Table 2 shows several examples prepared as part of the amine exploration. Aniline **7**, 2,6-diethylaniline **10**, 4-ethylaniline **12**, and naphthyl derivative **14** appeared equipotent with **1**. Five- to ten-fold potency improvements were observed with the introduction of linear or branched aliphatic substituents to the ortho position of aniline **7** (e.g., **8**, **11**) or branched aliphatic groups at the aniline para position (**13**). Comparing the activity of **16** with 5-indolyl analog **15** and 1-methyl-6-indolyl derivative **18** suggested that the indole NH in **16** formed an important interaction, possibly a hydrogen bond, that improved the activity by 60-fold, and therefore, this

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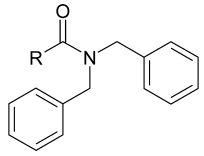
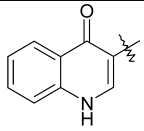
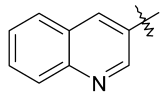
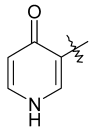
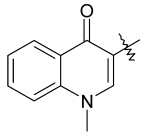
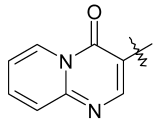
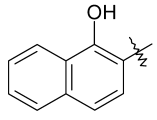
Table 1. Effect of Quinolinone Modifications on Potentiator Activity in NIH-3T3 Cells Expressing F508del-CFTR[16,a]



Compd	R	EC_{50} (μM)*
1		2.1 ± 1.4
2		16 ± 4
3		2% @ 30 μM#
4		37% @ 30 μM#
5		10% @ 30 μM#
6		3.5 ± 0.6

[a]Footnotes: *Mean and standard deviation EC_{50} values from at least three determinations are reported. #% activity of VRT-532 at the specified concentration.

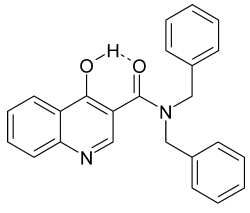
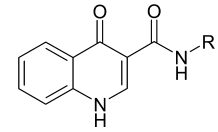


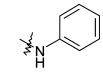
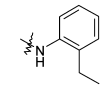
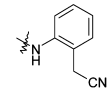
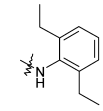
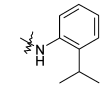
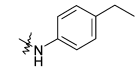
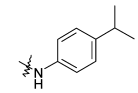
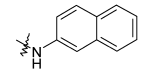
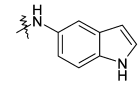
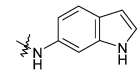
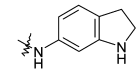
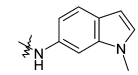
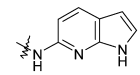
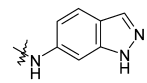
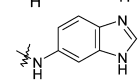
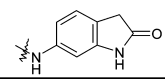
Figure 2. Proposed active tautomer of compound **1**.

position was left unsubstituted for the remainder of our studies. Reduction of indole **16** to indoline **17** retained activity. Further attempts to increase the polarity of the molecule by replacing the indole ring with other heterocycles while retaining the orientation of the NH (azaindole **19**, indazole **20**, benzimidazole **21**, and oxyindole **22**) or interchanging alkyl groups with polar moieties (**9**) resulted in reduction of potency.

After the identification of lead compound **16**, it was profiled more broadly. Compound **16** had good selectivity against a

Table 2. Effect of Amine Modifications on Potentiator Activity in NIH-3T3 Cells Expressing F508del-CFTR[a]



Compd	R	EC_{50} (μM)*
7		1.3 ± 0.3
8		0.2 ± 0.1
9		8.5 ± 1.1
10		1.5 ± 0.4
11		0.2 ± 0.05
12		0.8 ± 0.02
13		0.2 ± 0.05
14		0.8 ± 0.3
15		6.1 ± 0.9
16		0.1 ± 0.06
17		0.4 ± 0.08
18		6.4 ± 1.1
19		13% @ 30 μM#
20		5.6 ± 0.2
21		12% @ 30 μM#
22		21% @ 30 μM#

[a]Footnotes: *Mean and standard deviation EC_{50} values from at least three determinations are reported. #% activity of VRT-532 at the specified concentration.

panel of over 60 different targets, part of the Ricerca lead profiling screen, only hitting the $GABA_A$ benzodiazepine receptor, a ligand-gated chloride channel, with an IC_{50} of ~0.1 μM.[17] It was found that **16** had low solubility (aqueous and organic)[18] despite its low molecular weight of 303 and

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cLog P of 1.6, due to its planarity and hydrogen bonding that results in a tightly packed crystal lattice. In addition, indole **16** displayed low oral bioavailability in rats (11%) and a short iv half-life in dogs (0.9 h), mostly due to moderate to high clearance (Table 3).

Table 3. Pharmacokinetic Parameters of Indole 16[a]

compd	species	dose (mg/kg)	CL (mL min^{-1} kg^{-1})	$t_{1/2}$ (h)	V_{ss} (L/kg)	F (%)
16	rat	2.5	28	1.3	2.4	11
16	dog	1	17	0.9	1.6	ND

[a]ND = not determined.

It was proposed that the formation of an intramolecular hydrogen bond between the amide hydrogen and the lone pair of electrons on the carbonyl oxygen of the quinolinone imposed a planar conformation to the whole structure that exposed the indole NH to form a key hydrogen bond interaction. Molecular mechanics calculations revealed that the most favorable conformation was one with an intramolecular hydrogen bond and that a rotational barrier for the amidequinolinone dihedral exceeded 10 kcal/mol.[19] Single crystal X-ray crystallography of **16** (Figure 3) confirmed that, in

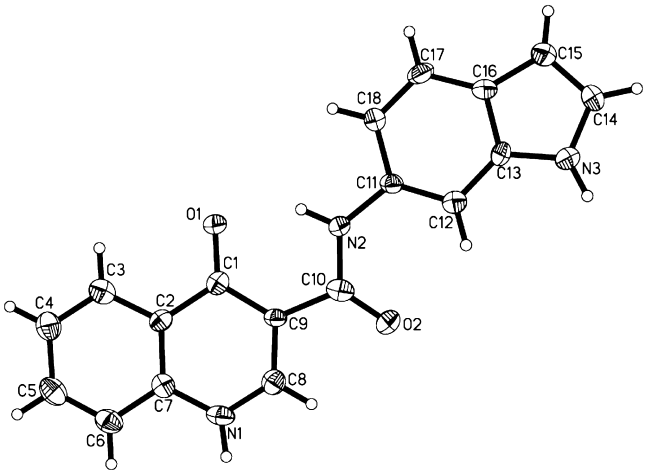


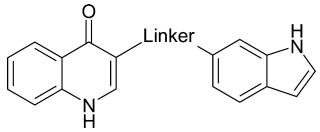
Figure 3. X-ray structure of compound **16**. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

the solid state, **16** adopted essentially a planar overall conformation, with the quinolinone moiety displaying the keto tautomeric form engaged in an intramolecular hydrogen bond with the amide hydrogen.

In order to disrupt the planarity of compound **16** and improve solubility, changes to the amide linker were investigated with the intent to modify the formation of the intramolecular hydrogen bond while recognizing that this may negatively affect activity. Representative examples of this approach are shown in Table 4.

Ester **23**, sulfonamide **24**,[20] reduced analog **25**, and reverse amide **26** displayed lower potencies. These results highlighted the importance of the planar conformation for potentiator activity, and therefore, the quinolinone amide portion of **16** was kept unchanged. Subsequent efforts targeted further modifications to the aniline portion with the purpose of increasing

Table 4. Effect of Linker Modification in the Activity of 16 in NIH-3T3 Cells Expressing F508del-CFTR

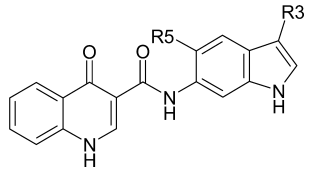


compd	linker	EC_{50} (μM)[a]
16	-CONH-	0.1 ± 0.06
23	-CO_2-	19% at 30 μM[b]
24	-SO_2NH-	1% at 30 μM[b]
25	-CH_2NH-	1% at 30 μM[b]
26	-NHCO-	8% at 30 μM[b]

[a]Mean and standard deviation EC_{50} values from at least three determinations are reported. [b]% activity of VRT-532 at the specified concentration.

potentiator potency by 10-fold, reducing $GABA_A$ activity, and adding three-dimensionality to the molecule to improve organic solubility. To that extent, a two-prong approach was undertaken. On one side, following up on our initial observations, substituents were introduced at positions 3 and 5 of indole derivative **16** (Table 5). On the other side encouraged by the

Table 5. In Vitro Potentiator Activity in NIH-3T3 Cells Expressing F508del-CFTR of Indole Derivatives



compd	R_3	R_5	EC_{50} (μM)[a]
27	Et	H	0.1 ± 0.02
28	^{t}Bu	H	0.009 ± 0.004
29	H	Et	0.020 ± 0.003
30	H	^{t}Bu	0.011 ± 0.002

[a]Mean and standard deviation EC_{50} values from at least three determinations are reported.

activity of indoline **17**, several ring opened analogs were prepared (Table 6). Consistent with our initial observations, potency enhancements over **16** were observed with 5-ethyl analog **29** (5-fold), 3-*tert*-butyl derivative **28**, and 5-*tert*-butylindole **30** (10-fold) whereas 3-ethyl analog **27** remained equipotent with **16**.

The second approach focused on the preparation of analogs of indoline **17**. Ring opened aniline **31** was prepared and showed 15-fold reduction in activity (Table 6). Introduction of alkyl groups of varying size and volume at the 4-position (compounds **32**–**36**) resulted in improved potency with increasing size (**32** vs **35**) and branching (**33** vs **36**) and culminated with compound **36**, which is equipotent with indole derivative **16**.

The next step in the evaluation targeted the replacement of the 1,3-dianiline motif of compound **36**. In an effort to understand the role of the aniline moiety at the 3 position, it was removed and replaced with hydrogen bond donating groups and aniline bioisosteres (Table 7).[21] Whereas the removal of the amine group (**43**) retained activity, both amine acylation (**37**) and carbamate derivatization (**38**) had a

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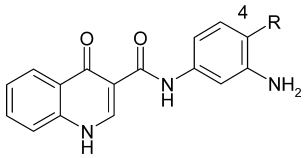
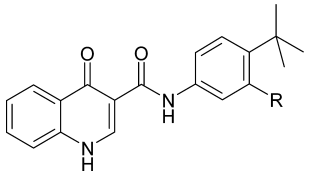
Table 6. In Vitro Potentiator Activity in NIH-3T3 Cells Expressing F508del-CFTR of 4-Substituted Aniline Derivatives



compd	R	cLogP	EC_{50} (μM)[a]
31	H	0.4	6.9 ± 1.2
32	Me	0.8	6.0 ± 1.5
33	Et	1.3	1.7 ± 0.3
34	^{i}Pr	1.5	0.3 ± 0.08
35	*n*-Pr	1.9	0.4 ± 0.05
36	^{t}Bu	2.1	0.1 ± 0.05

[a]Mean and standard deviation EC_{50} values from at least three determinations are reported.

Table 7. In Vitro Potentiator Activity in NIH-3T3 Cells Expressing F508del-CFTR of Aniline Replacements

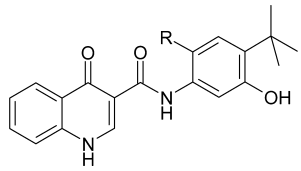


compd	R	EC_{50} (μM)[a]
37	$NHCOCH_3$	24% at 30 μM[b]
38	$NHCO_2CH_3$	3.5 ± 1.5
39	CH_2NH_2	20% at 30 μM[b]
40	CH_2OH	0.1 ± 0.02
41	CO_2H	13% at 30 μM[b]
42	SO_2NH_2	5.1 ± 1.8
43	H	0.1 ± 0.1
44	F	0.1 ± 0.09
45	OH	0.003 ± 0.002

[a]Mean and standard deviation EC_{50} values from at least three determinations are reported. [b]% activity of VRT-532 at the specified concentration.

detrimental effect on activity. Similarly, substitution of the aniline moiety with a benzylamine (**39**), a carboxylic acid (**41**), or a sulfonamide group (**42**) significantly reduced activities. Substitution of the aniline with a hydroxymethyl group (**40**) or a fluorine atom (**44**) was tolerated, while replacement with a phenolic group (**45**) increased the activity by 40-fold. Although the concept of indole−phenol bioisosterism was reported by Asselin et al. in the context of the dopamine receptor,[22] the improved activity of phenol **45** was unexpected. We speculated that it was due to the combination of the flat conformation induced by the intramolecular hydrogen bond, the hydrophobic interaction created by the 4-*tert*-butyl group, and the 3-phenol moiety acting as a hydrogen-bond donor. As phenol **45** potentiated CFTR in F508del-CFTR HBE with a potency of 5 nM, a 300-fold increase over the starting point **1** (Table 8), 2-fluoro (**46**), 2-trifluoromethyl (**47**), and 2-*tert*-butyl (**48**) derivatives were then selected for synthesis with the purpose of introducing a substituent of a varying molecular volume and lipophilic character at the para position of phenol **45** (Table 8). From an activity point of view, all three compounds appeared

Table 8. In Vitro Potency of Phenol Derivatives 45−48



compd	R	EC_{50} (nM)[a]
45	H	3 ± 2
46	F	2 ± 1
47	CF_3	3 ± 1
48	^{t}Bu	3 ± 1

[a]Mean and standard deviation EC_{50} values from at least three determinations are reported.

equipotent with **45** in the optical assay. Further profiling of compounds **28**, **30**, and **45**−**48** in F508del-HBEs and rat iv pharmacokinetic parameters is shown in Table 9.

Although compounds **28**, **30**, **45**−**48** retained excellent potencies in the F508del-CFTR HBE assay, phenol **48** displayed the best rat iv pharmacokinetic profile with low clearance and long half-life. Further profiling of **48** revealed that it had an improved organic solubility[23] and no major CYP_{450} activity (1A2, 2C9, 2C19, 2E1, 3A4, and 2D6 IC_{50} > 20 μM) and did not inhibit the cardiac potassium channel hERG (IC_{50} >10 μM). In the Ricerca spectrum screen, **48** showed no significant activity against 160 targets tested including the $GABA_A$ benzodiazepine receptor. The ability of compound **48** to increase the gating activity of G551D-CFTR was evaluated in HBE (F508del/G551D) epithelia using Ussing chambers recording techniques. Compound **48** increased the chloride secretion with an EC_{50} of 0.236 ± 0.200 μM, a 10-fold shift in potency compared to the F508del HBEs. Further, the maximum Cl^- secretion in F508del/G551D HBE reached levels close to 50% of the levels observed in HBE from individuals without CF, suggesting an excellent potential for clinical efficacy. These data also highlighted that the compound was able to potentiate two different CFTR mutations. More recently it was shown that **48** potentiates multiple mutant CFTR forms with defects in CFTR gating (including G551D), suggesting that **48** is not mutation-specific but may improve the underlying molecular cause of gating defects caused by several mutations.[24,25]

Further characterization in male mice, beagle dogs, and cynomolgus monkeys indicated that compound **48** had prolonged half-lives, moderate to low clearance values, and good oral bioavailability in rat and dog (Table 10).

In a rat dose proportionality study, the AUC and C_{max} were increased linearly after oral administration of **48** in a suspension vehicle at doses from 1 to 200 mg/kg (3, 10, 30, and 100 were the intermediate doses). A similar trend was observed in beagle dogs increasing the oral dose from 3 to 80 mg/kg (10, 30, and 60 were the intermediate doses), confirming high levels of oral absorption.

The predicted human hepatic clearance of **48** using allometric scaling from four species was 4.7 mL min^{-1} kg^{-1}, which is approximately 23% of hepatic blood flow. On the basis of its potency, selectivity, and favorable pharmacokinetic profile, compound **48** (VX-770, ivacaftor) was selected for further (pre)clinical evaluation and eventually was approved by the FDA for the treatment of CF patients 6 years and older carrying the G551D mutation.

