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Generation of Highly Selective, Potent, and Covalent G Protein-Coupled Receptor Kinase 5 Inhibitors

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Abstract

The ability of G protein-coupled receptor (GPCR) kinases (GRKs) to regulate the desensitization of GPCRs has made GRK2 and GRK5 attractive targets for treating diseases such as heart failure and cancer. Previously, our work showed that Cys474, a GRK5 subfamily-specific residue located on a flexible loop adjacent to the active site, can be used as a covalent handle to achieve selective inhibition of GRK5 over GRK2 subfamily members. However, the potency of the most selective inhibitors remained modest. Herein, we describe a successful campaign to adapt an indolinone scaffold with covalent warheads, resulting in a series of 2-haloacetyl containing compounds that react quickly and exhibit three orders of magnitude selectivity for GRK5 over GRK2 and low nanomolar potency. They however retain a similar selectivity profile across the kinome as the core scaffold, which was based on Sunitinib.

Graphical Abstract

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Author Contributions

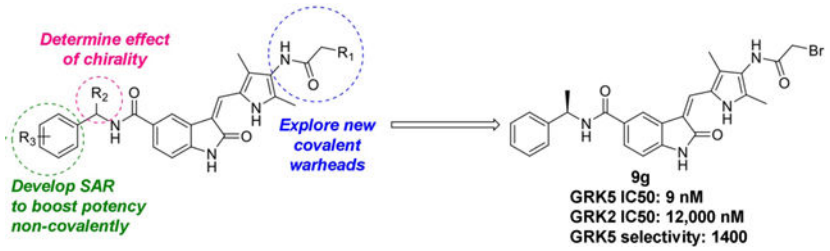
The manuscript was written primarily by RAR, QC, and JJGT. All authors have given approval to the final version of the manuscript. RAR synthesized all compounds and performed MS experiments. QC performed covalent kinetic analysis, and QC, LA, and RAB determined IC_{50} values for GRK2, GRK5, GRK5-C474S, and PKA. RAB expressed and purified GRK5 and GRK5-C474S. JJGT, QC, and LA analyzed IC_{50} data.

Supporting Information

Supporting figures S1–3, Table S1, Molecular Strings File, and coordinates for docked ligand complexes are provided in the Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

The authors declare no competing financial interest.



Keywords

covalent inhibitor; kinase inhibitor; GPCR; GPCR kinase; cardiac hypertrophy; cancer; selectivity

INTRODUCTION

G protein-coupled receptor (GPCR) kinases (GRKs) selectively recognize and phosphorylate activated GPCRs, leading to their desensitization and internalization, a process critical for maintaining cellular homeostasis. The seven human GRKs (GRK1–7), are classified via structural and sequence similarity into three subfamilies: GRK1 (GRK1 and 7), GRK2 (GRK2 and 3), and GRK4 (GRK4, 5, and 6).[1] GRK1 and 7 are found primarily in the retina and GRK4 in the testes, whereas GRK2, 3, 5, and 6 are more ubiquitously expressed. Of these kinases, GRK2 and GRK5 are the two isoforms with the highest concentration in cardiovascular tissue. Because they are overexpressed in the diseased heart and their inhibition or ablation has been shown to prevent heart failure and hypertrophic cardiomyopathy, they have become important therapeutic targets.[2, 3] GRK2 and GRK5 are also potential targets for treatment of cancer[4, 5] and other pathophysiological conditions[1]. GRK5 is further unique among GRKs because it undergoes Ca^{2+}-calmodulin-dependent nuclear localization, where it phosphorylates histone deacetylase 5 (HDAC5) induces an increase in transcription of associated genes[6]. In studies where GRK5 was knocked down, cardiomyocytes were protected from hypertrophic cardiomyopathy[7]. The influence of GRK5 in progressive heart failure and hypertrophic cardiomyopathy however remains unclear, in part because GRK2 can also mediate hypertrophic responses[8] and there are few compounds known to have clear GRK5 selectivity that could be used to test mechanistic hypotheses in physiologically relevant cells or animals.

