Discovery and Optimization of a Novel Series of *N*-Arylamide Oxadiazoles as Potent, Highly Selective and Orally Bioavailable Cannabinoid Receptor 2 (CB_2) Agonists

Yuan Cheng,*[,†] Brian K. Albrecht,[§] James Brown,[†] John L. Buchanan,[§] William H. Buckner,[§] Erin F. DiMauro,[§] Renee Emkey,[⊥] Robert T. Fremeau, Jr.,[||] Jean-Christophe Harmange,[§] Beth J. Hoffman,[||] Liyue Huang,[#] Ming Huang,[||] Josie Han Lee,[⊥] Fen-Fen Lin,[▽] Matthew W. Martin,[§] Hung Q. Nguyen,[▽] Vinod F. Patel,[§] Susan A. Tomlinson,[§] Ryan D. White,[§] Xiaoyang Xia,[‡] and Stephen A. Hitchcock[†]

Chemistry Research and Discovery, Department of Molecular Structure, Department of Neuroscience, Department of Protein Science, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, Chemistry Research and Discovery, Department of HTS and Molecular Pharmocology, Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., 1 Kendall Square Building 1000, Cambridge, Massachusetts 02139

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The CB_2 receptor is an attractive therapeutic target for analgesic and anti-inflammatory agents. Herein we describe the discovery of a novel class of oxadiazole derivatives from which potent and selective CB_2 agonist leads were developed. Initial hit **7** was identified from a cannabinoid target-biased library generated by virtual screening of sample collections using a pharmacophore model in combination with a series of physicochemical filters. **7** was demonstrated to be a selective CB_2 agonist (CB_2 EC_{50} = 93 nM, E_{max} = 98%, CB_1 EC_{50} > 10 μM). However, this compound exhibited poor solubility and relatively high clearance in rat, resulting in low oral bioavailability. In this paper, we report detailed SAR studies on **7** en route toward improving potency, physicochemical properties, and solubility. This effort resulted in identification of **63** that is a potent and selective agonist at CB_2 (EC_{50} = 2 nM, E_{max} = 110%) with excellent pharmacokinetic properties.

Introduction

Two distinct receptors have been identified that mediate the biologic effect of plant-derived, synthetic, and endogenous cannabinoids, the CB_1[1] and CB_2[2] receptors. These receptors are composed of seven transmembrane proteins that couple to inhibitory G_i/G_o proteins and thus inhibit adenylate cyclase and activate MAP kinases. The CB_1 and CB_2 receptors share 44% amino acid sequence identity and exhibit different pharmacologic profiles and distinct tissue expression patterns.[2] The CB_1 receptor is highly expressed in the central nervous system (CNS),[a] where it is primarily localized on axons and nerve terminals.[3] Activation of presynaptic CB_1 receptors inhibits neurotransmitter release and produces a characteristic tetrad of behavioral effects including hypomotility, hypothermia, catalepsy, and acute analgesia. It is likely that Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**, Figure 1), the active ingredient in marijuana produces most, if not all of its psychoactive behavioral effects through action of the CB_1 receptor.[4] In contrast, the CB_2 receptor is primarily expressed by immune cells and tissues.[5] Activation of the CB_2 receptor is thought to mediate the immunosuppressive effects of cannabinoids. It has been recently reported that CB_2

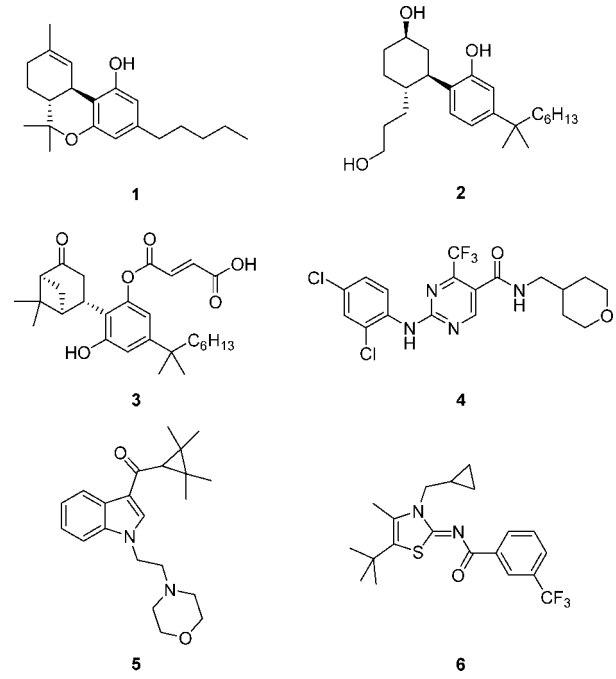

Figure 1. Structure of **1** (Δ^9-THC), **2** (CP-55,940),[11] **3** (Cannabinor),[12] **4** (GW842166X),[13] **5** (A-796260),[14] and **6**.

* To whom correspondence should be addressed. Phone: 805-447-3543. Fax: 805-480-3016. E-mail:yuanc@amgen.com.

† Chemistry Research and Discovery, Amgen Inc., Thousand Oaks, CA.

‡ Department of Molecular Structure, Amgen Inc., Thousand Oaks, CA.

§ Chemistry Research and Discovery, Amgen Inc., Cambridge, MA.

|| Department of Neuroscience, Amgen Inc., Thousand Oaks, CA.

⊥ Department of HTS and Molecular Pharmocology, Amgen Inc., Cambridge, MA.

Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., Cambridge, MA.

▽ Department of Protein Science, Amgen Inc., Thousand Oaks, CA.

[a] Abbreviations: CB_2, cannabinoid receptor 2; CB_1, cannabinoid receptor 1; SAR, structure−activity relationship; FLAME, fexibly align molecules; MAP kinases, mitogen-activated protein kinases; CNS, central nervous system; Δ^9-THC, Δ^9-tetrahydrocannabinol; HLM, human liver microsomes; RLM, rat liver microsomes; *F*, bioavailability; CL, in vivo clearance; AUC, the area under the curve; C_{max}, the maximum concentration of a compound observed after its administration; SIF, simulated intestinal fluid solubility; ACD-SC, available chemicals directory-screening compounds; PSA, polar surface area; DMF, *N*,*N*-dimethylformamide; DMA, *N*,*N*-dimethylacetamide; EtOAc, ethyl acetate; NMP, *N*-methylpyrrolidone, EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, 1-hydroxylbenzotrizole; HATU, 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium; DMSO, dimethyl sulfoxide; PS-carbodiimide, *N*-cyclohexylcarbodiimide-*N*′-propyloxymethylpolystyrene; MP-carbonate, macroporous triethylammonium methylpolystyrene carbonate; Ph_3P, triphenylphosphine; cAMP, cyclic adenosine monophosphate; GTP, guanosine-5′-triphosphate.

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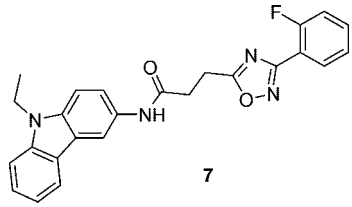


7

Figure 2. Initial lead structure as CB2 agonist.

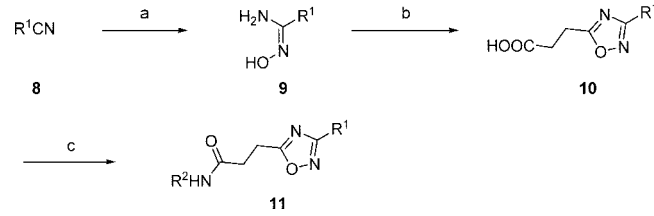
is also found within the CNS.[6] Given the predominant peripheral distribution of the CB_2 receptor, we hypothesized that selective activation of CB_2 should be devoid of undesired psychoactive effects that are believed to be mediated through central CB_1.

The role of CB_1 and CB_2 in the transduction and perception of pain is supported by an emerging body of evidence.[7] The role of CB_1 receptors in pain response is more thoroughly understood than that of CB_2, however CB_1 agonists typically illicit problematic psychotropic effects and are consequently hampered by the potential for abuse.[3,4] Since its identification and cloning in 1993, understanding of the pharmacology associated with the CB_2 receptor subtype continues to evolve. The expression pattern of CB_2 receptors on cells and in tissues that modulate the immune system renders CB_2 an attractive potential therapeutic target for analgesic and anti-inflammatory agents. Recent studies indicate that the CB_2 receptor could be involved in the modulation of inflammation,[8] neuropathic, and inflammatory pain.[9] Multiple CB_2 agonists of different structural classes have been reported to reduce inflammatory and neuropathic pain. In addition, CB_2 agonists have been reported to elicit anti-inflammatory effects and to promote functional recovery in animal models of multiple sclerosis.[10] These factors, as well as the need for safe and effective anti-inflammatory agents and analgesics, make the CB_2 receptor an attractive target. The development of highly selective CB_2 agonists with desirable physical and pharmacokinetic properties is necessary for both understanding the biological role of the CB_2 receptors and evaluation of their therapeutic potential.

In recent years, there has been growing interest from a number of groups focused on developing selective CB_2 agonists.[9] However, only a few compounds are known in the literature to be selective for the CB_2 receptor (**3**−**6**, Figure 1).[9,11−15] For example, compound **6** was recently disclosed to be potent (CB_2 K_i = 13 nM) and selective (CB_1 K_i = 3500 nM).[15] However there are even fewer examples of CB_2 agonists that have acceptable oral bioavailability (**4**, formulation dependent F = 25−60% in rats and dogs; **6**, F = 52% in rats).[13,15] Cannabinoid ligands are typically highly lipophilic, often leading to low aqueous solubility and poor physicochemical properties. Therefore, design of selective cannabinoid agonists that have good oral bioavailability and retain high affinity remains a major challenge.

Our efforts to identify effective CB_2 agonists have revealed a novel class of aryl oxadiazoles that are potent and highly selective. Our program began with the generation of a pharmacophore model, based on known cannabinoid ligands, which was used to conduct a virtual screen of our in-house and commercial compound databases. This method led to a cannabinoid target-biased library, which was then screened in both CB_1 and CB_2 binding assays. Hits showing IC_{50} < 10 μM at CB_2 and >10-fold selectivity over CB_1 were evaluated for their agonist activity in functional assays. Those that demonstrated selective CB_2 functional agonist activity were selected for their physicochemical and pharmacokinetic profiles. This line of investigation led to identification of oxadiazole **7** (Figure 2) as a full CB_2 agonist (CB_2 EC_{50} = 93 nM, E_{max} = 98%) with high selectivity over the CB_1 receptor (CB_1 EC_{50} > 10 μM). Full agonist activity was defined as E_{max} equal to 100% efficacy of **2**, a known agonist at CB_1 and CB_2 receptor.[11] Broader selectivity of compound **7** for the CB_2 receptor was further demonstrated by absence of activity when screened against a panel of 141 receptors/enzymes/ion channels. Compound **7** showed moderate microsomal stability (HLM = 274 μL/min/mg and RLM = 198 μL/min/mg) and moderate in vivo clearance (CL = 1.2 L/h/kg, iv dose of 0.5 mg/kg in rats).

Scheme 1. Synthesis of Oadiazole Aide Drivatives[a]



[a] Reagents and conditions: (a) NH_2OH, MeOH, reflux; (b) succinimide, DMF, 120 °C; (c) EDC, CH_2Cl_2, R^2NH_2, 0−25 °C; or thionyl chloride, then R^2NH_2, *N*,*N*-diisopropylethylamine, CH_2Cl_2.

However, compound **7** suffered from poor solubility (simulated intestinal fluid solubility, SIF = 2 μg/mL) and low and variable oral bioavailability (F = 13−24% in rats). Overall the results indicated that compound **7** represented a promising profile as a lead compound. We therefore carried out SAR studies on this compound, particularly focused on improving solubility and physicochemical properties. Herein we describe the discovery and systematic SAR investigation on the oxadiazole structural class that has revealed CB_2 agonists that exhibit subnanomolar activity and excellent oral bioavailability.

Chemistry. The representative synthesis of oxadiazole amide analogues is shown in Scheme 1. Commercially available or readily prepared nitriles **8** were treated with hydroxylamine in methanol to give amide oxime **9**, which upon heating with succinimide in DMF gave oxadiazole acid **10**.[16] The acid was subjected to standard coupling under standard conditions with commercially available or readily made amines to afford formal target **11**.

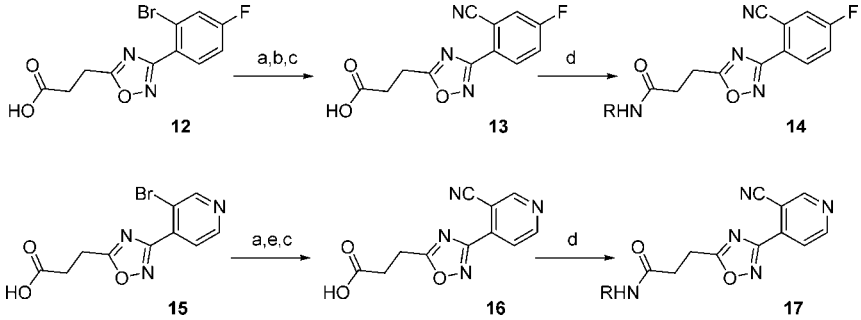
Specifically, 2-cyano-4-fluoro-phenyl **14** and 2-cyano-4-pyridyl **17** derivatives were prepared as illustrated in Scheme 2. Protection of the carboxylic acid functionality was necessary to allow convertion of the bromide to the correspondent nitrile by heating with copper(I) cyanide under microwave conditions. Hydrolysis to acids (**13** and **16**) and coupling with the appropriate amines gave **14** and **17**.

