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Discovery of Anthranilamides as a Novel Class of Inhibitors of Neurotropic Alphavirus Replication

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Abstract

Neurotropic alphaviruses are debilitating pathogens that infect the central nervous system (CNS) and are transmitted to humans via mosquitoes. There exist no effective human vaccines against these viruses, underlining the need for effective antivirals, but no antiviral drugs are available for treating infection once the viruses have invaded the CNS. Previously, we reported the development of novel indole-2-carboxamide-based inhibitors of alphavirus replication that demonstrate significant reduction of viral titer and achieve measurable brain permeation in a pharmacokinetic mouse model. Herein we report our continued efforts to improve physicochemical properties predictive of *in vivo* blood-brain barrier (BBB) permeability through reduction of overall molecular weight, replacing the indole core with a variety of aromatic and non-aromatic monocyclics. These studies culminated in the identification of simple anthranilamides that retain excellent potency with improved metabolic stability and significantly greater aqueous solubility. Furthermore, in a live virus study, we showed that two new compounds were capable of reducing viral titer by two orders of magnitude and that these compounds likely exert their effects through a mechanism similar to that of our indole-2-carboxamide inhibitors.

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1. Introduction

Emerging and re-emerging viruses pose a serious health risk to the world population. Mosquito-borne viruses (arboviruses) are of particular concern with the increase of urban environments that facilitate mosquito reproduction and range expansion.^{1–3} Among the arboviruses, the genus *Alphavirus* within the family *Togaviridae* contains approximately 10 viruses that cause significant disease in animals and humans.⁴ Of notable concern are the neurotropic alphaviruses eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and Venezuelan equine encephalitis virus (VEEV), which are NIAID and CDC category B priority pathogens due to their re-emerging status and potential use in bioterrorism.^{5, 6} In humans, these neurotropic alphaviruses replicate in neurons causing inflammation of the central nervous system (CNS).^{7–9} Several days post-exposure, this infection can result in debilitating encephalitis with fatality rates as high as 70%.^{3, 5, 10–14} Currently, there are no clinically available therapeutics for alphavirus infection, signifying a need to develop novel antiviral agents.

Neurotropic alphaviruses replicate to high titer within the CNS,¹⁵ necessitating development of CNS-penetrant antiviral agents. This is especially important because clinical manifestations may appear well after the systemic virus titer has dropped to immeasurable levels, days after initial transmission.^{16–18} This places an enhanced emphasis upon physical properties predictive of both good pharmacokinetics and CNS penetration when optimizing both *in vitro* and *in vivo* activity. The most challenging barrier to CNS entry is the blood-brain barrier (BBB). The BBB is structurally distinct from other membrane obstacles; due to diminished pinocytosis and the presence of tight-junctions, most drugs must cross the BBB via transcellular passive diffusion alone.^{19, 20} However, there are a number of common features among successful CNS-active drugs that enhance passive BBB transit, including low molecular weight (< 400–450), low polar surface area (< 60–70 Å²), and positive logD (~1–3).^{19, 21–24} Molecular weight (MW) is of particular interest to us because weight reduction improves passive permeability into membranes non-linearly²⁵ and reduces recognition by xenobiotic efflux transporters – such as P-glycoprotein (PgP or MDR1) – that are highly expressed at the BBB.^{26–28}

We have previously reported the discovery of a lead alphavirus RNA replication inhibitor, CCG-32091,²⁹ and initial optimization leading to indole **1** (Figure 1).^{30, 31} During our SAR development, we prepared pyrrole **2** in an effort to reduce molecular weight and identify the minimal pharmacophore.³¹ Replacement of the indole with pyrrole afforded a significant 50 Da reduction in MW with retention of antiviral potency and reduced cytotoxicity, which suggested that the indole core was not a requisite feature of the pharmacophore. Replacement of the central piperidine with azetidine was another MW-reduction strategy, but potency of a preliminary prototype (analogue **3**, Figure 1) was significantly diminished. This was likely due to the size difference between the two rings, since our previous work³¹ had established an apparent dependence of potency on overall length. Thus, we elected to focus our efforts on additional low MW replacements for the central pyrrole of **2** that retain the inhibitor length, including pyrrolidine, phenyl, and acyclic amides and ureas, the results of which are described herein.

2. Chemistry

The azetidine-containing pyrrole **3** was prepared through the union of the previously reported *N*-benzyl pyrrole **4**³¹ and methyl azetidine-3-carboxylate, followed by base hydrolysis and amidation with 4-(2-aminoethyl)pyridine (Scheme 1). Urea **8a** was synthesized in a manner similar to our previous report³¹ by the addition of ethyl isonipecotate to 4-chlorobenzylisocyanate, followed by hydrolysis and amidation with 4-(2-aminoethyl)pyridine (Scheme 2). The acyclic amides **11a** and **11b** were synthesized from the amide coupling of the appropriate carboxylic acid and ethyl isonipecotate, followed by hydrolysis/amidation similar to the previous syntheses (Scheme 2).

N-benzylpyrrolidine enantiomers **15** were prepared through base-catalyzed alkylation of proline with 4-chlorobenzylchloride to protect stereochemical integrity via the carboxylate anion,³² followed by amide coupling with *N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide dihydrochloride **14**³¹ to yield the appropriate (*R*)- and (*S*)- analogues (Scheme 3). Enantiomers **18** were synthesized in a similar fashion, first by acylating proline under classical Schotten-Baumann conditions^{33, 34} followed by amide coupling with ethyl isonipecotate (Scheme 3). Hydrolysis of the ethyl ester through a non-racemizing protocol³⁵ provided the carboxylic acid, and amidation with 4-(2-aminoethyl)pyridine gave the desired (*R*)- and (*S*)- benzoyl analogues.

Salicylamide analogue **21** was prepared from the Chan-Lam coupling³⁶ of methyl salicylate and 4-chlorophenyl boronic acid, providing the ether intermediate **20** in low yield, followed by ester hydrolysis and amidation (Scheme 4). Secondary phenyl anthranilamides **23a** and **23b** were assembled from Buchwald amination^{37, 38} of triflated methyl salicylate and the appropriate aniline, followed by saponification/amidation. The *N*-methyl anthranilamide derivative **25** was accessed through methylation of coupling intermediate **22a**, followed by the usual saponification and amide coupling. Benzyl anthranilamides **28a** and **28b** were obtained from alkylation of methyl 2-aminobenzoate with the corresponding benzyl halide, followed by the base hydrolysis and amidation (Scheme 5).

Phenethyl anthranilamide **31** could not be prepared through direct *N*-alkylation of methyl 2-aminobenzoate because the various electrophiles employed (phenethyltosylates, mesylates, and halides) were highly prone to elimination. Conveniently, the transformation could be achieved through a Jourdan-Ulmann coupling³⁹ of 2-bromobenzoic acid and 1-(2-aminoethyl)-4-chlorophenyl amine in ethylene glycol dimethyl ether (Scheme 6). Base hydrolysis and amidation then provided the desired analogue **31**.

The preparation of benzyl- and benzoyl benzamide derivatives entailed the known Friedel-Crafts acylation of chlorobenzene with phthalic anhydride,⁴⁰ which delivered ketone intermediate **33** from which a simple amidation with **14** provided the benzoylbenzamide analogue **34** (Scheme 7). The related benzylbenzamide analogue **36** was accessed through a dissolving metal reduction of the ketone intermediate **33**^{41, 42} to afford benzyl benzoic acid **35**, which was followed by amidation with amine **14**. Interestingly, the ketone could not be reduced without concomitant reduction of the chloride under several heterogenous hydrogenation conditions.^{40, 43} Also, attempts to reduce the ketone to the respective alcohol

with NaBH₄ failed,⁴⁴ even with excess borohydride and vigorous conditions. Thionation with Lawesson's Reagent and subsequent Raney-Ni reduction also could be used to generate the desired benzyl intermediate **35**, but the dissolving metal reduction in Scheme 7 was found to be more direct and efficient.

Carbazole analogues were synthesized as shown in Scheme 8. Ortho-directed lithiation of carbazole **37** followed by carboxylation with carbon dioxide in a manner similar to that reported by Katritzky provided key intermediate **38**.⁴⁵ Direct coupling with amine **14** provided analogue **41**. The carbazole carboxylic acid **38** could not be chlorinated with NCS under a variety of conditions,^{46, 47} while the methyl ester **39** yielded a complex mixture of variously chlorinated carbazoles. Ultimately, sulfuryl chloride⁴⁸ gave clean regioselective conversion to chloro-substituted carbazole ester **40** as confirmed by ¹HNMR. This ester was then hydrolyzed to the corresponding carboxylic acid and coupled with amine **14** to give the desired analogue **42**.

Finally, quinolone analogues were accessed as shown in Scheme 9. Hydrolysis of 2,4-dichloroquinoline^{49, 50} and subsequent addition/elimination with ethyl isonipecotate gave intermediate **44** which was subsequently N-arylated via a Chan-Lam coupling,⁵¹ then saponified and coupled with 4-(2-aminoethyl)pyridine to give phenylquinolone **46**. Conversely, **44** was alkylated with 4-chlorobenzyl chloride, hydrolyzed and coupled under similar conditions to the phenylquinolone to provide the benzylquinolone **48**.

3. Results and discussion

3.1. WEEV Replicon SAR

We used a previously described WEEV genome replication reporter system^{29, 52} as a phenotypic cell-based assay to evaluate new candidate compound antiviral activity, the results from which are shown in Tables 1–5. The average CNS drug has a molecular weight of about 319 Da.¹⁹ Table 1 summarizes our attempts to reduce the molecular weight of the indole core to the maximum extent by replacing it with simple acyclic linkers. Only ureas **8a** and **8b** retained any activity in the replicon assay, and those were greatly diminished relative to pyrrole **2**. Amides **11a** and **11b** were completely inactive. These results suggested that some degree of rigidity is likely necessary to retain good antiviral activity. Although the pyrrole analogue **2** indicates that the indole left-hand core is not a necessary component of the SAR, it is immediately evident in Table 1 that complete removal of the heterocycle eliminates activity.

We therefore turned our attention to cyclic, but non-aromatic, pyrrolidine analogs (Table 2). While significantly less active than pyrrole **2**, *N*-benzyl pyrrolidines (*S*)-**15** and (*R*)-**15** still retain some activity. Worth noting is the small but notable chiral preference for the *S*-enantiomer, which is four times more potent than the *R*-enantiomer. This is consistent with our previous observation that the unknown molecular target for this series of antiviral compounds possesses some degree of enantiospecificity.^{30, 31} *N*-benzoyl pyrrolidines (*S*)-**18** and (*R*)-**18** by contrast were completely inactive. This could suggest a need for a basic nitrogen in the linker, but more likely reflects an unfavorable conformational bias (vide infra).

We then synthesized and evaluated a series of arene derivatives (Table 3) to more closely mimic the pyrrole scaffold but with greater predicted stability to oxidative metabolism. Several of these compounds possessed low micromolar activity in the replicon assay, including salicylamide **21** and anthranilamide **23a**. We anticipated that the aniline moiety might be the most suitable bioisosteric replacement for pyrrole based on its electronic similarity, and indeed **23a** was only two-fold less potent than **2**. The benzyl aniline homologue **28a** proved to be equipotent with the lead pyrrole **2**, while further homologation of the anthranilamide (analogue **31**) resulted in a diminishment of activity. This observation of an optimal length is consistent with what we observed while developing **1**³¹ and suggests either a well-defined limit to the size of the unknown binding site, or simply reflects an excessive loss of entropy needed for binding a long acyclic substituent. Removal of the chloride (intended to improve metabolic stability by reducing lipophilicity) resulted in compounds (**23b** and **28b**) with greatly reduced potency. Replacement of the aniline nitrogen of **23a** with oxygen (analogue **21**) resulted in a modest loss of activity, while replacement with methylene (analogue **36**) provided an equipotent analogue.

Interestingly, the *N*-methylated analogue **25** was ten times less active than its parent anthranilamide **23a**, suggesting that the aniline nitrogen may be engaged in internal hydrogen bonding with the ortho carbonyl oxygen, resulting in a specific conformation favorable for activity. Further evidence for this conformational hypothesis is provided by comparing benzylbenzamide analogue **36**, with inactive benzoylbenzamide **34**. The latter would not be expected to be able to achieve a conformation similar to the internally hydrogen bonded conformation of **23a**.

Several rigid analogues were designed to evaluate our conformational hypothesis (Figure 2 and Table 4). Carbazole derivatives (**41** and **42**) were intended to tether the two arenes in the molecule and prevent rotation around the two C-NH bonds. Surprisingly, these compounds were remarkably less active than their more flexible parent, which may indicate that the putative internal hydrogen may not be sufficient for achieving the biologically active conformation. The quinolone derivatives (**46** and **48**), on the other hand, were designed to freeze rotation about the aryl carboxamide C-C bond (Figure 2). Their poor activity, however, indicates either a poor match for the biologically active conformation, or a key role for the benzamide carbonyl of **23a** that has been disrupted.

3.2. Potential for CNS Permeability

In its protective role, the blood-brain barrier (BBB) has a number of efflux transporters to facilitate removal of xenobiotics from the CNS, of which P-glycoprotein (Pgp or MDR1) is generally the most important.^{26, 27} Neurotropic alphaviruses replicate to high titer in the CNS, thereby necessitating the development of transporter-evasive inhibitors to elicit the greatest antiviral effect. We previously described the use of a straightforward assay for predicting the interaction of novel analogs with MDR1 using MDCK cells that express human MDR1 (MDR1-MDCKII cells).³¹ In this assay, cellular efflux of the MDR1 substrate rhodamine 123 is measured in the absence or presence of a test compound. If the test compound interacts with MDR1, it will impede efflux of rhodamine 123, thereby increasing its intracellular concentration. The known MDR1 inhibitor tariquidar is included

as a control, and results for selected new analogs are reported as percent effect on Rho123 intracellular concentration relative to tariquidar, where lower values indicate lesser interaction with MDR1 and therefore less potential for efflux (Table 3).

Replacement of the pyrrole of **2** with phenyl (**23a**) markedly increased recognition by MDR1. Surprisingly, simple N-methylation of **23a** (**25**) almost completely abated MDR1 recognition. Reductions in MDR1 recognition were also observed upon replacement of the NH of **23a** with O, C=O, or CH₂ (**21**, **34** and **36**, respectively) suggesting that the NH may be a key factor for recognition. This is supported by the strong MDR1 recognition of NH homologs **28a** and **31**. However, it is notable that simple removal of the aromatic chloro group strongly attenuated MDR1 recognition (**23b** and **28b**), indicating that overall lipophilicity may also be a factor.

Passive permeability is also an excellent predictor of successful BBB penetration, and high permeability may also overcome the effect of efflux transporters.²³ Therefore, compounds with good passive BBB permeability are of central importance to our development of CNS active inhibitors. Selected compounds were evaluated using pION's BBB-PAMPA assay and PAMPA Explorer program as previously described.³¹ Compounds with log $P_{\text{eff}} > -4.7$ are considered to be highly permeable, whereas those with log $P_{\text{eff}} < -6$ are considered poorly permeable. In general, our compounds exhibit moderate to high BBB permeability, and all but one (**34**) possess modestly better permeability than pyrrole lead **2** (Table 3).