RESULTS AND DISCUSSION

Previously, we developed a set of GRK5/6-selective pyrrolopyrimidine inhibitors using a covalent strategy[9]. This effort established that Cys474, located on a loop known as the active site tether (AST) that packs over the ATP binding site in AGC kinases, can serve as a covalent handle to achieve GRK5 selectivity. However, the affinities of the best compounds were low μM at best. Because the intrinsic affinity of the compound plays an important role in dictating the concentration of the protein-inhibitor covalent complex, which must persist long enough for a covalent reaction to occur[10], the discovery of a more intrinsically potent scaffold for GRK5 inhibition was prioritized. To this end, we considered a set of known GRK5 modulators derived from an indolinone scaffold present in the FDA approved receptor tyrosine kinase (RTK) inhibitor Sunitinib (**1**) (Figure 1), a compound that targets

both GRK5 and multiple RTKs including the platelet-derived growth factor receptor and vascular endothelial growth factor receptor[4, 11]. We focused on Ullrich-57 (**5a**), which was reported to have low nanomolar activity against GRK5 (GRK5 IC_{50} = < 0.1 μM), although its selectivity was not reported[12]. We independently synthesized **5a**, and also tested its parent compound, Sunitinib and showed that **5a** has orders of magnitude more potency against GRK5 than our previous pyrrolopyrimidine scaffold, and that both exhibit 1–2 orders of magnitude selectivity for GRK5 over GRK2 (Figure 1, Table 1). Modeling the **5a** complex with GRK5 was performed by docking in the program MOE. The highest scoring pose predicted that **5a** would bind in the active site of GRK5 in a fashion that would allow the formation of three hydrogen bonds with the hinge, which would in turn project its diethylamine moiety towards the AST loop (Figure 2A). We hypothesized that replacing the diethylamine arm of the scaffold with thiol reactive warheads could allow for covalent attachment to Cys474 and generation of even more selective and potent covalent GRK5 inhibitors. Thus, the goal for our first series of compounds was to identify a covalent warhead that affords the highest incorporation and the most selectivity for GRK5 over GRK2. As a control, potency against the canonical AGC kinase protein kinase A (PKA) was also evaluated, however all but one of our compounds had negligible effects against this enzyme.

Synthesis for this series began with an amide coupling to give common intermediate **3** (Scheme 1). In a convergent line of synthesis, a secondary amide coupling with the starting material **6**, gave intermediates **4a-e**. Combining the two lines of synthesis, a Knoevenagel condensation yielded compounds **5a-f**, wherein **5a** and **5b** represent the (R) and (S) enantiomers of Ullrich-57. We found that the (S)-enantiomer **5b** was over 1000-fold less potent (Table 1), indicating that the (R)-enantiomer (**5a**) places the methyl and benzyl pendants in a more ideal position. The structural rationale for this based on the dock remains elusive because of unknowns about the overall conformation of the GRK5 kinase domain (see Figure 2). All other compounds were therefore synthesized with the same stereo-configuration as **5a**.

Initial diversification tested a series of alkenyl or alkynyl amines as covalent modifiers. The propargyl analogue, **5c**, demonstrated similar potency to **5a** (GRK5 IC_{50} = 21 nM) but exhibited over a magnitude higher selectivity over GRK2 (2100-fold). We speculate this high level of selectivity is due to a potential clash of the propargyl warhead with the GRK2 AST and/or large lobe given the predicted vector for the alkynyl and alkenyl groups (Figure 2A). For all other amide linked compounds with an alkenyl or alkynyl warhead (**5d-f**), the selectivity for GRK5 over GRK2 remained between 330–1400 fold, but in each case the IC_{50} for GRK5 was higher than that of **5c** (Table 1). Linker length also contributed to GRK5 affinity, as demonstrated by comparison of **5c** with **5e**, and **5d** with **5f**, wherein the latter compounds have homologated alkenyl and alkynyl warheads and somewhat less potency. Thus, a single methylene linker to the reactive center seems optimal for maintaining GRK5 activity among these trial compounds.

By reversing the warhead amide, the covalent warheads used in our previous work[9] would become synthetically accessible. To accomplish this, a Knoevenagel with 3,5-dimethyl-4-nitro-1-pyrrolocarb-aldehyde yielded common intermediate, **7a** (Scheme 2). **7a** was itself