As part of our SAR investigation, we replaced the amide linker with ether and amine linkers using the sequence described in Scheme 3. Reduction of the ester **18** to alcohol **19** and Mitsunobu reaction or converting the alcohol to bromide followed by displacement with the appropriate alcohols afforded the ether-linked analogues **20**. Alternatively, half-reduction of ester **18** could also provide aldehyde **21**, which underwent reductive amination with various amines to produce oxadiazole amine analogues **22**.

Intermediate nicotinonitrile **26**, used in the preparation of **56** and **60**, was synthesized as described in Scheme 4. Displacement of the chloride in **23** with trifluoroethanol gave intermediate **24**. Subsequent conversion of bromide in **24** to the nitrile group followed by selective reduction of the nitro group to amine yielded **26**.

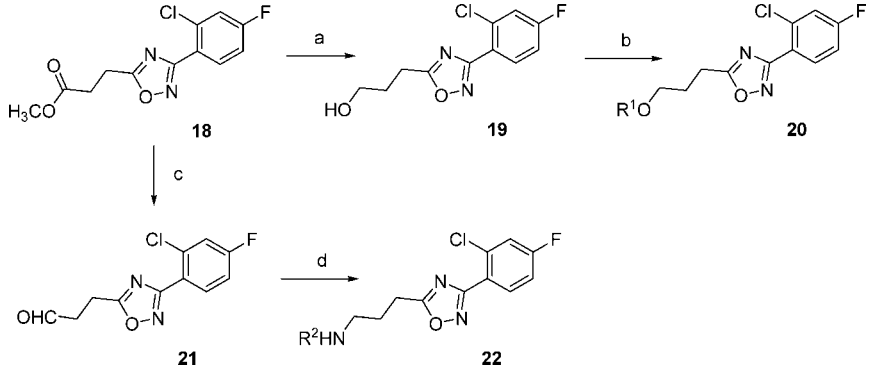
Intermediates 6-chloroquinolin-3-amine for **47** and **63**, 6-trifluoromethoxyquinolin-3-amine for **64**, and 6-chloro-1,8-naph-

Scheme 2. Synthesis of 2-Cyano-4-fluoro-phenyl and 2-Cyano-4-pyridyl Derivatives[a]



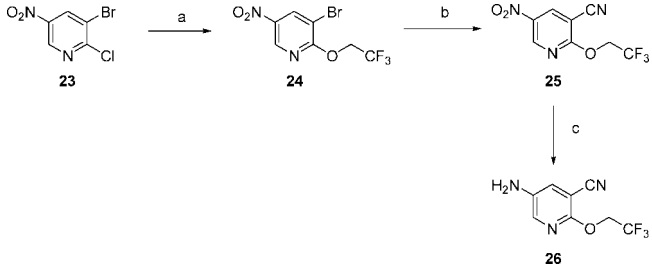
[a] Reagents and conditions: (a) trimethylsilyldiazomethane, toluene/methanol (9:1), rt; (b) KCN, CuCl, DMA, 130 °C; (c) LiOH, methanol or DME, rt; (d) EDC, CH_2Cl_2, RNH_2, 0−25 °C. (e) CuCN, DMF, microwave, 180 °C.

Scheme 3. Synthesis of Oxadiazole Ether and Amine Analogues[a]



[a] Reagents and conditions: (a) diisobutylaluminum hydride, dichloromethane, −78−0 °C; (b) R^1OH, Ph_3P, diisopropyl azodicarboxylate, benzene, 25 °C, or Br_2, THF, then R^1OH, K_2CO_3, DMF, 60 °C; (c) diisobutylaluminum hydride, dichloromethane, −78 °C; (d) titanium isopropoxide, R^2NH_2, $NaBH_4$.

Scheme 4. Synthesis of 5-Amino-2-(2,2,2-trifluoroethoxy)nicotinonitrile[a]



[a] Reagents and conditions: (a) Sodium hydride, 2,2,2-trifluoroethanol, 0−25 °C; (b) copper(I) cyanide, *N*-methylpyrrolidone, microwave, 195 °C; (c) iron, acetic acid, H_2O, ethanol, reflux.

thyridin-3-amine for **48** were prepared by cyclization of methazonic acid with correspondent 2-amino-5-chlorobenzaldehyde, 2-amino-5-(trifluoromethoxy)benzaldehyde, and 2-amino-5-chloropyridine-3-carboxaldehyde, respectively, under acidic conditions. Intermediate 6-chloroquinolin-3-ol for **67** was synthesized by diazotizing 6-chloroquinolin-3-amine with sodium nitrite, and the diazonium compound was decomposed to 6-chloroquinolin-3-ol (see Experimental Section). Compounds (**30**−**36**, Table 1) with alternative heterocycles were prepared using known procedures to form the heterocycle cores (see Experimental Section).

Results and Discussion

FLAME Model and Screening. The cannabinoid pharmacophore model was designed by employing three known cannabinoid ligands, **27** (WIN 55,212-2),[17] 1,8-naphthyridin-4(1*H*)-on-3-carboxamide **28**,[18] and indolopyridone **29**,[19] (Figure 3) using an in-house developed software (FLAME, flexibly align molecules).[20] These ligands have high affinity for the CB_2 receptor but limited selectivity over CB_1. The FLAME program aligns three molecules by first finding maximum common pharmacophores among them using a generic algorithm. The resulting alignments were then subjected to simultaneous optimizations of their internal energies and given an alignment score. Figure 3 shows the 3-feature pharmacophore model generated by the three cannabinoid ligands. The best alignment was then used to search the in-house sample collection (363416 compounds) and commercial compound databases (prefiltered ACD-SC database by MRL, 778296 compounds). Top 1000 scored compounds from each database search were extracted and were further refined by applying physicochemical filters, such as calculated logP (ClogP) < 5, polar surface area (PSA) < 110 Å^2, number of rotatable bonds ≤ 10, and hydrogen bond donors ≤ 2. This exercise led to a cannabinoid target-biased library with 1092 compounds. After screening this library in CB_1 and CB_2 binding assays, 74 compounds met the criteria of CB_2 binding affinity less than 10 μM with greater than 10-fold selectivity over the CB_1 receptor. These compounds were next tested in GTP-Eu binding functional assays for CB_2 and CB_1 receptors, respectively. Eighteen compounds were confirmed as full CB_2 agonists with EC_{50} values less than 1 μM and greater than 25-fold selectivity over the CB_1 receptor. The structural diversity of the resulting hits compared to the three original ligands used the build the pharmacophore model, demonstrates the utility of the FLAME model to generate novel starting points. Figure 4 shows the alignments of compound **7** and the pharmacophore model.

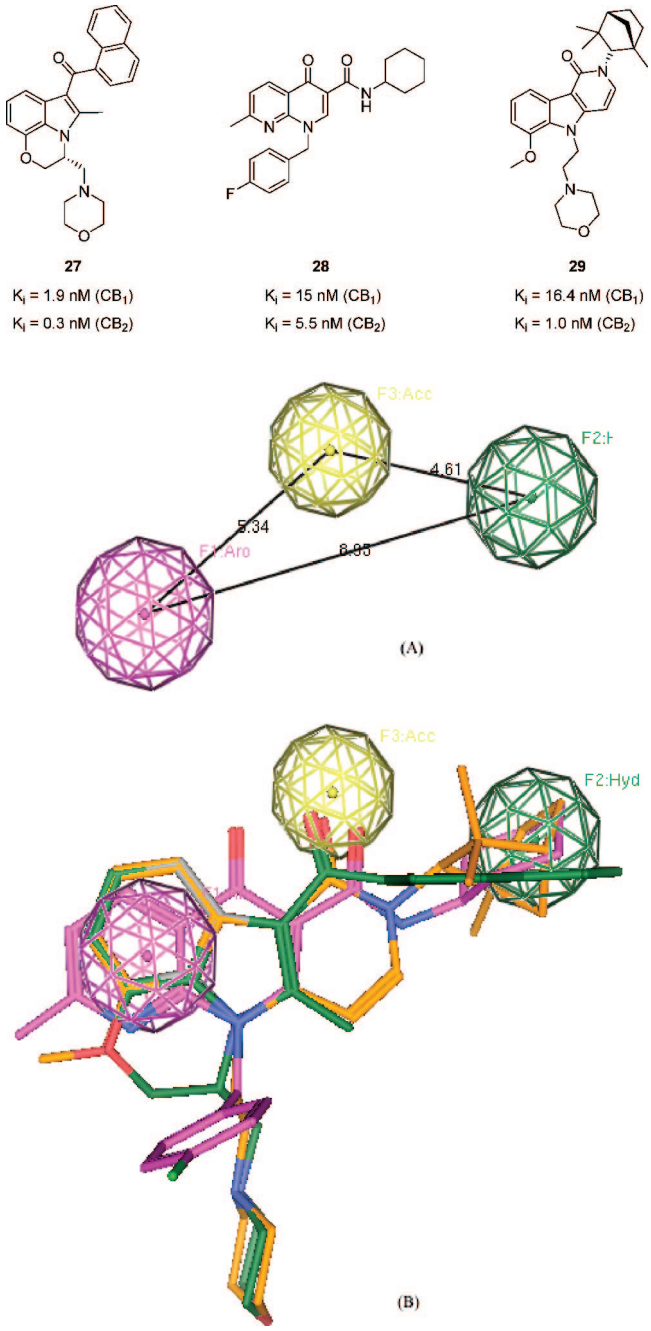


Figure 3. The pharmacophore model generated by canabinoid ligands, **27** in green, **28** in magenta, and **29** in orange. (A) Geometrical relationships among the pharmacophore features. F1 in magenta, aromatic; F2 in green, hydrophobic; F3 in yellow, hydrogen bond acceptor. The distances (in Å) among the centers of the features are labeled. (B) Mapping of **27**, **28**, and **29** to the pharmacophore model.

We confirmed the structures of 18 lead compounds and evaluated their physicochemical properties, solubility, melting point, permeability, rat (RLM) and human (HLM) microsomal stability, and broader selectivity profile with pharmacology screening. RLM and HLM were used as a filter to select compounds for in vivo pharmacokinetic studies in rats. In vivo clearance was determined by intravenous (iv) administration in rats with compounds that had HLM/RLM less than 300 μL/min/mg. This investigation allowed us to prioritize oxadiazole **7** that showed in vivo clearance less than 2 L/h/kg. Although we derived compound **7** from three relatively nonselective CB_2 ligands, it not only showed good agonist activity on the CB_2 receptor but also displayed excellent selectivity over CB_1. This lead identification process demonstrated that combining pharmacophore model screening and physicochemical filters could provide a powerful and efficient tool for generating high quality leads.

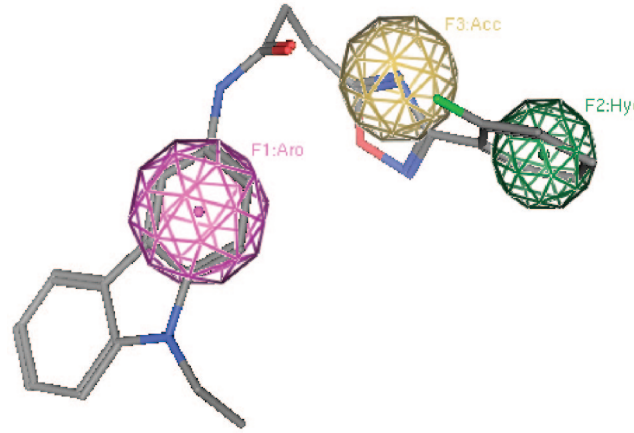


Figure 4. Mapping of compound **7** to the pharmacophore model. F1 in magenta, aromatic; F2 in green, hydrophobic; F3 in yellow, hydrogen bond acceptor.

Pharmacology. The CB receptor activity and selectivity of the compounds reported in this paper were evaluated in two functional assays, a GTP binding assay and a cAMP assay. Membranes prepared from cells expressing either human CB_1 or CB_2 receptor were used in the GTP binding assay. This is a proximal assay that measures activation of G-protein coupled receptors by the ability of the associated G_α-protein to bind GTP. Compound **2** (Figure 1) was used to define 100% efficacy (Figure 5).

Compounds that exhibited full agonism with good potency in the GTP binding assay were progressed for evaluation in the cell-based cAMP assay. This assay monitors a distal signaling event that emanates from the CB_1 and CB_2 receptors. Activation of CB_1 and CB_2 receptors results in inhibition of adenylate cyclase. Compounds were assessed for their ability to inhibit forskolin-induced increase in intracellular cAMP. Species difference in agonist activity was also investigated in this assay by using cells expressing human CB_2 receptor, human CB_1 receptor, rat CB_2 receptor, and rat CB_1 receptor. Although the CB_2 receptor exhibits 80% sequence identity between human and rodent,[21] the analogues of the oxadiazole class showed consistent activity in both human and rat CB_2 receptor (Tables 1−4).

SAR Studies. We first examined the oxadiazole core of **7** by replacing it with a number of alternative heterocycles shown in Table 1. Removing either one of the nitrogens of the oxadiazole, giving isoxazole **30** and oxazole **31**, resulted in a drop in activity (over 2-fold and 17-fold, respectively) and a decrease in efficacy (E_{max}). Pyrazole **32** and methylated pyrazole **33** showed no CB_2 agonist activity up to 10 μM, while triazole **34** also registered as a 0.5 μM partial agonist of CB_2. Although the isomeric oxadiazole **35** and tetrazole **36** were similar and displayed comparable potency and efficacy to compound **7**, none had better potency than compound **7**, suggesting that the oxadiazole was optimal.