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Table 9. In Vitro Potency and Rat iv Pharmacokinetic Parameters of Indole Analogs 28 and 30 and Phenol Derivatives 45−48

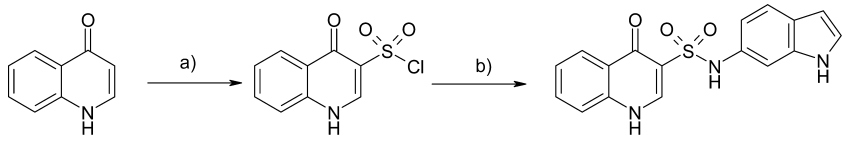
compd	EC_{50} (nM),[a] F508del-HBE	dose (mg/kg)	$AUC_{0-\infty}$ (μg·h/mL)	CL (mL min^{-1} kg^{-1})	$t_{1/2}$ (h)	V_{ss} (L/kg)
28	42	2.45	0.70	60.7	1.3	4.4
30	97 ± 63	2.49	2.64	15.8	2.9	3.3
45	5 ± 2	1.29	0.26	85.6	0.7	2.9
46	45 ± 61	1.57	0.52	62.9	1.1	2.9
47	22 ± 14	0.65	0.65	17.7	2.9	3.2
48	22 ± 10	1.3	3.04	5.5	9.5	3.6

[a]Mean and standard deviation EC_{50} values from at least three determinations are reported.

Table 10. Mouse, Rat, Dog, and Cynomolgus Monkey Pharmacokinetic Parameters of 48

	iv				po		
species	dose (mg/kg)	CL (mL min^{-1} kg^{-1})	$t_{1/2}$ (h)	V_{ss} (L/kg)	dose (mg/kg)	F (%)	$AUC_{0-\infty}$ (μg·h/mL)
mouse	3	20.0	1.3	2.8		not determined	
rat	2.5	5.5	9.5	3.6	3	55	4.8
dog	0.9	0.7	13	0.7	3.8	43	13.5
monkey	0.8	7.4	6.7	2.2		not determined	

Scheme 1. Synthesis of Quinolinone-4-oxo-3-sulfonamide 24[a]



[a]Reagents and conditions: (a) $ClSO_3H$, 125 °C, 100%; (b) 6-indolylamine, pyridine, DCE, ΔT, 3%.

CONCLUSION AND PERSPECTIVE

The quinolinone-3-carboxamides were discovered as a new class of CFTR potentiators with activity in NIH-3T3 cells expressing the F508del-CFTR mutation and in human bronchial epithelial cells isolated from CF subjects with F508del/F508del and G551D/F508del-CFTR. This article summarized the efforts toward the evaluation of screening hit dibenzylamide **1**. This work demonstrated that the quinolinone-3-carboxamide moiety played an important role satisfying the requirements of planar conformation for potentiator activity through intramolecular hydrogen-bonding. Variation of the amine moiety indicated that 6-indolinyl and 6-indolyl derivatives improved activity by 5- to 20-fold. Ring opening to 3-anilino-4-alkyl derivatives followed by replacement of the aniline with a phenol group resulted in an additional 40-fold improvement in potentiator activity. Introduction of an alkyl group at the 4-position to the aniline had a surprising and dramatic improvement in organic solubility (while retaining poor aqueous solubility). Incorporation of a substituent at the 2-position that varied molecular volume and lipophilicity culminated with the identification of *N*-(2,4-di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**48**). This compound potentiates more than one CFTR mutation in human bronchial epithelial cells with greater potency than genistein (70-fold in G551D/F508del, 200-fold in F508del/F508del), has favorable pharmacokinetic properties in rodents and non-rodents, and was selected for clinical evaluation for the treatment of cystic fibrosis.

The discovery of VX-770 (**48**) is an example of a personalized medicine drug discovery effort.[26] The excellent in vitro activity of **48** for the G551D mutation (see above) guided us to first study the compound in CF patients with this particular mutation. Thus, the efficacy and safety of **48** in CF patients carrying the G551D mutation in the *CFTR* gene was demonstrated in two randomized, double-blind, placebo-controlled trials.[12,13] Significant improvements in lung function as measured by FEV^1 were observed in the CF patients who received VX-770 (**48**), compared to the patients who received placebo. In addition the VX-770 (**48**)-treated patients generally showed improved body weight parameters and reduced frequency of pulmonary exacerbations. VX-770 (**48**) was generally well tolerated. VX-770 (**48**) was first approved for the treatment of CF patients aged 6 years and older who carry the G551D mutation in their *CFTR* gene. Subsequently, VX-770 (**48**) has been approved in the U.S. for the treatment of eight additional CFTR mutations. Additional clinical studies are underway or planned to assess the clinical benefit of VX-770 (**48**) in other groups of CF patients carrying different CFTR mutations. In retrospect, it took over 22 years from the discovery of the CFTR gene in 1989[27] until approval of the first drug that targets the defective CFTR mutant protein, which is the underlying cause of cystic fibrosis. Looking forward, it is likely that additional CFTR modulators will eventually be approved to treat other mutations, either alone or in combination with VX-770 (**48**).

CHEMISTRY

4-Oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) was synthesized from aniline and diethyl 2-(ethoxymethylene)malonate following a reported procedure.[28] Sulfonamide **24** was prepared in two synthetic steps from quinolin-4-one (**50**) following a procedure previously described by Davis et al.[29] (Scheme 1). Briefly, electrophilic addition of chlorosulfonic acid to **50** afforded 3-sulfonyl chloride intermediate **51** that was reacted under standard conditions to yield desired product **24**.

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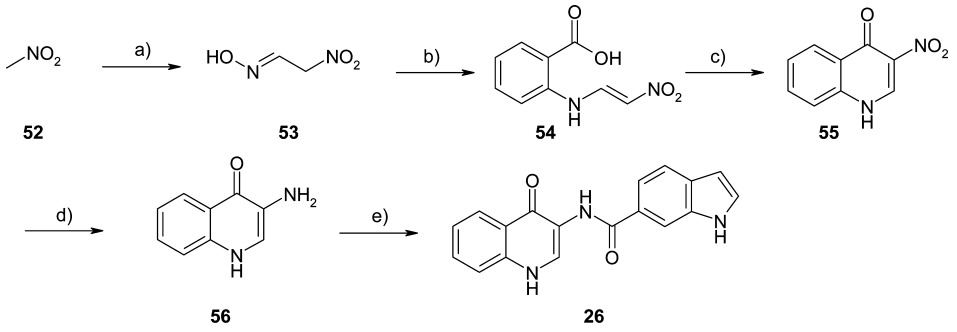
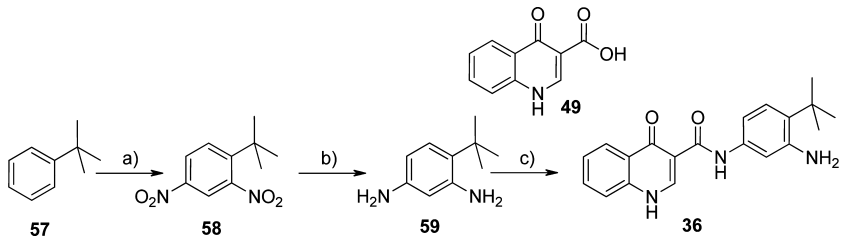
Scheme 2. Synthesis of Reverse Amide 26[a]



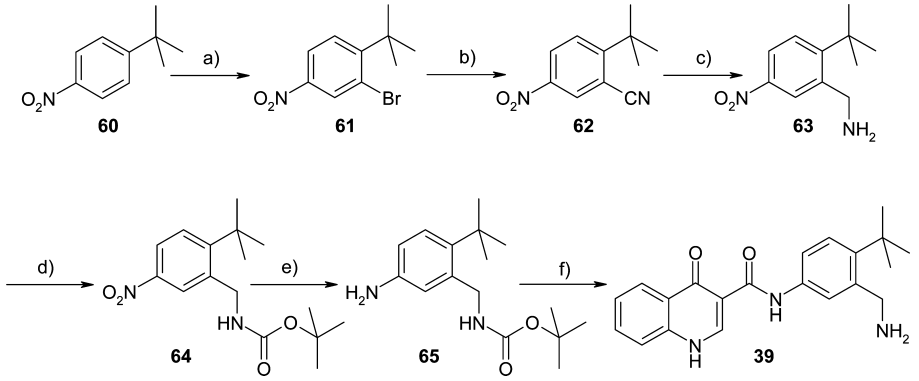
[a]Reagents and conditions: (a) NaOH, H_2O, rt to 45 °C; (b) anthranilic acid, HCl, H_2O, 96% over two steps; (c) AcONa anhydrous, Ac_2O, 120 °C, 41%; (d) ammonium formate, Pd/C, THF, 70 °C, 99%; (e) 6-indolylcarboxylic acid, HATU, DIEA, DMF, rt, 21%.

Scheme 3. Preparation of Compound 36[31,a]



[a]Reagents and conditions: (a) fuming HNO_3, H_2SO_4, 0 °C to rt, 95% ; (b) H_2, Pd/C, MeOH, 51%; (c) HATU, DIEA, DMF, 62%.

Scheme 4. Preparation of 49[a]



[a]Reagents and conditions: (a) Ag_2SO_4, Br_2, 90% H_2SO_4, 98%; (b) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 200 °C, 80%; (c) BH_3, THF, 70 °C, 43%; (d) Boc_2O, THF, 70 °C, 78%; (e) H_2, Pd/C, AcOH, MeOH, 92%; (f) (i) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), DMF, TEA, HBTU; (ii) CH_2Cl_2, TFA, 53% over two steps.

Compound **26** was prepared in modest yield by coupling of 3-aminoquinoline-4-one (**56**) and commercially available indole-6-carboxylic acid (Scheme 2). Amine **56** was synthesized in four steps and 39% overall yield following a modified route reported by Reich et al.[30] Condensation of nitromethane (**52**) yielded acetaldehyde oxime **53** that was trapped with antranilic acid to afford benzoic acid **54**. Cyclization promoted by acetic anhydride and sodium acetate followed by reduction yielded **56**.

4-*tert*-Butyl-3-amino derivative **36** was prepared following the three synthetic steps shown in Scheme 3. Starting with the corresponding *tert*-butylbenzene **57**, nitration with fuming HNO_3/H_2SO_4 yielded the 1,3-dinitrophenyl derivative **58** that was reduced to afford the dianiline intermediate **59**. Coupling with 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) yielded a mixture of regioisomers in moderate yields that were separated by chromatography. It was noted that as the size of the alkyl group at the 4 position increased, the coupling favored the desired and less sterically hindered amine isomer.

Compounds **39**–**41** were prepared from common intermediate **62** (Scheme 4) obtained from bromination[32] of commercially available **60** followed by palladium mediated cyanation.[33] Selective reduction of the cyano group with BH_3·THF yielded benzylamine **63** in moderate yield. Protection and reduction of the nitro group followed by amide coupling and deprotection under acidic conditions afforded compound **39**.

Acidic hydrolysis of cyano intermediate **62** proceeded smoothly under microwave irradiation to afford the corresponding carboxylic acid **66** (Scheme 5). Esterification of **66** by treatment with methyl iodide and K_2CO_3 followed by reduction of the nitro group afforded ester **67** that was reduced with LAH to yield benzyl alcohol **68**. Coupling with 4-oxo-1,4-

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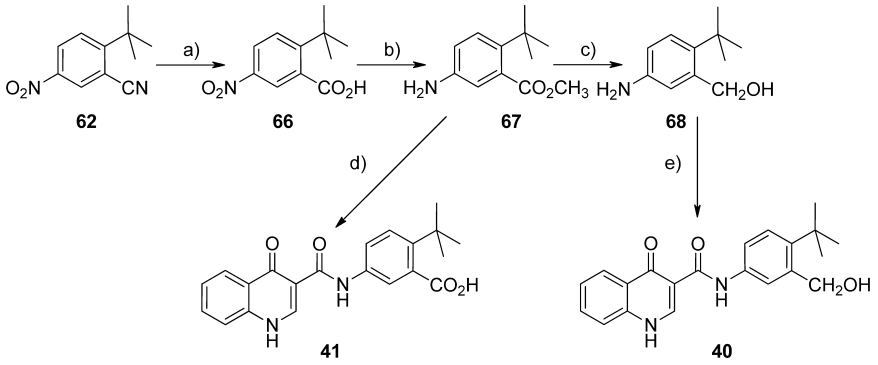
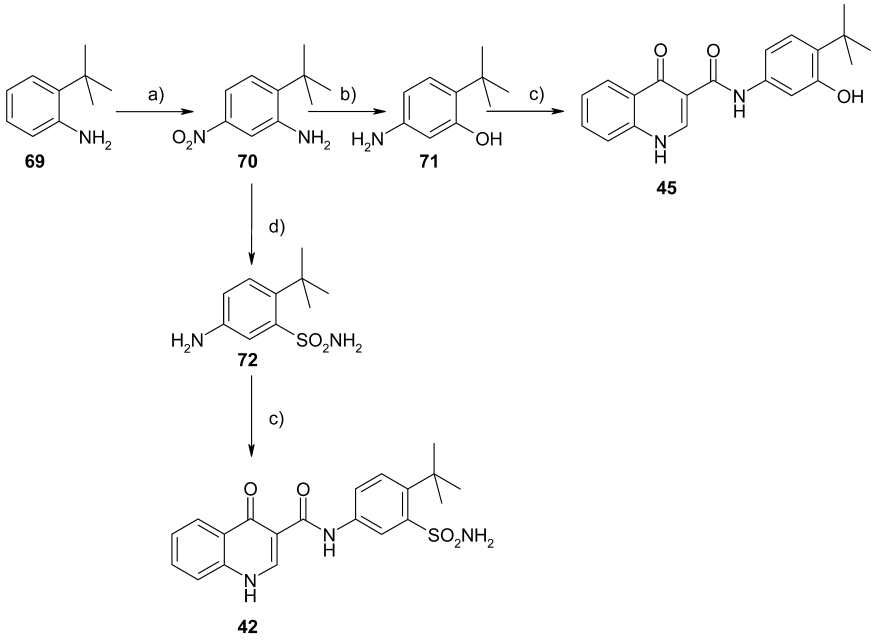
Scheme 5. Preparation of 40 and 41[a]



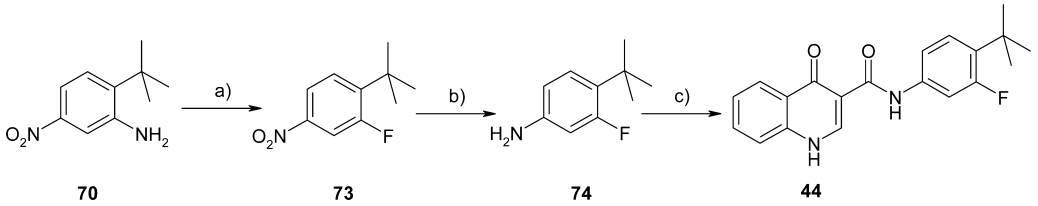
[a]Reagents and conditions: (a) 75% H_2SO_4, microwave, 200 °C, 90%; (b) (i) K_2CO_3, MeI, DMF, 71%; (ii) Pd/C, HCO_2H, EtOH, 95%; (c) LAH, THF, 70 °C, 20%; (d) (i) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HATU, Et_3N, DMF, 60 °C, 80%; (ii) LiOH, MeOH, THF, microwave 140 °C, 66%; (e) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HBTU, Et_3N, DMF, 5%.

Scheme 6. Preparation of 42 and 45[a]



[a]Reagents and conditions: (a) KNO_3, H_2SO_4, 64%; (b) (i) $NaNO_2$, H_2SO_4–H_2O, 62%; (ii) NH_4CO_2H, Pd–C, 87%; (c) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HBTU, Et_3N, DMF, 47% (**45**), 33% (**42**); (d) (i) $NaNO_2$, HCl, Na_2SO_3, $CuSO_4$, H_2O, 17%; (ii) NH_4OH, diethyl ether, 34% ; (iii) $SnCl_2$, EtOH, 100%.

Scheme 7. Preparation of 44[a]



[a]Reagents and conditions: (a) $NaNO_2$, HPF_6, 12%; (b) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH, 75%; (c) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HATU, Et_3N, THF, 21%.

dihydroquinoline-3-carboxylic acid (**49**) under standard conditions yielded **40**. Coupling ester **67** with 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) under standard conditions followed by ester hydrolysis afforded carboxylic acid **41** in moderate yield.