tested for inhibitory activity because it possesses the ability to release nitric oxide, a known vasodilator, rendering it a possible dual mechanism compound[13]. The nitro group was however poorly tolerated (GRK5 IC_{50} = 730 nM) and only 10-fold selective for GRK5. A zinc-catalyzed reduction of the nitro group of **7a** to the free amine **8a** allowed for rapid derivatization through amide coupling to yield final compounds **9a-h** (Scheme 2). **9a**, which features a 2-butynyl acid warhead that reacted with Cys474 in our prior study[9] exhibited low μM potency (Table 1). The commonly used acrylamide and vinyl sulfonamide variants, **9b** and **9c**, respectively, showed high nanomolar activity against GRK5 and retained >100 fold selectivity over GRK2. The 2-chloroacetylamido containing compound **9e** showed moderate potency (GRK5 IC_{50} = 220 nM) but 1500-fold selectivity over GRK2. Because **5a** had low-nanomolar potency, we introduced a similar reactive appendage, dimethylaminobutenoic acid, in **9f**. This compound showed an increased IC_{50} (360 nM, thus 24-fold higher than **5a**), but only 50-fold selectivity over GRK2. The 2-bromoacetylamido compound, **9g** was found to have a greater potency against GRK5 (IC_{50} = 8.6 nM) than its chloro analog, **9e** while retaining a high level of selectivity against GRK2 (1400-fold). Thus, we concluded that the bromo group of **9g** must be better filling a lipophilic pocket than the chloro group, but it is not possible to model this because the structure of the AST loop is uncertain in our GRK5/6 overlay model (Figure 2). It was also observed that **9g** took longer to fully engage Cys474 within the 30 min incubation time frame (Figure 3). **9h** was also potent against GRK5 (IC_{50} = 80 nM) whereas compounds **10a-b** exhibited only moderate potency.

We evaluated adduct formation in this series at 30 min and 3 hours by intact mass spectrometry (MS) (Table 1, Figure 3, Figure S1). Only a few compounds exhibited signal for GRK5 at the 3 hour timepoint. **5c**, which exhibited similar potency to the parent compound **5a** and over 2000-fold selectivity against GRK2 was, surprisingly, not covalent under our conditions. Neither were compounds **5d-f**. **9a** and **9b** were likewise unreactive at the 30 minute timepoint. The vinyl sulfonamide **9c** was able to react after a 3 hour incubation (Figure S1) despite its moderate affinity (740 nM). The chloroacetyl **9e** however had nearly complete covalent engagement by 30 min (Figure 3A). **9e** was unreactive against GRK5-C474S, consistent with Cys474 being the covalent handle (Figure 3D). Its bromoacetyl analog **9g** also rapidly reacted with GRK5 (Figure 3B), but **9h**, **10a** and **10b** were unreactive at 30 minutes. Based on the sum of the data, we rationalize that compounds in the **5c-f** series were unreactive because of constraints placed on the warhead by increased hydrogen bond interactions with the GRK5 hinge relative to **9c**, **9e** and **9g** where the attaching amide was flipped, affording a different vector for the attached warhead and less conformational constraints (Figure 2 A,B).

At this point, **9e** and **9g** provided the most reactivity combined with the most selectivity for GRK5 over GRK2 (Table 1). Therefore, we initialized structure-activity relationships (SAR) around indolinone scaffolds bearing 2-haloacetylamido warheads (Schemes 3–4, Table 2). Benzyl and pyridyl pendants in the R_1 position (Table 2) were explored first. Overall, in terms of potency GRK5 seemed to have a strong preference for smaller electron withdrawing substituents in the *para*-position of the benzyl groups: F (**9j**) > Cl (**9o**) > H (**9e**) > CH_3 (**9n**). The MOE docked models do not explain this preference, but such behavior would be expected if the benzyl pendant packs instead under the P loop of the active site. In

fact, most known GRK inhibitors, including CCG215022, tend to have benzyl groups that pack in this site (Figure 2C)[14]. The enhancement in potency of 3-Me (**9i**) is large compared to **9e**, suggesting that a 3-Me, 4-F analog would be even more potent. However, a lack of appropriate chemical precursors prevented us from studying such combinations. We note that we took advantage of the *meta* position of the fluorobenzyl group of paroxetine to make a highly successful series of GRK2 selective inhibitors[15]. The position of the nitrogen in pyridyl pendants also appeared to make a small difference. In **9k**, the potency was 3.5-fold higher than that of **9l** with an *ortho*-nitrogen, suggesting once again that the *para*-nitrogen in **9k** fulfills an electronic deficiency. **9j**, with a 4-fluoro substituent, had 55-fold more potency (IC_{50} = 4 nM) relative to the parent compound **9e**. It was also able to rapidly form a covalent interaction with Cys474 within 30 min (Figure 3C), **9j** was thus the most intriguing lead of the second series (Table 2, Figure 2C). When the 4-fluoro substituent was maintained, and a 2-bromoacetylamido warhead was used, the resulting compound, **9p**, showed slightly lower potency (IC_{50} = 15 nM) than **9j** or the des-fluoro compound **9g** (Table 1). This is different than expected from comparison of **9g** and **9e** (Table 1), where the bromo substitution rendered much higher potency. The structural explanation for this is unclear.