3.3. In vitro ADME Properties

Based on their WEEV replicon activities, selected compounds were advanced into a mouse liver microsome (MLM) assay to assess their stabilities towards Phase I oxidative metabolism (Table 3). The stability of aniline **23a** was improved with a half-life of 19 minutes, a six-fold improvement over the slightly more electron-rich pyrrole **2** (3 minutes) and a two-fold improvement over the indole **1** (9 minutes). The chlorobenzyl homologue **28a** was slightly less stable towards metabolism than **23a**, perhaps because of the introduction of a benzylic carbon and increased lipophilicity. Phenolic ether **21** was as stable towards microsomal metabolism as aniline **23a**. Interestingly, replacement of the NH of **23a** with CH₂ (**36**) markedly attenuated metabolic stability, due either to increased lipophilicity or the creation of an additional site for oxidation.

3.4. Aqueous Solubility

Compounds derived from high-throughput screens tend to be lipophilic with low aqueous solubility,⁵³ and problems associated with poor aqueous solubility include compound aggregation and precipitation, which may lead to poor oral absorption and diminished efficacy.^{53–56} Therefore, we used a previously described protocol³¹ to assess the kinetic aqueous solubility of select compounds (Table 3). Overall, solubility is excellent (> 250 µg/mL) for the aniline analogues, exceeding the indole and pyrrole leads (31–63 and 125–250 µg/mL, respectively). This is particularly notable because BBB-PAMPA permeabilities of the anilines are also significantly greater than that of the pyrrole lead **2**. On the other hand, benzyl phenyl analogue **36** was less soluble, likely due to reduced polar surface area

and increased lipophilicity. Interestingly, the ether **21** was similarly soluble to **36** despite the presence of the phenolic oxygen and greater polar surface area.

3.5. Antiviral Activity

Based on their WEEV replicon potencies, **23a** and **28a** were advanced into assays to evaluate their ability to inhibit cellular replication of infectious live virus. We used two parallel assays to measure activity against infectious virus: reduction in cytopathic effect (CPE) and extracellular virus titers, and examined activity against both WEEV and Fort Morgan Virus (FMV), a WEEV-serogroup alphavirus that can be used safely under reduced biosafety conditions compared to WEEV.³¹ Against FMV, both **23a** and **28a** reduced virus titers by ten-fold when used at 25 μ M, and also reduced FMV-induced CPE similar to previous lead **1** (Table 5). Furthermore, both **23a** and **28a** also reduced WEEV titers by at least 10-fold when used at 25 μ M. Although we did not do full titration curves with these experiments, **28a** maintained antiviral activity against both FMV and WEEV when used at 5 μ M, whereas **23a** had reduced activity at this lower concentration (Table 5). A third analog, salicylamide **21**, was also evaluated for antiviral activity due to its much lower recognition by MDR1 (and thus greater potential for CNS penetration) despite its reduced potency in the replicon assay. However, **21** was observed to have no significant effect on viral titer (data not shown).

To further evaluate the antiviral potency and breadth of activity for **23a** and **28a**, we completed both full titration studies with FMV in HEK293 cells (Figure 3A) and examined their inhibitory activity against viruses derived from three different families: Venezuelan equine encephalitis virus (VEEV, TC-83 vaccine strain; *Togaviridae*), California encephalitis virus (CEV; *Bunyaviridae*), and encephalomyocarditis virus (EMCV; *Picornaviridae*) (Figure 3B). Both **23a** and **28a** had dose-dependent antiviral activity against FMV, where **28a** had an IC₅₀ similar to previous lead **1** (Figure 3A). In addition, both **23a** and **28a** had demonstrable antiviral activity against VEEV, CEV, and EMCV in cultured cells, with an approximate 10-fold reduction in infectious virus titers for all three pathogens, similar to previous lead **1** (Figure 3B).

3.6. Mechanism of Action

The use of the WEEV replicon assay, a phenotypic cell-based assay, increases the probability of selecting compounds having a cellular rather than a viral target for antiviral activity. There are several advantages of targeting host factors over viral proteins. The most significant advantage is a decreased probability of the targeted virus developing resistance to therapeutics.⁵⁷ Our studies with the lead **1** and related carboxamide analogs indicated that the mechanism of action for their antiviral activity is through the modulation of cellular cap-dependent translation.⁵⁸ To examine whether the current generation of compounds described in this report potentially used a similar mechanism, we evaluated the ability of **23a** and **28a** to modulate cellular cap-dependent translation (Figure 4). For these experiments, we used BSR-T7 cells and two reporter plasmids: pSV40-LUC, which produces a capped mRNA through nuclear transcription and export, and pCITE-LUC, which produces an uncapped mRNA containing a cap-independent translational element (CITE) through cytoplasmic transcription that is driven by a T7 promoter (Figure 4A).⁵⁹ Similar to lead **1** and previous

generation compounds, both **23a** and **28a** had no effect upon cap-independent translation, but potently inhibited cellular cap-dependent translation of transcripts produced by the pSV40-LUC reporter plasmid (Figure 4B). These results suggest that the anthranilamide analogs **23a** and **28a** also function as antiviral compounds, in part, through suppression of host cell cap-dependent translation.

4. Conclusions

Our goals in this work were to continue the development of our novel series of neurotropic alphavirus replication inhibitors with emphasis on reducing MW and enhancing ADME properties in order to maximize their potential for CNS penetration and *in vivo* activity against neurotropic alphavirus infection. Towards reduction of molecular weight, our previous work had established that the indole template of our lead could be replaced by pyrrole without loss of activity. Thus, we focused on further exploring monocyclic and acyclic replacements for the indole. Complete removal of the ring system resulted in poorly active analogs (*e.g.* **8a** and **11a**). Replacement of the pyrrole with an aliphatic pyrrolidine also yielded analogs with reduced activity, indicating that a flat, aromatic ring was critical to activity. It was also noted that *N*-benzyl substitution was preferred over *N*-benzoyl substitution, presumably because the more flexible *N*-benzyl analog can more easily access the biological conformation.

Ultimately, we found that replacing the pyrrole of **2** with a simple phenyl ring could be accomplished without sacrificing antiviral potency. The nature of the linker between the new phenyl ring and the former indole *N*-benzyl substituent proved to be critical. NH was optimal, while both O and CH₂ also were effective. Significantly, these new arene analogs were generally more stable to oxidative metabolism by mouse liver microsomes than the previous heterocyclic leads **1** and **2**. As in our previous studies, we found that recognition by MDR1, critical to predicting efflux at the BBB, was highly variable. Both the presence of a linking NH group and overall lipophilicity seemed to be key factors in enhancing recognition by MDR1. Also important to any future therapeutics development was the observation that these monocyclic arene analogs exhibited much higher solubility than indole lead **1**, consistent with what we had observed with pyrrole analog **2**.

From this work, two new arene analogs have been selected for advancement to *in vivo* WEEV infection studies: anthranilamide analogues **23a** and **28a**. Both of these analogs possess greater metabolic stability than **1** (Table 3), much improved aqueous solubility, and nearly equivalent passive permeability as measured by BBB-PAMPA. **23a** and **28a** have nearly equivalent potency in the replicon assay and strong antiviral activity in cell infection assays. An obvious caveat is that both compounds are predicted to have greater recognition by MDR1 than **1**, which may impede penetration of the CNS. However, we believe it is still important to test these analogs in an infection model for several reasons: 1) Our MDR1 recognition assay cannot differentiate between substrates and inhibitors of MDR1. Although compounds with low values are obviously ideal, we cannot say for certain whether higher values will preclude CNS penetration; 2) lead **1** is active *in vivo* despite a relatively high value (31%) in the MDR1 assay, and we don't have a large enough database to predict whether the higher values for **23a** and **28a** (48% and 60%, respectively) are too high for in

vivo activity; and 3) the greater metabolic stability and solubility of the new anthranilamide analogs could result in significantly higher overall exposure levels that could compensate for a greater efflux rate. Results from *in vivo* WEEV infection studies in mice will be reported in due course.

5. Experimental Section

5.1. Biological and Physicochemical Characterization

5.1.1. Kinetic Solubility—Solubility was determined in the manner previously reported.^{30, 31}

5.1.2. Antiviral, Viability, and Cellular Translation Assays—WEEV replicon, MTT, infectious virus, and cellular reporter translation assays were done as previously described.^{30, 31, 58}

5.1.3. MDR1 Recognition Assay—Assay was performed as previously described.^{30, 31}

5.1.4. Mouse Liver Microsome (MLM $T_{1/2}$) Stability Assay—Balb-C mouse liver microsomes were purchased from Invitrogen. $MgCl_2$ and NADPH were obtained from Sigma. Solvents were of HPLC grade or better. The HPLC-MS/MS system consisted of a ThermoElectron Finnigan TSQ Quantum Ultra AM.

Microsomal incubations were done in triplicate. Incubation mixtures contained 2 μ L of 20mg/mL microsomes (approximately 0.04 mg microsomal protein), 4 μ L of 100 μ M DMSO-dissolved substrate (1.0 μ M final), in 475 μ L potassium phosphate buffer (0.1 M, pH 7.5, containing 3.3 mM $MgCl_2$.) The incubation mixture was allowed to shake at 37 °C for 5 min, and a T=0 aliquot was removed. Reaction was initiated by the addition of 20 μ L of NaDPH (22 mM under the same buffer conditions) and the mixture was allowed to shake at 37 °C until the final aliquot was removed. 30 μ L aliquots were taken after 0 minutes, 5 minutes, and 15 minutes. All aliquots were quenched by dilution in 90 μ L MeCN containing an internal standard and the precipitate was pelleted via centrifugation. 20 μ L of supernatant was injected onto the HPLC-MS/MS. Mobile phase A was 95:5 H_2O :MeCN with 0.1% formic acid, and mobile phase B was MeCN with 0.1% formic acid. Column used was a Luna C18(2) 4.6 $\times$ 30 mm column with 3 μ M particle size. The flow rate was 2 mL/min and a gradient mobile phase composition was used: isocratic hold for 1 min at 90% A (10% B), 1 min gradient to 10% A (90% B), 1 min gradient back to 90% A (10% B), and 1 min isocratic hold at 90% A (10% B.) Ionization method consisted of positive electrospray. Source parameters were optimized for each individual substrate. Substrates and internal standards were followed by selected reaction monitoring. Substrate/internal standard area ratios were determined and converted into percent substrate remaining. The natural log of the percent remaining was plotted against time, the slope of the linear regression was determined, and the equation $T_{1/2} = -\ln(2)/k$ was used to calculate half-life.

5.2. Chemistry procedures

General Information—All reagents were used as received from commercial sources unless otherwise noted. 1H and ^{13}C spectra were obtained in DMSO- d_6 or $CDCl_3$ at RT,

unless otherwise noted, on a Varian Inova 400 MHz, Varian Inova 500 MHz, or Bruker Avance DRX 500 instruments. Chemical shifts for the ^1H NMR and ^{13}C NMR spectra were recorded in parts per million (ppm) on the δ scale from an internal standard of residual tetramethylsilane (0 ppm). Rotamers are described as a ratio of rotamer A and B if possible. Otherwise, if the rotamers cannot be distinguished, the NMR peaks are described as multiplets. Mass spectroscopy data were obtained on a Waters Corporation LCT. HPLC retention times (t_R) were recorded in minutes (min) using an Agilent 1100 Series with an Agilent Zorbax Eclipse Plus – C18 column (254 nm detection) with the gradient: 10% ACN/water (1 min), 10–90% ACN/water (6 min), and 90% ACN/water (2 min). Solvent abbreviations used: MeOH (methanol), DCM (dichloromethane), EtOAc (ethyl acetate), Hex (hexanes), DMSO (dimethylsulfoxide), DMF (dimethylformamide), H_2O (water), THF (tetrahydrofuran), ACN (acetonitrile). Reagent abbreviations used: HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), HOAT (1-hydroxy-7-azabenzotriazole), HOBt (1-hydroxy-1,2,3-benzotriazole), EDCI (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride), DIEA (diisopropylethylamine), TFA (trifluoroacetic acid), MgSO_4 (magnesium sulfate), Na_2SO_4 (sodium sulfate), NaHCO_3 (sodium bicarbonate), Na_2CO_3 (sodium carbonate), Cs_2CO_3 (cesium carbonate), NH_4Cl (ammonium chloride), K_2CO_3 (potassium carbonate), KOH (potassium hydroxide), HCl (hydrogen chloride), NaOH (sodium hydroxide), LiOH (lithium hydroxide), LAH (lithium aluminum hydride), EtOH (ethanol), NaCN (sodium cyanide), Et_2O (diethyl ether), CsF (cesium fluoride), NaCl (sodium chloride), TBSCl (tert-butyldimethylsilyl chloride), Ms_2O (methanesulfonic anhydride), MsCl (methanesulfonyl chloride), AcOH (acetic acid), NaBH_4 (sodium borohydride), NaBH_3CN (sodium cyanoborohydride), H_2 (hydrogen), N_2 (nitrogen), MS (molecular sieves). Assay abbreviations: LUC (luciferase), MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Additional abbreviations: Aq. (aqueous), sat. (saturated), RT (room temperature). All anhydrous reactions were run in oven- or flame-dried glassware under an atmosphere of dry nitrogen or argon.

5.2.1. 1-(1-(4-Chlorobenzyl)-1H-pyrrole-2-carbonyl)-N-(2-(pyridin-4-yl)ethyl)azetidine-3-carboxamide (3)—Methyl ester **5** (300 mg, 0.90 mmol) was dissolved in 200 proof EtOH (10 mL) and 10% aq. NaOH (10 mL) was added. The reaction was allowed to stir at RT for 24 h, at which time the solvent was stripped off *in vacuo* and the residue was taken up in H_2O and acidified to pH 1 by addition of conc. HCl at 0°C . The resulting precipitate was collected over a filter and dried by aspirator then high vacuum to afford 1-(1-(4-chlorobenzyl)-1H-pyrrole-2-carbonyl)azetidine-3-carboxylic acid as a white powder. Yield: 250 mg, 0.78 mmol, 87%.

The carboxylic acid (50 mg, 0.16 mmol) was added to DCM (5 mL), followed by 4-(2-aminoethyl)pyridine (20 μL , 0.17 mmol), TEA (66 μL , 0.47 mmol), EDC-HCl (33 mg, 0.17 mmol), and HOBt (26 mg, 0.17 mmol). The reaction was allowed to stir at RT for 17 h, at which time the reaction was concentrated and the residue taken up in EtOAc. This solution was then washed with H_2O (3 $\times$) and 10% aq. Na_2CO_3 (3 $\times$), and brine (1 $\times$), dried (MgSO_4), and concentrated. The residue was purified by silica flash chromatography (4 g silica, 1% to 30% 7 M methanolic ammonia/EtOAc) to provide the final compound **3** as a white powder.

Yield: 32 mg, 0.076 mmol, 48%. ¹H NMR (500 MHz, Chloroform-d) δ 8.48 (s, 2H), 7.25 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 4.1 Hz, 2H), 7.04 (d, J = 8.2 Hz, 2H), 6.80 (s, 1H), 6.50 (d, J = 3.9 Hz, 1H), 6.17 (t, J = 3.2 Hz, 1H), 5.97 (t, J = 5.2 Hz, 1H), 5.52 (s, 2H), 4.25 (bs, 4H), 3.58 (q, J = 6.6 Hz, 2H), 3.20 (p, J = 7.4 Hz, 1H), 2.84 (t, J = 6.9 Hz, 2H). TOF ES+ MS: (M + H) 423.1, (M + Na) 445.1. HPLC t_R = 5.09 min, >95% purity.