Scheme 6 outlines the preparation of sulfonamide **42** and phenol **45** from common intermediate **70** prepared by nitration of commercially available 2-*tert*-butylaniline **69**.[34] Diazotization followed by quenching with water[35] and nitro reduction yielded the anilinophenol intermediate **71** that was coupled with 4-oxo-

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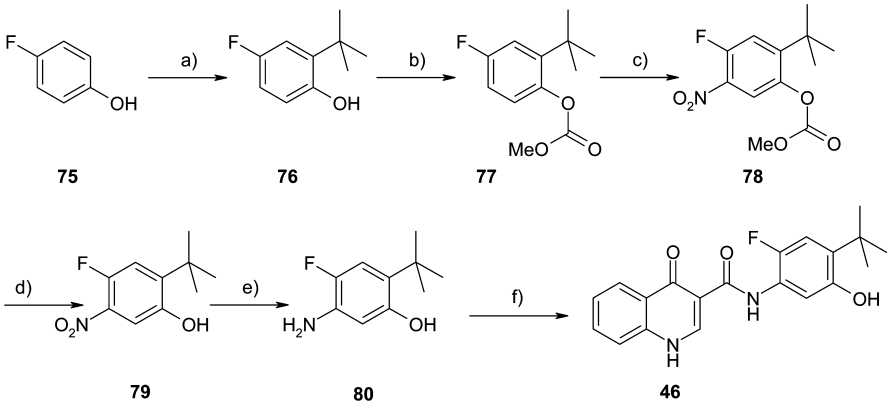
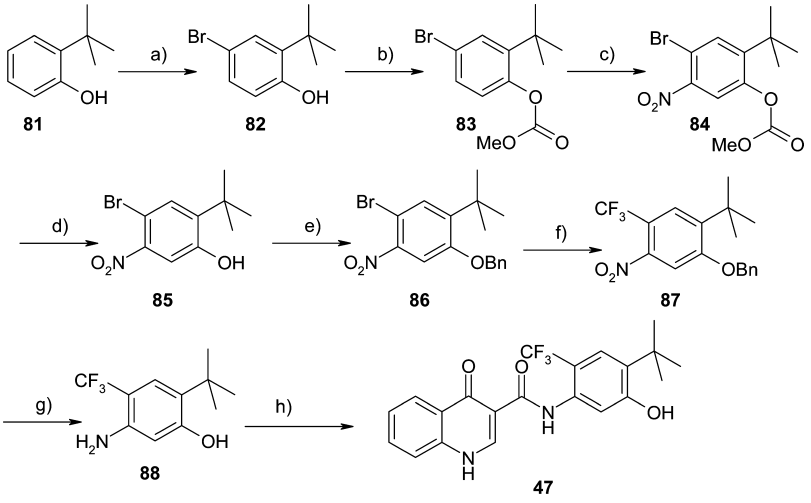
Scheme 8. Preparation of 46[a]



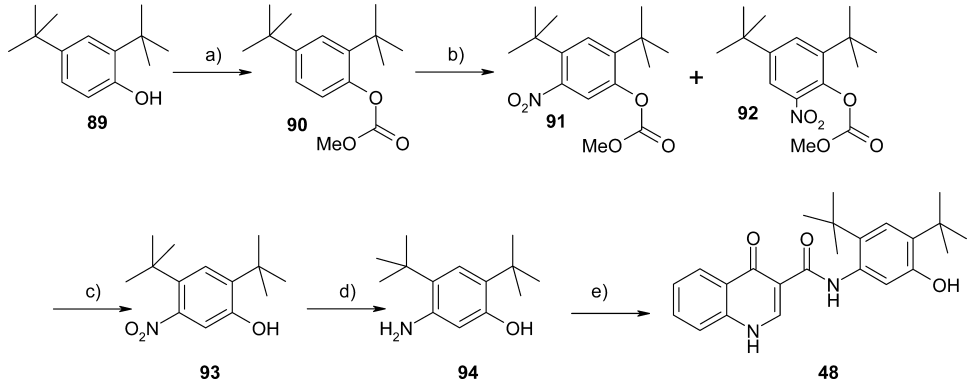
[a]Reagents and conditions: (a) ^{t}BuOH, H_2SO_4, $AlCl_3$, 42%; (b) CH_3CO_2Cl, Et_3N, CH_2Cl_2, 59%; (c) HNO_3, H_2SO_4, 55%; (d) piperidine, CH_2Cl_2, 62%; (e) ammonium formate, Pd/C, EtOH, 83%; (f) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HBTU, Et_3N, DMF, 23%.

Scheme 9. Preparation of 47[a]



[a]Reagents and conditions: (a) NBS, CH_3CN, 68%; (b) CH_3CO_2Cl, Et_3N, CH_2Cl_2, 72%; (c) HNO_3, H_2SO_4, 83%; (d) KOH, MeOH, 96%; (e) BnBr, Cs_2CO_3, DMF, 94%; (f) $ClCF_2CO_2Me$, KF, KBr, CuI, DMF, 67%; (g) ammonium formate, Pd/C, EtOH, 52%; (h) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HBTU, Et_3N, DMF, 8%.

Scheme 10. Preparation of 48[a]



[a]Reagents and conditions: (a) CH_3CO_2Cl, Et_3N, DMAP, CH_2Cl_2, quant; (b) HNO_3, H_2SO_4; (c) KOH, MeOH, 29% over two steps for the preparation of **91**; (d) ammonium formate, Pd/C, EtOH, quant; (e) 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**), HBTU, Et_3N, DMF, 71%.

1,4-dihydroquinoline-3-carboxylic acid (**49**) to afford **45** in moderate yields. Diazotization of **70** followed by sulfonylation[34] with Na_2SO_3 and $CuSO_4$, sulfonamide formation, and nitro reduction afforded aniline intermediate **72** that was coupled with quinoline-4-oxo-3-carboxylic acid (**49**) to afford desired sulfonamide **42**.

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Diazotization of **70** followed by fluorination[36] afforded nitro intermediate **73** that was reduced and coupled with 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) to afford **44** (Scheme 7).

Scheme 8 outlines the preparation of fluoro derivative **46** from 4-fluorophenol (**75**). Regioselective incorporation of the *tert*-butyl group was achieved by Friedel–Crafts alkylation in the presence of $AlCl_3$ as reported by Charpentier et al.[37] Phenol intermediate **76** was protected as a carbonate (**77**) and subjected to nitration conditions[38] to afford **78**. Deprotection proceeded smoothly at 25 °C using piperidine to yield phenol **79** that was reduced to aniline **80** and coupled with 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) to achieve desired compound **46** in moderate yields.

Trifluoromethyl derivative **47** was prepared in eight steps as shown in Scheme 9. Selective bromination of commercially available *tert*-butylphenol **81** with freshly crystallized NBS in acetonitrile afforded 4-bromophenol intermediate **82**. Protection of the phenol group as a carbamate, nitration, and deprotection afforded phenol intermediate **85** that was reprotected as benzyl derivative **86**. Trifluoromethylation to yield key intermediate **87** was achieved in moderate yield by treatment with $ClCF_2CO_2Me$, KF, KBr, CuI in DMF following the procedure reported by Su et al.[39] Finally, phenol deprotection and coupling with 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) afforded compound **47** in poor yield.

Compound **48** was prepared in five synthetic steps from commercially available 2,4-di-*tert*-butylphenol (**89**) as shown in Scheme 10. Phenol protection as a carbonate followed by nitration with nitric acid and sulfuric acid afforded an 8:1 mixture of nitro intermediates **91** and **92** that were separated by column chromatography. Deprotection under basic conditions afforded the desired nitrophenol **93** in 30% yield. Reduction followed by coupling with 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) afforded compound **48** in moderate yield.

EXPERIMENTAL SECTION

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid Dibenzylamide (1). To a solution of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**, 0.62 g, 3.0 mmol) and triethylamine (0.8 mL, 6.0 mmol) in dichloromethane (15 mL) was added thionyl chloride (0.5 mL, 6.0 mmol). The reaction mixture was heated at reflux for 1 h and concentrated to give the corresponding acid chloride.

A mixture of the acid chloride, triethylamine (0.8 mL, 6.0 mmol), and dibenzylamine (1.2 g, 6.0 mmol) was stirred at 25 °C in dichloromethane (15 mL) for 16 h. The crude reaction mixture was washed with 1 N HCl (10 mL), a saturated aqueous solution of $NaHCO_3$ (10 mL), and brine (10 mL). The organics were dried over $MgSO_4$ and evaporated under reduced pressure. The crude material was purified by silica gel chromatography (ethyl acetate–hexane, 50–100%) to give **1** (0.93 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 8.26 (s, 1H), 8.20 (dd, J = 8.1, 1.2 Hz, 1H), 7.70 (ddd, J = 8.4, 7.0, 1.5 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.51–7.43 (m, 2H), 7.43–7.33 (m, 3H), 7.33–7.21 (m, 4H), 7.21–7.13 (m, 2H), 4.59 (s, 2H), 4.42 (s, 2H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{24}H_{20}N_2O_2$, 369.1603; found, 369.1592.

Quinoline-3-carboxylic Acid Dibenzylamide (2). A mixture of quinoline-3-carboxylic acid (17 mg, 0.1 mmol), dibenzylamine (30 mg, 0.15 mmol), triethylamine (0.03 mL, 0.2 mmol), and HATU (38 mg, 0.1 mmol) in DMF (1 mL) was stirred for 3 h. The reaction mixture was filtered and purified by reverse-phase HPLC to give **2** (10 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (d, J = 2.1 Hz, 1H), 8.55 (d, J = 1.9 Hz, 1H), 8.03 (t, J = 8.1 Hz, 2H), 7.87–7.78 (m, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.49–7.23 (m, 8H), 7.23–7.07 (m, 2H), 4.69 (s, 2H), 4.53 (s, 2H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{24}H_{20}N_2O$, 353.1654; found, 3353.1652.

***N,N*-Dibenzyl-4-hydroxynicotinamide (3).** Compound **3** was prepared from 4-hydroxynicotinic acid (40 mg, 0.3 mmol) in a similar manner as described for compound **2**. Yield: 66 mg, 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.80 (d, J = 5.3 Hz, 1H), 7.43–7.21 (m, 9H), 7.17 (d, J = 6.7 Hz, 2H), 6.36 (d, J = 7.2 Hz, 1H), 4.54 (s, 2H), 4.34 (s, 2H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{18}N_2O_2$, 319.1446; found, 319.1431.

1-Methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Dibenzylamide (4). Compound **1** (74 mg, 0.2 mmol) and triethylamine (0.06 mL, 0.4 mmol) were dissolved in a mixture of toluene (0.5 mL) and methanol (0.5 mL). A 2 M solution of TMS-diazomethane (0.3 mL, 0.6 mmol) was added, and the reaction mixture was stirred at 25 °C for 16 h. The reaction mixture was evaporated to dryness, and the residue was purified by reverse-phase HPLC. Yield: 14 mg, 18%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.30 (d, J = 7.3 Hz, 1H), 7.80 (m, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.47 (m, 3H), 7.37 (t, J = 7.5 Hz, 2H), 7.28 (m, 4H), 7.17 (d, J = 7.1 Hz, 2H), 4.57 (s, 2H), 4.43 (s, 2H), 3.89 (s, 3H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{25}H_{22}N_2O_2$, 383.1759; found, 383.1758.

4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic Acid Dibenzylamide (5). Compound **5** was prepared from 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (38 mg, 0.2 mmol) in a similar manner as described for compound **2**. Yield: 58 mg, 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (d, J = 7.0 Hz, 1H), 8.63 (s, 1H), 7.89 (td, J = 7.8, 1.3 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.30 (m, 9H), 7.16 (d, J = 7.1 Hz, 2H), 4.76 (s, 2H), 4.48 (s, 2H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{23}H_{19}N_3O_2$, 370.1555; found, 370.1552.

1-Hydroxynaphthalene-2-carboxylic Acid Dibenzylamide (6). Compound **6** was prepared from 1-hydroxy-2-naphthoic acid (50 mg, 0.3 mmol) in a similar manner as described for compound **2**. Yield: 26 mg, 24%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.37–8.24 (m, 1H), 7.92–7.81 (m, 1H), 7.57–7.50 (m, 2H), 7.46 (d, J = 8.4 Hz, 1H), 7.41–7.18 (m, 11H), 4.52 (s, 4H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{25}H_{21}NO_2$, 368.1650; found, 368.1638.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid Phenylamide (7). Compound **7** was prepared from aniline (19 mg, 0.2 mmol) in a similar manner as described for compound **1**. Yield: 17 mg, 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 12.50 (s, 1H), 8.89 (s, 1H), 8.34 (dd, J = 8.1, 1.1 Hz, 1H), 7.83 (t, J = 8.3 Hz, 1H), 7.75 (m, 3H), 7.55 (t, J = 8.1 Hz, 1H), 7.37 (t, J = 7.9 Hz, 2H), 7.10 (t, J = 6.8 Hz, 1H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{16}H_{12}N_2O_2$, 265.0977; found, 265.0976.

***N*-(2-Ethylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8).** Compound **8** was prepared from 2-ethylaniline (60.6 mg, 0.5 mmol) in a similar manner as described for compound **1**. Yield: 16 mg, 11%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.93 (s, 1H), 12.37 (s, 1H), 8.90 (s, 1H), 8.36 (dd, J = 8.1, 1.4 Hz, 1H), 8.32 (dd, J = 8.1, 1.3 Hz, 1H), 7.86–7.79 (m, 1H), 7.76 (dd, J = 8.4, 1.1 Hz, 1H), 7.65–7.43 (m, 1H), 7.27 (dd, J = 7.6, 1.6 Hz, 1H), 7.25–7.18 (m, 1H), 7.15–6.98 (m, 1H), 2.80 (q, J = 7.5 Hz, 2H), 1.27 (t, J = 7.5 Hz, 3H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{16}N_2O_2$, 293.1285; found, 293.1296.

***N*-(2-(Cyanomethyl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (9).** Compound **9** was prepared from 2-ethylaniline (152.7 mg, 0.5 mmol) in a similar manner as described for compound **1**. Yield: 9.8 mg, 6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (s, 1H), 12.44 (s, 1H), 8.91 (s, 1H), 8.37 (dd, J = 8.2, 1.4 Hz, 1H), 8.16 (dd, J = 8.2, 1.3 Hz, 1H), 7.87–7.81 (m, 1H), 7.78 (dd, J = 8.3, 1.2 Hz, 1H), 7.60–7.52 (m, 1H), 7.49 (dd, J = 7.7, 1.5 Hz, 1H), 7.45–7.34 (m, 1H), 7.25–7.18 (m, 1H), 4.11 (s, 2H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{13}N_3O_2$, 304.1081; found, 304.1087.

***N*-(2,6-Diethylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (10).** Compound **10** was prepared from 2,6-diethylaniline (74.6 mg, 0.5 mmol) in a similar manner as described for compound **1**. Yield: 14.4 mg, 9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 11.64 (s, 1H), 8.86 (s, 1H), 8.34 (dd, J = 8.1, 1.4 Hz, 1H), 7.87–7.79 (m, 1H), 7.76 (dd, J = 8.4, 1.2 Hz, 1H), 7.64–7.46 (m, 1H), 7.25–7.19 (m, 1H), 7.19–7.11 (m, 2H), 2.57 (q, J = 7.6 Hz, 4H), 1.12 (t, J = 7.5 Hz, 6H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{20}N_2O_2$, 321.1598; found, 321.1609.

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***N*-(2-Isopropylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (11).** Compound **11** was prepared from 2-isopropylaniline (67.6 mg, 0.5 mmol) in a similar manner as described for compound **1**. Yield: 16.6 mg, 11%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 12.35 (s, 1H), 8.90 (s, 1H), 8.36 (dd, J = 8.1, 1.4 Hz, 1H), 8.20 (dd, J = 8.1, 1.4 Hz, 1H), 7.85−7.79 (m, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.61−7.46 (m, 1H), 7.35 (dd, J = 7.7, 1.6 Hz, 1H), 7.28−7.18 (m, 1H), 7.18−7.06 (m, 1H), 3.40−3.33 (m, 1H), 1.29 (d, J = 6.7 Hz, 6H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{19}H_{18}N_2O_2$, 307.1441; found, 307.1454.