Next, we investigated modification of the aryl group attached to the oxadizole (Table 2). The SAR proved very sensitive in this region of the molecule. Removing the 2-fluoro substituent, as with compound **37**, caused a reduction in activity. Replacement of the fluorine atom with chlorine (**38**) increased the

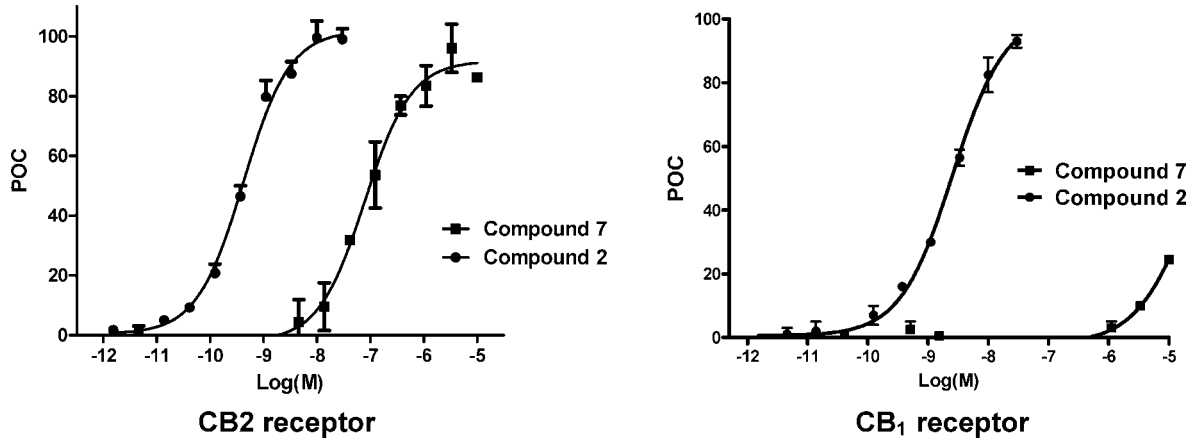
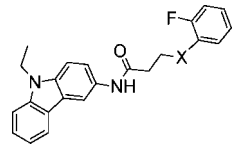


Figure 5. Compounds **2** and **7** in the GTP binding assay for CB_2 and CB_1.

potency over 18-fold without loss of efficacy, but larger *ortho* substituents such as isopropyl **39** were not well tolerated. Moving the chlorine atom to the *meta*-position resulted in compound **40** with a marked reduction in activity. However, fluoro substitution at the *para*-position **41** provided good potency. Combining both *ortho* and *para* substituents proved very productive, delivering compounds **42**−**44** that possessed excellent CB_2 activity with low nanomolar and picomolar CB_2 potency. Indeed, compound **43** exhibited an EC_{50} value of 89 pM in the GTP-Eu binding assay. Replacement of the phenyl group by 4-pyridyl **45** was well tolerated. In addition, saturated ring systems could also be tolerated. 4-Pyran **46**, for example, showed better potency than the corresponding phenyl analogue **37**. Gratifyingly, the excellent potency and selectivity of analogues **41**−**46** was consistent in the cAMP assay against cells expressing rat or human CB_2 receptors.

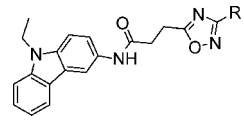
Table 1. Functional Activity for Oxadiazole Replacements

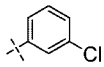
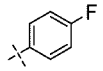
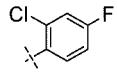
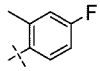
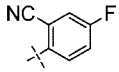
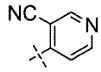


Compound	X	GTP binding				cAMP (human)				cAMP (rat)			
		CB_2		CB_1		CB_2		CB_1		CB_2		CB_1	
		EC_{50}[a] (nM)	E_{max}[b] (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
CP 55,940		0.43		2.28		0.88		0.26		0.34		0.16	
7		110	100	>10000	ND[c]	140	93	>1000	ND	33	91	>1000	ND
30		270	64	>10000	ND	ND		ND		ND		ND	
31		2000	46	>10000	ND	ND		ND		ND		ND	
32		>10000	ND	>10000	ND	ND		ND		ND		ND	
33		>10000	ND	>10000	ND	ND		ND		ND		ND	
34		470	15	>10000	ND	ND		ND		ND		ND	
35		190	76	>10000	ND	ND		ND		ND		ND	
36		200	93	>10000	ND	690	88	>1000	ND	97	90	>1000	ND

[a] The results are expressed as the means ± SEM for $n = 2-20$ independent measurements and were calculated in Prism by use of a logistic fit. [b] E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [c] ND = not determined.

Table 2. Functional Activity for Right Hand Side Variant Analogues



Compound	R	GTP binding				cAMP (human)				cAMP (rat)			
		CB_2		CB_1		CB_2		CB_1		CB_2		CB_1	
		EC_{50}[a] (nM)	E_{max}[b] (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
37		150	100	>10000	ND[c]	ND		ND		ND		ND	
38		6.0	100	>10000	ND	ND		ND		ND		ND	
39		670	88	>10000	ND	ND		ND		ND		ND	
40		410	87	>10000	ND	ND		ND		ND		ND	
41		9.8	95	1200	55	4.8	95	330	24	3.2	90	650	65
42		1.8	98	1200	65	0.6	100	>1000	20	0.4	98	330	79
43		0.089	120	1400	24	0.47	100	>1000	ND	0.27	110	280	81
44		0.85	120	709	39	0.33	110	>1000	12	0.13	130	240	84
45		3.8	110	>10000	ND	2.6	100	>1000	ND	1.3	110	>1000	ND
46		42	94	>10000	ND	2.4	98	>1000	23	0.38	100	150	110

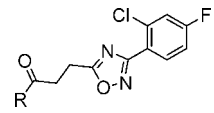
[a] The results are expressed as the mean of at least two determinations ± SEM and were calculated in Prism by use of a logistic fit. [b] E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [c] ND = not determined.

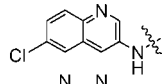
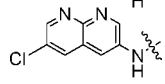
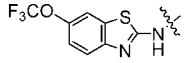
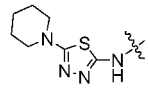
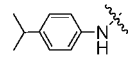
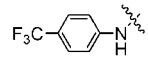
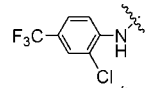
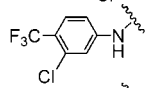
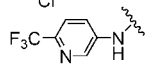
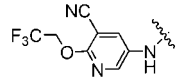
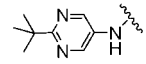
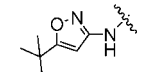
Using optimal aryl oxadiazoles, attention shifted to exploring the carbazole region of the lead molecule **7**. With a mindset to improve solubility and physicochemical properties of **7** by lowering molecular weight and decreasing hydrophobicity and the precedent for mutagenic potential among certain carbazole containing compounds,[22] we elected to search for carbazole replacements. The results of this endeavor are shown in Table 3. This region of the molecule appears to be fairly accommodating, accepting a variety of aromatic ring systems such as quinoline **47**, naphthyridine **48**, benzothiazole **49**, and thiadiazole **50**, all of which showed better potency and selectivity than **7**. In addition, they all demonstrated improved metabolic stability in human and rat liver microsomes (HLM/RLM < 100 μL/min/mg). However, these compounds **47**−**50** showed little improvement in solubility and oral bioavailability relative to **7**. A further reduction in hydrophobicity relative to **7** via replacement of the carbazole unit with a smaller isopropylphenyl group **51** demonstrated an impressive 10-fold increase in potency as compared to the lead compound **7**. Expanded SAR studies around additional aryl groups showed that *para*-substituted phenyl ring was important for CB_2 potency. Adding a chlorine atom at the *ortho*-position **53** led to 5-fold decrease in activity. On the other hand, chlorine substitution at the *meta*-position **54** was equipotent to **52**. To introduce polarity, we prepared heteroaryl derivative 6-substituted pyridin-3-amines **55**, 2-substituted pyrimidin-5-amines **57**, and 5-substituted isoxazole-3-amine **58**. Although compounds **57** and **58** were potent, they displayed CB_1 agonism in the cAMP assay. Further investigation yielded 2-trifluoroethoxy-3-cyano-pyridin-5-amine analogue **56**, which showed low nanomolar potency and good selectivity in the GTP binding and functional cAMP experiments. Compound **56** is more soluble than compound **7** with SIF solubility of 24 μg/mL and demonstrated more favorable pharmacokinetic properties in rat relative to compound **1** (Table 5). On oral dosing **56**, >30-fold higher levels in AUC and >40-fold C_{max} compared to **7** were achieved at an equivalent dose. The oral bioavailability of compound **56** (36%) was also superior to **7**.

As our work progressed, hydrolysis of the amide bond was observed as a major clearance pathway in the in vivo experiments in rat. Acid **59** was the major circulating metabolite (Figure 6). To suppress this metabolic pathway, we sought to remedy this problem by preparing suitable nonamide-linked analogues.

To this end, a number of amine-linked compounds were examined as shown in Table 4. We found, in general, the amine-linked monocyclic analogues gave lower activity as compared to their amide counterparts (cf. **60** and **56**, **61** and **58**). The loss

Table 3. Functional Activity for Left Hand Side Variant Analogues

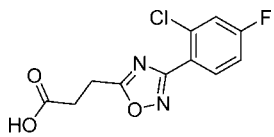


Compound	R	GTP binding				cAMP (human)				cAMP (rat)			
		CB_2		CB_1		CB_2		CB_1		CB_2		CB_1	
		EC_{50}[a] (nM)	E_{max}[b] (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
47		0.9	110	1400	82	0.5	99	710	57	11	97	130	99
48		3.3	110	2300	43	0.6	97	>1000	30	0.4	100	220	100
49		3.0	120	1900	36	6.5	99	>1000	36	6.9	98	>1000	21
50		1.2	98	211	47	0.8	98	>1000	38	1.1	98	220	87
51		10	130	2000	23	ND[c]		ND		ND		ND	
52		75	120	>10000	ND	38	94	>1000	ND	58	90	>1000	ND
53		370	41	>10000	ND	ND		ND		ND		ND	
54		62	120	>10000	ND	25	97	>1000	ND	79	100	>1000	ND
55		97	120	2800	17	78	96	>1000	ND	73	95	>1000	ND
56		14	110	>10000	ND	31	97	>1000	ND	3.1	100	>1000	ND
57		5.4	91	>10000	ND	2.9	97	>1000	ND	1.3	99	570	67
58		0.2	120	1600	103	0.7	96	>1000	48	0.4	100	170	110

[a] The results are expressed as the mean of at least two determinations ± SEM and were calculated in Prism by use of a logistic fit. [b] E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [c] ND = not determined.

in potency was also observed with the benzothiazole analogues such as compound **62**.

However, the amine-linked quinoline analogues exhibited a comparable potency and selectivity profile to corresponding amide analogues (cf. **63** and **47**). The 3-(trifluoromethoxy)quinolin-6-amine **64** was found to be one of the most potent analogues, with EC_{50} value at 15 pM in the GTP-Eu assay. Methylation of the amine (**66**) caused a large drop in potency. The amine-linked compounds also showed a significant improvement in solubility relative to amide congeners. As an example, SIF solubility of **63** and **64** was determined to be 87 and 49 μg/mL, respectively, an order of magnitude improved over compound **7**. Encouraged by these observations, we further evaluated the pharmacokinetic profile of several amine-linked analogues in rats of which compound **63** showed excellent in vivo properties (Table 5). The oral administration of **63** at a dose 10 mg/kg (po) in rats resulted in high plasma exposure (AUC at 43.1 μg h/mL), twice that of compound **56** and over 80-fold that of compound **7** at equivalent dose levels. Compound **63** also generated twice the C_{max} of **56** and over 90-fold that of **7**. The half-life of **63** was also improved over that of **56** and **7** (5.1, 3.1, and 1.6 h, respectively). More importantly, Compound **63** showed outstanding oral bioavailability (100%) in rats.

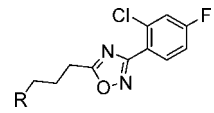


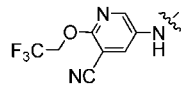
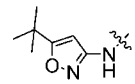
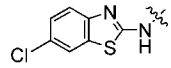
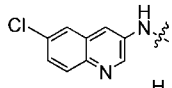
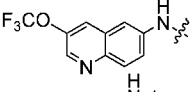
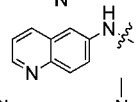
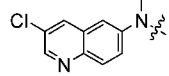
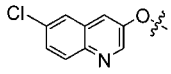
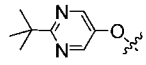
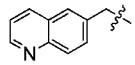
59

Figure 6. The acid metabolite of amide hydrolysis.

A number of ether-linked and carbon-linked analogues were also investigated. The ether-linked analogues, such as **67** and **68**, showed modestly lower but still respectable potency compared to amine-linked analogue **63** and amide-linked analogue **57**, respectively. However, solubility and the pharmacokinetic profile of these compounds were generally less desirable than the amine-linked analogues. Carbon-linked

Table 4. Functional Activity for Non-Amide Analogues



Compound	R	GTP binding				cAMP (human)				cAMP (rat)			
		CB_2		CB_1		CB_2		CB_1		CB_2		CB_1	
		EC_{50}[a] (nM)	E_{max}[b] (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
60		31	110	1300	24	360	87	>1000	ND[c]	130	85	>1000	ND
61		27	110	670	33	130	90	>1000	ND	190	82	>1000	ND
62		170	100	3300	16	110	87	>1000	25	290	66	>1000	45
63		1.7	110	220	51	2.2	94	560	88	1.3	90	77	85
64		0.015	110	1100	81	2.3	110	850	78	1.3	130	130	120
65		11	100	470	100	50	93	>1000	46	45	95	240	100
66		110	110	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
67		7.2	110	1100	92	3.6	99	990	48	5.1	99	83	98
68		4.9	110	610	99	24	91	1500	63	16	100	540	86
69		49	100	810	32	800	73	>1000	10	380	53	1600	28

[a] The results are expressed as the mean of at least two determinations ± SEM and were calculated in Prism by use of a logistic fit. [b] E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [c] ND = Not determined.