Given the profound effects of chirality on activity exhibited by compounds **5a** and **5b**, we also expanded SAR around the benzylic position of the scaffold (R_2 in Table 2). When the stereocenter was removed (**9q**), there was a small increase in potency (IC_{50} = 130 nM) relative to the parent compound **9e** (less than 2-fold). However, there were also similar increases in potency for **9s** (GRK5 IC_{50} = 87 nM) and **9t** (GRK5 IC_{50} = 95 nM). The geminal dimethyl of **9r** however had greatly reduced potency (IC_{50} = 12 μM), but the poor solubility of this compound under our assay conditions may have artifactually increased IC_{50} measurements. Therefore, we concluded that the benzylic position (R_2 in Table 2) is fairly insensitive to modification, at least when the groups concerned are the size of isopropyl or smaller. Accordingly, when **9q**, **9r**, and **9s** were incubated for 8 hours, covalent interaction with GRK5 was observed as in **9e** (Figure S2). It is not clear why **9t** did not also react.

Finally, although the indolinone scaffold offers high potency and selectivity for GRK5 over GRK2, there were a few anticipated drawbacks in terms of its pharmacokinetic properties. First, **9a-t** are less soluble due to the number of aromatic rings and their rigid, linear conformation. The pyridyl pendants of **9k** and **9l** were a first attempt to address this issue. **9l** was found to improve solubility 3.5 fold over **9e** to 125 μg/ml (Table S1), which is still ~3-fold less than reported for Sunitinib (350 μg/ml). Metabolic liability was also a concern because of the high lipophilicity of our compounds and thus their potential metabolism by CYP3A4. Pyridyl pendants are also known to limit metabolic liability relative to benzyl rings, as are fluorine containing pendants, as in **9j** (Figure S4). We indeed found that **9j** indeed had a longer half-life (HLM $t_{1/2}$ =18.9 min) compared to the des-fluoro compound **9e** (HLM $t_{1/2}$ = 13.3 min). However, there was an opposite trend in MLMs. Limited commercial availability of fluorine substituted benzylic amines from the chiral pool however limited our ability to explore the additional *ortho* and *meta*-substitution patterns.

Ullrich-57 (**5a**) is already a potent (15 nM) and moderately selective GRK5 inhibitor relative to GRK2 (74-fold). Our overarching goal here was to ascertain whether potency and

selectivity could be improved by covalent modification of a residue unique to the GRK5 subfamily of kinases. Interestingly, although **9e, 9g,** and **9i-t** all had 2-haloacetylamido warheads, only a few were able to label Cys474 within a 30 minute time period. The absence of reactivity could be explained by the higher IC_{50} exhibited by many of these analogs. Those compounds with lower affinity for GRK5, for example **9c**, will bind in the active site of GRK5 more transiently than **9e**, resulting in a longer incubation period being needed to detect covalent engagement (Figure S1). **9e**, **9g**, and **9j** were not only the most efficacious at modifying GRK5 (Figure 3), but also they spanned a range of IC_{50} values (220–4 nM, respectively), and exhibited selectivity for GRK5 over GRK2 by over three orders of magnitude, 20 fold-more than exhibited by the noncovalent compound **5a**. We therefore performed a detailed kinetic analysis on these compounds to gauge the impact of their reactivity on measured potency and selectivity. We first showed that all three compounds exhibited 3–5 fold less potency against GRK5-C474S (Table 3), whereas our control compound CCG215022 was essentially unchanged. We then determined K_I and k_{inact} for these compounds (Figure 4, Table 3) which showed that **9e**, **9g**, and **9j** fully reacted with GRK5 before the first time point (hence $k_{inact}>1\ min^{-1}$) and that K_I scaled with the observed IC_{50} values reported for these compounds (Tables 1–2). This shared reactivity is consistent with the fact that they all contain haloacetyl warheads. The results further suggest that a direct comparison of the SAR among compounds in Table 2 along with **9e** and **9g** from Table 1 is reasonable. It also implies that in this situation, where the reactive cysteine in on a flexible loop near the ligand binding site, K_I dominates the IC_{50} measurements. More reactive warheads like 2-haloacetyls may also be required to take advantage of a more transiently associated cysteine side chain given the anticipated conformational variability of the AST loop.