5.2.2. *N*₁-(4-Chlorobenzyl)-*N*₄-(2-(pyridin-4-yl)ethyl)piperidine-1,4-dicarboxamide (8a)—Ethyl ester **7** (810 mg, 2.50 mmol) was dissolved in 200 proof EtOH (20 mL) at RT, followed by the addition of 10% aq. NaOH, which elicited precipitation. The mixture was then stirred at 50 °C for 2 h over which time it became a solution. The reaction was then allowed to cool to RT and the solvent was then removed *in vacuo*. The residue was taken up in H₂O and acidified to pH 1 with conc. HCl at 0 °C and the resulting precipitate was collected over a filter, washed with cold 1 M aq. HCl, and dried over the filter then under high vacuum to give the desired 1-((4-chlorobenzyl)carbonyl)piperidine-4-carboxylic acid as a white powder. No further purification necessary. Yield: 675 mg, 2.27 mmol, 91%.

The carboxylic acid (60 mg, 0.20 mmol) and TEA (93 μ L, 0.61 mmol) were dissolved in DCM (5 mL), followed by EDC·HCl (47 mg, 0.22 mmol), HOBT (37 mg, 0.22 mmol), and 4-(2-aminoethyl)pyridine (30 μ L, 0.20 mmol). The reaction was allowed to stir at RT for 15 h. After this time, the reaction was diluted with EtOAc and washed with H₂O (3 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), then dried with MgSO₄ and concentrated. The residue was then crystallized from EtOAc and washed with Et₂O to provide **8a** as an off-white, slightly tanned solid. Yield: 42 mg, 0.11 mmol, 52%. ¹H NMR (500 MHz, Chloroform-d) δ 8.52 (d, J = 4.4 Hz, 2H), 7.32 – 7.20 (m, 4H), 7.11 (d, J = 4.4 Hz, 2H), 5.56 (s, 1H), 4.79 (d, J = 5.9 Hz, 1H), 4.37 (d, J = 5.2 Hz, 2H), 3.96 (d, J = 13.2 Hz, 2H), 3.55 (q, J = 6.4, 5.8 Hz, 2H), 2.85 – 2.78 (m, 4H), 2.19 (t, J = 11.5 Hz, 1H), 1.77 (d, J = 13.0 Hz, 2H), 1.63 (dd, J = 18.8, 5.5 Hz, 2H). TOF ES+ MS: (M + H) 401.2, (M + Na) 423.2. HPLC t_R = 4.61 min, > 95% purity.

5.2.3. 1-(2-(4-Chlorophenyl)acetyl)-*N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (11a)—Ethyl ester **10a** (811 mg, 2.62 mmol) was added to EtOH (15 mL) and 10% aq. NaOH (5 mL). This mixture was heated to 50 °C and stirred for 5 h, then allowed to cool to RT. Solvent was removed *in vacuo* until precipitation began to occur, at which point the material was cooled to 0 °C and acidified to pH<1 by the dropwise addition of conc. HCl. The solution was decanted off the resulting gum, and the residue was air dried, then dried under high vacuum to afford a highly gummy material that could be solidified by sonication in Et₂O to provide 1-(2-(4-chlorophenyl)acetyl)piperidine-4-carboxylic acid as a white powder without further purification. Yield: 723 mg, 2.57 mmol, 98%.

The following were added sequentially to DCM (5 mL) at RT: the carboxylic acid (60 mg, 0.22 mmol), TEA (93 μ L, 0.67 mmol), EDC·HCl (47 mg, 0.25 mmol), HOBT (37 mg, 0.25 mmol), and 4-(2-aminoethyl)pyridine (29 μ L, 0.25 mmol). This solution was stirred a RT for 18 h, at which time the reaction was diluted with EtOAc and washed with H₂O (3 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The crude residue was purified by crystallization from the dropwise addition of EtOAc to boiling hexanes, then

cooling of the solution, to give **11a** as a colorless oil. Yield: 40 mg, 0.105 mmol, 54%. ¹H NMR (500 MHz, Chloroform-d) δ 8.52 (d, J = 4.7 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 7.10 (d, J = 4.8 Hz, 2H), 5.50 (bs, 1H), 4.57 (d, J = 13.3 Hz, 1H), 3.86 (d, J = 13.5 Hz, 1H), 3.68 (s, 2H), 3.54 (q, J = 6.6 Hz, 2H), 3.03 – 2.96 (m, 1H), 2.82 (t, J = 6.9 Hz, 2H), 2.70 – 2.62 (m, 1H), 2.21 (tt, J = 11.1, 3.6 Hz, 1H), 1.77 (d, J = 12.3 Hz, 2H), 1.57 (qd, J = 12.2, 4.1 Hz, 1H), 1.46 (qd, J = 12.3, 3.9 Hz, 1H). TOF ES+ MS: 386.2 (M + H), 408.2 (M + Na). HPLC t_R = 4.35 min, > 95% purity.

5.2.4. 1-(3-(4-Chlorophenyl)propanoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (11b)—Ethyl ester **10b** (850 mg, 2.62 mmol) was dissolved in EtOH (10 mL), followed by the addition of 10% aq. NaOH (10 mL). This was stirred 11 h at RT, after which time a majority of the solvent was stripped off *in vacuo*. The remaining concentrated aq. solution was chilled to 0 °C and acidified to pH<1 with conc. HCl. The aq. solution was decanted off of the resulting gummy residue. H₂O was then added, sonicated, and decanted. The residue was then dried under high vacuum, and the thick viscous residue was sonicated in Et₂O to afford 1-(3-(4-chlorophenyl)propanoyl)piperidine-4-carboxylic acid as a white solid that was collected over a filter. Yield: 730 mg, 2.47 mmol, 94%.

The carboxylic acid (50 mg, 0.17 mmol) was added to DCM (2 mL) at RT followed by TEA (71 μ L, 0.51 mmol), EDC $\cdot$ HCl (36 mg, 0.19 mmol), HOBT (28 mg, 0.19 mmol), and 4-(2-aminoethyl)pyridine (22 μ L, 0.19 mmol). The reaction was allowed to stir at RT for 14 h, after which time the DCM was removed *in vacuo* and the residue taken up in EtOAc, which was subsequently washed with 0.5 M aq. HCl (1 $\times$), H₂O (2 $\times$), 10% aq. Na₂CO₃ (2 $\times$), and brine (1 $\times$), then dried (MgSO₄) and decanted. Compound **11b** was crystallized from the dropwise addition of EtOAc to boiling hexanes, then cooling of the solution. Yield: 42 mg, 0.11 mmol, 62%. ¹H NMR (500 MHz, Chloroform-d) δ 8.52 – 8.48 (m, 2H), 7.29 – 7.20 (m, 2H), 7.17 – 7.07 (m, 4H), 5.66 (s, 1H), 4.57 (d, J = 12.9 Hz, 1H), 3.81 (d, J = 13.2 Hz, 1H), 3.54 (q, J = 5.9 Hz, 2H), 2.96 – 2.89 (m, 3H), 2.82 (t, J = 6.1 Hz, 2H), 2.63 – 2.54 (m, 3H), 2.22 (dt, J = 11.2, 5.8 Hz, 1H), 1.79 – 1.69 (m, 2H), 1.60 – 1.46 (m, 2H). TOF ES+ MS: 400.2 (M + H), 422.2 (M + Na). HPLC t_R = 4.89 min, > 95% purity.

5.2.5. (R)-1-(1-(4-Chlorobenzyl)pyrrolidine-2-carbonyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (R-15)—(R)-1-(4-chlorobenzyl)pyrrolidine-2-carboxylic acid (**R**)-**13** (64 mg, 0.27 mmol) was added to DCM (2 mL), followed by the sequential addition of TEA (150 μ L, 1.06 mmol), HATU (111 mg, 0.29 mmol), and amine dihydrochloride **14** (75 mg, 0.28 mmol). The reaction was stirred for 20 h at RT, after which time the reaction was diluted in sat. aq. NH₄Cl and extracted with EtOAc. The organic extract was then washed with 10% aq. NH₄Cl (2 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and sat. aq. Na₂CO₃ (1 $\times$), then dried with MgSO₄ and concentrated. The residue was purified via silica flash chromatography (4 g silica, 5% to 20% 7 M methanolic ammonia/EtOAc) to give a slightly yellow oil that was triturated from Et₂O/Hex to provide a white powder. Yield: 75 mg, 0.17 mmol, 62%. ¹H NMR (500 MHz, Chloroform-d) δ 8.52 (d, J = 5.1 Hz, 2H), 7.26 (s, 4H), 7.12 (s, 2H), 5.57 (s, 1H), 4.55 (d, J = 11.7 Hz, 1H), 4.15 (bs, 1H), 3.90 (dd, J = 18.8, 13.1 Hz, 1H), 3.61 – 3.50 (m, 2H), 3.39 (d, J = 12.0 Hz, 2H), 3.06 (d, J = 7.0 Hz, 1H), 2.98 – 2.91 (m, 1H), 2.84 (q, J = 6.2 Hz, 2H), 2.59 (q, J = 13.9, 13.3 Hz, 1H), 2.33 – 2.21

(m, 2H), 2.14 (s, 1H), 1.80 – 1.77 (m, 2H), 1.59 – 1.48 (m, 2H). TOF ES+ MS: 455.2 (M + H), 477.2 (M + Na). HPLC t_R = 3.44 min, > 90% purity.

5.2.6. (S)-1-(1-(4-Chlorobenzyl)pyrrolidine-2-carbonyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (S-15)—(S)-1-(4-chlorobenzyl)pyrrolidine-2-carboxylic acid (S)-13 (63 mg, 0.27 mmol) was added to DCM (2 mL), followed by the sequential addition of TEA (150 μ L, 1.06 mmol), HATU (111 mg, 0.29 mmol), and N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide dihydrochloride (75 mg, 0.28 mmol). The reaction was stirred for 24 h at RT, after which time the reaction was diluted in sat. aq. NH_4Cl and extracted with EtOAc. The organic extract was then washed with 10% aq. NH_4Cl (2 $\times$), 10% aq. Na_2CO_3 (3 $\times$), and sat. aq. Na_2CO_3 (1 $\times$), then dried with MgSO_4 and concentrated. The residue was purified via silica flash chromatography (4 g silica, 5% to 20% 7 M methanolic ammonia/EtOAc) to give a slightly yellow oil that was triturated from Et_2O /Hex to provide a white powder. Yield: 64 mg, 0.14 mmol, 53%. ^1H NMR (500 MHz, Chloroform- d) δ 8.48 (d, J = 4.6 Hz, 2H), 7.31 – 7.22 (m, 4H), 7.11 (bs, 2H), 5.82 (bs, 1H), 4.52 (bs, 1H), 4.13 (d, J = 11.9 Hz, 1H), 3.89 (dd, J = 18.3, 13.1 Hz, 1H), 3.58 – 3.49 (m, 2H), 3.46 – 3.34 (m, 2H), 3.10 – 3.00 (m, 1H), 2.94 (d, J = 9.3 Hz, 1H), 2.87 – 2.79 (m, 2H), 2.63 – 2.52 (m, 1H), 2.33 – 2.26 (m, 2H), 2.18 – 2.08 (m, 1H), 1.89 – 1.75 (m, 4H), 1.60 – 1.50 (m, 2H). TOF ES+ MS: 455.2 (M + H), 477.2 (M + Na). HPLC t_R = 3.39 min, > 90% purity.

5.2.7. (R)-1-(1-(4-Chlorobenzoyl)pyrrolidine-2-carbonyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (R-18)—The ethyl ester (R)-17 (300 mg, 0.76 mmol) was dissolved in a 9:1 DCM:MeOH solution (20 mL), and a solution of methanolic NaOH (92 mg NaOH/2mL MeOH) was added. This was stirred at RT for 2 h, after which time the solvent was stripped off *in vacuo* to give a white solid that was dissolved in a small amount of H_2O and acidified by the addition of conc. HCl, and the material was extracted with EtOAc (3 $\times$). The combined organic extracts were dried (MgSO_4) and concentrated. The clear oily residue was then dissolved in minimum EtOAc and precipitated with Hex to afford (R)-1-(4-chlorobenzoyl)pyrrolidine-2-carboxylic acid as a white powder. No further purification necessary. Yield: 260 mg, 0.71 mmol, 93%.

The carboxylic acid (50 mg, 0.14 mmol) and TEA (57 μ L, 0.41 mmol) were dissolved in DCM (5 mL) followed by HATU (57 mg, 0.15 mmol), 4-(2-aminoethyl)pyridine (18 μ L, 0.15 mmol). The reaction was allowed to stir at RT for 15 h, at which time the DCM was removed and the residue taken up in EtOAc and washed with H_2O (3 $\times$), 10% aq. Na_2CO_3 (3 $\times$), and brine (1 $\times$). The solution was then dried (MgSO_4) and concentrated, and the residue was purified via silica flash chromatography (4 g silica, 1% to 20% methanolic ammonia:EtOAc) to give the desired product (R)-18 as a slightly yellow clear oil. Yield: 23 mg, 0.048 mmol, 35%. ^1H NMR (500 MHz, Chloroform- d) δ 8.59 – 8.47 (m, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.12 (dd, J = 9.5, 4.8 Hz, 2H), 5.90 (s, 0.5H), 5.50 (s, 0.5H), 5.10 – 4.99 (m, 1H), 4.63 (d, J = 13.3 Hz, 0.5H), 4.48 (d, J = 13.2 Hz, 1H), 4.09 (dd, J = 40.4, 14.2 Hz, 2H), 3.70 (t, J = 8.6 Hz, 1H), 3.54 (m, 3H), 3.29 (t, J = 12.8 Hz, 0.5H), 3.12 (t, J = 12.7 Hz, 0.5H), 2.83 (dt, J = 14.7, 6.6 Hz, 3H), 2.64 (t, J = 12.6 Hz, 0.5H), 2.40–

1.20 (m, 7H). TOF ES+ MS: (M + H) 469.3, (M + Na) 491.3. HPLC t_R = 4.26 min, > 95% purity.

5.2.8. (S)-1-(1-(4-Chlorobenzoyl)pyrrolidine-2-carbonyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (S-18)—The ethyl ester (S)-17 (300 mg, 0.76 mmol) was dissolved in a 9:1 DCM:MeOH solution (20 mL), and a solution of methanolic NaOH (92 mg NaOH/2mL MeOH) was added. This was stirred at RT for 2 h, after which time the solvent was stripped off *in vacuo* to give a white solid that was dissolved in a small amount of H₂O and acidified by the addition of conc. HCl, and the material was extracted with EtOAc (3×). The combined organic extracts were dried (MgSO₄) and concentrated. The clear oily residue was then dissolved in minimum EtOAc and precipitated with Hex to afford a white powder. No further purification necessary. Yield: 265 mg, 0.73 mmol, 95%.