Table 5. Pharmacokinetics in Rats (dosed at 10 mpk)

compd	C_{max}(μg/mL)	AUC (μg h/mL)	F (%)	$t_{1/2}$ (h)
7	0.1	0.46	13	1.6
56	3.4	21	36	3.1
63	8.4	43	<100	5.1

analogues generally suffered from weaker CB_2 activity, such as compound **69** vs **65**.

Summary

Targeted library screening of a cannabinoid agonist-biased library and subsequent hit assessment has identified a novel class of potent and selective CB_2 agonists. SAR investigation of lead compound **7** showed that the oxadiazole core was essential and substitutions at the 2- and 4- position of the phenyl ring appended to the oxadiazole were important for desired CB_2 potency. Subsequent optimization involving replacement of the carbazole group revealed that a variety of aromatic groups, especially simple ring systems, such as phenyl and pyridyl rings, provided excellent CB_2 potency. This effort led to compounds with greatly improved physical properties and pharmacokinetics (i.e., **56**). Subsequently, we incorporated an amine linker in an effort suppress plasma accumulation of an acid metabolite resulting from amide hydrolysis. This study led to highly potent, selective CB_2 agonists, as exemplified by **63**, which displayed an excellent pharmacokinetic profile with 100% oral bioavailability in rats.

Experimental Section

FLAME Model and Screening. The pharmacophore model was designed using three known cannabinoid ligands selected from the literature using FLAME following this procedure: CONCORD was used to generate their three-dimensional structure. No further modification was made to their conformation. For each compound, 100 different conformations were generated using FLAME. They were combined as the probe molecules. Each conformation of the probe molecules was used as a static probe to align all target molecules. In other words, multiple conformations of all molecules were used as fixed probes, one at a time, to align all other molecules. For each resulting alignment, the total score (energies and alignment scores of all molecules) was minimized with respect to the coordinates of all atoms of all molecules. All alignments were saved and sorted by the total scores for visual inspection. A reasonable active conformation was derived from the alignment and used for

subsequent database searching against in-house compound database and commercial database. Top 1000 scored compounds from each database search were kept and further filtered by physicochemical criteria (ClogP < 5, PSA < 110 Å, number of rotatable bonds ≤ 10, and hydrogen bond donors ≤ 2) to give 1092 compounds.

Preparation of rCB$_1$ or rCB$_2$-Transfected CHO Cell Lines. One day before transfection, approximately 3−4 × 10^6 AM1 cells, derived from CHO-K1 cells, were plated in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1× MEM nonessential amino acids solution, 1× HT supplement, and 1× penicillin-streptomycin-glutamate (Gibco/Invitrogen, Carlsbad, CA). The cells were transfected with 5.6 μg of pcDNA3.1hyg-rCB$_1$ or 5.6 μg of pcDNA3.1hyg-rCB$_2$ using 16.8 μL FuGENE 6 transfection reagent following manufacturer's protocol (Roche Applied Science, Indianapolis, IN). One day later, the transfected cells were split at 1:10 dilution and were placed under antibiotic selection in the medium containing 250 μg/mL hygromycin B (Roche Applied Science, Indianapolis, IN) and colonies were picked after two weeks of antibiotic selection.

GTP-Europium Binding Assay. Two membrane preparations (B_{max}: 1.7 pmol/mL for both) from Sf9 insect cells cotransfected with CB$_1$ or CB$_2$ and Gai3 were assessed by monitoring binding of a nonhydrolyzable GTP analogue, GTP-europium. Three μM of **2** was used to define 100% efficacy. The procedure was modified from a commercial kit (Perkin-Elmer, AD-0167) in that 25 μL of buffer A (50 mM Hepes, 0.1% BSA) containing a certain concentration (0.01 nM to 10 μM) of tested compound was added to 125 μL of buffer B (50 mM Hepes, 0.1% BSA, 10 mM $MgCl_2$, 150 mM NaCl, 5 μM GDP, 10 nM GTP-europium, 0.0005% saponin, and CB$_1$ (4.5 μg/well) or CB$_2$ (14 μg/well) membranes) in a 96-well filtration assay plate that came with the kit. The final DMSO concentration was 1%. The plate was incubated at room temperature for 45 min, followed by filtering and washing twice with 300 μL of cold GTP wash buffer (supplied with the kit) on a vacuum manifold device (Millipore, MAVM 096 0R). The plate was then read in a fluorescent plate reader (Perkin-Elmer Envision) at 615 nm and data analyzed with ActivityBase.

Whole Cell cAMP Assay. Intracellular cAMP levels were measured using the Lance cAMP kit from Perkin-Elmer (Waltham, MA). CHO-K1 cells stably expressing human CB$_1$-receptor (Euroscreen, Belgium), human CB$_2$-receptor (Euroscreen), rat CB$_1$-receptor, or rat CB$_2$-receptor were used. Compound concentration curves were prepared in DMSO at 100 times the desired final concentration in the assay. The compounds were diluted 50-fold into assay buffer (HBSS/5 mM HEPES/0.1% BSA) containing 60 μM forskolin (EMD Biosciences/Calbiochem; San Diego, CA). The compound/forskolin mixture was transferred to the assay plate (96-well half area, white opaque plate; No. 3693 Corning), 12 μL/well. Cells were harvested, pelletted at 1000 rpm for 5 min, and resuspended in assay buffer to a density of 416667 cells/mL. The Alexa-fluor 647 labeled anti-cAMP antibody supplied with the Lance cAMP kit was added to the cells at a dilution of 1:100. The cells/antibody mixture was added to the assay plate (12 μL/well, which is 5000 cells/well) containing compound/forskolin. The assay plate was incubated at room temperature for 1 h. The detection mix, which included biotinylated cAMP, was prepared per manufacturer's instructions and was added to the assay plate (24 μL/well) and the plate was incubated at room temperature for an additional hour. The plate was read on the RUBYstar (BMG LabTech, Durham, NC). The emission at 665 nm was used to plot the concentration response curves of the compounds and calculate their EC_{50}. A CB$_1$ or a CB$_2$ agonist results in inhibition of the forskolin-induction production of intracellular cAMP, which results in an increase in emission at 665 nm. Compound **2** was used to define 100% efficacy.

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich or EM Science and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. All microwave assisted reactions were conducted with a Smith synthesizer from Personal Chemistry, Uppsala, Sweden. All final compounds were purified to >95% purity, as determined by LC/MS obtained on an Agilent 1100 or HP 1100 spectrometer. Silica gel chromatography was performed using either glass columns packed with silica gel (230−400 mesh, EMD Chemicals, Gibbstown, NJ) or prepacked silica gel cartridges (Biotage or RediSep). ^{1}H NMR spectra were determined with a Bruker 300 MHz or DRX 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ units). Low-resolution mass spectral (MS) data were determined on a Perkin-Elmer-SCIEX API 165 mass spectrometer using ES ionization modes (positive or negative).

3-(3-(2-Cyano-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (13). 3-(3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (0.88 g, 2.8 mmol, prepared in the same manner as in **37**) was suspended in toluene/methanol (9:1) 30 mL. Trimethylsilyldiazomethane (2 M in hexanes, 2.09 mL, 4.2 mmol) was added dropwise with stirring until the solution remained pale yellow in color. The solution was evaporated to dryness under reduced pressure. Dry dimethylacetamide (20 mL) was added to dissolve the crude ester followed by copper(I) chloride (0.102 g, 1 mmol) and potassium cyanide (0.726 g, 11 mmol). The mixture was heated under nitrogen at 130 °C for 14 h before cooling to room temperature. Saturated ammonium chloride (20 mL), EtOAc (80 mL), and water (70 mL) were added and the phases were mixed and separated. The organic layer was then dried ($MgSO_4$), and the solvent was concentrated in vacuo. The crude product was purified by column chromatography on silica gel (eluting with 10−100% EtOAc/hexane) to give methyl 3-(3-(2-cyano-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoate (0.104 g, 0.38 mmol, 14% yield). The solid was dissolved in methanol (15 mL) and treated with a solution of lithium hydroxide monohydrate (0.079 g, 1.9 mmol) in water (2 mL). The mixture was stirred for 30 min. EtOAc (60 mL) and 5N HCl (20 mL) were added, and the phases were mixed and separated. The organic layer was then dried ($MgSO_4$), and the solvent was concentrated in vacuo to give **13**. This material was used without further purification. MS (ESI, pos. ion) *m/z*: 262.0 (M + 1).

3-(3-(3-Cyanopyridin-4-yl)-1,2,4-oxadiazol-5-yl)propanoic acid (16). 3-(3-(3-Bromopyridin-4-yl)-1,2,4-oxadiazol-5-yl)propanoic acid (3.12 g, 10 mmol, prepared in the same manner as in **37**) was suspended in toluene/methanol (9:1) 70 mL. The solution was cooled in a ice bath. Trimethylsilyldiazomethane (2 M in hexane, 5 mL, 10 mmol) was added dropwise until the solution remained pale yellow in color. The solvent was removed, and the residue was purified by column chromatograph on silica gel (eluting with 0−100% EtOAc/hexane). The resulting material (2.50 g, 8.01 mmol), copper(I) cyanide (2.87 g, 32.0 mmol) and DMF 30 mL were combined and divided into two microwave tubes. The reaction was heated at 180 °C for 10 min in microwave reactor. After cooling to room temperature, the reaction mixture was poured into water and extracted with EtOAc three times. The combined organic layers were washed with water and brine, dried on Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane). The resulting material (1.23 g, 5 mmol) was dissoved in dimethoxyethane (25 mL) and 1 M lithium hydroxide aqueous solution (7 mL, 7 mmol) was added. The mixture was stirred at room temperature for 16 h. The solvent was evaporated and 1N HCl was added. The solution was extracted with EtOAc. The combined organic layers were washed with water and brine, dried on Na_2SO_4, filtered, and concentrated. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give **16** (0.68 g, 28% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.25 (s, 1H), 9.05 (d, J = 5.02 Hz, 1H), 8.12 (d, J = 5.52 Hz, 1H), 3.27 (t, J = 6.78 Hz, 2H), 2.85 (t, J = 6.78 Hz, 2H). MS (ESI, pos. ion) *m/z*: 245.0 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propan-1-ol (19). Methyl 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoate (0.501 g, 1.85 mmol, esterification as in **13** from the acid prepared in the same manner as in **37**) was dissolved in dry dichloromethane (40 mL) and cooled in a dry ice bath under nitrogen. Diisobutylaluminum hydride (1.0 M in toluene, 4.0 mL,

4.0 mmol) was added dropwise and the solution was allowed to warm up to room temperature overnight. The reaction was quenched by addition of 1N HCl (50 mL). Dichloromethane (50 mL) was added, and the phases were mixed for 30 min, then separated and the organic layer was dried with $MgSO_4$ before evaporating to dryness. The crude material was purified by column chromatography on silica gel (eluting with 30−100% EtOAc/hexane) to give **19** (0.40 g, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.8, 6.1 Hz, 1H), 7.28 (dd, J = 8.4, 2.5 Hz, 1H), 7.11 (m, 1H), 3.80 (t, J = 6.0 Hz, 2H), 3.12 (t, J = 7.3 Hz, 2H), 2.25 (br s, 1H), 2.13 (m, 2H). MS (ESI, pos. ion) m/z: 257.0 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanal (21). 3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (1.54 g, 5.7 mmol, prepared in the same manner as in **37** using 2-chloro-4-fluorobenzonitrile) was dissolved in dichloromethane (80 mL) and methanol (10 mL) and treated with (trimethylsilyl)diazomethane (2 M in hexanes, 4.3 mL, 8.5 mmol). The mixture was stirred for 15 min, after which nitrogen evolution ceased and the solution was evaporated to dryness under reduced pressure. The crude ester was then dissolved in dry dichloromethane (80 mL) and cooled to −78 °C under nitrogen. Diisobutylaluminum hydride (1.0 M in toluene, 8.5 mL, 8.5 mmol) was added slowly, and the solution was stirred for 60 min, then methanol (20 mL) was added to quench the reaction. The mixture was added water and 1N HCl. The phases were separated. The organic layer was dried ($MgSO_4$), and the solvent was concentrated in vacuo. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give **21** (0.88 g, 61% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 9.89 (s, 1H), 7.92 (dd, J = 8.6, 6.4 Hz, 1H), 7.26 (dd, J = 8.6, 2.4 Hz, 1H), 7.11 (dd, J = 8.5, 2.5 Hz, 1H), 3.28 (t, J = 6.1 Hz, 2H), 3.15 (t, J = 6.2 Hz, 2H). MS (ESI, pos. ion) m/z: 255.1 (M + 1).

3-Bromo-5-nitro-2-(2,2,2-trifluoroethoxy)pyridine (24). 2,2,2-Trifluoroethanol (300 mL) was stirred at 0 °C and sodium hydride (15 g, 379 mmol) was added by small portions carefully to the solution. The mixture was then stirred at room temperature for 30 min. 3-Bromo-2-chloro-5-nitropyridine (10 g, 42 mmol) in trifluoroethanol was added, and the reaction was refluxed overnight. The solvent was removed, and the residue was taken up in water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated. The product was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give **24** (11.2 g, 88% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (d, J = 2.48 Hz, 1H), 8.70 (d, J = 2.48 Hz, 1H), 4.92 (q, J = 8.18 Hz, 2H).