***N*-(4-Ethylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (12).** Compound **12** was prepared from 4-ethylaniline (60.6 mg, 0.5 mmol) in a similar manner as described for compound **1**. Yield: 5.6 mg, 4%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.83 (s, 1H), 8.29 (d, J = 7.8 Hz, 1H), 7.78−7.70 (m, 2H), 7.61 (d, J = 7.8 Hz, 2H), 7.51 (t, 1H), 7.17 (d, J = 8.1 Hz, 2H), 2.57 (q, J = 7.5 Hz, 2H), 1.17 (t, J = 7.5 Hz,1H), 0.92 (t, J = 7.8 Hz, 3H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{18}H_{16}N_2O_2$, 293.1285; found, 293.1292.

***N*-(4-Isopropylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (13).** Compound **13** was prepared from 4-isopropylaniline (67.6 mg, 0.5 mmol) in a similar manner as described for compound **1**. Yield: 5.5 mg, 3%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (s, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.78−7.70 (m, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.50 (t, J = 7.8 Hz, 1H), 7.20 (d, J = 8.7 Hz, 2H), 2.85 (h, J = 6.9 Hz, 1H), 1.19 (d, J = 6.9 Hz, 6H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{19}H_{18}N_2O_2$, 307.1441; found, 307.1439.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid Naphthalen-2-ylamide (14). Compound **14** was prepared from 2-naphthylamine (28 mg, 0.2 mmol) in a similar manner as described for compound **1**. Yield: 20 mg, 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 8.92 (s, 1H), 8.45 (d, J = 1.7 Hz, 1H), 8.37 (dd, J = 8.1, 1.0 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.90−7.80 (m, 3H), 7.80−7.72 (m, 2H), 7.60−7.53 (m, 1H), 7.53−7.46 (m, 1H), 7.46−7.39 (m, 1H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{20}H_{14}N_2O_2$, 315.1133; found, 315.1141.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid (1*H*-Indol-5-yl)amide (15). Compound **15** was prepared from 5-aminoindole (26 mg, 0.2 mmol) in a similar manner as described for compound **1**. Yield: 31 mg, 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (s, 1H), 12.34 (s, 1H), 11.05 (s, 1H), 8.88 (s, 1H), 8.35 (d, J = 7.1 Hz, 1H), 8.08 (s, 1H), 7.81 (dd, J = 11.7, 4.8 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.37 (d, J = 8.6 Hz, 1H), 7.34 (t, J = 2.7 Hz, 1H), 7.29 (dd, J = 8.6, 1.9 Hz, 1H), 6.41 (s, 1H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{18}H_{13}N_3O_2$, 304.1086; found, 304.1086.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid (1*H*-Indol-6-yl)amide (16). Compound **16** was prepared from 6-aminoindole (45 mg, 0.34 mmol) in a similar manner as described for compound **1**. Yield: 54 mg, 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 12.47 (s, 1H), 11.08 (s, 1H), 8.90 (s, 1H), 8.35 (dd, J = 8.1, 1.1 Hz, 1H), 8.20 (t, J = 0.8 Hz, 1H), 7.83 (t, J = 8.3 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.55 (t, J = 8.1 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.30 (t, J = 2.7 Hz, 1H), 7.06 (dd, J = 8.4, 1.8 Hz, 1H), 6.39 (m, 1H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{18}H_{13}N_3O_2$, 304.1086; found, 304.1078.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid (2,3-Dihydro-1*H*-indol-6-yl)amide (17). *Step 1.* 4-Oxo-1,4-dihydroquinoline-3-carboxylic acid (1-acetyl-2,3-dihydro-1*H*-indol-6-yl)amide was prepared from 1-(6-amino-2,3-dihydroindol-1-yl)ethanone (70 mg, 0.4 mmol) in a similar manner as described for compound **1**. Yield: 43 mg, 30%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (d, J = 13.6 Hz, 1H), 8.87 (s, 1H), 8.32−8.28 (m, 2H), 7.76−7.70 (m, 2H), 7.60 (d, J = 7.8 Hz, 1H), 7.49−7.45 (m, 1H), 7.18 (d, J = 8.4 Hz, 1H), 4.11 (t, J = 8.3 Hz, 2H), 3.10 (t, J = 7.7 Hz, 2H), 2.18 (s, 3H).

Step 2. 4-Oxo-1,4-dihydroquinoline-3-carboxylic acid (1-acetyl-2,3-dihydro-1*H*-indol-6-yl)amide (43 mg, 0.12 mmol) was heated at reflux in a mixture of ethanol (0.5 mL) and 1 N NaOH (0.5 mL) for 48 h. The reaction mixture was evaporated under reduced pressure, and the residue was purified by reverse-phase HPLC. Yield: 10 mg, 27%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27 (d, J = 6.6 Hz, 1H), 12.73 (s, 1H), 11.36 (s, 1H), 8.87 (d, J = 6.7 Hz, 1H), 8.33 (d, J = 8.1 Hz, 1H), 8.15 (s, 1H), 7.83 (q, J = 8.1 Hz, 2H), 7.65−7.29 (m, 3H), 3.75 (t, J = 7.7 Hz, 2H), 3.18 (t, J = 7.7 Hz, 2H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{18}H_{15}N_3O_2$, 306.1242; found, 306.1237.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid (1-Methyl-1*H*-indol-6-yl)amide (18). Compound **18** was prepared from 1-methyl-1*H*-indol-6-ylamine (55 mg, 0.3 mmol) in a similar manner as described for compound **1**. Yield: 30 mg, 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (d, J = 5.3 Hz, 1H), 12.51 (s, 1H), 8.89 (d, J = 6.3 Hz, 1H), 8.36 (dd, J = 8.1, 1.1 Hz, 1H), 8.06 (t, J = 0.7 Hz, 1H), 7.85−7.75 (m, 2H), 7.57−7.51 (m, 2H), 7.28 (d, J = 3.1 Hz, 1H), 7.24 (dd, J = 8.4, 1.8 Hz, 1H), 6.39 (dd, J = 3.1, 0.8 Hz, 1H), 3.78 (s, 3H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{19}H_{15}N_3O_2$, 318.1242; found, 318.1231.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid (1*H*-Pyrrolo[2,3-*b*]pyridin-6-yl)amide (19). Compound **19** was prepared from 1*H*-pyrrolo[2,3-*b*]pyridin-6-ylamine (53 mg, 0.4 mmol) in a similar manner as described for compound **1**. Yield: 18 mg, 15%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 12.02 (s, 1H), 10.46 (s, 1H), 9.00 (s, 1H), 8.48 (d, J = 8.4 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 8.7 Hz, 1H), 7.82 (m, 2H), 1H), 7.37 (s, 1H), 6.46 (s, 1H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{17}H_{12}N_4O_2$, 305.1038; found, 305.1039.

***N*-(1*H*-Indazol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (20).** Compound **20** was prepared from 1*H*-indazol-6-amine (26 mg, 0.2 mmol) in a similar manner as described for compound **1**. Yield: 27 mg, 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (s, 1H), 12.78 (s, 1H), 8.91 (s, 1H), 8.40−8.31 (m, 2H), 7.99 (s, 1H), 7.84−7.69 (m, 3H), 7.53 (t, J = 7.4 Hz, 1H), 7.09 (dd, J = 8.6, 1.6 Hz, 1H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{17}H_{12}N_4O_2$, 305.1038; found, 305.1031.

***N*-(1*H*-Benzo[*d*]imidazol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (21).** Compound **21** was prepared from 1*H*-benzo[*d*]imidazol-6-amine (27 mg, 0.2 mmol) in a similar manner as described for compound **1**. Yield: 28 mg, 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (d, J = 6.4 Hz, 1H), 12.77 (s, 1H), 9.09 (s, 1H), 8.92 (d, J = 6.7 Hz, 1H), 8.52 (d, J = 1.6 Hz, 1H), 8.35 (d, J = 7.4 Hz, 1H), 7.87−7.81 (m, 1H), 7.77 (dd, J = 8.3, 6.1 Hz, 2H), 7.60−7.49 (m, 2H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{17}H_{12}N_4O_2$, 305.1038; found, 305.1043.

4-Oxo-*N*-(2-oxoindolin-6-yl)-1,4-dihydroquinoline-3-carboxamide (22). Compound **22** was prepared from 6-aminoindolin-2-one (60 mg, 0.4 mmol) in a similar manner as described for compound **1**. Yield: 4 mg, 3%. ^{1}H NMR (400 MHz, DMSO) δ 12.95 (s, 1H), 12.51 (s, 1H), 10.43 (s, 1H), 8.86 (s, 1H), 8.33 (d, J = 8.1 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.58−7.50 (m, 2H), 7.17 (d, J = 8.0 Hz, 1H), 7.06 (dd, J = 8.0, 1.2 Hz, 1H), 3.44 (s, 2H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{18}H_{13}N_3O_3$, 320.1035; found, 320.1041.

1*H*-Indol-6-yl 4-oxo-1,4-dihydroquinoline 3-Carboxylate (23). In a round-bottom flask 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**, 57 mg, 0.3 mmol), benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate (133 mg, 0.3 mmol), 1*H*-indol-6-ol (40 mg, 0.3 mmol), DMF (1.0 mL), and DIEA (116.3 mg, 0.16 mL, 0.9 mmol) were added and the mixture was stirred at 25 °C for 18 h. The solvent was evaporated under reduced pressure. The crude material was dissolved in DMSO, filtered, and purified by reverse-phase HPLC to provide compound **23**. Yield: 27 mg, 30%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.58 (s, 1H), 11.17 (s, 1H), 8.83 (d, J = 3.5 Hz, 1H), 8.21 (dd, J = 8.1, 1.0 Hz, 1H), 7.79−7.72 (m, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.49−7.43 (m, 1H), 7.39−7.34 (m, 1H), 7.22 (d, J = 1.7 Hz, 1H), 6.83 (dd, J = 8.5, 2.0 Hz, 1H), 6.49−6.42 (m, 1H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{18}H_{12}N_2O_3$, 305.0926; found, 305.0921.

4-Oxo-1,4-dihydroquinoline-3-sulfonyl Chloride (51). In a round-bottom flask, 4-hydroxyquinoline (**50**, 1.13 g, 7.78 mmol) was treated with chlorosulfonic acid (3.1 mL, 47 mmol), and the mixture was heated at 125 °C for 3 h. The brownish clear solution was then poured into ice−water, and a yellow precipitate was formed. The solid was collected by filtration and washed with cold water (5 × 50 mL). The solid was lyophilized to provide compound **51** (1.9 g, 100%) as a beige solid. ^{1}H NMR (300 MH, DMSO-d_6,) δ 9.20 (s, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.12−8.04 (m, 2H), 7.81 (t, J = 6.9 Hz, 1H).

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***N*-(1*H*-Indol-6-yl)-4-oxo-1,4-dihydroquinoline-3-sulfonamide (24).** To a solution of **51** (85 mg, 0.34 mmol) in pyridine (0.06 mL, 0.69 mmol) was added 1*H*-indol-6-amine (59 mg, 0.45 mmol) and the solution was heated at reflux for 3 h. The solvent was evaporated under reduced pressure and the crude residue was purified by reverse-phase HPLC to provide **24** (3.5 mg, 3% yield). ^{1}H NMR (400 MHz, DMSO) δ 12.43 (d, J = 6.6 Hz, 1H), 10.92 (s, 1H), 9.42 (s, 1H), 8.36 (d, J = 6.6 Hz, 1H), 8.19 (d, J = 8.1 Hz, 1H), 7.72 (t, J = 7.7 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 8.5 Hz, 1H), 7.24–7.10 (m, 2H), 6.81 (dd, J = 8.5, 1.6 Hz, 1H), 6.25 (s, 1H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{17}H_{13}N_3O_3S$, 340.0756; found, 340.0742.

3-((1*H*-Indol-6-ylamino)methyl)quinolin-4(1*H*)-one (25). A mixture of 4-hydroxyquinoline-3-carbaldehyde (10 mg, 0.06 mmol), 6-aminoindole (8 mg, 0.06 mmol), and sodium borohydride (4 mg, 0.07 mmol) was heated in toluene for 16 h at 100 °C. The reaction mixture was evaporated to dryness, and the residue was purified by reverse-phase HPLC. Yield: 7 mg, 40%. ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 10.46 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.80 (s, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 6.98–6.90 (m, 1H), 6.49 (d, J = 8.5 Hz, 1H), 6.44 (s, 1H), 6.17 (s, 1H), 5.66 (t, J = 5.1 Hz, 1H), 4.15 (d, J = 5.0 Hz, 2H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{15}N_3O$, 290.1293; found, 290.1288.

(*E*)-2-(2-Nitrovinylamino)benzoic Acid (54). In a round-bottom flask, a solution of NaOH (6.9 g in 15 mL of water) was cooled and stirred while nitromethane (6 mL, 112 mmol) was added dropwise maintaining the internal temperature at 30 °C. The mixture was allowed to warm to 40 °C and then cooled again while nitromethane (6 mL) was added slowly. The mixture was warmed to 45 °C until the solid dissolved and then to 50–55 °C for 5 min. The mixture was cooled to 30 °C, poured over ice (30 g), and acidified with concentrated HCl (30 mL). The resulting solution of (*E*)-2-nitroacetaldehyde oxime (**53**) was immediately added to a filtered solution of anthranilic acid (7 g, 51 mmol) and 4.6 mL of concentrated HCl in 102 mL of water. After a few minutes a yellow precipitate formed and the mixture was allowed to stand at 25 °C for 12 h. The solid was collected by filtration and washed with cold water (6 × 60 mL). The residue was lyophilized to give compound **54** as a yellow solid (10.2 g, 96% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.06 (d, J = 6.6 Hz, 1H), 8.02 (m, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.65 (td, J = 8.2 Hz, 1H), 7.21 (td, J = 8.1 Hz, 1H), 6.74 (d, J = 6.0 Hz, 1H).

3-Nitroquinolin-4-ol (55). To compound **54** (1 g, 4.8 mmol) acetic anhydride (5 mL, 4.8 mmol) was added, and the mixture was heated for 1 h at 100 °C. The heating was then withdrawn, and sodium acetate (0.4 g, 4.95 mmol), which was dried in an oven at 120 °C for 14 h, was added. The mixture was heated at reflux for 15 min, and a second equivalent of sodium acetate (0.4 g, 4.95 mmol) was added. The mixture was heated for an additional 1 h. The mixture was cooled to 25 °C and filtered. The beige precipitate was collected, washed with acetic acid (60 mL), water (2 × 20 mL), and dried under vacuum. Compound **55** was obtained as a beige solid (0.37 g, 41% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.17 (s, 1H), 8.23 (m, 1H), 7.82–7.69 (m, 2H), 7.50 (td, J = 7.2 Hz, 1H).

3-Aminoquinolin-4-ol (56). To a solution of compound **55** (0.74 g, 3.9 mmol) in THF (10 mL) was added ammonium formate (0.49 g, 7.8 mmol) followed by 10% Pd/C (cat.). After heating at reflux for 4 h, an additional equivalent of ammonium formate (0.49 g, 7.8 mmol) and Pd/C were added. After heating for an additional 2.5 h, the mixture was filtered through a bed of Celite, which was washed with THF (10 mL) and MeOH (10 mL). The combined filtrate was concentrated under reduced pressure, and the residue was dried under vacuum to provide **56** (0.62 g, 99% yield) as a yellow foam that was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (d, J = 8.7 Hz, 1H), 7.51 (s, 1H), 7.45–7.43 (m, 2H), 7.15 (m, 1H), 4.36 (br s, 2H).

***N*-(4-Hydroxyquinolin-3-yl)-1*H*-indole-6-carboxamide (26).** A mixture of compound **56** (65 mg, 0.40 mmol), 1*H*-indole-6-carboxylic acid (68 mg, 0.4 mmol), HATU (0.23 g, 0.61 mmol), and DIEA (0.14 mL, 0.8 mmol) in DMF (0.8 mL) was stirred for 6 h at 25 °C. At this point, PS-trisamine (0.1 g, 4.1 mmol/g, Argonaut Technologies) was added and the mixture was heated at 70 °C for 2 h. The resin was filtered and the filtrate concentrated under reduced pressure. The residue was purified by reverse-phase HPLC to provide compound **26** (25.5 mg, 21% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (d, J = 6.1, 1H), 11.48 (s, 1H), 9.33 (s, 1H), 9.10 (d, J = 6.2, 1H), 8.23 (d, J = 7.6, 1H), 8.07 (s, 1H), 7.72–7.55 (m, 5H), 7.37 (t, J = 7.4, 1H), 6.62–6.46 (m, 1H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{13}N_3O_2$, 304.1086; found, 304.1071.