(S)-1-(4-Chlorobenzoyl)pyrrolidine-2-carboxylic acid (50 mg, 0.14 mmol) was added to DCM (5 mL), followed by the sequential addition of TEA (76 μ L, 0.55 mmol), HATU (63 mg, 0.16 mmol), and 4-(2-aminoethyl)pyridine (20 μ L, 0.16 mmol). The reaction was stirred for 17 h at RT, after which time the reaction was diluted in sat. aq. NH₄Cl and extracted with EtOAc. The organic extract was then washed with 10% aq. NH₄Cl (2×), 10% aq. Na₂CO₃ (3×), and sat. aq. Na₂CO₃ (1×), then dried with MgSO₄ and concentrated. The residue was purified via silica flash chromatography (4 g silica, 5% to 20% 7 M methanolic ammonia:EtOAc) to give the desired compound (S)-18 as a white powder. Yield: 42 mg, 0.09 mmol, 65%. ¹H NMR (500 MHz, Chloroform-d) δ 8.52 (m, 2H), 7.53 (d, J = 8.0 Hz, 2H), 7.37 (t, J = 7.0 Hz, 2H), 7.16 – 7.09 (m, 2H), 5.91 (d, J = 2.1 Hz, 0.5H), 5.58 – 5.49 (m, 0.5H), 5.07 – 4.97 (m, 1H), 4.63 (d, J = 14.5 Hz, 0.5H), 4.48 (d, J = 13.9 Hz, 1H), 4.09 (dd, J = 41.8, 14.7 Hz, 2H), 3.68 (t, J = 9.9 Hz, 1H), 3.54 (m, 3H), 3.32 – 3.21 (m, 0.5H), 3.12 (t, J = 12.8 Hz, 0.5H), 2.83 (dt, J = 14.6, 6.8 Hz, 3H), 2.64 (t, J = 12.6 Hz, 0.5H), 2.47 – 1.15 (m, 7H). TOF ES+ MS: 469.3(M + H), 491.2 (M + Na). HPLC t_R = 4.36 min, > 95% purity.

5.2.9. 1-(2-(4-Chlorophenoxy)benzoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (21)—Methyl ester 20 was dissolved in EtOH (5 mL), followed by the addition of 10% aq. NaOH (2 mL). The reaction was allowed to stir for 14 h, after which time the solvent was stripped off *in vacuo* and the residue re-dissolved in H₂O. This aqueous solution was then cooled to 0 °C and acidified to pH<1 with conc. HCl, and the resulting precipitate was collected over a filter, then dried via aspirator and high vacuum to provide the desired carboxylic acid as a white powder. No further purification necessary. Yield: 92 mg, 0.37 mmol, 97%.

All of the previously prepared carboxylic acid (92 mg, 0.37 mmol) was added to DCM (5 mL), followed by the amine dihydrochloride 14 (136 mg, 0.44 mmol), TEA (310 μ L, 2.22 mmol), EDC·HCl (85 mg, 0.44 mmol), and HOBT (68 mg, 0.44 mmol). The solution was allowed to stir at RT for 18 h, after which time the reaction was concentrated *in vacuo* and the resulting residue was taken up in EtOAc and washed with H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. The resulting residue was purified by silica flash chromatography (10 g silica, 1% to 20% methanolic ammonia:EtOAc) to give the desired compound 21 as a thick oil. ¹H NMR (500 MHz,

Chloroform-*d*) δ 8.43 (d, J = 4.7 Hz, 2H), 7.28 (dt, J = 29.2, 7.9 Hz, 4H), 7.14 (t, J = 7.4 Hz, 1H), 7.06 (d, J = 4.8 Hz, 2H), 6.97 – 6.76 (m, 3H), 6.15 (d, J = 47.8 Hz, 1H), 4.57 (d, J = 12.6 Hz, 1H), 3.63 (bs, 1H), 3.48 (q, J = 6.5 Hz, 2H), 3.04 – 2.88 (m, 1H), 2.77 (t, J = 6.9 Hz, 2H), 2.75 – 2.62 (m, 1H), 2.24 (t, J = 11.0 Hz, 1H), 1.81 – 1.73 (m, 1H), 1.72 – 1.61 (m, 2H), 1.61 – 1.51 (m, 1H). TOF ES+ MS: 464.2 ($M + H$), 486.2 ($M + Na$). HPLC t_R = 5.13 min, > 90% purity.

5.2.10. 1-(2-((4-Chlorophenyl)amino)benzoyl)-*N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (23a)—Methyl 2-((4-chlorophenyl)amino)benzoate **22a** (650 mg, 2.48 mmol) was dissolved in 200 proof EtOH (15 mL) at RT and 10% aq. NaOH (6 mL) was added. The reaction was allowed to stir at RT for 5 h, at which time the solvent was stripped off *in vacuo* and 1M aq. HCl was added to the residue. The solution was then chilled in an ice bath and acidified to pH 1, and the resulting white precipitate was collected over a filter, washed with 1M aq. HCl, and dried via aspirator and high vacuum to yield 2-((4-chlorophenyl)amino)benzoic acid as a white powder. No further purification necessary. Yield: 581 mg, 2.35 mmol, 94%. 1H NMR (500 MHz, Chloroform-*d*) δ 9.27 (s, 1H), 8.06 (dd, J = 8.1, 1.5 Hz, 1H), 7.38 (ddd, J = 8.6, 7.1, 1.6 Hz, 1H), 7.35 – 7.31 (m, 2H), 7.23 – 7.19 (m, 2H), 7.17 (d, J = 8.5 Hz, 1H), 6.79 (t, J = 7.5 Hz, 1H).

The carboxylic acid (370 mg, 1.49 mmol) was then dissolved in DCM (15 mL), followed by ethyl isonipecotatate (253 μ L, 1.64 mmol), TEA (625 μ L, 4.48 mmol), EDC·HCl (315 mg, 1.64 mmol), and HOBT (252 mg, 1.64 mmol). The reaction was stirred at RT for 16 h, at which time the reaction was diluted with 2:1 EtOAc:Et₂O and washed with 1 M aq. HCl (2 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (12 g silica, 5% to 20% EtOAc:Hex) to provide ethyl 1-(2-((4-chlorophenyl)amino)benzoyl)piperidine-4-carboxylate as a yellow oil. Yield: 129 mg, 0.333 mmol, 22%. 1H NMR (500 MHz, Chloroform-*d*) δ 7.32 (d, J = 8.2 Hz, 1H), 7.25 – 7.15 (m, 4H), 7.02 (d, J = 8.7 Hz, 2H), 6.89 (t, J = 7.4 Hz, 1H), 4.30 (bs, 2H), 4.15 (q, J = 7.1 Hz, 2H), 3.09 (t, J = 11.1 Hz, 2H), 2.56 (tt, J = 10.6, 4.0 Hz, 1H), 1.95 (bs, 2H), 1.75 – 1.67 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H).

Ethyl 1-(2-((4-chlorophenyl)amino)benzoyl)piperidine-4-carboxylate (72 mg, 0.19 mmol) was dissolved in EtOH (5 mL), followed by the addition of 10% aq. NaOH (2 mL). This reaction was allowed to stir at RT for 24 h, after which time the solvent was stripped off *in vacuo* and the residue was taken up in H₂O and acidified to pH<1 with conc. HCl at 0 °C. The resulting white precipitate was collected over a filter, dried via aspirator then high vacuum to afford 1-(2-((4-chlorophenyl)amino)benzoyl)piperidine-4-carboxylic acid as a white powder requiring no further purification. Yield: 47 mg, 0.13 mmol, 70%.

The carboxylic acid (36 mg, 0.10 mmol), 4-(2-aminoethyl)pyridine (13 μ L, 0.11 mmol), TEA (42 μ L, 0.30 mmol), EDC·HCl (21 mg, 0.11 mmol), and HOBT (17 mg, 0.11 mmol) were all added to DCM (5 mL) in the listed order and stirred at RT for 20 h, at which time the reaction was diluted with 1:1 EtOAc:Et₂O and washed with 0.5 M aq. HCl (1 $\times$), H₂O (1 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$). The organic phase was then dried (MgSO₄) and concentrated, and the residue was purified via silica flash chromatography (4 g silica, 80% to 100% EtOAc:Hex gradient) to give the title compound **23a** as a clear, colorless oil. Yield:

36 mg, 0.078 mmol, 78%. ¹H NMR (500 MHz, Chloroform-d) δ 8.51 (d, *J* = 4.9 Hz, 2H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.26 – 7.19 (m, 3H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.13 – 7.09 (m, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.89 (t, *J* = 7.4 Hz, 1H), 5.56 (s, 1H), 4.23 (bs, 2H), 3.53 (q, *J* = 6.6 Hz, 2H), 2.93 (s, 2H), 2.82 (t, *J* = 6.9 Hz, 2H), 2.28 (tt, *J* = 11.2, 3.7 Hz, 1H), 1.80 – 1.66 (m, 4H). TOF ES+ MS: 463.2 (M + H), 485.2 (M + Na). HPLC *t*_R = 5.23 min, > 95% purity.

5.2.11. 1-(2-(Phenylamino)benzoyl)-*N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (23b)—

Methyl 2-(phenylamino)benzoate **22b** (74 mg, 0.33 mmol) was dissolved in 10% aq. NaOH (1 mL), THF (5 mL), and EtOH (2 mL), and stirred at RT for 5 h. At this time, the solvent was stripped off *in vacuo* and the residue was dissolved in H₂O and acidified with conc. HCl to pH < 1. Material was collected over a filter and air-dried then dried under high vacuum to give 2-(phenylamino)benzoic acid as a white powder. No further purification necessary. Yield: 62 mg, 0.29 mmol, 89%.

The carboxylic acid (56 mg, 0.26 mmol), amine dihydrochloride **14** (97 mg, 0.32 mmol), EDC·HCl (60 mg, 0.32 mmol), HOBT (48 mg, 0.32 mmol), and TEA (220 μL, 1.58 mmol) were dissolved in DCM (10 mL) and stirred at RT for 17 h. After this time, the DCM was removed *in vacuo*. The residue was taken up in EtOAc and washed with H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×), and dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (10 g silica, 1% to 20% methanolic NH₃/EtOAc) to give the desired compound **23b** as a colorless oil. Yield: 53 mg, 0.12 mmol, 47%. ¹H NMR (500 MHz, Chloroform-d) δ 8.47 (d, *J* = 5.0 Hz, 2H), 7.37 (d, *J* = 8.3 Hz, 1H), 7.28 – 7.21 (m, 3H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.10 – 7.02 (m, 4H), 6.93 (t, *J* = 7.8 Hz, 1H), 6.86 (t, *J* = 7.4 Hz, 1H), 5.78 (s, 1H), 4.26 (s, 2H), 3.50 (q, *J* = 6.5 Hz, 2H), 2.92 (bs, 2H), 2.80 (t, *J* = 6.9 Hz, 2H), 2.28 (ddd, *J* = 14.7, 11.0, 3.4 Hz, 1H), 1.77 (bs, 2H), 1.70 – 1.57 (m, 2H). TOF ES+ MS: 429.2 (M + H), 451.2 (M + Na). HPLC *t*_R = 5.05 min, > 95% purity.

5.2.12. 1-(2-((4-Chlorophenyl)(methyl)amino)benzoyl)-*N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (25)—

Methyl 2-((4-chlorophenyl)(methyl)amino)benzoate **24** (120 mg, 0.44 mmol) was dissolved in EtOH (8 mL), followed by the addition of 10% aq. NaOH (3 mL). The reaction was allowed to stir at RT for 6 h, after which time the solvent was removed and the residue was taken up in H₂O, cooled to 0 °C, and acidified to pH < 1. The resulting white precipitate was collected over a filter, and dried under high vacuum. 2-((4-chlorophenyl)(methyl)amino)benzoic acid was used without further purification. Yield: 108 mg, 0.41 mmol, 95%. ¹H NMR (400 MHz, Chloroform-d) δ 9.44 (s, 1H), 7.96 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.35 – 7.24 (m, 3H), 7.17 (t, *J* = 9.0 Hz, 3H), 6.79 – 6.72 (m, 1H), 3.89 (s, 3H).

The carboxylic acid (85 mg, 0.33 mmol), amine dihydrochloride **14** (119 mg, 0.39 mmol), TEA (270 μL, 1.95 mmol), EDC·HCl (75 mg, 0.39 mmol), and HOBT (60 mg, 0.39 mmol) were added to DCM (5 mL) sequentially, and the solution was allowed to stir at RT for 20 h. After this time, the solvent was removed *in vacuo* and the residue was dissolved in EtOAc and washed with H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. The residue was then purified via silica flash chromatography (10 g silica, 1% to 20% methanolic ammonia:EtOAc) to provide the desired compound **10** as a thick colorless oil. Yield: 90 mg, 0.19 mmol, 58%. ¹H NMR (400 MHz, Chloroform-d, mixture of

rotamers) δ 8.48 (m, 2H), 7.39 (t, J = 8.1 Hz, 1H), 7.34 – 7.19 (m, 3H), 7.09 (d, J = 7.9 Hz, 4H), 6.63 (t, J = 9.6 Hz, 2H), 5.75 (t, J = 5.4 Hz, 0.5H), 5.41 (t, J = 5.4 Hz, 0.5H), 4.64 – 4.44 (m, 1H), 3.61 – 3.39 (m, 3H), 3.22 (s, 1.5H), 3.17 (s, 1.5H), 2.93 – 2.84 (m, 0.5H), 2.83 – 2.74 (m, 2H), 2.74 – 2.63 (m, 1H), 2.53 – 2.44 (m, 0.5H), 2.22 – 2.09 (m, 1H), 1.82 (bd, J = 13.1 Hz, 0.5H), 1.78 – 1.51 (m, 3.5H), 1.41 (bd, J = 13.2 Hz, 0.5H), 1.30 (qd, J = 12.9, 4.2 Hz, 0.5H). TOF ES+ MS: 477.2 (M + H), 499.2 (M + Na). HPLC t_R = 5.13 min, > 90% purity.

5.2.13. 1-(2-((4-Chlorobenzyl)amino)benzoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (28a)—Methyl ester **27a** (80 mg, 0.29 mmol) was

dissolved in MeOH (8 mL) and 10% aq. NaOH (4 mL) was added and the reaction was stirred at RT for 24 h. At this time, the solvent was removed *in vacuo* and the residue was dissolved in H₂O and acidified with conc. HCl to pH<1 at 0 °C. The resulting precipitate was collected over a filter and dried via aspirator then high vacuum to afford 2-((4-chlorobenzyl)amino)benzoic acid as a white powder. No further purification necessary. Yield: 75 mg, 0.29 mmol, 99%.

The carboxylic acid (75 mg, 0.29 mmol) was dissolved in DCM (12 mL) along with TEA (325 μ L, 2.32 mmol), EDC·HCl (67 mg, 0.35 mmol), HOBT (53 mg, 0.35 mmol), and amine dihydrochloride **14** (133 mg, 0.44 mmol). The solution was stirred at RT for 20 h, after which time the DCM was stripped off *in vacuo* and the resulting residue was taken up in EtOAc and washed with H₂O (3 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was then purified via silica flash chromatography (10 g silica, 1% to 30% methanolic ammonia:EtOAc) to give the desired compound **28a**. Yield: 63 mg, 0.132 mmol, 46%. ¹H NMR (500 MHz, Chloroform-d) δ 8.60 – 8.51 (m, 2H), 7.30 – 7.25 (m, 4H), 7.22 – 7.15 (m, 1H), 7.14 – 7.10 (m, 2H), 7.08 (dd, J = 7.6, 1.6 Hz, 1H), 6.67 (t, J = 7.4 Hz, 1H), 6.58 (d, J = 8.4 Hz, 1H), 5.58 (t, J = 5.2 Hz, 1H), 5.53 (t, J = 5.7 Hz, 1H), 4.33 (m, 4H), 3.57 (q, J = 6.7 Hz, 2H), 2.95 (t, J = 12.8 Hz, 2H), 2.85 (t, J = 6.9 Hz, 2H), 2.30 (tt, J = 11.1, 3.8 Hz, 1H), 1.82 (m, 2H), 1.74 (dt, J = 15.9, 7.9 Hz, 2H). TOF ES+ MS: 477.2 (M + H), 499.2 (M + Na). HPLC t_R = 5.33 min, > 90% purity.