5-Nitro-2-(2,2,2-trifluoroethoxy)nicotinonitrile (25). Compound **24** (4.8 g, 16 mmol) was divided into eight 20 mL microwave vials and copper(I) cyanide (5.7 g, 64 mmol), divided into 8 portions, was added to each vial. The vials were diluted with 20 mL NMP and sealed. Each reaction vial was heated to 195 °C for 5 min. The crude material was combined, diluted with water and EtOAc, and filtered through celite. The filtrate was extracted with EtOAc (4 × 50 mL). The combined organic fractions were washed with water and brine, dried over Na_2SO_4, and concentrated. The crude residue was purified by column chromatography on silica gel (eluting with 15−50% EtOAc/hexane). The pure fractions were combined and concentrated to give the product (2.4 g, 61%) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (d, J = 2.78 Hz, 1H), 8.78 (d, J = 2.63 Hz, 1H), 5.02 (q, J = 8.04 Hz, 2H). MS (ESI, pos. ion) m/z: 248.1 (M + 1).

5-Amino-2-(2,2,2-trifluoroethoxy)nicotinonitrile (26). Compound **25** (1.8 g, 7.4 mmol) was dissolved in ethanol 50 mL. Acetic acid (5 mL) and water (1 mL) were added, followed by addition of iron powder (0.82 g, 24 mmol). The reaction was refluxed for 1 h. The mixture was filtered through a pad of celite and the solvent was removed. The residue was taken in saturated aqueous $NaHCO_3$ solution and extracted with dichromethane. The organic layers were combined and washed with water and brine, dried on Na_2SO_4, filtered, and concentrated under reduced pressure to give **26** (1.03 g, 65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.27 (s, 1H), 4.77 (q, J = 8.18 Hz, 2H), 3.66 (s br, 2H). MS (ESI, pos. ion) m/z: 218.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(3-(2-fluorophenyl)isoxazol-5-yl) propanamide (30).** 2-Fluorobenzaldehyde (2.0 mL, 19 mmol) was dissolved in dry dichloromethane (50 mL) and treated with hydroxylamine (50% in water, 1.16 mL, 19 mmol). $MgSO_4$ (1.75 g) was added, and the reaction was stirred for 7 h. The mixture was filtered and evaporated to dryness to give an off-white solid. The imine was dissolved in dry DMF (20 mL) under nitrogen and treated with *N*-chlorosuccinimide (2.53 g, 19 mmol). The reaction was stirred for 90 min, then poured into a separatory funnel containing water (300 mL) and diethyl ether (100 mL). The phases were mixed and separated and the organic layer was washed twice with water (100 mL each). The organic layer was then dried ($MgSO_4$), and the solvent was concentrated in vacuo to give 2-fluorobenzoyl chloride oxime that was used without further purification.

Methyl 4-pentynoate (1.80 g, 16 mmol) was dissolved in THF (20 mL) and added to the 2-fluorobenzoyl chloride oxime. Triethylamine (2.6 mL, 19 mmol) was added, and the reaction was stirred under nitrogen overnight. The reaction was evaporated to dryness under reduced pressure. The crude residue was redissolved in EtOAc (60 mL) and washed with 1N HCl (20 mL), water (20 mL), and 1N NaOH (20 mL). The organic layer was then dried ($MgSO_4$), and the solvent was concentrated in vacuo to give methyl 3-(3-(2-fluorophenyl)isoxazol-5-yl)propanoate.

The crude methyl 3-(3-(2-fluorophenyl)isoxazol-5-yl)propanoate was dissolved in methanol (30 mL) and treated with a solution of lithium hydroxide monohydrate (0.98 g, 23 mmol) in water (5 mL). The mixture was stirred for 3 h. EtOAc (80 mL) and water (80 mL) were added, and the phases were separated. The aqueous layer was acidified with 5N HCl (20 mL) and extracted with EtOAc (90 mL). The organic layer was then dried ($MgSO_4$), and the solvent was concentrated in vacuo to give 3-(3-(2-fluorophenyl)isoxazol-5-yl)propanoic acid (2.14 g, 48% yield), which was used without further purification.

General Procedure for Amide Coupling Method A. 3-(3-(2-Fluorophenyl)isoxazol-5-yl)propanoic acid (0.50 g, 2.1 mmol) and 3-amino-9-ethylcarbazole (0.45 g, 2.1 mmol) were dissolved in dichloromethane (10 mL). The reaction mixture was cooled in an ice bath. A suspension of EDC (0.41 g, 2.1 mmol) in dichloromethane (10 mL) was added slowly, and the reaction was stirred for 15 min. The mixture was removed from the ice bath and stirred for 2 h. The reaction was quenched by addition of water (50 mL) and EtOAc (80 mL), and the phases were separated. The organic layer was dried with $MgSO_4$, and the solvent was concentrated in vacuo. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give compound **30** (0.29 g, 31% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.31 (d, J = 1.9 Hz, 1H), 8.03 (d, J = 7.9 Hz, 1H), 7.88 (dt, J = 1.7, 7.5 Hz, 1H), 7.35−7.55 (m, 5H), 7.50−7.15 (m, 3H), 6.65 (d, J = 3.2 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 3.30 (t, J = 7.4 Hz, 2H), 2.90 (t, J = 7.4 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). MS (ESI, pos. ion) m/z: 428.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(3-(2-fluorophenyl)isoxazol-5-yl) propanamide (31).** Methyl succinimate (0.54 g, 4.1 mmol) and 2-bromo-1-(2-fluorophenyl)ethanone (0.95 g, 2.1 mmol) were dissolved in NMP (20 mL). The mixture was heated to 120 °C for 6 h, then allowed to cool to room temperature. The reaction was diluted with EtOAc (80 mL) and water (70 mL) and the phases were separated. The organic layer was dried ($MgSO_4$), and the solvent was concentrated in vacuo. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give methyl 3-(4-(2-fluorophenyl)oxazol-2-yl)propanoate (0.35 g, 34% yield).

Hydrolysis of 3-(4-(2-fluorophenyl)oxazol-2-yl)propanoate (in the same manner as in **30**) followed by amide coupling method A (in the same manner as **30**) gave compound **31** (0.16 g, 18% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35−8.25 (m, 2H), 8.1 (dt, J = 1.5, 7.3 Hz, 1H), 7.95−8.05 (m, 2H), 7.05−7.55 (m, 7H), 4.31 (q, J = 7.0 Hz, 2H), 3.30 (t, J = 6.9 Hz, 2H), 2.98 (t, J = 6.9 Hz,

2H), 1.7−1.5 (br s, 1H), 1.40 (t, J = 7.1 Hz, 3H). MS (ESI, pos. ion) m/z: 428.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(5-(2-fluorophenyl)-1*H*-pyrazol-3-yl)propanamide (32).** 2′-Fluoroacetophenone (2.0 mL, 16 mmol) was dissolved in dry THF (60 mL) under nitrogen and cooled in an ice bath. Lithium bis(trimethylsilyl)amide (1 M in THF, 16.2 mL, 16 mmol) was added slowly and the reaction stirred for 20 min. Succinic anhydride (1.32 mL, 16 mmol) was added in one portion, and the reaction was stirred overnight. Water (50 mL), 1N HCl (30 mL), and ethyl acetate (70 mL) were added, and the phases were separated. The organic was dried with $MgSO_4$ and concentrated to 10 mL of the crude solution of 6-(2-fluorophenyl)-4,6-dioxohexanoic acid under reduced pressure.

The 6-(2-fluorophenyl)-4,6-dioxohexanoic acid solution (8 mmol) was diluted with ethanol (20 mL) and treated with anhydrous hydrazine (0.26 mL, 8.1 mmol). The reaction was stirred overnight. Water (50 mL), 1N NaOH (10 mL), and EtOAc (50 mL) were added, and the phases were separated. The aqueous layer was acidified with 1N HCl (30 mL) and extracted with EtOAc (80 mL). The organic layer was dried ($MgSO_4$), and the solvent was concentrated in vacuo to give the crude material of 3-(5-(2-fluorophenyl)-1*H*-pyrazol-3-yl)propanoic acid. Using the crude acid and following amide coupling method A (as in **30**) gave **32** (0.18 g, 3.3% yield over three steps) as an off white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (d, J = 1.6 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.79 (dd, J = 8.6, 1.8 Hz, 1H), 7.50 (dd, J = 8.6, 1.8 Hz, 1H), 7.38−7.47 (m, 3H), 7.28−7.36 (m, 1H), 7.10−7.25 (m, 3H), 6.62 (d, J = 2.7 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 3.15 (t, J = 7.5 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H). MS (ESI, pos. ion) m/z: 427.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(5-(2-fluorophenyl)-1-methyl-1*H*-pyrazol-3-yl)propanamide (33).** The 6-(2-fluorophenyl)-4,6-dioxohexanoic acid (8 mmol, prepared in the same manner as in **32**) was diluted with ethanol (20 mL) and treated with 1-methylhydrazine (0.47 mL, 8.9 mmol). The reaction was stirred overnight. The reaction was diluted with water (50 mL), ethyl acetate (60 mL), and 1N NaOH (20 mL). The phases were separated and the aqueous layer was acidified with 5N HCl (10 mL) and extracted with ethyl acetate (70 mL). The organic layer was then dried ($MgSO_4$), and the solvent was concentrated in vacuo to give the crude 3-(5-(2-fluorophenyl)-1-methyl-1*H*-pyrazol-3-yl)propanoic acid. Using the crude acid and following amide coupling method A (as in **30**) gave **33** (0.047 g, 1.4% yield over three steps). ^{1}H NMR (400 MHz, CD_3Cl) δ 8.2−8.3 (m, 2H), 8.05 (d, J = 7.7 Hz, 1H), 7.3−7.55 (m, 6H), 7.15−7.25 (m, 3H), 6.21 (s, 1H), 4.33 (q, J = 7.3 Hz, 2H), 3.80 (s, 3H), 3.15 (t, J = 7.0 Hz, 2H), 2.85 (t, J = 7.1 Hz, 2H), 1.40 (t, J = 7.3 Hz, 3H). MS (ESI, pos. ion) m/z: 441.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(4-(2-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)propanamide (34).** To a solution of 3-amino-9-ethylcarbazole (0.50 g, 2.38 mmol) and *N,N*-diisopropylethylamine (0.54 mL, 3.09 mmol) in DMF (10 mL) was added 3-chloropropionyl chloride (0.25 mL, 2.62 mmol). The resulting mixture was allowed to stir for 1 h, at which time sodium azide (0.42 mL, 11.9 mmol) was added. The resulting mixture was heated to 60 °C overnight. The reaction was cooled to room temperature and poured onto water (100 mL) and extracted with ether (3 × 25 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to afford 3-azido-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide, which was used without further purification.

To a slurry of 1-ethynyl-2-fluorobenzene (0.053 mL, 0.47 mmol) and 3-azido-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (0.15 g, 0.47 mmol) in *t*-BuOH (0.5 mL) and water (0.5 mL) was added a freshly prepared solution of sodium ascorbate (0.094 mL, 0.047 mmol) and a freshly prepared solution of copper sulfate pentahydrate (0.0094 mL, 0.0047 mmol). The mixture was allowed to stir at room temperature. After 5 h, the reaction was diluted with water (0.75 mL) and *t*-butanol (0.75 mL) and continued to stir overnight. Then the mixture was diluted with water and filtered. The solid material was washed with water, then reconstituted in acetonitrile and dichloromethane. The crude mixture was purified via flash chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to afford **34** as an off-white solid (0.079 g, 39% yield over two steps). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.32 (s, 1H), 8.47 (d, J = 1.89 Hz, 1H), 8.33 (d, J = 3.92 Hz, 1H), 8.24 (dt, J = 7.71, 1.64 Hz, 1H), 8.07 (d, J = 7.71 Hz, 1H), 7.60 (dd, J = 8.65, 1.96 Hz, 1H), 7.51−7.55 (m, 1 H), 7.41−7.50 (m, 2H), 7.34−7.40 (m, 1H), 7.26 - 7.32 (m, 1H), 7.15 - 7.25 (m, 2H), 4.90 (t, J = 6.63 Hz, 2H), 4.45 (q, J = 7.20 Hz, 2H), 4.45 (q, J = 7.20 Hz, 2H), 1.37 (t, J = 7.14 Hz, 3H). MS (ESI, pos. ion) m/z: 428 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl)propanamide (35).** A mixture of 3-cyanopropanoic acid (1.0 g, 10 mmol), HOBt (2.0 g, 17 mmol), and PS-carbodiimide (16 g, 20 mmol, Argonaut Technologies) in methylene chloride was stirred at room temperature for 10 min, at which time 3-amino-9-ethylcarbazole (2 g, 10 mmol) was added. The resulting mixture was stirred overnight at room temperature. MP-carbonate (17 g, 50 mmol, Argonaut Technologies) was then added, and the reaction mixture was stirred an additional 1 h. The reaction mixture was filtered and the resins washed with dichloromethane. The filtrate was concentrated in vacuo and the residue was triturated with methanol and filtered, affording 3-cyano-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (1.44 g, 49% yield) as a yellow solid.