***N*-(3-(Ethyl)-1*H*-indol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27).** Compound **27** was prepared from 3-ethyl-1*H*-indol-6-ylamine (112.2 mg, 0.7 mmol) in a similar manner as described for compound **28** (vide infra). Yield: 5 mg, 2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 12.46 (s, 1H), 10.73 (s, 1H), 8.89 (d, J = 6.7 Hz, 1H), 8.35 (d, J = 6.9 Hz, 1H), 7.83 (t, J = 8.3 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.55 (t, J = 8.1 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.05 (m, 2H), 2.70 (q, J = 7.6 Hz, 2H), 1.27 (t, J = 7.6 Hz, 3H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{17}N_3O_2$, 332.1394; found, 332.1385.

***N*-(3-(*tert*-Butyl)-1*H*-indol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (28).** *Step 1: 3-tert-Butyl-6-nitro-1H-indole.* To a mixture of 6-nitroindole (1.0 g, 6.2 mmol), zinc triflate (2.1 g, 5.7 mmol), and tetrabutylamonium iodide (1.7 g, 5.16 mmol) in anhydrous toluene (11.0 mL) was added diisopropylethylamine (1.47 g, 11.4 mmol) at room temperature under nitrogen. The reaction mixture was stirred for 10 min at 120 °C, followed by addition of *tert*-butyl bromide (0.71 g, 5.16 mmol). The resulting mixture was stirred for 45 min at 120 °C. The solid was filtered off and the filtrate was concentrated to dryness and purified by column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (20:1) to give 3-*tert*-butyl-6-nitro-1*H*-indole as a yellow solid (0.25 g, 19%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.1 Hz, 1H), 8.00 (dd, J = 2.1, 14.4 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.25 (s, 1H), 1.46 (s, 9H).

Step 2: 3-tert-Butyl-1H-indol-6-ylamine. A suspension of 3-*tert*-butyl-6-nitro-1*H*-indole (3.0 g, 13.7 mmol) and Raney Ni (0.5 g) in ethanol was stirred at room temperature under H_2 (1 atm) for 3 h. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (4:1) to give 3-*tert*-butyl-1*H*-indol-6-ylamine (2.0 g, 77%) as a gray solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (m, 2H), 6.73 (d, J = 1.2 Hz, 1H), 6.66 (s, 1H), 6.57(dd, J = 8.6, 0.8 Hz, 1H), 3.60 (br s, 2H), 1.42 (s, 9H).

*Step 3: N-(3-(tert-Butyl)-1H-indol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**28**).* To 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (135.0 mg, 0.7 mmol) and HBTU (270 mg, 0.7 mmol) in DMF (1.2 mL) was added triethylamine (298 μL, 2.1 mmol), and the mixture was stirred at room temperature for 5 min. A solution of 3-*tert*-butyl-1*H*-indol-6-ylamine (131.7 mg, 0.7 mmol) in DMF (0.7 mL) was added to the mixture, and the mixture was heated in the microwave instrument at 150 °C for 20 min. The mixture was cooled to room temperature, filtered, and purified by reverse phase HPLC to yield **28** (42.0 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (br s, 1H), 12.45 (s, 1H), 10.73 (d, J = 1.8 Hz, 1H), 8.89 (s, 1H), 8.35 (dd, J = 8.2, 1.0 Hz, 1H), 8.13 (d, J = 1.7 Hz, 1H), 7.82 (t, J = 8.3 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.54 (t, J = 8.1 Hz, 1H), 7.04 (dd, J = 8.6, 1.9 Hz, 1H), 6.98 (d, J = 2.4 Hz, 1H), 1.40 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{21}N_3O_2$, 360.1707; found, 360.1700.

***N*-(5-Ethyl-1*H*-indol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (29).** To a mixture of DMAP (1.5 g, 12.3 mmol), benzenesulfonyl chloride (24.0 g, 136 mmol), and 2,3-dihydro-1*H*-indole (14.7 g, 124 mmol) in CH_2Cl_2 (200.0 mL) in ice–water bath was added dropwise Et_3N (19 g, 186 mmol). After addition, the mixture was stirred at room temperature overnight. The organic layer was washed with water twice, then dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to obtain 1-benzenesulfonyl-2, 3-dihydro-1*H*-indole (30.9 g, 96%) that was used in the next step without further purification.

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To a magnetically stirred suspension of $AlCl_3$ (144.0 g, 1.08 mol) in CH_2Cl_2 (1070 mL) was added acetic anhydride (54 mL, 0.57 mol), and the mixture was stirred for 15 min. A solution of 1-benzenesulfonyl-2, 3-dihydro-1*H*-indole (46.9 g, 0.18 mol) in CH_2Cl_2 (1070 mL) was then added dropwise. The mixture was stirred for 5 h and quenched by slow addition of crushed ice to the reaction. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 two additional times. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under vacuum to obtain 1-(1-benzenesulfonyl-2, 3-dihydro-1*H*-indol-5-yl)ethanone (42.6 g) that was taken into the next step without further purification.

To magnetically stirred TFA (1600 mL) at 0 °C was added sodium borohydride (64 g, 1.69 mol) over 1 h. To this mixture was added dropwise a solution of 1-(1-benzenesulfonyl-2,3-dihydro-1*H*-indol-5-yl)ethanone (40.0 g, 0.13 mol) in TFA (700 mL) over a period of 1 h. The mixture was then stirred overnight at 25 °C. After dilution with H_2O (1600 mL), the mixture was made basic by the addition of sodium hydroxide pellets at 0 °C. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica column using a gradient of ethyl acetate in hexanes (1−99%) to give 1-benzenesulfonyl-5-ethyl-2,3-dihydro-1*H*-indole (16.2 g, 47% over two steps).

A mixture of 1-benzenesulfonyl-5-ethyl-2,3-dihydro-1*H*-indole (15 g, 0.05 mol) in HBr (48%, 162 mL) was heated at reflux for 6 h. The mixture was basified to pH ≈ 9 with saturated NaOH, and then it was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a residue that was purified by silica column using a gradient of ethyl acetate in hexanes (1−99%) to give 5-ethyl-2,3-dihydro-1*H*-indole (2.5 g, 32%).

To a solution of 5-ethyl-2,3-dihydro-1*H*-indole (2.5 g, 17 mmol) in H_2SO_4 (98%, 20 mL) was slowly added KNO_3 (1.7 g, 17 mmol) at 0 °C. After addition, the mixture was stirred at 0−10 °C for 10 min. The mixture was then carefully poured into ice, basified with NaOH solution to pH ≈ 9, and extracted with ethyl acetate. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica column using a gradient of ethyl acetate in hexanes (1−99%) to give 5-ethyl-6-nitro-2,3-dihydro-1*H*-indole (1.9 g, 58%).

To a solution of 5-ethyl-6-nitro-2,3-dihydro-1*H*-indole (1.9 g, 9.9 mmol) in CH_2Cl_2 (30 mL) was added MnO_2 (4 g, 46 mmol). After addition, the mixture was stirred at 25 °C for 8 h. The solid was filtered off, and the filtrate was concentrated to dryness to give crude 5-ethyl-6-nitro-1*H*-indole (1.9 g). A suspension of 5-ethyl-6-nitro-1*H*-indole (1.9 g, 10 mmol) and Ni (1 g) in methanol (10 mL) was hydrogenated under H_2 (1 atm) at room temperature for 2 h. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was purified by silica gel column using a gradient of ethyl acetate in hexanes (1−99%) to give 5-ethyl-1*H*-indol-6-amine (760 mg, 48% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (br s, 1H), 7.41 (s, 1H), 7.00 (s, 1H), 6.78 (s, 2H), 6.39 (s, 1H), 3.39 (br s, 2H), 2.63 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 6.9 Hz, 3H). MS (ESI) m/e ($M + H^+$): 161.05.

To a mixture of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (95.0 mg, 0.5 mmol) and 5-ethyl-1*H*-indol-6-amine (84 mg, 0.52 mmol) in CH_2Cl_2 (5 mL) was added HBTU (227 mg, 0.6 mmol) followed by triethylamine (167 μL, 1.2 mmol). The mixture was heated at 60 °C for 24 h and cooled to 25 °C. The solid was collected via filtration and washed with CH_2Cl_2 to provide **29** (58.0 mg, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 12.34 (s, 1H), 10.96 (s, 1H), 8.91 (s, 1H), 8.48 (s, 1H), 8.37 (d, J = 8.1 Hz, 1H), 7.84−7.76 (m, 2H), 7.53 (t, J = 7.4 Hz, 1H), 7.39 (s, 1H), 7.26 (t, J = 2.6 Hz, 1H), 6.34 (s, 1H), 2.89−2.84 (m, 2H), 1.29 (t, J = 7.4 Hz, 3H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{17}N_3O_2$, 332.1394; found, 332.1384.

***N*-(5-(*tert*-Butyl)-1*H*-indol-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (30).** To a suspension of NaH (60% in mineral oil, 8.4 g, 0.21 mol) in THF (200 mL) was added dropwise a solution of 2-*tert*-butyl-4-methylphenol (32.8 g, 0.2 mol) in THF (100 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min, and then phosphorochloridic acid diethyl ester (37.2 g, 0.21 mol) was added dropwise at 0 °C. After addition, the mixture was stirred at 25 °C for 30 min. The reaction was quenched with sat. NH_4Cl (300 mL) and then extracted with Et_2O (350 mL × 2). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and then evaporated under vacuum to give crude compound as a colorless oil (60 g, 100%), which was used directly in the next step.

To NH_3 (liquid, 1000 mL) was added a solution of phosphoric acid 2-*tert*-butyl-4-methylphenyl ester diethyl ester (60 g, crude from last step, ∼0.2 mol) in Et_2O (anhydrous, 500 mL) at −78 °C under N_2 atmosphere. Lithium metal was added to the solution in small chunks until the blue color persisted. The reaction mixture was stirred at −78 °C for 15 min and then quenched with a saturated solution of NH_4Cl until the mixture turned to colorless. The liquid NH_3 was evaporated, and the residue was dissolved in water and extracted with Et_2O (400 mL × 2). The combined organic phases were dried over Na_2SO_4 and evaporated to give 1-(*tert*-butyl)-3-methylbenzene (crude, contaminated some mineral oil) as a colorless oil (27 g, 91%), which was used directly in the next step.

To HNO_3 (95%, 13.8 mL) was added H_2SO_4 (98%, 20 mL) at 0 °C followed by dropwise addition of 1-*tert*-butyl-3-methylbenzene (7.4 g, 50 mmol, crude from last step) with the temperature being controlled under 30 °C. The reaction mixture was stirred at 25 °C for 30 min, then poured onto crushed ice (100 g), and extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with water and brine, evaporated to give a brown oil, which was purified by column chromatography using a gradient of ethyl acetate in hexanes (0−20%) to give a mixture of 1-(*tert*-butyl)-5-methyl-2,4-dinitrobenzene and 1-(*tert*-butyl)-3-methyl-2,4-dinitrobenzene (2:1 based on ^{1}H NMR) as a yellow oil (9 g, 61%).

To this mixture (9 g, 37.8 mmol) dissolved in DMF (50 mL) was added 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (5.4 g, 45.4 mmol), and the mixture was heated to reflux for 2 h. After cooling to room temperature, the mixture was poured into ice−water and was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered, and evaporated to give a brown oil, which was purified by column chromatography to give (*E*)-2-(5-(*tert*-butyl)-2,4-dinitrophenyl)-*N*,*N*-dimethylethenamine (5 g, 68%).

A solution of (*E*)-2-(5-(*tert*-butyl)-2,4-dinitrophenyl)-*N*,*N*-dimethylethenamine (5.3 g, 18.1 mmol) and tin(II) chloride dihydrate (37.4 g, 0.18 mol) in ethanol (200 mL) was refluxed overnight. It was then cooled to room temperature, and the solvent was removed under vacuum. The residual slurry was diluted with water (500 mL), and a 10% aqueous solution of Na_2CO_3 was added until pH ≈ 8 was obtained. The resulting suspension was extracted with ethyl acetate (100 × 3 mL). The ethyl acetate extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residual solid was washed with CH_2Cl_2 to afford a yellow powder, which was purified by column chromatography using a gradient of ethyl acetate in hexanes (10−40%) to give 5-(*tert*-butyl)-1*H*-indol-6-amine (0.4 g, 12%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (br s, 1 H), 7.23 (s, 1 H), 6.92 (s, 1 H), 6.65 (s, 1H), 6.14 (s, 1 H), 4.43 (br s, 2 H), 2.48 (s, 9 H).

To a mixture of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (38 mg, 0.2 mmol) and 5-(*tert*-butyl)-1*H*-indol-6-amine (38 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) was added HBTU (92 mg, 0.24 mmol) followed by the addition of triethylamine (66 μL, 0.48 mmol). The mixture was heated at 60 °C for 24 h and cooled to 25 °C. The solid was collected via filtration and washed with EtOH to provide **30** (43 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (br s, 1H), 11.82 (s, 1H), 10.86 (s, 1H), 8.83 (s, 1H), 8.28 (dd, J = 8.1, 1.0 Hz, 1H), 7.75 (t, J = 8.3 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.49−7.43 (m, 3H), 7.23 (m, 1H), 6.32 (m, 1H), 1.39 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{21}N_3O_2$, 360.1707; found, 360.1708.

***N*-(3-Aminophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (31).** Compound **31** was prepared from benzene-1,3-diamine dihydrochloride (36 mg, 0.2 mmol) in a similar manner as described for compound **1**. Yield: 9.8 mg, 35%. ^{1}H NMR (400 MHz, MeOD) δ

8.86 (s, 1H), 8.42 (d, J = 8.2 Hz, 1H), 7.81 (t, J = 7.7 Hz, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.19−7.14 (m, 1H), 7.09 (t, J = 8.0 Hz, 1H), 7.00 (d, J = 7.7 Hz, 1H), 6.52 (d, J = 7.8 Hz, 1H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{16}H_{13}N_3O_2$, 280.1086; found, 280.1082.

***N*-(3-Amino-4-methylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (32).** To a solution of **49** (0.21 g, 1.11 mmol), HBTU (0.42 g, 1.11 mmol), and DIEA (0.58 mL, 3.3 mmol) in THF (10 mL) was added 4-methylbenzene-1,3-diamine (0.15 g, 1.2 mmol). The mixture was heated at 60 °C for 18 h, and then the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (0−10% methanol−dichloromethane) to yield **32** (0.14 g, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 12.19 (s, 1H), 8.84 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.89−7.71 (m, 2H), 7.55- 7.45 (m, 1H), 7.03 (s, 1H), 6.87 (s, 2H), 4.91 (s, 2H), 2.03 (s, 3H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{17}H_{15}N_3O_2$, 294.1242; found, 294.1247.

***N*-(3-Amino-4-ethylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (33).** Compound **33** was prepared from 4-ethylbenzene-1,3-diamine[40] (27 mg, 0.2 mmol) in a similar manner as described for compound **32**. Yield: 16 mg, 29%. ^{1}H NMR (300 MHz, CD_3OD) δ 8.86 (s, 1H), 8.42 (d, J = 8.5 Hz, 1H), 7.94 (s, 1H), 7.81 (t, J = 8.3 Hz, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.54−7.47 (m, 2H), 7.38 (d, J = 8.5 Hz, 1H), 2.71 (q, J = 7.7 Hz, 2H), 1.30 (t, J = 7.4 Hz, 3H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{17}N_3O_2$, 308.1399; found, 308.1386.

***N*-(3-Amino-4-isopropylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (34).** Compound **34** was prepared from 4-isopropylbenzene-1,3-diamine (30 mg, 0.2 mmol) in a similar manner as described for compound **32**. Yield: 16 mg, 28%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (d, J = 6.5 Hz, 1H), 12.51 (s, 1H), 8.88 (d, J = 6.6 Hz, 1H), 8.33 (dd, J = 8.1, 1.0 Hz, 1H), 7.85−7.74 (m, 3H), 7.55 (t, J = 8.1 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 3.03 (septet, J = 6.8 Hz, 1H), 1.20 (d, J = 6.7 Hz, 6H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{19}H_{19}N_3O_2$, 322.1555; found, 322.1548.