5.2.14. 1-(2-(Benzylamino)benzoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (28b)—Methyl 2-(benzylamino)benzoate **27b** (50 mg, 0.21 mmol) was

dissolved in EtOH (2 mL) and THF (5 mL), then 10% aq. NaOH (2 mL) was added and the reaction was stirred at RT for 15 h. At this time, the solvent was removed *in vacuo* and the residue was dissolved in H₂O and acidified with conc. HCl to pH<1. The resulting precipitate was collected over a filter and dried via aspirator then high vacuum to afford the desired 2-(benzylamino)benzoic acid as a white powder. No further purification necessary. Yield: 41 mg, 0.18 mmol, 88%.

The carboxylic acid (30 mg, 0.13 mmol) was dissolved in DCM (5 mL), followed by amine dihydrochloride **14** (45 mg, 0.15 mmol), TEA (0.11 mL, 0.79 mmol), EDC·HCl (30 mg, 0.16 mmol), and HOBT (24 mg, 0.16 mmol). The reaction was then stirred at RT for 16 h, at which time the reaction was diluted with EtOAc and washed with H₂O (2 $\times$), 10% aq. Na₂CO₃ (2 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated *in vacuo*. The resulting residue was then purified by silica flash chromatography (10 g silica, 1% to 20% methanolic

ammonia:EtOAc) to provide the desired product **28b** as a colorless oil. Yield: 26 mg, 0.06 mmol, 48%. ¹H NMR (500 MHz, Chloroform-d) δ 8.48 (d, J = 5.7 Hz, 2H), 7.32 (d, J = 6.7 Hz, 4H), 7.26 – 7.21 (m, 1H), 7.17 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 5.7 Hz, 2H), 7.07 – 7.02 (m, 1H), 6.63 (dd, J = 7.8, 3.8 Hz, 2H), 5.92 (t, J = 5.6 Hz, 1H), 5.44 (t, J = 5.4 Hz, 1H), 4.33 (d, J = 5.3 Hz, 2H), 4.26 (bs, 2H), 3.52 (q, J = 6.7 Hz, 2H), 2.90 (t, J = 11.7 Hz, 2H), 2.81 (t, J = 6.9 Hz, 2H), 2.28 (tt, J = 11.2, 3.5 Hz, 1H), 1.78 (d, J = 12.1 Hz, 2H), 1.69 (qd, J = 12.9, 12.4, 4.0 Hz, 2H). TOF ES+ MS: 443.2 (M + H), 465.2 (M + Na). HPLC t_R = 4.92 min, > 95% purity.

5.2.15. 1-(2-((4-Chlorophenethyl)amino)benzoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (31)—2-((4-Chlorophenethyl)amino)benzoic acid (40 mg, 0.15 mmol), amine dihydrochloride **14** (53 mg, 0.17 mmol), EDC·HCl (33 mg, 0.17 mmol), HOBT (26 mg, 0.17 mmol), and TEA (0.12 mL, 0.87 mmol) were dissolved in DCM (10 mL) and stirred at RT for 24 h, at which time the DCM was removed *in vacuo* and the residue was taken up in EtOAc and washed with H₂O (2 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$). The organic extract was dried (MgSO₄) and concentrated, and the residue purified via silica flash chromatography (10 g silica, 1% to 20% methanolic ammonia:EtOAc) to give **31** as a colorless oil. Yield: 41 mg, 0.083 mmol, 57%. HPLC t_R = 5.55 min, > 95% purity. ¹H NMR (500 MHz, Chloroform-d) δ 8.52 (d, J = 5.2 Hz, 2H), 7.30 – 7.21 (m, 3H), 7.16 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 5.2 Hz, 2H), 7.05 (d, J = 7.5 Hz, 1H), 6.69 (d, J = 8.3 Hz, 1H), 6.65 (d, J = 7.4 Hz, 1H), 5.57 (t, J = 5.6 Hz, 1H), 4.97 (bs, 1H), 4.21 (bs, 2H), 3.56 (q, J = 6.5 Hz, 2H), 3.34 (t, J = 7.2 Hz, 2H), 2.89 (t, J = 7.2 Hz, 2H), 2.83 (q, J = 12.6, 9.7 Hz, 4H), 2.24 (tt, J = 11.2, 3.7 Hz, 1H), 1.77 – 1.62 (m, 7H). TOF ES+ MS: 491.2 (M + H), 513.2 (M + Na). HPLC t_R = 5.44 min, >95% purity.

5.2.16. 1-(2-(4-Chlorobenzoyl)benzoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (34)—2-(4-Chlorobenzoyl)benzoic acid **33** (366 mg, 1.404 mmol) was added to DCM (20 mL), followed by N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide dihydrochloride (473 mg, 1.544 mmol), TEA (978 μ L, 7.02 mmol), EDC·HCl (296 mg, 1.544 mmol), and HOBT (237 mg, 1.544 mmol.) The reaction was allowed to stir at RT for 20 h, at which time the solvent was removed *in vacuo*. The residue was taken up in EtOAc and washed with H₂O (1 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was then purified by silica flash chromatography (25 g silica, 1% to 20% 7 M methanolic ammonia:EtOAc.) ¹H NMR (400 MHz, Chloroform-d) δ 8.47 (d, J = 5.5 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 7.61 – 7.53 (m, 1H), 7.52 – 7.24 (m, 5H), 7.10 (d, J = 5.4 Hz, 2H), 5.98 (t, J = 5.5 Hz, 1H), 4.47 (d, J = 12.9 Hz, 1H), 3.62 (d, J = 13.5 Hz, 1H), 3.51 (q, J = 6.6 Hz, 2H), 3.02 (t, J = 11.6 Hz, 1H), 2.81 (t, J = 6.9 Hz, 2H), 2.71 (t, J = 11.9 Hz, 1H), 2.28 (ddd, J = 14.9, 11.1, 3.9 Hz, 1H), 1.91 – 1.52 (m, 4H). TOF ES+ MS: (M + H) 476.2, (M + Na) 498.2. HPLC t_R = 4.83 min, > 95% purity.

5.2.17. 1-(2-(4-Chlorobenzyl)benzoyl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (36)—2-(4-Chlorobenzyl)benzoic acid **35** (40 mg, 0.16 mmol), amine dihydrochloride **14** (55 mg, 0.18 mmol), TEA (225 μ L, 1.62 mmol), EDC·HCl (37 mg, 0.20 mmol), and HOBT (30 mg, 0.20 mmol) were dissolved in DCM (10 mL) and stirred at RT for 24 h. The DCM was then removed *in vacuo* and the residue was taken up in EtOAc, then

washed with H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. Purification via silica flash chromatography (4 g silica, 1% to 20% methanolic ammonia:EtOAc) provided the desired compound as white microcrystals upon concentration. Yield: 23 mg, 0.05 mmol, 28%. ¹H NMR (500 MHz, Chloroform-d, 3:2 mixture of rotamers) δ 8.51 (bs, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 7.27 – 7.18 (m, 4H), 7.17 – 7.05 (m, 5H), 5.61 – 5.42 (m, 1H), 4.73 – 4.59 (m, 1H), 4.18 – 4.03 (m, 1H), 3.93 – 3.80 (m, 1H), 3.53 (q, *J* = 6.5 Hz, 2H), 3.37 – 3.15 (m, 1H), 2.86 – 2.81 (m, 2H), 2.50 (td, *J* = 12.7, 3.1 Hz, 1H), 2.23 – 1.99 (m, 2H), 1.90 – 1.75 (m, 2H), 1.73 – 1.49 (m, 2H). TOF ES+ MS: 462.2 (M + H). HPLC *t*_R = 4.93 min, > 90% purity.

5.2.18. 1-(9*H*-Carbazole-1-carbonyl)-*N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (41)—9*H*-Carbazole-1-carboxylic acid **38** was prepared in a manner similar to that reported by Katritzky.⁴⁵

9*H*-Carbazole-1-carboxylic acid **38** (120 mg, 0.57 mmol), amine dihydrochloride **14** (190 mg, 0.63 mmol), TEA (475 μL, 3.41 mmol), EDC·HCl (120 mg, 0.63 mmol) and HOBT (96 mg, 0.63 mmol) were dissolved in DCM (10 mL) and stirred at RT for 14 h. At this time, the reaction was diluted with EtOAc and washed with H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (10 g silica, 10% to 30% methanolic ammonia/EtOAc) to give **41** as a tan powder. Yield: 170 mg, 0.40 mmol, 70%. ¹H NMR (500 MHz, Chloroform-d) δ 9.13 (s, 1H), 8.53 (d, *J* = 5.6 Hz, 2H), 8.14 (d, *J* = 7.7 Hz, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.50 – 7.42 (m, 2H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.26 – 7.23 (m, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.11 (d, *J* = 5.3 Hz, 2H), 5.59 (t, *J* = 5.5 Hz, 1H), 4.46 (s, 2H), 3.56 (q, *J* = 6.6 Hz, 2H), 3.04 (t, *J* = 12.3 Hz, 2H), 2.84 (t, *J* = 6.9 Hz, 2H), 2.32 (ddd, *J* = 15.0, 10.9, 4.1 Hz, 1H), 1.91 – 1.72 (m, 4H). TOF ES+ MS: 427.2 (M + H), 449.2 (M + Na). HPLC *t*_R = 4.80 min, >95% purity.

5.2.19. 1-(6-Chloro-9*H*-carbazole-1-carbonyl)-*N*-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (42)—Methyl 6-chloro-9*H*-carbazole-1-carboxylate **40** (54 mg, 0.21 mmol) was dissolved in EtOH (4 mL) and THF (5 mL), then 10% aq. NaOH (2 mL) was added. The reaction was allowed to stir at RT for 22 h, after which time the solvent was stripped off *in vacuo*. The residue was taken up in H₂O and acidified to pH < 1 with conc. HCl, and the resulting white precipitate was collected over a filter, air dried, then high vacuum dried to give 6-chloro-9*H*-carbazole-1-carboxylic acid as a white powder with no further purification. Yield: 51 mg, 0.21 mmol, 100%.

The carboxylic acid (51 mg, 0.21 mmol) was added to DCM (5 mL), followed by amine dihydrochloride **14** (76 mg, 0.25 mmol), TEA (145 μL, 1.04 mmol), EDC·HCl (48 mg, 0.25 mmol) and HOBT (38 mg, 0.25 mmol). This solution was stirred at RT for 16 h, at which time the solvent was removed *in vacuo* and the residue taken up in EtOAc and washed with H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×). The extract was dried (MgSO₄) and concentrated, and the residue purified via silica flash chromatography (10 g silica, 1% to 20% methanolic ammonia: EtOAc). Yield: 48 mg, 0.10 mmol, 50%. ¹H NMR (500 MHz, Chloroform-d) δ 8.53 (d, *J* = 4.7 Hz, 2H), 8.10 – 8.06 (m, 1H), 8.04 – 7.98 (m, 1H), 7.48 – 7.36 (m, 3H), 7.22 (ddd, *J* = 7.6, 5.9, 1.0 Hz, 1H), 7.13 (d, *J* = 5.1 Hz, 2H), 5.61 – 5.52 (m, 1H), 4.45 (bs, 2H), 3.57 (q, *J* = 6.6 Hz, 2H), 3.06 (s, 2H), 2.85 (t, *J* = 6.9 Hz, 2H), 2.34 (tt, *J*

= 10.9, 4.6 Hz, 1H), 1.91 – 1.69 (m, 4H). TOF ES+ MS: 461.2 (M + H), 483.2 (M + Na). HPLC t_R = 5.23 min, > 90% purity.

5.2.20. 1-(1-(4-Chlorophenyl)-2-oxo-1,2-dihydroquinolin-4-yl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (46)—Ethyl ester **45** (150 mg, 0.37 mmol) was dissolved in THF (10 mL) followed by the addition of 10% aq. NaOH (5 mL). This biphasic reaction was stirred at RT for 20 h. The solvent was then stripped off *in vacuo* and the resulting residue was dissolved in H₂O, chilled to 0°C, then acidified to pH < 1. The resulting precipitate was collected over a filter, air dried, then high vacuum dried to provide 1-(1-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylic acid as a white powder. No further purification necessary. Yield: 130 mg, 0.33 mmol, 91%.

The carboxylic acid (66 mg, 0.17 mmol), 4-(2-aminoethyl)pyridine (25 μ L, 0.21 mmol), TEA (72 μ L, 0.52 mmol), ED C (40 mg, 0.21 mmol), and HOBt (32 mg, 0.21 mmol) were dissolved in DCM (5 mL) and stirred at RT for 19 h. The reaction was then diluted with EtOAc and washed with H₂O (3 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (10 g silica, 1 to 17% methanolic ammonia/EtOAc) to give the title compound **46** as an off-white powder. Yield: 47 mg, 0.10 mmol, 56%. ¹H NMR (500 MHz, Chloroform-d) δ 8.55 (d, J = 5.1 Hz, 2H), 7.79 (d, J = 7.9 Hz, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.32 (t, J = 7.5 Hz, 1H), 7.25 – 7.18 (m, 3H), 7.15 (d, J = 4.9 Hz, 2H), 6.66 (d, J = 8.4 Hz, 1H), 6.20 (s, 1H), 5.78 (t, J = 6.0 Hz, 1H), 5.30 (s, 1H), 3.58 (q, J = 9.6, 6.8 Hz, 4H), 2.85 (t, J = 6.8 Hz, 2H), 2.77 (t, J = 11.9 Hz, 2H), 2.34 – 2.21 (m, 2H), 2.07 (q, J = 13.3 Hz, 2H), 1.95 (d, J = 13.5 Hz, 2H). TOF ES+ MS: 487.2 (M + H), 509.2 (M + Na). HPLC t_R = 5.09 min, >95% purity.

5.2.21. 1-(1-(4-Chlorobenzyl)-2-oxo-1,2-dihydroquinolin-4-yl)-N-(2-(pyridin-4-yl)ethyl)piperidine-4-carboxamide (48)—Ethyl 1-(1-(4-chlorobenzyl)-2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylate **47** (75 mg, 0.18 mmol) was dissolved into a mixture of EtOH (4 mL), THF (4 mL), and 10% aq. NaOH (2 mL) and stirred at RT for 18 h. After this time, the reaction was concentrated and the residue dissolved in H₂O, chilled to 0 °C, and acidified to pH<1 with conc. HCl. The resulting precipitate was collected over a filter and washed with cold 1M HCl, air dried via aspirator, then dried under high vacuum to give 1-(1-(4-chlorobenzyl)-2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylic acid as a white solid. No further purification necessary. Yield: 67 mg, 0.17 mmol, 95%.

The carboxylic acid (48 mg, 0.12 mmol), 4-(2-aminoethyl)pyridine (17 μ L, 0.15 mmol), TEA (51 μ L, 0.36 mmol), EDC·HCl (28 mg, 0.15 mmol), and HOBt (22 mg, 0.15 mmol) were dissolved in DCM (5 mL) and stirred at RT for 20 h. DCM was then removed *in vacuo* and the residue taken up in EtOAc and washed with H₂O (3 $\times$), 10% aq. Na₂CO₃ (3 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was purified by silica flash chromatography (10 g silica, 1% to 20% methanolic ammonia:EtOAc). Yield: 45 mg, 0.05 mmol, 45%. ¹H NMR (500 MHz, Chloroform-d) δ 8.54 (s, 2H), 7.78 (d, J = 8.1 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.26 (d, J = 8.0 Hz, 2H), 7.21 – 7.00 (m, 6H), 6.22 (s, 1H), 5.81 (t, J = 5.4 Hz, 1H), 5.46 (s, 2H), 3.58 (dt, J = 16.9, 8.7 Hz, 4H), 2.87 (t, J = 6.9 Hz, 2H), 2.74 (t, J = 11.6 Hz, 2H), 2.28 (tt, J = 11.4, 3.7 Hz, 1H), 2.05 (qd, J = 12.5, 12.0, 3.5 Hz, 2H), 1.94

(d, $J = 11.2$ Hz, 2H). TOF ES+ MS: 501.2 (M + H), 523.2 (M + Na). HPLC $t_R = 5.25$ min, >95% purity.