Having confirmed that **5c** and **9g** were potent and highly GRK5 selective relative to GRK2, we explored their kinome wide selectivity. When tested at 1 μM (~1000-fold higher than their IC_{50} values), **5c** inhibits GRK5 at 92%, and GRK6 at 94%, whereas **9g** inhibits GRK5 at 93%, and GRK6 at 97%. GRK3, a very close homolog of GRK2, was inhibited at only 21%. Both compounds had many off-target effects across the kinome (Figure 5 and Figure S3), which is not surprising because the indolinone series is derived from Sunitinib, which can similarly inhibit the activity of many RTKs. We confirmed that selectivity improved at lower concentrations by testing **9g** at 100 and 10 nM (Figure 4). **9g** however continued to inhibit a small number of tyrosine kinases and Ca^{2+}/calmodulin-dependent protein kinases at the lowest dose, at which GRK5 was 50% inhibited, consistent with the IC_{50} value measured in our assays (Table 1).

CONCLUSIONS

In summary, we have developed a potent series of covalent inhibitors based on the indolinone scaffold that show higher potency and three orders of magnitude selectivity for GRK5 over the GRK2 subfamily, improving on the potency and selectivity of the parent compound Ullrich-57 (4-fold and 74-fold, respectively). Our MS and kinetic results further suggested that their ability to covalently engage a cysteine unique to the GRK5 subfamily, which contributes 3–5 fold to their IC_{50} values under our assay conditions, is responsible for these improved characteristics. The compounds thus set the stage for cell based assays that

would allow one to tease apart the roles of GRK5 versus GRK2 in cellular processes linked to cardiovascular disease and cancer. They also set the stage for a future generation of GRK5 subfamily selective therapeutics that would address potential toxicity issues associated with the use of strongly reactive 2-haloacetyls, further improve selectivity versus other subfamilies of protein kinases and their moderate solubility and metabolic stability. Such would facilitate their transition into *in vivo* trials. While this paper was under review, a new GRK5 inhibitor, KR-39038, with 20 nM IC_{50} was reported, although experiments addressing its selectivity versus GRK2 and other kinases and its toxicology were not provided[16]. This compound showed mild positive effects in a rodent model of hypertrophy but exhibited low bioavailability and a short half-life (<1 hour). These results reinforce the potential utility of covalent GRK5 inhibitors that could long-lasting effects even if the circulating compound is rapidly cleared, which would in turn mitigate off-target toxicity[10].

EXPERIMENTAL SECTION

General Chemistry.

All reagents from commercial sources were used without further purification unless otherwise noted. ^{1}H-NMR spectra were taken in DMSO-d6, MeOD or $CDCl_3$ at room temperature on Varian MR 400 MHz, Varian Vnmrs 500 MHz, and Varian Vnmrs 700 MHz instruments. The reported chemical shifts for the ^{1}HNMR spectra were recorded in parts per million (ppm) on the δ scale from an internal tetramethylsilane standard (0.0 ppm). Small molecule mass spectrometry data was measured using a Waters Corporation Micromass LCT or Agilent6230 Q-TOF instrument. HPLC was used to determine purity of compounds on an Agilent 1100 series with an Agilent Zorbax Eclipse Plus-C18 column. A gradient of 10–90% acetonitrile/water over 6 minutes followed by 90% acetonitrile/water for 7 minutes was used with detection at 254 nm. Purity of all compounds was > 95% as determined by HPLC. The Sunitinib scaffold is well known to photoisomerize in solution[17], and thus our compounds represent a 70:30 mix of active versus inactive isomers. All figures in the paper depict the active isomer.

Intact Protein MS and Tandem MS/MS.

Intact protein MS was acquired with a Phenomenex C4 column paired with an Agilent 6545 Q-TOF LC/MS. For intact MS and Tandem MS, all samples were prepared with 20 μM GRK in assay buffer (see below), 1 mM compound, and incubated at 4 °C for 3 hr before being quenched with 1.0 μL of formic acid. In Tandem MS/MS, we chose Glu-C as the restricting enzyme to avoid small fragments with mass-to-charge ratios below the limit of detection. All samples were digested with Glu-C sequencing enzyme, procured from Sigma Aldrich (Roche Life Sciences subsidiary) and used without further purification. MS/MS experiments were run on a nano-LC (Dionex RSLC-nano) with an Orbitrap Fusion Tribrid ETD mass spectrometer. This work was conducted by the Proteomics Resource Facility at the University of Michigan.

Structural Models and Docking.