***N*-(3-Amino-4-propylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (35).** Compound **35** was prepared from 4-propylbenzene-1,3-diamine (30 mg, 0.2 mmol) in a similar manner as described for compound **32**. Yield: 3 mg, 5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (d, J = 6.5 Hz, 1H), 12.51 (s, 1H), 8.88 (d, J = 6.6 Hz, 1H), 8.33 (dd, J = 8.1, 1.0 Hz, 1H), 7.85−7.74 (m, 3H), 7.55 (t, J = 8.1 Hz, 1H), 7.38 (dd, J = 8.4, 1.9 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 3.03 (septet, J = 6.8 Hz, 1H), 1.20 (d, J = 6.7 Hz, 6H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{19}H_{19}N_3O_2$, 322.1555; found, 322.1570

1-*tert*-Butyl-2,4-dinitrobenzene (58). A prechilled mixture of fuming nitric acid (30 mL) and concentrated sulfuric acid (70 mL) was added portionwise with stirring to a mixture of *tert*-butylbenzene (10.0 g, 75 mmol) and concentrated sulfuric acid (100 mL) at 0 °C over 20 min. The cloudy yellow mixture was stirred for 30 min before warming to 25 °C. After 3 h, the mixture was poured over ice (400 g), and the green mixture was extracted with ether (2 × 100 mL). (*Caution*: During the workup, a violent gas evolution was observed from the aqueous phase during the draining of the aqueous phase from the first ether extraction.) The combined ether extracts were washed with water (100 mL) and brine (100 mL). The organics were dried over $MgSO_4$ and concentrated under reduced pressure to afford **58** as a yellow oil (16.0 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29, (dd, J = 2.4, 9.0 Hz, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.79 (d, J = 9.0 Hz, 1H), 1.46 (s, 9H).

1-*tert*-Butyl-2,4-diaminobenzene (59). A 250 mL flask was charged with **58** (2.24 g, 10 mmol), 10% Pd/C (0.3 g), and methanol (100 mL), and the mixture was hydrogenated at 25 °C for 16 h. The mixture was filtered through a plug of Celite and concentrated under reduced pressure to afford a crude residue that was purified by silica gel chromatography (0−50% ethyl acetate−hexanes) to afford the dianiline **59** as a red-brown oil (0.68 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, J = 8.3 Hz, 1H), 6.10 (dd, J = 2.4, 8.3 Hz, 1H), 6.01 (d, J = 2.4 Hz, 1H), 3.59 (br s, 4H), 1.37 (s, 9H); ESI-MS m/z 164.9 $(M + H)^+$.

4-Oxo-1,4-dihydroquinoline-3-carboxylic Acid (4-*tert*-Butyl-3-aminophenyl)amide (36). DIEA (1.05 mL, 6 mmol, 3 equiv) was added to a suspension of **49** (0.38 g, 2.0 mmol) and HATU (0.76 g, 1 mmol) in DMF (5 mL). The mixture was stirred for 15 min and added dropwise over 5 min to a solution of **59** (0.4 g, 2.4 mmol) in a mixture of dichloromethane/methanol (8/1, 4.5 mL). The mixture was diluted with ethyl acetate (50 mL) and washed with brine (3 × 25 mL), a saturated aqueous solution of K_2CO_3 (10 mL), and 0.5 M citric acid (10 mL). The organics were dried over Na_2SO_4, filtered, and concentrated to afford a brown solid that was suspended in dichloromethane (50 mL) and sonicated. The mixture was filtered, and the filter cake was washed with dichloromethane (100 mL). The filter cake was then dissolved in methanol (150 mL), filtered, and concentrated to afford the **36** as a pale yellow solid (0.42 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.5 (br s, 1H), 12.20 (s, 1H), 8.85 (s, 1H), 8.33 (dd, J = 1.3, 8.1 Hz, 1H), 7.81 (app dt, J = 1.4, 7.6 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.53 (app t with fine str, J = 7.9 Hz, 1H), 7.03 (d, J = 5.3 Hz, 1H), 7.02 (s, 1H), 6.94 (dd, J = 2.1, 8.5 Hz, 1H), 4.83 (s, 2H), 1.33 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{21}N_3O_2$, 336.1712; found, 336.1709.

***N*-(3-Acetamido-4-*tert*-butylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (37).** To a solution of **36** (74 mg, 0.22 mmol) in THF (1.0 mL) was added acetyl chloride (0.015 mL, 0.20 mmol), followed by DIEA (0.1 mL, 0.28 mmol), and the mixture was stirred at 25 °C for 40 min. The solvent was removed and the residue was purified by reverse-phase HPLC to yield **37** (10 mg, 12%) . ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (d, J = 6.6 Hz, 1H), 12.43 (s, 1H), 9.27 (s, 1H), 8.86 (d, J = 6.8 Hz, 1H), 8.33 (d, J = 6.9 Hz, 1H), 7.82 (dd, J = 6.9, 1.4 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.55 (t, J = 7.5 Hz, 2H), 7.44 (d, J = 2.0 Hz, 1H), 7.36 (d, J = 8.7 Hz, 1H), 2.06 (s, 3H), 1.32 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{23}N_3O_3$, 378.1817; found, 378.1802.

Methyl 2-*tert*-Butyl-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenylcarbamate (38). To a solution of **36** (34 mg, 0.10 mmol) in THF (1.0 mL) was added methyl chloroformate (0.01 mL, 0.15 mmol), followed by DIEA (0.04 mL, 0.20 mmol), and the mixture was stirred at 25 °C for 40 min. The mixture was dissolved in dichloromethane (0.5 mL) and treated with piperidine (7.5 equiv) for 30 min. The solvent was removed and the compound was purified by reverse-phase HPLC to yield **38** (3 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 12.45 (s, 1H), 8.87 (d, J = 6.7 Hz, 1H), 8.69 (s, 1H), 8.33 (d, J = 7.0 Hz, 1H), 7.85−7.71 (m, 2H), 7.57−7.48 (m, 3H), 7.36 (d, J = 8.4 Hz, 1H), 3.64 (s, 3H), 1.32 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{23}N_3O_4$, 394.1767; found, 394.1754.

2-Bromo-1-*tert*-butyl-4-nitrobenzene (61). To a solution of 1-*tert*-butyl-4-nitrobenzene (8.9 g, 50 mmol) and silver sulfate (10 g, 32 mmol) in 50 mL of 90% sulfuric acid was added bromine (7.95 g, 50 mmol) dropwise. Stirring was continued at 25 °C for 16 h, and then the mixture was poured into a dilute $NaHSO_3$ solution and was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, and concentrated to give **61** (12.7 g, 98%) that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 2.5 Hz, 1H), 8.11 (dd, J = 8.8, 2.5 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 1.57 (s, 9H).

2-*tert*-Butyl-5-nitrobenzonitrile (62). To a solution of **61** (2.1 g, 8.2 mmol) and $Zn(CN)_2$ (0.77 g, 6.6 mmol) in DMF (10 mL) was added $Pd(PPh_3)_4$ (0.5 g, 0.4 mmol) under nitrogen. The mixture was heated in a sealed vessel at 205 °C for 5 h. After cooling to 25 °C, the mixture was diluted with water and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel chromatography (0−10% ethyl acetate−hexane) to give **62** (1.3 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, J = 2.3 Hz, 1H), 8.36 (dd, J = 8.8, 2.2 Hz, 1H), 7.73 (d, J = 8.9 Hz, 1H), 1.60 (s, 9H).

(2-*tert*-Butyl-5-nitrophenyl)methanamine (63). To a solution of **62** (0.6 g, 3.0 mmol) in THF (10 mL) was added a 1 M solution of BH_3 in THF (12 mL, 12.0 mmol), and the reaction mixture was stirred

at 70 °C for 16 h under nitrogen. The mixture was cooled to 0 °C, and methanol (2 mL) was added followed by 1 N HCl (2 mL). After heating at reflux for 30 min, the solution was diluted with water (10 mL) and extracted with ethyl acetate (20 mL). The aqueous layer was made basic by the addition of 1 N NaOH and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel chromatography (0−10% methanol−dichloromethane) to give **63** (0.3 g, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J = 2.7 Hz, 1H), 7.99 (dd, J = 8.8, 2.8 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 4.03 (s, 2H), 2.00 (t, J = 2.1 Hz, 2H), 1.40 (s, 9H).

***tert*-Butyl 2-*tert*-Butyl-5-nitrobenzylcarbamate (64).** A solution of **63** (0.2 g, 1.0 mmol) and $(Boc)_2O$ (0.23 g, 1.1 mmol) in THF (5 mL) was heated at reflux for 30 min. After cooling to 25 °C, the solution was diluted with water (10 mL) and extracted with ethyl acetate (20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, and evaporated to dryness to give **64** (0.24 g, 78%) that was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, J = 2.3 Hz, 1H), 8.09 (dd, J = 8.8, 2.5 Hz, 1H), 7.79 (t, J = 5.9 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 4.52 (d, J = 6.0 Hz, 2H), 1.48 (s, 18H).

***tert*-Butyl 2-*tert*-Butyl-5-aminobenzylcarbamate (65).** To a solution of **64** (20 mg, 0.07 mmol) in 5% acetic acid in methanol (1 mL), 10% Pd/C (14 mg) was added under nitrogen. The mixture was hydrogenated at 25 °C using a hydrogen filled balloon for 1 h. The mixture was filtered through a plug of Celite, and the filtrate was evaporated to dryness to give **65** (18 mg, 92% yield) that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.5 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 6.47 (dd, J = 8.5, 2.6 Hz, 1H), 4.61 (br s, 1H), 4.40 (d, J = 5.1 Hz, 2H), 4.15 (br s, 2H), 1.39 (s, 9H), 1.29 (s, 9H).

***N*-(3-(Aminomethyl)-4-*tert*-butylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (39).** *Step 1.* A mixture of **49** (10 mg, 0.05 mmol), **65** (18 mg, 0.065 mmol), triethylamine (0.02 mL, 0.12 mmol), and HATU (23 mg, 0.06 mmol) in dichloromethane (1 mL) was heated at 60 °C for 16 h. The crude material was purified by reverse-phase HPLC to give *tert*-butyl 2-*tert*-butyl-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)benzylcarbamate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (d, J = 6.0 Hz, 1H), 12.40 (s, 1H), 8.87 (d, J = 6.8 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 7.84−7.75 (m, 3H), 7.57−7.43 (m, 2H), 7.31 (d, J = 8.6 Hz, 1H), 4.40 (d, J = 5.8 Hz, 2H), 1.44 (s, 9H), 1.38 (s, 9H).

Step 2. To a solution of *tert*-butyl 2-*tert*-butyl-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)benzylcarbamate in dichloromethane (1 mL) was added trifluoroacetic acid (0.2 mL), and the mixture was stirred at 25 °C for 30 min. The solvent was evaporated to afford **29** as a TFA salt (16 mg, 53% over two steps). ^{1}H NMR (400 MHz, DMSO) δ 13.08 (d, J = 6.5 Hz, 1H), 12.56 (s, 1H), 8.89 (d, J = 6.7 Hz, 1H), 8.33 (d, J = 8.1 Hz, 1H), 8.27 (s, 3H), 7.98 (dd, J = 8.7, 1.9 Hz, 1H), 7.87−7.81 (m, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.59−7.53 (m, 1H), 7.43 (d, J = 8.7 Hz, 1H), 4.33−4.27 (m, 2H), 1.38 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{23}N_3O_2$, 350.1868; found, 350.186.

2-*tert*-Butyl-5-nitrobenzoic Acid (66). A solution of **62** (0.2 g, 1.0 mmol) in 5 mL of 75% H_2SO_4 was heated in a microwave oven at 200 °C for 30 min. The reaction mixture was poured over ice and extracted with ethyl acetate. The organics were washed with brine (5 mL), dried over $MgSO_4$, and evaporated to dryness to give **66** (0.2 g, 90%) that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 2.6 Hz, 1H), 8.24 (dd, J = 8.9, 2.6 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H) 1.51 (s, 9H).

Methyl 2-*tert*-Butyl-5-aminobenzoate (67). Step 1. To a mixture of **66** (0.12 g, 0.53 mmol) and K_2CO_3 (0.15 g, 1.1 mmol) in DMF (5.0 mL) was added iodomethane (0.04 mL, 0.64 mmol). The reaction mixture was stirred at 25 °C for 10 min, diluted with water (5 mL), and extracted with ethyl acetate (10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, and evaporated to dryness to give methyl 2-*tert*-butyl-5-nitrobenzoate (90 mg, 71%) that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 2.6 Hz, 1H), 8.17 (t, J = 1.8 Hz, 1H), 7.66 (d, J = 8.6 Hz, 1H), 4.11 (s, 3H), 1.43 (s, 9H).

Step 2. To a solution of methyl 2-*tert*-butyl-5-nitrobenzoate (90 mg, 0.38 mmol) in ethanol (2 mL) was added a solution of potassium formate (0.4 g, 4.8 mmol) in water (1 mL), followed by 10% Pd/C (20 mg). The mixture was heated at reflux for 40 min, cooled to 25 °C and filtered though a plug of Celite. The filtrate was concentrated to dryness to give **67** (76 mg, 95%) that was used in the next step without further purification. ^{1}H NMR H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 8.6 Hz, 1H), 6.67 (dd, J = 8.6, 2.7 Hz, 1H), 6.60 (d, J = 2.7 Hz, 1H), 3.86 (s, 3H), 1.34 (s, 9H).

(2-*tert*-Butyl-5-aminophenyl)methanol (68). To a solution of compound **67** (0.16 g, 0.72 mmol) in THF (5 mL) at 0 °C was added a 1 M solution of lithium aluminum hydride in THF (1.4 mL, 1.4 mmol). The reaction mixture was refluxed for 2 h, diluted with water (5 mL), and extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, and evaporated to give **68** (25 mg, 20%) that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 2.6 Hz, 1H), 6.56 (dd, J = 8.4, 2.7 Hz, 1H), 4.83 (s, 2H), 1.36 (s, 9H).

***N*-(4-*tert*-Butyl-3-(hydroxymethyl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (40).** To a solution of compound **49** (26 mg, 0.14 mmol), HATU (54 mg, 0.14 mmol), and triethylamine (0.06 mL, 0.4 mmol) in DMF (1 mL) was added compound **68** (25 mg, 0.14 mmol). The mixture was heated at 60 °C for 18 h, filtered, and purified by reverse-phase HPLC to give **40** (3 mg, 5%). ^{1}H NMR (400 MHz, DMSO) δ 12.96 (s, 1H), 12.43 (s, 1H), 8.88 (s, 1H), 8.35 (d, J = 8.0 Hz, 1H), 7.86−7.81 (m, 1H), 7.78−7.70 (m, 3H), 7.56 (t, J = 7.5 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 5.24 (t, J = 5.5 Hz, 1H), 4.74 (d, J = 5.4 Hz, 2H), 1.37 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{22}N_2O_3$, 351.1708; found, 351.1704.

2-*tert*-Butyl-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)-benzoic Acid (41). *Step 1.* To 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**) (62 mg, 0.3 mmol), methyl 5-amino-2-*tert*-butylbenzoate (**67**) (68 mg, 0.3 mmol), HATU (126 mg, 0.3 mmol), DMF (2.4 mL), and Et_3N (138 μL, 1 mmol) were added, and the mixture was stirred at 60 °C for 18 h. DMF was evaporated under reduced pressure. The compound was dissolved in EtOAc (6 mL) and washed with 1 M HCl (3 mL × 2), a saturated aqueous solution of $NaHCO_3$ (3 mL × 2), and a saturated aqueous solution of NaCl (3 mL × 1). The organic layer was dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (0−30% ethyl acetate in dichloromethane) to yield methyl 2-*tert*-butyl-5-[(4-oxo-1*H*-quinoline-3-carbonyl)amino]benzoate (0.10 g, 80%) .