5.2.22. Methyl 1-(1-(4-chlorobenzyl)-1H-pyrrole-2-carbonyl)azetidine-3-carboxylate (5)—Carboxylic acid **4** (500 mg, 2.12 mmol) was added to DCM (20 mL), followed sequentially by DIPEA (1.1 mL, 6.36 mmol), EDC·HCl (447 mg, 2.33 mmol), HOBT (357 mg, 2.33 mmol), and methyl azetidine-3-carboxylate hydrochloride (387 mg, 2.55 mmol). The reaction was allowed to stir at RT for 18 h, at which time the DCM was removed *in vacuo* and the residue was taken up in 2:1 EtOAc:Et₂O and washed with 1 M aq. HCl (5×), H₂O (5×), 10% aq. Na₂CO₃ (5×), and brine (1×), then dried (MgSO₄) and concentrated. The resulting residue was purified by silica flash chromatography (25 g silica, 5% to 30% EtOAc/Hex) to give the desired compound as a clear, slightly yellow oil. Yield: 508 mg, 1.53 mmol, 72%. ¹H NMR (500 MHz, Chloroform-d) δ 7.25 (d, $J = 8.3$ Hz, 2H), 7.04 (d, $J = 8.2$ Hz, 2H), 6.81 (s, 1H), 6.58–6.44 (m, 1H), 6.20–6.13 (m, 1H), 5.52 (s, 2H), 4.36 (bs, 4H), 3.75 (s, 3H), 3.43 (p, $J = 7.5$ Hz, 1H).

5.2.23. Ethyl 1-((4-chlorobenzyl)carbamoyl)piperidine-4-carboxylate (7)—(4-Chlorobenzyl)isocyanate **6** (0.30 mL, 2.26 mmol) was dissolved in DCM (10 mL) at RT, then ethyl isonipecotate (0.38 mL, 2.49 mmol) was added dropwise with stirring, resulting in precipitation and discoloration. This slurry was allowed to stir at RT for 1 h, at which time the precipitate was collected over a filter and dried via aspirator to afford an off-white granular solid. No further purification was necessary. Yield: 690 mg, 2.13 mmol, 94%. ¹H NMR (500 MHz, Chloroform-d) δ 7.30–7.25 (m, 2H), 7.25–7.20 (m, 2H), 4.84 (s, 1H), 4.37 (d, $J = 5.6$ Hz, 2H), 4.14 (q, $J = 7.1$ Hz, 2H), 3.87 (dt, $J = 13.3, 3.7$ Hz, 2H), 2.96–2.87 (m, 2H), 2.46 (ddd, $J = 14.6, 10.7, 3.8$ Hz, 1H), 1.91 (dd, $J = 13.4, 3.1$ Hz, 2H), 1.71–1.62 (m, 2H), 1.25 (t, $J = 7.2$ Hz, 3H).

5.2.24. Ethyl 1-(2-(4-chlorophenyl)acetyl)piperidine-4-carboxylate (10a)—4-Chlorophenylacetic acid **9a** (900 mg, 5.28 mmol) was dissolved in DCM (20 mL), followed by the sequential addition of TEA (2.21 mL, 15.83 mmol), EDC·HCl (1110 mg, 5.80 mmol), HOBT (889 mg, 5.80 mmol), and ethyl isonipecotate (0.894 mL, 5.80 mmol). The reaction was stirred at RT for 28 h, after which time the DCM was evaporated off. The residue was taken up in EtOAc and washed with 1M HCl (3×), H₂O (5×), 10% aq. Na₂CO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. The residue was then purified through a plug of silica (30 g silica, 5% EtOAc:Hex). Yield: 1.31 g, 4.22 mmol, 80%. ¹H NMR (500 MHz, Chloroform-d) δ 7.29 (d, $J = 8.3$ Hz, 2H), 7.17 (d, $J = 8.2$ Hz, 2H), 4.40 (dt, $J = 12.5, 4.0$ Hz, 1H), 4.13 (q, $J = 7.1$ Hz, 2H), 3.79 (dt, $J = 13.5, 3.7$ Hz, 1H), 3.69 (s, 2H), 3.12–3.05 (m, 1H), 2.89–2.82 (m, 1H), 2.52–2.45 (m, 1H), 1.96–1.87 (m, 2H), 1.83–1.77 (m, 1H), 1.61–1.57 (m, 1H), 1.49 (ddd, $J = 24.1, 10.9, 3.8$ Hz, 1H), 1.25 (t, $J = 7.1$ Hz, 3H).

5.2.25. Ethyl 1-(3-(4-chlorophenyl)propanoyl)piperidine-4-carboxylate (10b)—3-(4-Chlorophenyl)propionic acid **9b** (0.90 g, 4.87 mmol) was dissolved in DCM (20 mL), followed by TEA (2.04 mL, 14.62 mmol), EDC·HCl (1.03 g, 5.36 mmol), HOBT (0.82 g, 5.36 mmol), and ethyl isonipecotate (0.83 mL, 5.36 mmol). The solution was stirred at RT for 28 h, after which time the DCM was removed *in vacuo* and the residue was dissolved in

2:1 EtOAc:Et₂O and washed with 1 M aq. HCl (3×), H₂O (2×), 10% aq. Na₂SO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. The residue was then purified by silica flash chromatography (100 g silica, 5% to 50% EtOAc:Hex). ¹H NMR (500 MHz, Chloroform-d) δ 7.20 (d, *J* = 8.2 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 4.41 – 4.34 (m, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.71 (d, *J* = 13.7 Hz, 1H), 3.04 – 2.96 (m, 1H), 2.89 (t, *J* = 7.8 Hz, 2H), 2.80 – 2.72 (m, 1H), 2.58 – 2.53 (m, 2H), 2.46 (tt, *J* = 10.7, 4.0 Hz, 1H), 1.90 – 1.80 (m, 2H), 1.54 (ddd, *J* = 24.1, 13.3, 3.5 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H).

5.2.26. (*R*)-1-(4-Chlorobenzyl)pyrrolidine-2-carboxylic acid (*R*-13)—*L*-Proline (*R*)-12 (500 mg, 4.34 mmol) was dissolved in IPA (20 mL) in a dry flask, followed by the addition of KOH pellets (730 mg, 13.03 mmol). Reaction was stirred at 40 °C while an IPA (5 mL) solution of 4-chlorobenzyl chloride (770 mg, 4.78 mmol) was added via syringe pump over 3 h. Reaction was allowed to stir an additional 6 h, after which time the reaction was allowed to cool to RT. The solution was then acidified to pH 5 and DCM was added and the reaction stirred for 16 hours at RT. After this time, the precipitate was filtered off and washed with DCM (2×), and the combined organic filtrate extracts were concentrated to provide a light yellow solid. Dried under high vacuum. Yield: 956 mg, 3.77 mmol, 87%. ¹H NMR (400 MHz, Chloroform-d) δ 10.24 (s, 1H), 7.89 (d, *J* = 9.0 Hz, 1H), 7.35 (s, 4H), 6.62 (s, 2H), 4.75 (s, 4H), 1.58 (s, 2H). TOF ES– MS: 238.1 (M – H).

5.2.27. (*S*)-1-(4-Chlorobenzyl)pyrrolidine-2-carboxylic acid (*S*-13)—*D*-Proline (*S*)-12 (500 mg, 4.34 mmol) was dissolved in IPA (20 mL) in a dry flask, followed by the addition of KOH pellets (730 mg, 13.03 mmol). Reaction was stirred at 40 °C while an IPA (5 mL) solution of 4-chlorobenzyl chloride (770 mg, 4.78 mmol) was added via syringe pump over 3 h. Reaction was allowed to stir an additional 6 h, after which time the reaction was allowed to cool to RT. The solution was then acidified to pH 5 and DCM was added and the reaction stirred for 16 hours at RT. After this time, the precipitate was filtered off and washed with DCM (2×), and the combined organic filtrate extracts were concentrated to provide a light yellow solid. Yield: 923 mg, 3.64 mmol, 84%. TOF ES– MS: 238.1 (M – H).

5.2.28. (*R*)-1-(4-Chlorobenzoyl)pyrrolidine-2-carboxylic acid (*R*-16)—*L*-Proline (*R*)-12 (800 mg, 6.95 mmol) was dissolved in IPA (30 mL), followed by the addition of KOH pellets (1.17 g, 20.85 mmol) and H₂O (10 mL). Reaction was stirred at RT while an IPA solution of 4-chlorobenzoyl chloride (1.34 g, 7.64 mmol) was added via syringe pump over 1 h. Reaction was allowed to stir an additional 1 h, after which time the reaction was cooled to 0°C. The solution was then acidified to pH 5, DCM was added, and the reaction stirred for 12 hours at RT. After this time, the precipitate was filtered off and washed with DCM (2×), and the combined organic filtrate extracts were concentrated to provide an off-white solid without further purification. Yield: 1.58 g, 6.23 mmol, 90%. TOF ES– MS: 252.0 (M – H).

5.2.29. (*R*)-Ethyl 1-(1-(4-chlorobenzoyl)pyrrolidine-2-carbonyl)piperidine-4-carboxylate (*R*-17)—(*R*)-1-(4-Chlorobenzoyl)pyrrolidine-2-carboxylic acid (*R*)-16 (430 mg, 1.79 mmol) and TEA (750 μL, 5.38 mmol) were dissolved in DCM (10 mL) followed by HATU (720 mg, 1.88 mmol), ethyl isonipecotate (290 μL, 1.88 mmol), and 3 Å MS. The

reaction was allowed to stir at RT for 17 h, at which time the DCM was removed and the residue taken up in a 1:1 solution of EtOAc:Et₂O and washed with 0.5 M aq. HCl (2×), H₂O (1×), 10% aq. Na₂CO₃ (3×), and brine (1×). The solution was then dried (MgSO₄) and concentrated, and the residue was purified via silica flash chromatography (100 g silica, 50% to 100% EtOAc:Hex) to give the desired product as a clear oil. Yield: 420 mg, 1.11 mmol, 62%. ¹H NMR (500 MHz, Chloroform-d) δ 7.52 (d, *J* = 8.1 Hz, 2H), 7.35 – 7.30 (m, 2H), 5.04 (dt, *J* = 14.6, 7.4 Hz, 1H), 4.50 – 4.44 (m, 1H), 4.40 (m, 0.5H), 4.27 (dd, *J* = 9.6, 3.9 Hz, 0.5H), 4.17 – 4.09 (m, 2H), 4.04 (d, *J* = 13.6 Hz, 0.5H), 3.94 (d, *J* = 13.7 Hz, 0.5H), 3.80 (tt, *J* = 11.5, 5.9 Hz, 0.5H), 3.67 (q, *J* = 7.8 Hz, 1H), 3.50 (ddd, *J* = 10.5, 7.2, 4.1 Hz, 1H), 3.41 – 3.30 (m, 0.5H), 3.17 (dt, *J* = 13.8, 6.9 Hz, 0.5H), 3.05 – 2.95 (m, 0.5H), 2.90 – 2.76 (m, 0.5H), 2.62 – 2.48 (m, 1H), 2.22 (ddt, *J* = 22.4, 15.3, 7.9 Hz, 1H), 2.06 – 1.81 (m, 7H), 1.64 (ddd, *J* = 27.7, 15.2, 8.6 Hz, 2H), 1.27 – 1.21 (m, 3H).

5.2.30. (S)-1-(4-Chlorobenzoyl)pyrrolidine-2-carboxylic acid (S-16)—*D*-Proline (S)-12 (800 mg, 6.95 mmol) was dissolved in IPA (30 mL), followed by the addition of KOH pellets (1.17 g, 20.85 mmol) and H₂O (10 mL). Reaction was stirred at RT while an IPA solution of 4-chlorobenzoyl chloride (1.34 g, 7.64 mmol) was added via syringe pump over 1 h. Reaction was allowed to stir an additional 1 h, after which time the reaction was cooled to 0°C. The solution was then acidified to pH 5, DCM was added, and the reaction stirred for 13 hours at RT. After this time, the precipitate was filtered off and washed with DCM (2×), and the combined organic filtrate extracts were concentrated to provide an off-white solid without further purification. Yield: 1.62 g, 6.39 mmol, 92%. TOF ES– MS: 252.0 (M – H).

5.2.31. (S)-Ethyl 1-(1-(4-chlorobenzoyl)pyrrolidine-2-carbonyl)piperidine-4-carboxylate (S-17)—The benzoyl carboxylic acid (S)-17 (490 mg, 2.05 mmol) and TEA (860 μL, 6.15 mmol) were dissolved in DCM (10 mL) followed by HATU (820 mg, 2.15 mmol), ethyl isonipecotate (330 μL, 2.15 mmol), and 3 Å MS. The reaction was allowed to stir at RT for 16 h, at which time the DCM was removed and the residue taken up in a 1:1 solution of EtOAc:Et₂O and washed with 0.5 M aq. HCl (2×), H₂O (1×), 10% aq. Na₂CO₃ (3×), and brine (1×). The solution was then dried (MgSO₄) and concentrated, and the residue was purified via silica flash chromatography (100 g silica, 50% to 100% EtOAc:Hex) to give the desired product as a clear oil. Yield: 340 mg, 0.91 mmol, 44%. ¹H NMR (500 MHz, Chloroform-d) δ 7.52 (d, *J* = 8.1 Hz, 2H), 7.33 (m, 2H), 5.04 (dt, *J* = 14.6, 7.4 Hz, 1H), 4.49 – 4.40 (m, 1H), 4.27 (d, *J* = 9.6 Hz, 0.5H), 4.13 (dp, *J* = 13.9, 7.4 Hz, 2H), 4.04 (d, *J* = 13.6 Hz, 0.5H), 3.94 (d, *J* = 13.7 Hz, 0.5H), 3.80 (tt, *J* = 11.5, 5.9 Hz, 0.5H), 3.67 (q, *J* = 7.8 Hz, 1H), 3.50 (ddd, *J* = 10.5, 7.2, 4.1 Hz, 1H), 3.39 – 3.29 (m, 0.5H), 3.17 (dt, *J* = 13.8, 6.9 Hz, 0.5H), 3.05 – 2.94 (m, 0.5H), 2.88 – 2.80 (m, 0.5H), 2.61 – 2.48 (m, 1H), 2.22 (ddt, *J* = 22.4, 15.3, 7.9 Hz, 1H), 2.10 – 1.80 (m, 4H), 1.64 (m, 2H), 1.30 – 1.19 (m, 3H).

5.2.32. Methyl 2-(4-chlorophenoxy)benzoate (20)—Methyl salicylate **19** (0.40 mL, 3.86 mmol), pyridine (0.75 mL, 9.26 mmol), (4-chlorophenyl)boronic acid (531 mg, 3.40 mmol), and Cu(OAc)₂ (617 mg, 3.40 mmol) were added to MeOH (10 mL) with 4 Å MS and allowed to stir at RT for 5 d. After this time, the reaction mixture was filtered over celite and the filtrate was concentrated. The resulting residue was taken up in EtOAc and washed with

5% aq. NaOH (3×) to remove residual copper, then H₂O (3×), 1 M aq. HCl (3×), and brine (1×), then the organic phase as dried (MgSO₄) and concentrated, and the resulting residue was purified via silica flash chromatography (1% to 10% EtOAc:Hex) to provide the desired ether as colorless clear oil. Yield: 233 mg, 0.89 mmol, 23%. ¹H NMR (500 MHz, Chloroform-d) δ 7.90 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.46 (ddd, *J* = 9.0, 7.9, 1.7 Hz, 1H), 7.29 – 7.24 (m, 2H), 7.23 – 7.17 (m, 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 6.92 – 6.84 (m, 2H), 3.94 (s, 3H).