A flask containing 3-cyano-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (0.23 g, 0.79 mmol) was dissolved in methanol MeOH (8 mL). Hydroxylamine hydrochloride (0.060 g, 0.87 mmol), sodium bicarbonate (0.080 g, 0.95 mmol), and 0.62 mL of water were added. The reaction mixture was refluxed for 48 h. An additional 80 mg of sodium bicarbonate and 60 mg of hydroxylamine hydrochloride was added to the reaction, and reflux was continued overnight. The reaction mixture was cooled to room temperature, and the solvents were removed. To the crude reaction product was added dichloromethane and water. The organic layers were separated and washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (eluting with 0−10% methanol/dichloromethane) providing (*Z*)-4-amino-*N*-(9-ethyl-9*H*-carbazol-3-yl)-4-(hydroxyimino)butanamide as a light purple solid (72 mg, 28% yield).

A vial containing (*Z*)-4-amino-*N*-(9-ethyl-9*H*-carbazol-3-yl)-4-(hydroxyimino)butanamide (0.072 g, 0.22 mmol) was charged with dichloromethane (1.7 mL). To the stirring solution was added 2-fluorobenzoic acid (0.031 g, 0.22 mmol), HOBt (0.034 g, 0.22 mmol), EDC (0.043 g, 0.22 mmol), and *N,N*-diisopropylethylamine (0.078 mL, 0.44 mmol). The reaction was stirred at room temperature for 6 h then diluted with dichloromethane and transferred to a separation funnel. 1N HCl was added to wash the organic layer. The phases were separated, and the aqueous layer was extracted with dichloromethane. The organic fractions were combined and washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated to afford a brown film, (*Z*)-4-(2-fluorobenzoyloxyimino)-4-amino-*N*-(9-ethyl-9*H*-carbazol-3-yl)butanamide (0.045 g, 46% yield), which was used without further purification.

A vacuum-dried flask containing (*Z*)-4-(2-fluorobenzoyloxyimino)-4-amino-*N*-(9-ethyl-9*H*-carbazol-3-yl)butanamide (0.045 g, 0.1 mmol) was added pyridine (1.0 mL). The reaction mixture was heated to reflux overnight, then it was cooled and pyridine was removed. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane). The product-containing fractions were combined and purified by column chromatography on silica gel (eluting 0−5% methanol/dichloromethane). The impure fractions were combined and purified by column chromatography on silica gel (eluting with 3:1 EtOAc/hexanes followed by 1:1 EtOAc/hexanes) to afford additional material. The combined product fractions were concentrated to afford **35** (9.3 mg, 21.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.13 (t, J = 6.82 Hz, 1H), 8.06 (d, J = 7.83 Hz, 1H), 7.75 (s, 1H), 7.63−7.57 (m, 1H), 7.53 (d, J = 7.20 Hz, 1H), 7.50−7.44 (m, 1H), 7.42−7.37 (m, 1H), 7.37−7.25 (m, 3H), 7.21 (t, J = 7.33 Hz, 1H), 4.35 (q, J = 7.16 Hz, 2H), 3.36 (t, J = 7.26

Hz, 2H), 2.99 (t, $J = 7.26$ Hz, 2H), 1.42 (t, $J = 7.20$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 429.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(5-(2-fluorophenyl)-2*H*-tetrazol-2-yl)propanamide (36).** 2-Fluorobenzonitrile (0.80 mL, 7.4 mmol), dibutyltin oxide (0.092 g, 0.37 mmol), and azidotrimethylsilane (1.47 mL, 11 mmol) were added to a microwave reaction vessel containing dimethoxyethane (2 mL). The reaction was heated to 150 °C in the microwave for 10 min, then cooled to room temperature. The solution was transferred to a separatory funnel and diluted with water (100 mL), 1N HCl (20 mL), and EtOAc (80 mL). The phases were separated, and the organic layer was dried with $MgSO_4$ before evaporating to dryness under reduced pressure to give 5-(2-fluorophenyl)-2*H*-tetrazole, which was used without purification. The crude 5-(2-fluorophenyl)-2*H*-tetrazole (7.4 mmol) was dissolved in dimethoxyethane (3 mL) and transferred to a microwave reaction vial. Methyl 3-bromopropanoate (0.81 mL, 7.4 mmol) was added, and the vial was heated to 150 °C in a microwave reactor for 10 min. The crude material was concentrated and redissolved in methanol and treated with an aqueous solution of lithium hydroxide (1M, 10 mL, 37 mmol). After stirring for 15 min, the mixture was acidified with 5N HCl (10 mL) and diluted with water (100 mL) and EtOAc (100 mL). The phases were separated, and the organic layer was dried with $MgSO_4$ before evaporating to dryness under reduced pressure. The crude material was purified by column chromatography on silica gel (eluting with 0−10% methanol/dichromethane) to give 3-(5-(2-fluorophenyl)-2*H*-tetrazol-2-yl)propanoic acid (0.20 g, 12% yield).

3-(5-(2-Fluorophenyl)-2*H*-tetrazol-2-yl)propanoic acid (0.20 g, 0.86 mmol) and 3-amino-9-ethylcarbazole (0.20 g, 0.95 mmol) dissolved in a minimal amount of dry DMF (2 mL) and treated with HATU (0.36 g, 0.95 mmol). The reaction was stirred for 10 min. Water (60 mL) and EtOAc (60 mL) were added, and the phases were separated. The organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give **36** (0.11 g, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, $J = 1.8$ Hz, 1H), 8.13 (dt, $J = 7.5$, 1.8 Hz, 1H), 8.02 (d, $J = 7.7$ Hz, 1H), 7.72 (br s, 1H), 7.50−7.35 (m, 4H), 7.1−7.3 (m, 4H), 5.15 (t, $J = 6.9$ Hz, 2H), 4.30 (q, $J = 7.2$ Hz, 2H), 3.25 (t, $J = 6.9$ Hz, 2H), 1.38 (t, $J = 7.2$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 428.9 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(3-phenyl-1,2,4-oxadiazol-5-yl) propanamide (37).** To a mixture of sodium carbonate (1.0 g, 10 mmol) and hydroxylamine hydrochloride (1.0 g, 19 mmol) in methanol/H_2O was added benzonitrile (2 mL, 19 mmol). The mixture was heated to 70 °C for 1 h. The cooled reaction mixture was concentrated, and the residue was taken up in dichloromethane. The organic layer was washed with water and concentrated to give (*Z*)-*N*′-hydroxybenzamidine (1.85 g, 70% yield), which was used without further purification.

A mixture of succinic anhydride (1.1 mL, 13.6 mmol) and (*Z*)-*N*′-hydroxybenzamidine (1.85 g, 13.6 mmol) was heated to 120 °C for 30 min. The reaction was cooled to room temperature, concentrated to dryness, and triturated with methanol. The white solid was filtered, washed with water, and dried under high vacuum to give 3-(3-phenyl-1,2,4-oxadiazol-5-yl)propanoic acid (2.11 g, 71.2% yield), which was used without further purification.

A mixture of 3-(3-phenyl-1,2,4-oxadiazol-5-yl)propanoic acid (0.15 g, 0.69 mmol), HOBt (0.11 g, 0.78 mmol), and PS-carbodiimide (0.72 g, 0.92 mmol) in dichloromethane was mixed for 10 min, at which time 9-ethyl-9*H*-carbazol-3-amine (0.096 g, 0.46 mmol) was added. The resulting mixture was mixed for overnight at room temperature. MP-carbonate (0.76 g, 2.29 mmol) was then added, and the mixture was stirred for an additional hour. The reaction mixture was filtered and the resins washed with dichloromethane. The filtrate was concentrated down and the residue triturated with methanol and filtered to give **37** (93.2 mg, 50% yield) as a off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.96 (d, $J = 1.89$ Hz, 1H), 8.46−8.55 (m, 3H), 8.08 (dd, $J = 8.78$, 1.83 Hz, 1H), 7.84−8.02 (m, 6H), 7.61 (t, $J = 7.26$ Hz, 1H), 4.89 (q, $J = 7.07$ Hz, 2H), 3.84 (t, $J = 7.07$ Hz, 2H), 3.55 (t, $J = 7.07$ Hz, 2H), 1.82 (t, $J = 7.14$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 411.1 (M + 1).

3-(3-(2-Chlorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (38). Using 2-chlorobenzonitrile and following the procedure used to prepare **37** gave **38**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 8.41 (s, 1H), 8.05 (d, $J = 7.73$ Hz, 1H), 7.90 (dd, $J = 7.63$, 1.66 Hz, 1H), 7.68 (dd, 1H), 7.49−7.63 (m, 5H), 7.44 (t, $J = 7.19$ Hz, 1H), 7.17 (t, $J = 7.43$ Hz, 1H), 4.42 (q, $J = 7.04$ Hz, 2H), 3.32−3.44 (m, 2H), 3.01 (t, $J = 6.94$ Hz, 2H), 1.30 (t, $J = 7.09$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 445.0 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(3-(2-isopropylphenyl)-1,2,4-oxadiazol-5-yl)propanamide (39).** Using 2-isopropylbenzonitrile and following the procedure in **37** to prepare 3-(3-(2-isopropylphenyl)-1,2,4-oxadiazol-5-yl)propanoic acid. Then **39** was prepared by amide coupling method A (as in **30**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, $J = 1.88$ Hz, 1H), 7.93 (d, $J = 7.91$ Hz, 1H), 7.68 (m, 2H), 7.37 (m, 6H), 7.20 (m, 2H), 7.11 (t, $J = 7.44$ Hz, 1H), 4.24 (q, $J = 7.22$ Hz, 2H), 3.58 (m, 1H), 3.35 (t. $J = 7.06$ Hz, 2H), 2.94 (t, $J = 7.06$ Hz, 2H), 1.31 (t, $J = 7.25$ Hz, 3H), 1.15 (d, $J = 6.78$ Hz, 6H). MS (ESI, pos. ion) *m/z*: 452.8 (M + 1).

3-(3-(3-Chlorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (40). Using 2-chlorobenzonitrile and following the procedure used to prepare **37** gave **40**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.96 (s, 1H), 8.55−8.44 (m, 2H), 8.12−8.02 (m, 3H), 8.01−7.92 (m, 2H), 7.89 (t, $J = 7.39$ Hz, 1H), 7.62 (t, $J = 7.45$ Hz, 1H), 4.90 (q, $J = 7.03$ Hz, 2H), 3.86 (t, $J = 6.95$ Hz, 2H), 3.86 (t, $J = 6.95$ Hz, 2H), 3.86 (t, $J = 6.95$ Hz, 2H). MS (ESI, pos. ion) *m/z*: 445.1 (M + 1).

***N*-(9-ethyl-9*H*-carbazol-3-yl)-3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanamide (41).** Using 2-chlorobenzonitrile and following the procedure used to prepare **37** gave **41**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 8.41 (t, 1H), 7.98−8.11 (m, 3H), 7.51−7.62 (m, 3H), 7.36−7.48 (m, 3H), 7.16 (t, 1H), 4.41 (q, $J = 7.11$ Hz, 2H), 3.33 (t, 2H), 3.01 (t, $J = 6.99$ Hz, 2H), 1.29 (t, $J = 7.14$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 429.1 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (42). Using 2-chloro-4-fluorobenzonitrile and following the procedure used to prepare **37** gave **42**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, $J = 1.64$ Hz, 1H), 8.06 (d, $J = 7.58$ Hz, 1H), 7.94 (dd, $J = 8.65$, 6.00 Hz, 1H), 7.59 (s, 1H), 7.48 (t, $J = 8.02$ Hz, 2H), 7.33−7.43 (m, 2H), 7.28−7.31 (m, 2H), 7.06−7.14 (m, 1H), 3.47 (t, $J = 7.14$ Hz, 2H), 3.05 (t, $J = 7.20$ Hz, 2H), 1.43 (t, $J = 7.14$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 463.0 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(3-(4-fluoro-2-methylphenyl)-1,2,4-oxadiazol-5-yl)propanamide (43).** Using 2-chlorobenzonitrile and following the procedure used to prepare **37** gave **43**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.41 (s, 1H), 8.04 (d, $J = 7.96$ Hz, 1H), 7.97 (dd, $J = 8.53$, 6.13 Hz, 1H), 7.57 (m, 3H), 7.44 (t, $J = 7.58$ Hz, 1H), 7.30 (dd, $J = 10.0$, 2.46 Hz, 1H), 7.20 (m, 2H), 4.42 (q, $J = 6.91$ Hz, 2H), 3.34 (m, 2H), 3.00 (t, $J = 7.01$ Hz, 2H), 2.56 (s, 3H), 1.30 (t, $J = 7.01$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 443.0 (M + 1).

3-(3-(2-Cyano-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (44). 44 was prepared by amide coupling method A (as in **30**) using **13** (90 mg, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, $J = 1.88$ Hz, 1H), 8.17 (dd, $J = 5.3$, 8.8 Hz, 1H), 8.03 (d, $J = 7.7$ Hz, 1H), 7.79 (br s, 1H), 7.30−7.55 (m, 6H), 7.18 (t, $J = 7.0$ Hz, 1H), 4.32 (q, $J = 7.2$ Hz, 2H), 3.46 (t, $J = 6.9$ Hz, 2H), 3.05 (t, $J = 6.9$ Hz, 2H), 1.39 (t, $J = 7.2$ Hz, 3H). MS (ESI, pos. ion) *m/z*: 454.1 (M + 1).