Step 2. To methyl 2-*tert*-butyl-5-[(4-oxo-1*H*-quinoline-3-carbonyl)-amino]benzoate (50 mg, 0.13 mmol), THF (3 mL), MeOH (1.5 mL), and LiOH (1.52 mL of 2 M, 3.0 mmol) were added, and the mixture was heated at 140 °C for 1 h in the microwave instrument. The mixture was filtered, and the solvent was evaporated under reduced pressure. The compound was dissolved in MeOH, filtered, and purified by reverse-phase HPLC to give **41** (32 mg, 66%). ^{1} H NMR (400 MHz, MeOD) δ 8.84 (s, 1H), 8.41 (d, J = 8.2 Hz, 1H), 7.82−7.76 (m, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.62−7.57 (m, 1H), 7.57−7.49 (m, 2H), 7.39 (d, J = 8.7 Hz, 1H), 1.47 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{20}N_2O_4$, 365.1501; found, 365.1491.

2-*tert*-Butyl-5-nitroaniline (70). To a solution of 90% sulfuric acid (50 mL) at 0 °C was added 2-*tert*-butylphenylamine (4.5 g, 30.0 mmol) dropwise. Potassium nitrate (4.5 g, 45.0 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at 0−5 °C for 5 min, poured over ice−water, and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, filtered, and evaporated to yield a residue that was recrystallized from 70% EtOH−H_2O to give **70** (3.7 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, J = 8.7, 2.4 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.36 (d, J = 8.7 Hz, 1H), 4.17 (s, 2H), 1.46 (s, 9H).

2-*tert*-Butyl-5-aminophenol (71). *Step 1.* To a solution of **70** (1.9 g, 10.0 mmol) in 40 mL of 15% H_2SO_4 at 0 °C was added a solution of $NaNO_2$ (0.76 g, 11.0 mmol in 3 mL of water) dropwise.

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The resulting mixture was stirred at 0−5 °C for 5 min. Excess $NaNO_2$ was neutralized with urea. Then 5 mL of H_2SO_4−H_2O (v/v 1:2) was added and the mixture was heated at reflux for 5 min. Three additional 5 mL aliquots of H_2SO_4−H_2O (v/v 1:2) were added while heating at reflux. The reaction mixture was cooled to 25 °C and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and evaporated to yield a crude residue that was purified by silica gel chromatography (0−10% ethyl acetate−hexane) to afford 2-*tert*-butyl-5-nitrophenol (1.2 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, J = 8.6, 2.2 Hz, 1H), 7.58 (d, J = 2.1 Hz, 1H), 7.43 (d, J = 8.6 Hz, 1H), 5.41 (s, 1H), 1.45 (s, 9H).

Step 2. To a solution of 2-*tert*-butyl-5-nitrophenol (0.2 g, 1.0 mmol) in ethanol (10 mL) were added ammonium formate (0.2 g, 3.1 mmol) and 10% Pd/C (0.14 g). The reaction mixture was heated at reflux for 30 min, cooled to 25 °C, and filtered through a plug of Celite. The filtrate was concentrated to dryness and purified by silica gel chromatography (20−30% ethyl acetate−hexane) to give **71** (0.14 g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 6.74 (d, J = 8.3 Hz, 1H), 6.04 (d, J = 2.3 Hz, 1H), 5.93 (dd, J = 8.2, 2.3 Hz, 1H), 4.67 (s, 1H), 1.26 (s, 9H).

2-*tert*-Butyl-5-aminobenzene-1-sulfonamide (72). *Step 1: 2-tert-Butyl-5-nitrobenzene-1-sulfonyl Chloride.* To a suspension of 2-*tert*-butyl-5-nitrobenzenamine (**70**, 0.97 g, 5.0 mmol) in concentrated HCl (5 mL) cooled to 5−10 °C was added a solution of $NaNO_2$ (0.43g, 6.3 mmol in 0.8 mL of H_2O) dropwise. The mixture was stirred for 0.5 h and then vacuum filtered. The filtrate was added simultaneously with a solution of Na_2SO_3 (1.6 g, 12.4 mmol in 2.7 mL of H_2O) to a stirred solution of $CuSO_4$ (0.19 g, 0.76 mmol) and Na_2SO_3 (1.6 g, 12.4 mmol) in HCl (11.7 mL) and H_2O (2.7 mL) at 3−5 °C. Stirring was continued for 0.5 h and the resulting precipitate was filtered, washed with water and dried to give 2-*tert*-butyl-5-nitrobenzene-1-sulfonyl chloride (0.24 g, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, J = 2.5 Hz, 1H), 8.36 (dd, J = 8.9, 2.5 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 1.59 (s, 9H).

Step 2: 2-tert-Butyl-5-nitrobenzene-1-sulfonamide. To a solution of 2-*tert*-butyl-5-nitrobenzene-1-sulfonyl chloride (0.1 g, 0.36 mmol) in ether (2 mL) was added aqueous NH_4OH (0.13 mL, 3.6 mmol) at 0 °C. The mixture was stirred at 25 °C for 16 h, diluted with water, and extracted with ether (3 × 10 mL). The combined ether extracts were washed with brine (20 mL) and dried over Na_2SO_4. After removal of solvent, the residue was purified by silica gel chromatography (0−50% ethyl acetate−hexane) to give 2-*tert*-butyl-5-nitrobenzene-1-sulfonamide (32 mg, 34%).

Step 3: 2-tert-Butyl-5-aminobenzene-1-sulfonamide (72). A solution of 2-*tert*-butyl-5-nitrobenzene-1-sulfonamide (32 mg, 0.12 mmol) and $SnCl_2$·$2H_2O$ (0.14 g, 0.61 mmol) in ethanol (1.5 mL) was heated in a microwave oven at 100 °C for 30 min. The mixture was diluted with ethyl acetate (10 mL) and water (5 mL), basified with sat. $NaHCO_3$, and filtered through a plug of Celite. The organic layer was separated and dried over Na_2SO_4. The solvent was removed by evaporation to provide compound **72** (28 mg, 100%), which was used in the next step without further purification.

***N*-(4-*tert*-Butyl-3-sulfamoylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (42).** To a solution of **49** (23 mg, 0.12 mmol), HBTU (47 mg, 0.12 mmol), and triethylamine (0.05 mL, 0.37 mmol) in DMF (1.0 mL) was added **72** (28 mg, 0.12 mmol). The mixture was heated at 150 °C in the microwave oven for 50 min and the resulting solution was filtered and purified by reverse-phase HPLC to yield compound **42** (16 mg, 33%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H), 12.61 (s, 1H), 8.90 (s, 1H), 8.34 (dd, J = 8.1, 1.1 Hz, 1H), 8.22 (d, J = 2.4 Hz, 1H), 8.14 (dd, J = 8.8, 2.4 Hz, 1H), 7.86−7.80 (m, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.66−7.54 (m, 4H), 1.52 (s, 9H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{20}H_{21}N_3O_4S$, 400.1331; found, 400.1329.

***N*-(4-*tert*-Butylphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (43).** A mixture of **49** (38 mg, 0.2 mmol), 4-*tert*-butylaniline (60 mg, 0.4 mmol), DIEA (0.7 mL, 0.4 mmol), and HATU (76 mg, 0.2 mmol) in DMF (1 mL) was stirred for 24 h. The reaction mixture was filtered and purified by reverse-phase HPLC to give **43** (35 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (br s, 1H), 12.42 (s, 1H), 8.88 (s, 1H), 8.33 (dd, J = 8.2, 1.1 Hz, 1H), 7.82 (t, J = 8.3 Hz, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.54 (t, J = 8.1 Hz, 1H), 7.39 (d, J = 8.7 Hz, 2H), 1.29 (s, 9H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{20}H_{20}N_2O_2$, 321.1603; found, 321.1601.

1-*tert*-Butyl-2-fluoro-4-nitrobenzene (73). To a stirred solution of **70** (5.0 g, 25.8 mmol) in H_2O (20 mL) was added concentrated HCl (10 mL). The reaction mixture was cooled to 0 °C, and a solution of $NaNO_2$ (1.78 g, 25.8 mmol) in H_2O (10 mL) was added dropwise. The reaction mixture was stirred at 0 °C for another 0.5 h, and a solution of HPF_6 (40 mL) was added in two batches. The precipitate that formed was collected by filtration and heated under an infrared light at 130−150 °C (the conversion was monitored by TLC). The resulting dark oil was purified by silica gel chromatography to afford **73** (0.6 g, 12% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, J = 2.4, 8.8 Hz, 1 H), 7.87 (dd, J = 2.4, 12.0 Hz, 1 H), 7.48 (t, J = 8.0 Hz, 1 H), 1.43 (s, 9 H).

4-*tert*-Butyl-3-fluoroaniline (74). $NaBH_4$ (0.29 g, 7.6 mmol) was added to a solution of **73** (0.75 g, 3.8 mmol) and $NiCl_2$·$6H_2O$ (2.6 g, 11.4 mmol) in methanol (15 mL) at 15 °C. The mixture was stirred for 2 min, and then water was added to quench the reaction. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to afford **74** (0.47 g, 75%) that was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08−7.02 (m, 1 H), 6.42−6.34 (m, 2 H), 1.32 (s, 9 H).

***N*-(4-*tert*-Butyl-3-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (44).** To a solution of **49** (30 mg, 0.16 mmol), HATU (65 mg, 0.17 mmol), and triethylamine (0.07 mL, 0.47 mmol) in THF (1.0 mL) was added **74** (29 mg, 0.17 mmol). The mixture was heated at 150 °C in the microwave oven for 50 min and the resulting solution was filtered and purified by reverse-phase HPLC to give **44** (11 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 8.88 (s, 1H), 8.32 (d, J = 8.1, 1H), 7.83−7.71 (m, 3H), 7.52 (t, J = 7.5, 1H), 7.34−7.24 (m, 2H), 5.76 (s, 1H), 1.34 (s, 9H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{20}H_{19}FN_2O_2$, 339.1509; found, 339.1507.

2-*tert*-Butyl-5-aminophenol (71). To a solution of **70** (0.2 g, 1.0 mmol) in ethanol (10 mL) were added ammonium formate (0.2 g, 3.1 mmol) and 10% Pd/C (0.14 g), and the mixture was heated at reflux. After 30 min, the mixture was cooled to 25 °C and filtered through a plug of Celite. The filtrate was concentrated to dryness and was purified by silica gel chromatography (20−30% ethyl acetate−hexane) to give compound **71** (0.14 g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 6.74 (d, J = 8.3 Hz, 1H), 6.04 (d, J = 2.3 Hz, 1H), 5.93 (dd, J = 8.2, 2.3 Hz, 1H), 4.67 (s, 1H), 1.26 (s, 9H).

***N*-(4-*tert*-Butyl-3-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (45).** A mixture of **49** (57 mg, 0.30 mmol), **71** (59 mg, 0.36 mmol), triethylamine (0.1 mL, 0.72 mmol), and HATU (0.14 g, 0.36 mmol) in dichloromethane (3 mL) was heated at 70 °C for 12 h. The solvent was evaporated and the crude residue was purified by reverse-phase HPLC to give **45** (47 mg, 47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (br s, 1H), 12.31 (s, 1H), 9.44 (s, 1H), 8.86 (s, 1H), 8.33 (dd, J = 8.1, 1.1 Hz, 1H), 7.82 (td, J = 7.6, 1.4 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 6.96 (dd, J = 8.4, 2.1 Hz, 1H), 1.34 (s, 9H). HRMS-ESI (m/z): [M + H]$^+$ calcd for $C_{20}H_{20}N_2O_3$, 337.1552; found, 337.1564.

2-*tert*-Butyl-4-fluorophenol (76). 4-Fluorophenol (5.0 g, 45 mmol) and *tert*-butanol (5.9 mL, 63 mmol) were dissolved in dichloromethane (80 mL) and treated with concentrated sulfuric acid (3 mL). The mixture was stirred at 25 °C for 16 h. The organic layer was washed with water, neutralized with a saturated solution of $NaHCO_3$, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography (5−15% ethyl acetate−hexane) to give compound **76** (3.1 g, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H), 6.89 (dd, J = 11.1, 3.1 Hz, 1H), 6.84−6.79 (m, 1H), 6.74 (dd, J = 8.7, 5.3 Hz, 1H), 1.33 (s, 9H).

2-*tert*-Butyl-4-fluorophenyl Methyl Carbonate (77). To a solution of **76** (2.6 g, 15.7 mmol) and triethylamine (3.1 mL, 22.5

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mmol) in dioxane (45 mL) was added methyl chloroformate (1.3 mL, 16.5 mmol), and the mixture was stirred at 25 °C for 1 h. The precipitate was removed, and the filtrate was diluted with water and extracted with diethyl ether. The ether extract was washed with water and dried over $MgSO_4$. After removal of solvent, the residue was purified by silica gel chromatography to give compound **77** (2.1 g, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 (dd, J = 8.8, 5.4 Hz, 1H), 7.17−7.10 (m, 2H), 3.86 (s, 3H), 1.29 (s, 9H).

2-*tert*-Butyl-4-fluoro-5-nitrophenyl Methyl Carbonate (78). To a solution of **77** (1.8 g, 8.0 mmol) in concentrated H_2SO_4 (1 mL) at 0 °C was added a mixture of H_2SO_4 (1 mL) and HNO_3 (1 mL) dropwise. The mixture was stirred for 2 h while warming to 25 °C, before being poured over ice and extracted with diethyl ether (2 × 20 mL). The ether extract was washed with brine (10 mL), dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography (0−10% ethyl acetate−hexane) to give compound **78** (1.2 g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 7.1 Hz, 1H), 7.55 (d, J = 13.4 Hz, 1H), 3.90 (s, 3H), 1.32 (s, 9H).

2-*tert*-Butyl-4-fluoro-5-nitrophenol (79). To a solution of 2-*tert*-butyl-4-fluoro-5-nitrophenyl methyl carbonate (**78**) (1.1 g, 4.0 mmol) in dichloromethane (40 mL) was added piperidine (3.9 mL, 10 mmol). The mixture was stirred at 25 °C for 1 h and extracted with 1 N NaOH (3 × 10 mL). The aqueous layer was acidified with 1 N HCl and extracted with diethyl ether. The ether extract was washed with brine (20 mL), dried over $MgSO_4$, and concentrated to give compound **79** (0.53 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 7.49 (d, J = 6.8 Hz, 1H), 7.25 (d, J = 13.7 Hz, 1H), 1.36 (s, 9H).

2-*tert*-Butyl-5-amino-4-fluorophenol (80). To a solution of **79** (0.4 g, 1.9 mmol) and ammonium formate (0.4 g, 6.1 mmol) in ethanol (20 mL) was added 5% Pd/C (0.26 g). The mixture was heated at reflux for 1 h, cooled to 25 °C, and filtered through a plug of Celite. The solvent was removed by evaporation to give compound **80** (0.55 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (br s, 1H), 6.66 (d, J = 13.7 Hz, 1H), 6.22 (d, J = 8.5 Hz, 1H), 4.74 (br s, 2H), 1.26 (s, 9H).

***N*-(4-*tert*-Butyl-2-fluoro-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (46).** A mixture of **49** (0.19 g, 1 mmol), **80** (0.18 g, 1 mmol), triethylamine (0.28 mL, 2 mmol), and HATU (0.38 g, 1 mmol) in DMF (3 mL) was heated at 70 °C for 12 h. The solvent was evaporated, and the crude residue was suspended in water (5 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated. The crude residue was purified by silica gel chomatography (60−100% ethyl acetate−hexanes) to yield **46** (80 mg, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (d, J = 6.8 Hz, 1H), 12.56 (s, 1H), 9.44 (s, 1H), 8.87 (d, J = 6.8 Hz, 1H), 8.34 (dd, J = 8.2, 1.3 Hz, 1H), 8.08 (d, J = 7.4 Hz, 1H), 7.83 (t, J = 8.3 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.55 (t, J = 8.1 Hz, 1H), 7.00 (d, J = 13.3 Hz, 1H), 1.34 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{19}FN_2O_3$, 355.1458; found, 355.1452.