5.2.33. Methyl 2-((4-chlorophenyl)amino)benzoate (22a)—Methyl salicylate **19** (1.50 mL, 11.57 mmol), *N,N*-Bis(triflyl)aniline (4.76 g, 13.31 mmol), and DIPEA (6.06 mL, 34.70 mmol) were dissolved into anhydrous DMF (20 mL) in dry glassware under N₂ at RT. The solution was stirred for 18 h at RT, at which time the reaction was diluted with 2:1 EtOAc:Et₂O and washed with H₂O (5×) and brine (1×), then dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (80 g silica, 5% EtOAc:Hex) to give methyl 2-(((trifluoromethyl)sulfonyl)oxy)benzoate as a clear, colorless oil. Yield: 2.76 g, 9.72 mmol, 84%. ¹H NMR (500 MHz, Chloroform-d) δ 8.09 (d, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 3.97 (s, 3H).

A Schlenck flask was filled with toluene (60 mL, not anhydrous) and then charged sequentially with the following reagents: methyl 2-(((trifluoromethyl)sulfonyl)oxy)benzoate (900 mg, 3.17 mmol) from the previous step, 4-chloroaniline (485 mg, 3.80 mmol), Cs₂CO₃ (1444 mg, 4.43 mmol), Pd(OAc)₂ (36 mg, 0.16 mmol), and DPPP (104 mg, 0.25 mmol). The mixture was then thoroughly sparged with argon, and the flask was fitted with a condenser and then purged and filled with an argon atmosphere. The reaction was stirred at reflux under argon for 6 h, then allowed to cool to RT and stir for 12 h. At this time, the reaction was diluted with EtOAc and washed with 1M aq. HCl (3×) and brine (1×), dried (MgSO₄), and concentrated. The residue was purified via silica flash chromatography (40 g silica, 1% to 5% EtOAc:Hex gradient) to yield the desired compound **22a** as a clear, vaguely yellow oil. Yield: 710 mg, 2.71 mmol, 86%. ¹H NMR (500 MHz, Chloroform-d) δ 9.46 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.37 – 7.26 (m, 3H), 7.24 – 7.16 (m, 3H), 6.77 (t, *J* = 7.6 Hz, 1H), 3.92 (d, *J* = 1.1 Hz, 3H).

5.2.34. Methyl 2-(phenylamino)benzoate (22b)—A Schlenck flask was filled with toluene (30 mL, not anhydrous) and then charged sequentially with the following reagents: methyl 2-(((trifluoromethyl)sulfonyl)oxy)benzoate (440 mg, 1.63 mmol), aniline (178 μL, 1.94 mmol), Cs₂CO₃ (743 mg, 2.28 mmol), Pd(OAc)₂ (18 mg, 0.08 mmol), and DPPP (67 mg, 0.16 mmol). The mixture was then thoroughly sparged with argon, and the flask was fitted with a condenser and then purged and filled with an argon atmosphere. The reaction was stirred at reflux under argon for 6 h, then allowed to cool to RT and stir for 12 h. At this time, the reaction was diluted with EtOAc and washed with 1M aq. HCl (3×) and brine (1×), dried (MgSO₄), and concentrated. The residue was purified via silica flash chromatography (40 g silica, 1% to 5% EtOAc:Hex gradient) to yield the desired compound as a clear, somewhat yellow oil. Yield: 377 mg, 1.66 mmol, 57%. ¹H NMR (500 MHz, Chloroform-d)

δ 9.47 (s, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 16.2, 7.3 Hz, 3H), 7.26 (d, J = 7.8 Hz, 3H), 7.10 (t, J = 7.3 Hz, 1H), 6.74 (t, J = 7.5 Hz, 1H), 3.91 (s, 3H).

5.2.35. Methyl 2-((4-chlorophenyl)(methyl)amino)benzoate (24)—Methyl 2-((4-chlorophenyl)amino)benzoate **22a** (150 mg, 0.57 mmol) was dissolved in anhydrous DMF (8 mL) under N₂. The solution was then charged with granular K₂CO₃ (158 mg, 1.15 mmol). MeI (40 μ L, 0.63 mmol), dissolved in 1 mL anhydrous DMF, was added dropwise to the solution at RT under N₂, and the reaction was allowed to stir for 12 h at RT. After this time, the reaction was diluted in 2:1 EtOAc:Et₂O and washed with H₂O (3 $\times$), 10% aq. Na₂S₂O₃ (1 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (25 g silica, 1% to 10% EtOAc:Hex) to afford the desired compound as a colorless oil. Yield: 139 mg, 0.50 mmol, 88%. TOF ES+ MS: 276.1 (M + H).

5.2.36. Methyl 2-((4-chlorobenzyl)amino)benzoate (27a)—Methyl 2-aminobenzoate **35** (500 mg, 3.31 mmol) was dissolved in anhydrous DMF (15 mL) at RT under N₂. *Tert*-BuOK (408 mg, 3.64 mmol) was then added and the solution became yellow, and this was allowed to stir at RT for 30 min, at which point 4-chlorobenzyl chloride (0.45 mL, 3.47 mmol) was added, and the solution lost color. The reaction was allowed to stir for 18 h under N₂, at which time the reaction was diluted in EtOAc and washed with 10% aq. NaHCO₃ (1 $\times$), H₂O (5 $\times$), and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was purified by silica flash chromatography (40 g silica, 1% to 30% EtOAc:Hex) to provide the desired compound as a colorless oil. Yield: 1.37 g, 2.88 mmol, 87%. ¹H NMR (500 MHz, Chloroform-d) δ 8.20 (t, J = 5.7 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.31 (q, J = 7.8, 7.1 Hz, 5H), 6.63 (t, J = 7.6 Hz, 1H), 6.58 (d, J = 8.5 Hz, 1H), 4.43 (d, J = 5.7 Hz, 2H), 3.88 (s, 3H). TOF ES+ MS: 276.1 (M + H).

5.2.37. Methyl 2-(benzylamino)benzoate (27b)—To anhydrous DMF in dry glassware was added methyl 2-aminobenzoate **26** (280 mg, 1.85 mmol), benzyl bromide (330 mg, 1.94 mmol), and Cs₂CO₃ (1.50 g, 4.62 mmol). The reaction was stirred at 85 °C for 5 days, after which time the reaction was cooled to RT and diluted with 1:1 EtOAc:Et₂O and washed with H₂O (3 $\times$) and brine (1 $\times$), then dried (MgSO₄) and concentrated. The residue was purified via silica flash chromatography (5% EtOAc:Hex isochratic) to separate product from unreacted starting material. Yield: 138 mg, 0.57 mmol, 31%. ¹H NMR (500 MHz, Chloroform-d) δ 8.18 (s, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.40 – 7.25 (m, 6H), 6.65 (d, J = 8.5 Hz, 1H), 6.61 (t, J = 7.6 Hz, 1H), 4.46 (d, J = 5.6 Hz, 2H), 3.87 (s, 3H).

5.2.38. 2-((4-Chlorophenethyl)amino)benzoic acid (30)—2-Bromobenzoate **29** (200 mg, 1.00 mmol), 2-(4-chlorophenyl)ethanamine (290 mg, 1.86 mmol), K₂CO₃ (260 mg, 1.86 mmol), copper powder (12 mg, 0.19 mmol), and copper (I) oxide (13 mg, 0.09 mmol) were added to ethylene glycol dimethyl ether (5 mL) in a dry pressure tube. The mixture was sparged and the headspace purged with argon, and the reaction was stirred at 130 °C for 24 h. After cooling to RT, the reaction was diluted with EtOAc and H₂O and filtered through celite. The filtrate was acidified with conc. HCl and material was extracted with EtOAc (3 $\times$). The combined extracts were dried (MgSO₄) and concentrated. Purification of the

resulting residue was accomplished via silica flash chromatography (25 g silica, 10% EtOAc:Hex with 1% formic acid) to give a white powder. Yield: 120 mg, 0.44 mmol, 44%. ¹H NMR (500 MHz, Chloroform-d) δ 8.06 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.32 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 8.2 Hz, 2H), 6.71 (d, J = 8.5 Hz, 1H), 6.64 (t, J = 7.5 Hz, 1H), 3.47 (t, J = 7.1 Hz, 2H), 2.97 (t, J = 7.0 Hz, 2H).

5.2.39. 2-(4-Chlorobenzoyl)benzoic acid (33)—Aluminum chloride (9.0 g, 67.5 mmol) was carefully added at RT to chlorobenzene (50 mL) in dry glassware with a water separator for dense solvents, followed by phthalic anhydride (5.0 g, 33.8 mmol). The reaction was then refluxed under N₂ for 1.5 h, over which time it took on a deep red color. *Note:* longer reaction times result in rapid accumulation of a second, internal acylation. The reaction was then allowed to cool to RT over 1 h and was quenched by the slow and careful addition of 1 M aq. HCl and allowed to stir another 1 h, over which time color was lost and a white precipitate formed. The reaction was then taken up in EtOAc and washed with 1M aq. HCl (1 $\times$), then the solvent was removed *in vacuo*. The residue was then dissolved in 10% aq. NaOH, the resulting precipitate was filtered off, and the filtrate was washed with EtOAc (2 $\times$) before being chilled in an ice bath and acidified to pH 1 with conc. HCl. The resulting precipitate was collected over a filter to give the desired product as a white powder. No further purification necessary. Yield: 6.34 g, 24.31 mmol, 72%. ¹H NMR (500 MHz, Chloroform-d) δ 8.08 (d, J = 7.8 Hz, 1H), 7.70 – 7.61 (m, 3H), 7.58 (tt, J = 7.7, 1.2 Hz, 1H), 7.36 (dd, J = 12.2, 7.9 Hz, 3H). TOF ES– MS: 259.0 (M – H).

5.2.40. 2-(4-Chlorobenzyl)benzoic acid (35)—2-(4-Chlorobenzoyl)benzoic acid **33** (200 mg, 0.77 mmol) was added to 28% aq. ammonia (10 mL), followed by zinc (600 mg, 9.21 mmol) and CuSO₄·5H₂O (80 mg, 0.31 mmol). This mixture was stirred at reflux for 48 h, with 2 mL recharges of 28% aq. ammonia every 6 h, barring 12 h at night. After this time, the reaction was allowed to cool to RT and the solution was decanted off the solid precipitate and concentrated *in vacuo*. The residue was taken up in H₂O and acidified to pH < 1 by conc. HCl, then extracted 1 $\times$ with EtOAc. The extract was dried (MgSO₄) and concentrated, and the residue was crystallized from EtOAc to give tan crystals. Yield: 62 mg, 0.25 mmol, 33%. TOF ES– MS: 245.0 (M – H).

5.2.41. Methyl 9H-carbazole-1-carboxylate (39)—9H-Carbazole-1-carboxylic acid **38** (200 mg, 0.95 mmol) was stirred in refluxing sat. methanolic HCl for 1 h. The reaction was then allowed to cool and was concentrated. The residue was dissolved in EtOAc and washed with sat. aq. Na₂CO₃ (1 $\times$), then dried (MgSO₄), and concentrated. The residue was then purified by silica flash chromatography (10 g silica, 10% EtOAc/Hex) to give a white powder. Yield: 196 mg, 0.87 mmol, 92%. ¹H NMR (500 MHz, Chloroform-d) δ 9.94 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 8.14 – 8.04 (m, 2H), 7.57 – 7.46 (m, 2H), 7.34 – 7.28 (m, 1H), 7.26 (t, J = 7.7 Hz, 1H), 4.03 (s, 3H).

5.2.42. Methyl 6-chloro-9H-carbazole-1-carboxylate (40)—Methyl 9H-carbazole-1-carboxylate **39** (90 mg, 0.40 mmol) was stirred in DCM (15 mL) at 0 °C under N₂ while SO₂Cl₂ was added dropwise. The reaction was allowed to stir at 0 °C for 4 h. At this time, the reaction was diluted with DCM and washed with 10% aq. Na₂CO₃ (2 $\times$) and brine (1 $\times$),

then dried (Na₂SO₄) and concentrated. The residue was purified via silica flash chromatography (10 g silica, 10 to 30% EtOAc/Hex) to give a white powder. Yield: 75 mg, 0.29 mmol, 72%. ¹H NMR (500 MHz, Chloroform-d) δ 9.93 (s, 1H), 8.21 (d, *J* = 7.7 Hz, 1H), 8.10 (d, *J* = 7.6 Hz, 1H), 8.04 (s, 1H), 7.44 – 7.41 (m, 2H), 7.27 (t, *J* = 7.7 Hz, 1H), 4.03 (s, 3H). ¹H NMR (500 MHz, Benzene-d₆) δ 9.70 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 1.9 Hz, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.17 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.92 (t, *J* = 7.7 Hz, 1H), 6.46 (d, *J* = 8.6 Hz, 1H), 3.48 (s, 3H).

5.2.43. Ethyl 1-(2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylate (44)—4-Chloroquinolin-2(1*H*)-one prepared according to previous literature.⁵⁰

4-Chloroquinolin-2(1*H*)-one (500 mg, 2.78 mmol), ethyl isonipecotate (0.52 mL), NaI (420 mg, 2.78 mmol), and Na₂CO₃ (885 mg, 8.35 mmol) were added to DMF (10 mL) at RT under N₂ and stirred at 80 °C for 48 h. The reaction was then cooled to RT, at which time it was diluted in 1:1 EtOAc:Et₂O and washed with 1 M HCl (3×), H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×). The organic phase was dried (MgSO₄) and concentrated. The residue was purified by silica flash chromatography (45 g silica, 20 to 80% EtOAc/Hex) to give **44** as a white microcrystalline solid. Yield: 567 mg, 1.89 mmol, 68%. ¹H NMR (500 MHz, Chloroform-d) δ 12.43 (s, 1H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.48 – 7.40 (m, 2H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.13 (s, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.54 (d, *J* = 12.4 Hz, 2H), 2.82 (t, *J* = 10.8 Hz, 2H), 2.54 (ddd, *J* = 15.0, 10.8, 4.1 Hz, 1H), 2.13 – 2.07 (m, 2H), 2.06 – 1.98 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H).

5.2.44. Ethyl 1-(1-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylate (45)—Ethyl 1-(2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylate **44** (490 mg, 1.64 mmol) was dissolved in DCM (10 mL) with 4 Å MS, followed by the addition of 4-chlorophenylboronic acid (510 mg, 3.27 mmol), pyridine (0.26 mL, 3.27 mmol), TEA (0.46 mL, 3.27 mmol), and Cu(OAc)₂ (590 mg, 3.27 mmol). The reaction was stirred at RT for 4 d, over which time the reaction became increasingly green with precipitate. After this time, the reaction was diluted with more DCM and washed with 10% aq. NaOH (3×), 10% aq. NaHCO₃ (1×), and brine (1×), then dried (MgSO₄) and concentrated. The resulting residue was purified by silica flash chromatography (25 g, 5 to 50% EtOAc/Hex) to give **45** as a white solid. Yield: 480 mg, 1.18 mmol, 72%. ¹H NMR (500 MHz, Chloroform-d) δ 7.79 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 1H), 6.21 (s, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.55 (d, *J* = 12.2 Hz, 2H), 2.84 (t, *J* = 11.5 Hz, 2H), 2.57 (ddd, *J* = 15.1, 10.9, 4.1 Hz, 1H), 2.16 – 2.09 (m, 2H), 2.09 – 1.96 (m, 2H), 1.31 (t, *J* = 7.1 Hz, 3H).