3-(3-(3-Cyanopyridin-4-yl)-1,2,4-oxadiazol-5-yl)-*N*-(9-ethyl-9*H*-carbazol-3-yl)propanamide (45). 45 was prepared by amide coupling method A (as in **30**) using 3-(3-(3-cyanopyridin-4-yl)-1,2,4-oxadiazol-5-yl)propanoic acid **16** (142 mg, 78% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.16 (s, 1H), 9.25 (s, 1H), 9.05 (d, $J = 5.12$ Hz, 1H), 8.40 (s, 1H), 8.13 (d, $J = 5.12$ Hz, 1H), 8.04 (d, $J = 7.60$ Hz, 1H), 7.55 (m, 3H), 7.43 (t, $J = 7.67$ Hz, 1H), 7.16 (t, $J = 7.38$ Hz, 1H), 4.41 (q, $J = 7.02$ Hz, 2H), 3.41 (t, $J = 13.45$

Hz, 2H), 3.04 (t, J = 13.74 Hz, 2H), 1.28 (t, J = 6.94 Hz, 3H). MS (ESI, pos. ion) m/z: 437.1 (M + 1).

***N*-(9-Ethyl-9*H*-carbazol-3-yl)-3-(3-(tetrahydro-2*H*-pyran-4-yl)-1,2,4-oxadiazol-5-yl)propanamide (46).** Using tetrahydro-2*H*-pyran-4-carbonitrile and following the procedure in **37** to prepare 3-(3-(tetrahydro-2*H*-pyran-4-yl)-1,2,4-oxadiazol-5-yl)propanoic acid. Then **46** was prepared by amide coupling method A (as in **30**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 1.88 Hz, 1H), 8.04 (d, J = 7.72 Hz, 1H), 7.72 (s, 1H), 7.40 (m, 4H), 7.20 (m, 1H), 4.33 (q, J = 7.22 Hz, 2H), 4.03 (m, 2H), 3.54 (m, 2H), 3.34 (d, J = 7.06 Hz, 2H), 3.00 (m, 3H), 1.93 (m, 4H), 1.40 (t, J = 7.25 Hz, 3H). MS (ESI, pos. ion) m/z: 418.8 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(6-chloroquinolin-3-yl)propanamide (47). NaOH 10N (6.0 mL, 60 mmol) was cooled in an ice bath and nitromethane (1.0 mL, 18.5 mmol) was added dropwise. The reaction was stirred for 15 min, then removed from the cold bath and stirred for another 20 min. The solution was placed in a room temperature water bath, and nitromethane (1.0 mL, 18.5 mmol) was added slowly with vigorous stirring. The mixture was stirred for 25 min then poured into ice water (100 mL) with added concentrated HCl (6 mL) and diethyl ether (100 mL). The mixture was stirred until the ice melted, after which the phases were separated and the organic layer was dried with $MgSO_4$. The solution was filtered and concentrated under reduced pressure to give methazonic acid as a pale yellow solid (1.88 g, 98% yield, this compound is thermally sensitive!).

Methazonic acid (1.9 g, 19 mmol) solution (10 mL aqueous) was added to 5N HCl (10 mL) and ice (~50 mL). A solution of 2-amino-5-chlorobenzaldehyde (1.1 g, 7.2 mmol) in a mixture of ethanol (70 mL), water (60 mL), and 5N HCl (2 mL) was added slowly with stirring. The mixture was heated to 50 °C for 60 h, then concentrated under reduced pressure to ~10 mL solution that was cooled in an ice bath. The suspension was filtered through a fritted funnel and washed with water (10 mL) to give the intermediate 3-nitro-6-chloroquinoline. This intermediate was then suspended in isopropanol (50 mL) and water (5 mL) and heated to 50 °C. Saturated ammonium chloride (4 mL) and iron powder (4.03 g, 72 mmol) were added, and the mixture was stirred overnight. The reaction mixture was cooled and filtered through a pad of celite. The filtrate was evaporated to dryness under reduced pressure and redissolved in a mix of EtOAc (100 mL), 1N NaOH (20 mL), and water (80 mL). The phases were mixed and separated, and the organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure. The crude material was purified by column chromatography on silica gel (eluting with 0−100% EtOAc/hexane) to give 6-chloroquinolin-3-amine (0.40 g, 31% yield).

47 was prepared by 6-chloroquinolin-3-amine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method A (as in **30**). ^{1}H NMR (400 MHz, CD_3OD) δ 8.79 (d, J = 2.2 Hz, 1H), 8.70 (d, J = 2.4 Hz, 1H), 7.90−8.0 (m, 2H), 7.79 (d, J = 2.2 Hz, 1H), 7.56 (dd, J = 2.3, 8.9 Hz, 1H), 7.29 (dd, J = 8.4, 2.5 Hz, 1H), 7.12 (m, 1H), 3.43 (t, J = 7.2 Hz, 2H), 3.09 (t, J = 7.2 Hz, 2H). MS (ESI, pos. ion) m/z: 430.7 (M + 1).

***N*-(6-Chloro-1,8-naphthyridin-3-yl)-3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanamide (48).** 2-Amino-5-chloropyridine-3-carboxaldehyde (1.1 g, 7.0 mmol) was dissolved in a mixture of water (10 mL) and concentrated HCl (8 mL) and added to a solution of methazonic acid (1.9 g, 18 mmol, prepared as in **47**) in water (20 mL). The reaction was stirred overnight. The resulting suspension was filtered through a fritted funnel and dried in vacuo. The intermediate 3-chloro-6-nitro-1,8-naphthyridine (0.42 g, 2.0 mmol) was suspended in isopropanol (40 mL) and methanol (10 mL) and treated with saturated ammonium chloride (2 mL) and water (1 mL). Iron powder (1.1 g, 20 mmol) was added, and the mixture was heated to 60 °C with stirring. After 1 h, the mixture was cooled and filtered through a pad of celite. The filtrate was evaporated to dryness under reduced pressure. The crude material was dissolved in water (100 mL), 1N NaOH (20 mL), and EtOAc (100 mL), and the phases were separated. The organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure to give 6-chloro-1,8-naphthyridin-3-amine (0.18 g, 15% yield), which was used without further purification.

Amide Coupling Method B. 3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (0.69 g, 2.5 mmol, as in **42**) was dissolved in thionyl chloride (5 mL) and stirred for 2 h. The solution was evaporated to dryness under reduced pressure to give a pale yellow oil. The crude acid chloride was dissolved in dichloromethane (10 mL) and added to a solution of 6-chloro-1,8-naphthyridin-3-amine (0.46 g, 2.5 mmol) and diisopropylethylamine (0.44 mL, 2.5 mmol) in dichloromethane (30 mL). The reaction was stirred for 3 h, after which water (100 mL) was added and the phases were separated. The organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure. The crude material was purified using column chromatography on silica gel eluting with (0−10% methanol/dichloromethane) to give enriched material. This material was triturated with methanol (10 mL) and filtered to **48** (0.15 g, 14% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 10.88 (s, 1H), 9.04 (d, J = 2.1 Hz, 1H), 8.92 (s, J = 2.1, 1H), 8.83 (d, J = 2.3 Hz, 1H), 8.68 (d, J = 2.1 Hz, 1H), 7.96 (dd, J = 8.8, 6.3 Hz, 1H), 7.70 (dd, J = 8.7, 2.1 Hz, 1H), 7.43 (dt, J = 8.7, 1.9 Hz, 1H) 3.40 (t, J = 6.4 Hz partially obscured by MeOD, 1H), 3.10 (t, J = 6.4 Hz, 2H). MS (ESI, pos. ion) m/z: 432.0 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(6-(trifluoromethoxy)benzo[*d*]thiazol-2-yl)propanamide (49). 49 was prepared by riluzole and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method A (as in **30**, 88 mg, 33% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 8.12 (d, J = 1.51 Hz, 1H), 7.94 (dd, J = 8.85, 6.22 Hz, 1H), 7.83 (d, J = 8.85 Hz, 1H), 7.70 (dd, J = 8.85, 2.64 Hz, 1H), 7.41 (m, 2H), 3.39 (t, J = 6.78 Hz, 2H), 3.16 (t, J = 6.97 Hz, 2H). MS (ESI, pos. ion) m/z: 486.6 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(5-(piperidin-1-yl)-1,3,4-thiadiazol-2-yl)propanamide (50). 50 was prepared by 5-(piperidin-1-yl)-1,3,4-thiadiazol-2-amine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method B (as in **48**, 141 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.94 (t, 1H), 7.71 (d, J = 8.97 Hz, 1H), 7.43 (t, 1H), 3.25 - 3.42 (m, 8H), 1.50−1.64 (m, 6H). MS (ESI, pos. ion) m/z: 437.0 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(4-isopropylphenyl)propanamide (51). 51 was prepared by 4-isopropylbenzenamine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method A (as in **30**, 50 mg, 36% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.67, 6.03 Hz, 1H), 7.46 (s, 1H), 7.40 (d, J = 8.48 Hz, 2H), 7.28 (m, 1H), 7.17 (d, J = 8.29 Hz, 2H), 7.09 (m, 1H), 3.40 (t, J = 6.97 Hz, 2H), 2.97 (t, J = 7.06 Hz, 2H), 2.87 (m, 1H), 1.22 (d, J = 6.97 Hz, 6H). MS (ESI, pos. ion) m/z: 387.8 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(4-(trifluoromethyl)phenyl)propanamide (52). 52 was prepared by 4-(trifluoromethyl)benzenamine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method A (as in **30**, 29 mg, 37% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (dd, J = 8.67, 6.03 Hz, 1H), 7.61 (s br, 1H), 7.53 (q, J = 8.85 Hz, 4H), 7.21 (m, 1H), 7.02 (m, 1H), 3.34 (t, J = 6.88 Hz, 2H), 2.94 (t, J = 6.88 Hz, 2H). MS (ESI, pos. ion) m/z: 413.7 (M + 1).

***N*-(2-Chloro-4-(trifluoromethyl)phenyl)-3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanamide (53). 53** was prepared by 2-chloro-4-(trifluoromethyl)benzenamine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method A (as in **30**, 58 mg, 29% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 8.61 Hz, 1H), 8.01 (s br, 1H), 7.91 (dd, J = 8.80, 6.06 Hz, 1H), 7.64 (m, 1H), 7.52 (m, 1H), 7.28 (m, 1H), 7.10 (m, 1H), 3.43 (t, J = 6.94 Hz, 2H), 3.12 (t, J = 6.94 Hz, 2H). MS (ESI, pos. ion) m/z: 447.6 (M + 1).

***N*-(3-Chloro-4-(trifluoromethyl)phenyl)-3-(3-(2-chloro-4-fluo-**

rophenyl)-1,2,4-oxadiazol-5-yl)propanamide (54). 54 was prepared by 3-chloro-4-(trifluoromethyl)benzenamine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method A (as in **30**, 130 mg, 79% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, J = 8.67, 6.03 Hz, 1H), 7.84 (s br, 1H), 7.79 (d, J = 1.70 Hz, 1H), 7.61 (d, J = 8.48 Hz, 1H), 7.46 (dd, J = 8.57, 1.41 Hz, 1H), 7.29 (dd, J = 8.48, 2.45 Hz, 1H), 7.10 (m, 1H), 3.41 (t, J = 6.88 Hz, 2H), 3.02 (t, J = 6.78 Hz, 2H). MS (ESI, pos. ion) *m/z*: 447.6 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(6-(trifluoromethyl)pyridin-3-yl)propanamide (55). 55 was prepared by 6-(trifluoromethyl)pyridin-3-amine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method B (as in **48**, 95 mg, 21% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.86 (d, J = 2.0 Hz, 1H), 8.30 (dd, J = 8.2. 1.6 Hz, 1H), 7.95 (dd, J = 8.8, 6.3 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.71 (dd, J = 8.9, 2.5 Hz, 1H), 7.43 (m, 1H), 3.32 (m, 2H), 3.06 (t, J = 7.0 Hz 2H). MS (ESI, pos. ion) *m/z*: 415.0 (M + 1).

3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-*N*-(5-cyano-6-(2,2,2-trifluoroethoxy)pyridin-3-yl)propanamide (56). 56 was prepared by amide coupling method B (as in **48**, 1.2 g, 68% yield) using **26** and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (m, 2H), 7.91 (s br, 1H), 7.88 (m, 1H), 7.26 (m, 1H), 7.12 (m, 1H), 4.85 (q, J = 8.23 Hz, 2H), 3.42 (t, J = 6.78 Hz, 2H), 3.02 (t, J = 6.78 Hz, 2H). MS (ESI, pos. ion) *m/z*: 469.7 (M + 1).

***N*-(2-*tert*-Butylpyrimidin-5-yl)-3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanamide (57). 57** was prepared by 2-*tert*-butylpyrimidin-5-amine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method B (as in **48**, 127 mg, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.87 (s, 2H), 7.88 (m, 2H), 7.28 (m, 1H), 7.11 (m, 1H), 3.42 (t, J = 6.88 Hz, 2H), 3.03 (t, J = 6.88 Hz, 2H), 1.39 (s, 9H). MS (ESI, pos. ion) *m/z*: 403.8 (M + 1).

***N*-(5-*tert*-Butylisoxazol-3-yl)-3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanamide (58). 58** was prepared by 5-*tert*-butylisoxazol-3-amine and 3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propanoic acid (as in **42**) using amide coupling method B (as in **48**, 123 mg, 37% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.95 (dd, J = 8.84, 6.06 Hz, 1H), 7.71 (dd, J = 8.91, 2.59 Hz, 1H), 7.47−7.40 (m, 1H), 6.56 (s, 1H), 3.29 (t, J = 6.82 Hz, 2H), 2.97 (t, J = 6.82 Hz, 2H), 1.27 (s, 9H). MS (ESI, pos. ion) *m/z*: 393.1 (M + 1).