2-*tert*-Butyl-4-bromophenol (82).[41] To a solution of 2-*tert*-butylphenol (5.0 g, 33 mmol) in CCl_4 (25 mL) was added silica gel 60 (EMD 230−400 mesh). The solution was cooled to 0 °C, and bromine (5.3 g, 33 mmol) was added dropwise over 15 min. The solution was stirred for 1 h, filtered, and the solid was washed with dichloromethane. The organic layers were washed with a 1 N $Na_2S_2O_3$ solution (30 mL), brine (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (0−7% ethyl acetate−hexanes) to yield **72** (5.2 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J = 2 Hz, 1H), 7.16 (dd, J = 9 and 2 Hz, 1H), 6.55 (d, J = 9 Hz, 1H), 4.95 (br s, 1H), 1.38 (s, 9H).

Methyl 2-*tert*-Butyl-4-bromophenylcarbonate (83). To a solution of **82** (5.2 g, 22.7 mmol) in dichloromethane (200 mL) at 0 °C was added triethylamine (3.8 mL, 27.2 mmol) and methyl chloroformate (3.5 mL, 45.4 mmol). The solution was stirred for 2 h and quenched with a saturated aqueous solution of NH_4Cl. The organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield **83** (4.5 g 72%) that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 2 Hz, 1H), 7.35 (dd, J = 9 and 2 Hz, 1H), 6.98 (d, J = 9 Hz, 1H), 3.92 (s, 3H), 1.34 (s, 9H).

Methyl 2-*tert*-Butyl-4-bromo-5-nitrophenylcarbonate (84). To a solution of **83** (4.5 g, 16.4 mmol) in 10 mL of H_2SO_4 at 0 °C was added KNO_3 (2.5 g, 24.6 mmol) in small batches over 15 min. The solution was stirred for 1 h at 25 °C, and a yellow precipitate was formed. Ice (~20 g) was added to the solution, and the suspension was stirred for 15 min. The mixture was extracted with ethyl acetate (2 × 20 mL), and the organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure to yield a crude residue that was purified by silica gel chromatography (0−7% ethyl acetate−hexanes) to yield **84** (4.5 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.72 (s, 1H), 3.95 (s, 3H), 1.37 (s, 9H).

2-*tert*-Butyl-4-bromo-5-nitrophenol (85). To a solution of **84** (2.4 g, 7.25 mmol) in methanol (20 mL) was added KOH (0.6 g, 10.7 mmol). The mixture was stirred for 10 min at 25 °C and quenched with 1 N HCl. The reaction mixture was partitioned between ethyl acetate and H_2O. The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure to yield **85** (1.90 g, 96%) which was used in the next step without further purification.

1-*tert*-Butyl-2-(benzyloxy)-5-bromo-4-nitrobenzene (86). To a mixture of **85** (1.1 g, 4.0 mmol) and Cs_2CO_3 (1.6 g, 4.8 mmol) in DMF (8 mL) was added benzyl bromide (0.5 mL, 4.2 mmol). The mixture was stirred at 25 °C for 4 h, diluted with H_2O, and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried over $MgSO_4$. The crude material was purified by silica gel chromatography (0−5% ethyl acetate−hexane) to yield **86** (1.37 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.53 (s, 1H), 7.43 (m, 5H), 5.22 (s, 2H), 1.42 (s, 9H).

1-*tert*-Butyl-2-(benzyloxy)-5-(trifluoromethyl)-4-nitrobenzene (87). A mixture of **86** (0.9 g, 2.5 mmol), KF (0.3 g, 5 mmol), KBr (0.6 g, 5 mmol), CuI (0.6 g, 3 mmol), methyl chlorodifluoroacetate (1.6 mL, 15 mmol) in DMF (5 mL) was heated at 125 °C in a sealed tube overnight. After being cooled to 25 °C, the mixture was diluted with water and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (20 mL) and dried over $MgSO_4$. The residue was purified by silica gel chromatography (0−5% ethyl acetate−hexanes) to yield **87** (0.6 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.37 (m, 5H), 7.19 (s, 1H), 5.21 (s, 2H), 1.32 (s, 9H).

5-Amino-2-*tert*-butyl-4-(trifluoromethyl)phenol (88). To a solution of **87** (0.35 g, 1.0 mmol) and ammonium formate (0.35 g, 5.4 mmol) in ethanol (10 mL) was added 10% Pd/C (0.25 g), and the mixture was heated at reflux for 2 h. The mixture was cooled to 25 °C and filtered through a plug of Celite. After removal of the solvent, the residue was purified by silica gel chromatography (50−80% ethyl acetate−hexanes) to give **88** (0.12 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 6.05 (s, 1H), 1.28 (s, 9H).

***N*-(4-*tert*-Butyl-5-hydroxy-2-(trifluoromethyl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (47).** A mixture of **49** (9.5 mg, 0.05 mmol), **88** (14 mg, 0.06 mmol), triethylamine (0.02 mL, 0.12 mmol), and HATU (23 mg, 0.06 mmol) in dichloromethane (0.5 mL) was heated at 70 °C and was stirred for 24 h. The solvent was evaporated and the crude residue was purified by reverse-phase HPLC to give **47** (2 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (d, J = 6.6 Hz, 1H), 12.57 (s, 1H), 10.37 (s, 1H), 8.88 (d, J = 6.8 Hz, 1H), 8.34−8.32 (m, 1H), 7.99 (s, 1H), 7.85−7.81 (m, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.56−7.52 (m, 1H), 7.38 (s, 1H), 1.37 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{19}F_3N_2O_3$, 405.1426; found, 405.1429.

Carbonic Acid 2,4-Di-*tert*-butylphenyl Ester Methyl Ester (90). Methyl chloroformate (58 mL, 750 mmol) was added dropwise to a solution of 2,4-di-*tert*-butylphenol (**89**) (103.2 g, 500 mmol), triethylamine (139 mL, 1000 mmol), and DMAP (3.05 g, 25 mmol) in dichloromethane (400 mL) cooled to 0 °C in an ice−water bath. The mixture was allowed to warm to 25 °C over 16 h, then filtered through silica gel (approximately 1 L) using 10% ethyl acetate−hexanes (~4 L) as the eluent. The combined filtrates were concentrated to yield **90** as a yellow oil (132 g, quant). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35 (d,

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J = 2.4 Hz, 1H), 7.29 (dd, J = 8.5, 2.4 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 3.85 (s, 3H), 1.30 (s, 9H), 1.29 (s, 9H).

Carbonic Acid 2,4-Di-*tert*-butyl-5-nitrophenyl Ester Methyl Ester (91) and Carbonic Acid 2,4-Di-*tert*-butyl-6-nitrophenyl Ester Methyl Ester (92). To a stirring mixture of carbonic acid 2,4-di-*tert*-butylphenyl ester methyl ester (**90**) (4.76 g, 18 mmol) in concentrated H_2SO_4 (2 mL), cooled in an ice−water bath, was added a cooled mixture of H_2SO_4 (2 mL) and nitric acid (2 mL). The addition was done slowly so that the reaction temperature did not exceed 50 °C. The mixture was allowed to warm to 25 °C over a period of 2 h. The reaction mixture was then poured into ice−water and extracted into diethyl ether. The ether layer was dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (0−10% ethyl acetate−hexanes) to yield a mixture of carbonic acid 2,4-di-*tert*-butyl-5-nitrophenyl ester methyl ester (**91**) and carbonic acid 2,4-di-*tert*-butyl-6-nitrophenyl ester methyl ester (**92**) as a pale yellow solid (4.3 g), which was used directly in the next step without further purification.

5-Nitro-2,4-di-*tert*-butylphenol (94). The mixture of carbonic acid 2,4-di-*tert*-butyl-5-nitrophenyl ester methyl ester (**91**) and carbonic acid 2,4-di-*tert*-butyl-6-nitrophenyl ester methyl ester (**92**) (4.2 g, 12.9 mmol) was dissolved in methanol (65 mL), and KOH (2.0 g, 36 mmol) was added. The mixture was stirred at 25 °C for 2 h. The reaction mixture was then made acidic (pH 2−3) by adding concentrated HCl and partitioned between water and diethyl ether. The ether layer was dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (0−5% ethyl acetate−hexanes) to provide **93** (1.31 g, 29% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 7.34 (s, 1H), 6.83 (s, 1H), 1.36 (s, 9H), 1.30 (s, 9H).

5-Amino-2,4-di-*tert*-butylphenol (94). To a solution of **93** (1.86 g, 7.4 mmol) and ammonium formate (1.86 g) in ethanol (75 mL) was added 5% Pd/C (0.9 g). The reaction mixture was heated at reflux for 2 h, cooled to 25 °C, and filtered through a plug of Celite. The Celite was washed with methanol and the filtrate was concentrated to yield **94** as a gray solid (1.66 g, quant). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 6.84 (s, 1H), 6.08 (s, 1H), 4.39 (s, 2H, NH_2), 1.27 (m, 18H).

***N*-(2,4-Di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (48).** A mixture of **49** (0.14 g, 0.72 mmol), **95** (0.16 g, 0.72 mmol), triethylamine (2.0 mL, 14.4 mmol), and HATU (0.27 g, 0.72 mmol) in dichloromethane (0.5 mL) was stirred at 25 °C for 12 h. The solvent was evaporated, and the crude residue was diluted with H_2O and extracted twice with dichloromethane. The combined organic extracts were washed with 1 N HCl, a saturated solution of $NaHCO_3$ and dried over $MgSO_4$. After the removal of solvent, the residue was purified by silica gel chromatography (25−75% ethyl acetate−hexane) to yield **48** (0.2 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 11.82 (s, 1H), 9.20 (s, 1H), 8.87 (s, 1H), 8.33 (dd, J = 8.2, 1.0 Hz, 1H), 7.84−7.78 (m, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.56−7.45 (m, 1H), 7.17 (s, 1H), 7.10 (s, 1H), 1.38 (s, 9H), 1.37 (s, 9H). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{24}H_{28}N_2O_3$, 393.2178; found, 393.2164.

Biology. NIH-3T3 mouse fibroblasts stably expressing human F508del-CFTR (Michael J. Welsh, University of Iowa College of Medicine, Iowa City, IA) were cultured as previously described. All cells were maintained at 37 °C, unless otherwise indicated, in HyQ CCM5 (HyClone, Logan, UT) with 1% heat inactivated FBS.

Whole lungs provided by the National Disease Research Interchange (Philadelphia, PA) were obtained from CF subjects following autopsy or from subjects undergoing lung transplantation. After removal, the intact lung was packed in PBS and shipped on wet ice. The tissue was received and processed within 24 h after autopsy or surgery. CF airway epithelia were isolated from bronchial tissue, cultured as previously described,[42] and plated onto Costar Snapwell filters that were precoated with NIH3T3-conditioned medium. After 4 days the apical medium was removed and the cells were grown at an air−liquid interface for >14 days prior to use. This resulted in a monolayer of fully differentiated columnar cells that were ciliated.

Membrane Potential Optical Assay for Detecting F508del-CFTR Potentiator Activity.[8,11] To identify potentiators of F508del-CFTR, an HTS assay format utilizing fluorescent voltage sensing probes was developed using a FLIPR III (Molecular Devices Inc.) fluorescence plate reader. NIH-3T3 cells stably expressing F508del-CFTR were incubated for 16−24 h at 27 °C to correct the misfolded F508del-CFTR. Cells are then washed with a bath solution (160 mM NaCl, 4.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4 with NaOH) and treated with fluorescent voltage sensing dyes combined with test compounds (or DMSO vehicle control) for 30 min at room temperature. The assay is run on FLIPR III using a single liquid addition step of Cl^- free bath solution containing forskolin. Detected changes in membrane potential are due to the potentiator activity of test compounds on Cl^- anion flux through F508del-CFTR.

Ussing Chamber Recordings. All cells were grown on Costar Snapwell cell culture inserts maintained at 37 °C, unless otherwise indicated, prior to recording. The cell culture inserts were mounted into an Ussing chamber (VCC MC8; Physiologic Instruments, Inc., San Diego, CA) to record ISC in the voltage-clamp mode (V_{hold} = 0 mV). For measurement of ISC, the basolateral bath solution contained the following (in mM): 135 NaCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 2.4 K_2HPO_4, 0.6 KH_2PO_4, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 10 dextrose (titrated to pH 7.4 with NaOH). The apical NaCl was replaced by equimolar Na^+ gluconate (titrated to pH 7.4 with NaOH). For HBE cells, the ISC was measured in the presence of a basolateral to apical Cl^- gradient. The normal Cl^- solution contained the following (in mM): 145 NaCl, 0.83 K_2HPO_4, 3.3 KH_2PO_4, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, 10 HEPES (pH adjusted to 7.35 with NaOH). The low Cl^- solution contained the following (in mM): 145 Na gluconate, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, 10 HEPES (pH adjusted to 7.35 with NaOH). The ISCs were digitally acquired using Acquire and Analyze software (version 2; Physiologic Instruments, Inc., San Diego, CA).

cAMP Measurements. The total cAMP concentration (cellular and secreted) in FRT cells following test compound application was determined using a cAMP-Screen 96-well immunoassay system according the manufactures directions (Applied Biosystems, catalog number T1502). Briefly, FRT cells were incubated for 15 min with test compound and then lysed and transferred to a 96-well assay plate provided with the kit. The plate was incubated at room temperature for 1 h after which it was developed and luminescence emission was measured using the Acquest 384.1536 by LJL Biosystems. The cAMP concentrations were determined using a cAMP standard curve present in each plate.

In Vivo Pharmacokinetic Experiments. Male mouse, Sprague−Dawley rats, beagle dog, and cynomolgus monkeys (n = 3/group) were administered a single iv dose of compound formulated in dimethyl isosorbide/ethanol/PEG400/5% dextrose in water (D5W) (10%/15%/35%/40%) at the nominal dose indicated in a dose volume of 1 mL/kg. Blood samples (0.3 mL, sodium heparin anticoagulant) were collected from an indwelling carotid cannula at the following nominal time points: at predose, 5, 15, 30, and 45 min and 1, 2, 4, 6, 8, 12, 24, 36, and 48 h following iv administration and at predose, 0.25, 0.50, 1, 1.5, 2, 4, 8, 12, and 24 h following oral administration. The concentration of compound in the plasma samples was determined with a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method, which had a lowest limit of quantitation (LLOQ) of 1 ng/mL and a linearity range between 1 and 2500 ng/mL. The mean plasma concentration−time profiles and the measured dose values were used to estimate the pharmacokinetic parameters using noncompartmental analysis modules in WinNonlin Professional Edition software, version 4.0.1 (Pharsight Corporation, Mountain View, CA).

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures for compounds **27**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

$AUC_{0-\infty}$, area under the plasma concentration−time curve from zero to infinite time; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CL, clearance; F508del, deletion of Phe 508; G551D, mutation of Gly 551 into Asp; $GABA_A$, class A of the γ-aminobutyric acid receptors; HBE, human bronchial epithelia; ISC, short-circuit current; LLOQ, lowest limit of quantitation; NIH-3T3, National Institutes of Health 3T3 cells; P_o, open probability; V_{ss}, steady state volume of distribution

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(15) When compound **1** is drawn as the quinolin-4-ol, the cLogP is calculated to be 5.2, more than 2 log units higher than that of the corresponding quinolin-4(1*H*)-one. Experimental log *P* values were determined for compounds **16** and **45**. Experimental determinations were closer to the quinolin-4-ol value than the corresponding quinolin-4(1*H*)-one value. See the table below.

compd	cLogP, quinolin-4-ol	cLogP, quinolin-4(1*H*)-one	exptl log *P*
16	4.6	1.6	3.2
45	5.6	2.6	4.8

(16) Compounds showing less that 50% activity at 30 μM were not tested in a dose−response format; their activity is expressed as a percent response at that concentration.

(17) For additional information on the $GABA_A$ binding assay at Ricerca Biosciences, see www.ricerca.com. Percent activity at 10 μM was also measured for compounds **36** (47%), **45** (52%), and **48** (18%).

(18) Measured thermodynamic solubility of compound **16**: water, not detectable; ethanol, 1.2 mg/mL; miglyol 810, 0.057 mg/mL)

(19) Torsional potentials were evaluated using internally developed software that utilizes the Szybki Toolkit from OpenEye Scientific Software [OpenEye Scientific Software, Santa Fe, NM; http://www.eyesopen.com/products/applications/szybki.html]. Potentials were evaluated by restraining the torsion angle in question at 5° increments and energy minimizing the remainder of the molecule using the MMFF force field as reported by the following: Halgren, T. A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490−519. Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720−729.

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