5.2.45. Ethyl 1-(1-(4-chlorobenzyl)-2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylate (47)—Ethyl 1-(2-oxo-1,2-dihydroquinolin-4-yl)piperidine-4-carboxylate **44** (95 mg, 0.32 mmol) was dissolved in anhydrous DMF (5 mL) under nitrogen and cooled to 0 °C. *t* BuOK (43 mg, 0.38 mmol) was added, which elicited a slight color change. 4-chlorobenzyl chloride (49 μL, 0.38 mmol) was then added and the reaction was allowed to stir at RT for 15 h. At this time, the reaction was diluted with 2:1 EtOAc:Et₂O and washed

with 1M aq. HCl (3×), H₂O (3×), 10% aq. Na₂CO₃ (3×), and brine (1×), then dried (MgSO₄) and concentrated. Residue was purified via silica flash chromatography (10 g silica, 1% to 30% EtOAc:Hex). Yield: 117 mg, 0.28 mmol, 87%. ¹H NMR (500 MHz, Chloroform-d) δ 7.79 (d, *J* = 7.9 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.28 – 7.23 (m, 2H), 7.17 (dd, *J* = 13.3, 8.2 Hz, 4H), 6.24 (s, 1H), 5.48 (s, 2H), 4.20 (q, *J* = 7.3 Hz, 2H), 3.52 (d, *J* = 12.4 Hz, 2H), 2.82 (t, *J* = 11.8 Hz, 2H), 2.55 (tt, *J* = 11.0, 8.8, 4.0 Hz, 1H), 2.06 (ddd, *J* = 31.9, 19.5, 6.8 Hz, 4H), 1.30 (t, *J* = 7.4 Hz, 3H).

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Abbreviations Used

ADME	absorption, distribution, metabolism, excretion
BBB	blood-brain barrier
CEV	California encephalitis virus
CNS	central nervous system
CPE	cytopathic effect
ECMV	Encephalomyocarditis virus
fluc	firefly luciferase
FMV	Fort Morgan virus
hpi	hours after infection
MDCK	Madin-Darby canine kidney epithelial cells
MDR1	multiple drug resistance protein
MLM	mouse liver microsome
MOI	multiplicity of infection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
PAMPA	parallel artificial membrane permeability assay
Rho123	rhodamine 123
SAR	structure-activity relationship
WEEV	western equine encephalitis virus
VEEV	Venezuelan equine encephalitis virus

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Figure 1.

Figure 2.
Conformational restriction plan.

Figure 3. Spectrum of Antiviral Data for Selected Analogues.^a

^a**23a** and **28a** have potent and broad spectrum antiviral activity. (A) HEK293 cells were infected with FMV at an MOI of 1 and simultaneously treated with decreasing concentrations of the indicated compound. Virus titers in cell culture supernatants were determined at 24 hpi by plaque assay. The horizontal dashed line indicates FMV titers in cells treated with control DMSO. Calculated IC₅₀ values for **1**, **23a**, and **28a** were 1.0 ± 0.5 , 5.2 ± 1.0 , and 2.3 ± 1.2 μM, respectively. (B) HEK293 cells were infected with the indicated viruses at an MOI of 0.1, simultaneously treated with the indicated compound at 25 μM, and virus titers in tissue culture supernatants were determined by plaque assay at 24 hpi. P < 0.05* or 0.005** compared to DMSO-treated controls.

Figure 4.

Effect of Selected Analogues on Transfected Gene Expression in BSR-T7 Cells.^a

^a**23a** and **28a** modulate cellular gene expression. BSR-T7 cells were transfected with the expression plasmids pSV40-LUC (circles) or pT7/CITE-LUC (triangles), treated with decreasing concentrations of the indicated compound, and fLUC activity was measured 20 h later. Calculated IC₅₀ values for suppression of pSV40-LUC activity for **23a** and **28a** were 2.2 ± 0.2 and 1.0 ± 0.1 μM, respectively. **1** has an IC₅₀ value of 0.1 μM in this assay.⁵⁸ N=3 or 4 for all data, and results are the mean ± SEM.

Scheme 1. Preparation of Azetidine Analogue **3**.^a

^aReagents and conditions: (a) methyl 4-azetidinecarboxylate hydrochloride, EDC, HOBT, DIPEA, DCM, rt; (b) 10% aq. NaOH, EtOH; (c) 4-(2-aminoethyl)pyridine, EDC, HOBT, TEA, DCM, rt,.

Scheme 2. Preparation of Acyclic Amide and Urea Analogues.^a

^a Reagents and conditions: (a) ethyl isonipecotate, DCM, rt; (b) 10% aq. NaOH, EtOH, rt; (c) 4-(2-aminoethyl)pyridine, EDC, HOBT, TEA, DCM, rt; (d) ethyl isonipecotate, EDC, HOBT, TEA, DCM, rt.

Scheme 3. Preparation of *N*-Benzyl and *N*-Benzoyl Pyrrolidine Analogues.^a

^aReagents and conditions: (a) 4-chlorobenzylchloride, KOH, IPA, 40 °C; (b) **14**, HATU, TEA, DCM, rt; (c) 4-chlorobenzoylchloride, IPA, KOH, H₂O, 0 °C; (d) ethyl isonipecotate, HATU, TEA, DCM, rt; (e) 9:1 DCM:MeOH, NaOH, rt; (f) 4-(2-aminoethyl)pyridine, HATU, TEA, DCM, rt.

Scheme 4. Preparation of Anthranilamide and Salicylamide Analogues.^a

^aReagents and conditions: (a) Cu(OAc)₂, 4-chlorophenylboronic acid, TEA, pyridine, DCM, rt; (b) 10% aq. NaOH, EtOH, rt; (c) **14**, EDC·HCl, HOBT, TEA, DCM, rt; (d) PhN(Tf)₂, TEA, DMF, rt; (e) 4-chloroaniline or aniline, Pd(OAc)₂, DPPP, Cs₂CO₃, toluene, reflux; (f) ethyl isonipecotate, EDC·HCl, HOBT, TEA, DCM, rt; (g) 10% aq. NaOH, EtOH, THF, rt; (h) 4-(2-aminoethyl)pyridine, EDC, HOBT, TEA, DCM, rt; (i) MeI, Cs₂CO₃, DMF, rt.

Scheme 5. Preparation of Benzyl Anthranilamide Analogues.^a^aReagents and conditions: (a) 4-chlorobenzyl chloride or benzyl bromide, Cs₂CO₃, DMF;(b) 10% aq. NaOH, EtOH, rt; (c) **27**, EDC, HOBT, TEA, DCM, rt.

Scheme 6. Preparation of Phenethyl Anthranilamide Analogue **31**.^a

^aReagents and conditions: (a) 1-(2-aminoethyl)-4-chlorophenyl amine, Cu₂O, Cu⁰, K₂CO₃, EDME, 130 °C; (b) 10% aq. NaOH, EtOH, THF, rt; (c) **14**, EDC, HOBT, TEA, DCM, rt.

Scheme 7. Preparation of Benzyl and Benzoyl Benzoate Analogues.^a^aReagents and conditions: (a) AlCl₃, PhCl, reflux; (b) **14**, EDC, HOBT, TEA, DCM, rt; (c) Zn⁰, CuSO₄·5H₂O, 28% aq. NH₃, reflux.

Scheme 8. Preparation of Carbazole Analogues.^a

^aReagents and conditions: (a) pyrrolidine, 37% aq. HCHO, EtOH, reflux; (b) s-BuLi, THF, CO_{2(g)}, then 1 M aq. HCl, reflux; (c) sat. methanolic HCl, reflux; (d) SO₂Cl₂, DCM, 0 °C; (e) 10% aq. NaOH, EtOH, THF, rt; (f) **14**, EDC, HOBT, TEA, DCM, rt.

Scheme 9. Preparation of Quinolone Analogues.^a

^aReagents and conditions: (a) 6 M aq. HCl, 1,4-dioxane, reflux; (b) ethyl isonipecotate, NaI, Na₂CO₃, DMF; 80 °C; (c) 4-chlorophenylboronic acid, Cu(OAc)₂, TEA, pyridine, DCM, rt; (d) 10% aq. NaOH, EtOH, THF, rt; (e) 4-(2-aminoethyl)pyridine, EDC, HOBT, TEA, DCM, rt; (f) 4-chlorobenzyl chloride, KOtBu, DMF, 0 °C to rt.

Table 1

WEEV Replicon Data for Acyclic Analogs.

No.	G	IC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b	MW
2		0.68 ± 0.04	>100	451
8a		60.2 ± 4.6	>100	401
8b ^c		40.9 ± 5.0	>100	415
11a		>100	>100	386
11b		>100	>100	400

^a Inhibition of luciferase expression in WEEV replicon assay. Ribavirin (positive control) has an IC₅₀ of 16 μM in the assay. Values are mean ± SEM of at least n=3 independent experiments.

^b Cell viability determined by the MTT reduction assay. See Experimental Section for detailed methods and synthetic procedures.

^c Result for **8b** WEEV replicon activity have been previously published³¹ and are reproduced here for comparison.

Table 2

WEEV Replicon Data for Pyrrolidine Analogs.

No.	G	IC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b	MW
2		0.68 ± 0.04	>100	451
(S)-15		13.5 ± 2.0	91.6	455
(R)-15		53.5 ± 12.0	>100	455
(S)-18		>100	>100	467
(R)-18		>100	>100	467

^a Inhibition of luciferase expression in WEEV replicon assay.^b Cell viability determined by the MTT reduction assay.

* Stereogenic center. See Experimental Section for detailed methods and synthetic procedures.

Table 3

WEEV Replicon and In Vitro ADME Data for Arene Analogs.

No.	G	IC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b	BBB- PAMPA (log P _{eff}) ^c	MDR1 Recognition (% increase in Rho123 uptake) ^d	MLM T _{1/2} (min) ^e	Sol (μg/mL) ^f	MW
1		0.53 ± 0.08	49.9	-4.18 ± 0.06	30.9 ± 1.0	9	31–63	501
2		0.68 ± 0.04	>100	-5.01 ± 0.03	-1.2 ± 0.6	3	125–250	451
21		6.1 ± 0.8	56.0	-4.68 ± 0.09	9.3 ± 3.2	18	62–125	464
23a		1.6 ± 0.2	>100	-4.45 ± 0.11	47.7 ± 7.2	19	>250	463

No.	G	IC ₅₀ (μ M) ^a	CC ₅₀ (μ M) ^b	BBB- PAMPA (log P _{eff}) ^c	MDR1 Recognition (% increase in Rho123 uptake) ^d	MLM T _{1/2} (min) ^e	Sol (μ g/mL) ^f	MW
23b		28.8 ± 5.2	> 100	-4.75 ± 0.02	10.9 ± 1.7	---	>250	429
25		15.4 ± 2.8	>100	-4.71 ± 0.09	3.9 ± 1.5	---	>250	477
28a		0.56 ± 0.08	75.2	-4.39 ± 0.05	59.6 ± 7.5	15	>250	477
28b		19.0 ± 1.7	92.2	-4.93 ± 0.03	2.5 ± 0.3	---	>250	443
31		3.49 ± 0.10	86.0	-4.49 ± 0.35	30.7 ± 4.7	---	>250	491

No.	G	IC ₅₀ (μ M) ^a	CC ₅₀ (μ M) ^b	BBB- PAMPA (log P _{eff}) ^c	MDR1 Recognition (% increase in Rho123 uptake) ^d	MLM T _{1/2} (min) ^e	Sol (μ g/mL) ^f	MW
34		>50	>50	-5.08 $\pm$ 0.02	1.8 $\pm$ 1.3	---	>250	476
36		1.46 $\pm$ 0.09	70.7	-4.40 $\pm$ 0.06	22.6 $\pm$ 2.7	3	62–125	462

^a Inhibition of luciferase expression in WEEV replicon assay.

^b Cell viability determined by the MTT reduction assay. Values are mean of at least n=3 independent experiments.

^c Log of effective permeability (cm/s) determined using PAMPA Explorer (pION) with BBB lipid mixture measured at pH = 7.4.

^d Rhodamine 123 uptake was measured in MDR1-MDCKII cells utilizing Glomax Multi Detection System (Promega). 'MDR1 recognition' was assessed by measuring uptake of Rhodamine 123 in the presence of MDR inhibitor (30 μ M) and comparing the effect on Rhodamine 123 uptake to that induced by tariquidar (5 μ M), using the equation: $(C_{av} - C_{veh}) * 100 / (C_{tar} - C_{veh})$, where C_{av} = concentration of rhodamine 123 in the presence of anti-viral, C_{veh} = concentration in the presence of vehicle, C_{tar} = concentration of rhodamine 123 in the presence of tariquidar. In the presence of tariquidar, rhodamine 123 uptake was 1123 $\pm$ 54% of vehicle controls (n=44).

^e Half-life in BALB/c mouse liver microsomes incubation. Values are half-lives calculated from the equation $T_{1/2} = \ln(2) / \text{slope}$, where the slope was $\ln(\% \text{ detected})$ against time, plotted from the mean of at least n=3 independent experiments.

^f Kinetic solubility measured using the same assay media as WEEV replicon assay, except with 10% fetal bovine serum. See Experimental Section for detailed methods and synthetic procedures.

Table 4

WEEV Replicon Data for Conformationally Restricted Analogs.

No.	G	IC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b
41		59.2 ± 6.5	>100
42		18.7 ± 5.4	66.1
46		88.3 ± 11.7	>100
48		10.9 ± 2.0	34.1

^aInhibition of luciferase expression in WEEV replicon assay.^bCell viability determined by the MTT reduction assay. See Experimental Section for detailed methods and synthetic procedures.

Table 5Antiviral Data for Select Analogues^a

No.	Conc. (μM)	FMV		WEEV
		Titer (× 10 ⁶ pfu/ml)	Viability (% uninfected control)	Titer (× 10 ⁴ pfu/ml)
DMSO	NA	30.7 ± 9.0	35.6 ± 4.7	37.3 ± 7.3
1 ^b	25	ND	69.5 ± 10.0 [*]	1.1 ± 0.1 ^{**}
23a	25	3.7 ± 1.4 [*]	58.1 ± 9.0	3.2 ± 2.2 [*]
	5	10.6 ± 4.1	54.6 ± 9.2	49.2 ± 19.4
28a	25	3.2 ± 1.0 ^{**}	66.6 ± 9.5 [*]	1.6 ± 0.5 ^{**}
	5	9.4 ± 5.6 [*]	73.3 ± 9.1 [*]	11.7 ± 3.6 [*]

^a Assay utilized the alphaviruses Fort Morgan Virus (FMV) and western equine encephalitis virus (WEEV). Infections were done in cultured human BE(2)-C neuronal cells. Viability was measure using an MTT assay, and viral titers were measured using a plaque assay.

Values are mean ± SEM of n = 3–4 independent experiments. P value <0.05^{*} or 0.005^{**} compared to DMSO control.

^b Results for **1** (205432) antiviral activity have been previously reported,³¹ but were included in these experiments for direct comparison with newly generated analogues. NA, not applicable. ND, not determined.