5-(3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propylamino)-2-(2,2,2-trifluoroethoxy)nicotinonitrile (60). Compound **21** (0.080 g, 0.31 mmol) and compound **26** (0.068 g, 0.31 mmol) were combined in dichloroethane (1 mL). The mixture was stirred at room temperature. Titanium isopropoxide (1.4 mL, 4.7 mmol) was added, and the mixture was stirred for 45 min. The resulting solution was added to a stirring solution of sodium borohydride (0.066 mL, 1.9 mmol) in 5 mL of methanol. The reaction mixture was stirred for 30 min, and then the solvent was removed. The resulting material was taken in water and extracted with EtOAc three times. The combined organic layers were washed with water and brine, dried on sodium sulfate, filtered, and concentrated. The resulting residue was purified by column chromatography on silica gel eluting with 0−10% methanol/dichromethane to give **60** (0.10 g, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (dd, J = 8.70, 6.07 Hz, 1H), 7.74 (d, J = 2.92 Hz, 1H), 7.30 (dd, J = 8.48, 2.48 Hz, 1H), 7.21 (d, J = 2.92 Hz, 1H), 7.13 (m, 1H), 4.76 (q, J = 8.33 Hz, 2H), 3.88 (s br, 1H), 3.31 (q, J = 6.63 Hz, 2H), 3.12 (t, J = 7.02 Hz, 2H), 2.23 (m, 2H). MS (ESI, pos. ion) *m/z*: 456.0 (M + 1).

5-*tert*-Butyl-*N*-(3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propyl)isoxazol-3-amine (61). 77 mg, 35% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (s, 9H), 1.96−2.10 (m, 2H), 3.08 (t, J = 7.5 Hz, 2H), 3.15 (q, J = 6.6 Hz, 2H), 5.55 (s, 1H), 6.11 (t, J = 5.7 Hz, 1H), 7.38−7.49 (m, 1H), 7.71 (dd, J = 9.0, 2.5 Hz, 1H), 7.97 (dd, J = 8.7, 6.2 Hz, 1H). MS (ESI, pos. ion) *m/z*: 379 (M + 1).

6-Chloro-*N*-(3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propyl)benzo[*d*]thiazol-2-amine (62). 11 mg, 6.5% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.80−8.00 (m, 2H), 7.35−7.55 (m, 3H), 7.19 (dt, J = 7.8, 2.5 Hz, 1H), 3.77 (t, J = 6.6 Hz, 2H), 3.23 (t, J = 7.0 Hz, 2H), 2.40 (m, 2H). MS (ESI, pos. ion) *m/z*: 423.0 (M + 1).

6-Chloro-*N*-(3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propyl)quinolin-3-amine (63). 63 was prepared using 6-chloroquinolin-3-amine (prepared as in **47**); 1.2 g, 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 2.5 Hz, 1H), 7.74 (dd, J = 8.8, 6.1 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.10−7.20 (m, 2H), 6.98 (dt, J = 2.1, 8.1 Hz, 1H), 6.81 (d, J = 2.5 Hz, 1H), 4.05 (br s, 1H), 3.23 (t, J = 6.8 Hz, 2H), 3.01 (t, J = 7.2 Hz, 2H), 2.14 (m, 2H). MS (ESI, pos. ion) *m/z*: 417.1 (M + 1).

***N*-(3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propyl)-6-(trifluoromethoxy)quinolin-3-amine (64).** Butyl 2-formyl-4-(trifluoromethoxy)phenylcarbamate (1.28 g, 4.2 mmol) was dissolved in dry THF (20 mL) and added to a solution of methazonic acid (0.44 g, 4.2 mmol, prepared as in **47**) in water (20 mL). Concentrated HCl (2 mL) was added, and the mixture was heated to 80 °C. After 2 h, the mixture was concentrated under reduced pressure to about 20 mL that was diluted with water (50 mL) and filtered through a fritted funnel. The crude 3-nitro-6-(trifluoromethoxy)quinoline (0.52 g, 2.0 mmol) was dissolved in isopropanol (80 mL) and treated with saturated ammonium chloride (5 mL), water (5 mL), acetic acid (0.5 mL), and iron powder (0.57 g, 10 mmol). The reaction mixture was heated to 60 °C for 40 min. After cooling, the mixture was filtered through a pad of celite. The filtrate was evaporated to dryness under reduced pressure, and the crude material was resuspended in ethyl acetate (100 mL) before washing with 1N NaOH (20 mL) and water (60 mL). The organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure. The crude material was purified by reverse-phase HPLC to give 6-(trifluoromethoxy)quinolin-3-amine (0.38 g, 40% yield) as a yellow solid.

64 was prepared using 6-(trifluoromethoxy)quinolin-3-amine and compound **21** following the procedure used to prepare **60** (27 mg, 30% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 2.92 Hz, 1H), 7.92 (m, 2H), 7.40 (m, 1H), 7.30 (dd, J = 8.48, 2.48 Hz, 1H), 7.24 (m, 1H), 7.10 (m, 1H), 7.00 (d, J = 2.78 Hz, 1H), 4.36 (m, 1H), 3.44 (q, J = 6.72 Hz, 2H), 3.15 (t, J = 7.09 Hz, 2H), 2.31 (m, 2H). MS (ESI, pos. ion) *m/z*: 467.1 (M + 1).

***N*-(3-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propyl)quinolin-3-amine (65). 65** was prepared using quinolin-3-amine and compound **21** following the procedure used to prepare **60** (30 mg, 40% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09−2.22 (m, 2H), 3.19 (t, J = 7.2 Hz, 2H), 3.24−3.31 (m, 2H), 6.44 (t, J = 5.1 Hz, 1H), 7.07 (d, J = 2.4 Hz, 1H), 7.31 (t, J = 7.1 Hz, 1H), 7.35−7.45 (m, 2H), 7.63 (d, J = 7.5 Hz, 1H), 7.71 (dd, J = 8.8, 2.8 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.94 (dd, J = 8.6, 6.2 Hz, 1H), 8.45 (d, J = 2.5 Hz, 1H). MS (ESI, pos. ion) *m/z*: 383 (M + 1).

6-Chloro-*N*-(3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propyl)-*N*-methylquinolin-3-amine (66). Compound **63** (0.034 g, 0.08 mmol) was dissolved in dichloroethane (20 mL) and treated with acetic acid (2 mL) and formaldehyde, 37% in water (0.80 mL, 11 mmol). The mixture was heated to 60 °C for 2 h, then sodium triacetoxyborohydride (0.087 g, 0.4 mmol) was added and the mixture stirred at room temperature for 40 min. The reaction was evaporated to dryness and redissolved in acetic acid (5 mL). Another portion of formaldehyde (37% in water, 0.80 mL, 11 mmol) was added, and the mixture was stirred for another 45 min prior to addition of sodium triacetoxyborohydride (0.088 g, 0.4 mmol). The mixture was stirred for an additional 40 min then evaporated to dryness under reduced pressure. 1N NaOH (20 mL) and ethyl acetate (40 mL) were added, and the phases were mixed for 0.5 h. The organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure. The crude material was purified by column chromatography on silica gel (eluting with 30−100% EtOAc/

hexane) to give **66** (0.029 g, 81% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, J = 2.9 Hz, 1H), 7.85−8.00 (m, 2H), 7.56 (d, J = 2.2 Hz, 1H), 7.36 (dd, J = 8.9, 2.3, 1H), 7.30 (dd, J = 8.5, 2.5 Hz, 1H), 7.10−7.20 (m, 2H), 3.67 (t, J = 7.2 Hz, 2H), 3.12 (s, 3H), 3.07 (t, J = 7.1 Hz, 2H), 2.20−2.35 (m, 2H). MS (ESI, pos. ion) m/z: 431.1 (M + 1).

6-Chloro-3-(3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl) propoxy)quinoline (67). Compound **19** (0.33 g, 1.3 mmol) and triphenylphosphine (0.39 g, 1.5 mmol) were dissolved in dry THF (30 mL) prior to addition of bromine (0.085 mL, 1.6 mmol). The reaction was stirred at room temperature for 6 h then concentrated to dryness under reduced pressure. The crude was purified using silica chromatography (eluting with 0−50% EtOAc/hexane) to give 5-(2-bromoethyl)-3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazole (0.27 g, 65% yield).

6-Chloroquinolin-3-amine (0.34 g, 1.9 mmol, prepared as in **47**) was dissolved in 2N H_2SO_4 (40 mL) and cooled in an ice bath. A solution of sodium nitrite (0.15 g, 2.1 mmol) in water (10 mL) was added slowly and the mixture stirred for 30 min. The solution was added to 70 °C 5N H_2SO_4 (20 mL) and stirred at this temperature for 20 min. The mixture was cooled to room temperature, and EtOAc (100 mL) was added. Disodium hydrogen phosphate was added to adjust the pH to 5, and the phases were separated. The organic layer was dried with $MgSO_4$ then evaporated to dryness under reduced pressure to give 6-chloroquinolin-3-ol that was used without purification.

The crude 6-chloroquinolin-3-ol and 5-(3-bromopropyl)-3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazole (0.70 g, 2.2 mmol) were dissolved in dry DMF (5 mL) and treated with K_2CO_3 (0.45 g, 3.3 mmol). The reaction was heated to 60 °C for 20 min. Water (100 mL) and ethyl acetate (100 mL) were added. The phases were separated and the organic layer was dried with $MgSO_4$ before evaporating to dryness under reduced pressure. The crude material was purified by column chromatography on silica gel (eluting with 10−100% EtOAc/hexane) to give **67** (0.28 g, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 2.8 Hz, 1H), 7.87−8.00 (m, 2H), 7.67 (d, J = 2.3 Hz, 1H), 7.49 (dd, J = 9.0, 2.3 Hz, 1H), 7.25−7.32 (m, contains $CHCl_3$ peak, 2H), 7.10 (dt, J = 1.7, 7.1 Hz, 2H), 4.25 (t, J = 5.8 Hz, 2H), 3.26 (t, J = 7.3 Hz, 2H), 2.40−2.55 (m, 2H). MS (ESI, pos. ion) m/z: 418.1 (M + 1).

2-*tert*-Butyl-5-(3-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)propoxy)pyrimidine (68). Compound **19** (0.085 g, 0.33 mmol), 2-*tert*-butylpyrimidin-5-ol (0.050 g, 0.33 mmol), and triphenylphosphine (0.087 g, 0.33 mmol) were dissolved in benzene (10 mL) and treated with diisopropyl azodicarboxylate (0.065 mL, 0.33 mmol). After stirring for 2 h, the reaction was evaporated to dryness under reduced pressure and the crude material was purified by column chromatography on silica gel (eluting with 5−100% EtOAc/hexane). Further purification using reverse phase HPLC gave **68** (0.037 g, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 2H), 7.93 (dd, J = 8.6, 6.1 Hz, 1H), 7.29 (dd, J = 8.4, 2.6 Hz, 1H), 7.12 (dt, J = 2.6, 8.8 Hz, 1H), 4.25 (t, J = 5.9 Hz, 2H), 3.22 (t, J = 7.3 Hz, 2H), 2.40−2.50 (m, 2H). MS (ESI, pos. ion) m/z: 391.1 (M + 1).

3-(4-(3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)butyl)quinoline (69). To a solution of (*E*)-2-chloro-4-fluoro-*N*′-hydroxybenzamidine (4.4 g, 23 mmol, prepared in the same manner as in **37** using 2-chloro-4-fluorobenzonitrile) in THF (100 mL) at 0 °C was added pent-4-ynoyl chloride (3.26 g, 28 mmol) followed by triethylamine (4.9 mL, 35 mmol). The white mixture was allowed to warm to room temperature. After 1 h, the mixture was filtered and concentrated. The residue was taken up in EtOAc, washed with water and brine, and dried with Na_2SO_4. After concentration in vacuo, the tan oil was advanced without further purification. A solution of this material (6.2 g, 23.1 mmol) in toluene (100 mL) was heated to reflux. After 16 h, the solvent was removed in vacuo and the residue purified by column chromatography on silica gel eluting with 5−40% EtOAc/hexane to afford 5-(but-3-ynyl)-3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazole (4.1 g, 70% yield) as a white solid.

To a mixture of 5-(but-3-ynyl)-3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazole (0.15 g, 0.60 mmol), 3-bromoquinoline (0.10 mL, 0.78 mmol), copper(I) iodide (0.0057 g, 0.03 mmol), and dichlorobis(triphenylphosphine)palladium (II) (0.021 g, 0.03 mmol) was added acetonitrile (4 mL) and triethylamine (1.67 mL, 12 mmol). The mixture was heated to 80 °C in a sealed tube. After 16 h, the solvent was removed in vacuo and the residue purified by column chromatography on silica gel (eluting with 5−30% EtOAc/hexane) to afford 3-(4-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)but-1-ynyl)quinoline (0.12 g, 54% yield) as a light yellow solid. This material (0.052 g, 0.14 mmol) and palladium on activated carbon 10% (0.015 g) in benzene was exposed to an atmosphere of H_2. After 16 h, the mixture was filtered over a silica plug and concentrated in vacuo to afford **69** (0.040 g, 76% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 2.02 Hz, 1H), 8.16 (s, 1H), 7.88−8.00 (m, 3H), 7.66−7.72 (m, 2H), 7.54−7.60 (m, 1H), 7.38−7.45 (m, 1H), 3.10 (t, J = 6.88 Hz, 2H), 2.87 (t, J = 6.88 Hz, 2H), 1.76−1.92 (m, 4H). MS (ESI, pos. ion) m/z: 382.1 (M + 1).

Supporting Information Available: Detailed description of HPLC methods, HPLC purity analysis of all final compounds, and HPLC spectra of compound **56** and **63**. This material is available free of charge via the Internet at http://pubs.acs.org.

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