GRK5 (PDB ID 4WNK)[18] and GRK6 (PDB ID 3NYN)[19] were loaded into Molecular Operating Environment 2018.01 (Molecular Operating Environment (MOE), 2018.01;

Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2018) and proteins were prepared using QuickPrep function. The sequences of both proteins were aligned and used to create a super-position of the two proteins and a hybrid structure with the kinase domain of GRK5 and the AST region of GRK6 was created for docking analysis (Figure 1A). The highest scoring docked pose for each compound in Tables 1–2 are provided as Supporting Information.

Inhibition Assays.

For compounds **5a-9t**, IC_{50} values for human GRK5 and bovine GRK2 were determined using a radiometric assay, described as follows. 50 nM GRK was incubated for 3–5 minutes with 500 nM porcine brain tubulin (PurSolutions) and 0.01 – 50 μM inhibitor in 20 mM HEPES pH 7.0, 2 mM $MgCl_2$, 0.025% dodecylmaltoside (DDM), 1% DMSO, prior to initiation with the addition of 5 μM ATP supplemented with radioactive [γ-^{32}P]-ATP (PerkinElmer Life Sciences). Reactions were quenched at 8 minutes by addition of 5 μL of 4X SDS gel loading dye to the 10 μL reactions. 12 μL samples were separated on a 4–15% Criterion TGX precast gel (Bio-Rad). For potent inhibitors with low nanomolar IC50, the inhibitor concentration was adjusted to approximately 0 – 50x [IC_{50}] which was estimated from the first run to get more accurate measurements. Gels were dried, exposed to a storage phosphor screen overnight, and scanned using a Personal Molecular Imager (Bio-Rad). Bands corresponding to phosphorylated tubulin were quantified using ImageQuant, plotted as a function of log[inhibitor], and fit to the four-parameter log(inhibitor) vs. response model in GraphPad Prism 7.03 to determine the IC_{50}, and mean and standard deviation values. Outliner was eliminated automatically at 1% Q value. Experiments were performed at least three times.

PKA inhibition assays were performed with the ADP-Glo system (Promega Corporation) according to the manufactory's instruction. 500 nM of PKA was incubated with 1 μg of CREBtide (KRREILSRRPSYR) (Genscript Corporation) substrate, 50 μM ATP and inhibitor for 30 minutes in 20 mM HEPES pH 7.0, 2 mM $MgCl_2$, 0.025% dodecylmaltoside (DDM), 4% DMSO. The concentration range of each inhibitor varies depending on its solubility at 4% DMSO with the highest concentration from 100 μM to 500 μM. After the initial reaction, ADP-Glo reagent was added to the reaction and allowed to incubate for an additional 40 minutes. Lastly, the kinase detection reagent was added and allowed to incubate for 30 minutes, and the luminescence was measured with a FlexStation 3 Multi-mode Microplate Reader (Molecular Devices). All data was analyzed in the same way as GRK inhibition assay. Experiments were performed three times in duplicate.

Standard control compounds are run during each assay to assess consistency across time, experimenters, and subtle changes in assay conditions that are sometimes required to keep compounds soluble and dispersed (*e.g.* through addition of DDM or 3% DMSO). Paroxetine were used as controls for GRK2[20] and PKA, and CCG215022 for GRK5[18].

Covalent inhibition kinetic analysis.

For the covalent inhibitors **9e**, **9g**, and **9i,** K_I and k_{inact} were determined using a radiometric assay as follows. The reactions contained 50 nM GRK5 and 5 μM porcine brain tubulin

(PurSolutions) in 20 mM HEPES pH 7.0, 2 mM $MgCl_2$, 0.025% DDM, 1% DMSO. Inhibitors at different concentrations (0.02–1 μM for **9j** and **9g**, 0.3–20 μM for **9e**) were incubated with the reaction mix for 30 seconds, prior to initiation with the addition of 5 μM ATP supplemented with radioactive [γ-^{32}P]-ATP (PerkinElmer Life Sciences). Reactions were quenched at 1, 2 and 5 minutes by addition of 10 μL of 4X SDS gel loading dye to the 10 μL reactions. 12 μL samples were separated on a 4–15% Criterion TGX precast gel (Bio-Rad). Gels were dried, exposed to a storage phosphor screen overnight, and scanned using a Personal Molecular Imager (Bio-Rad). Bands corresponding to phosphorylated tubulin were quantified using ImageQuant, background corrected, normalized to the intensity level of phosphorylation tubulin in the absence of any inhibitor, and plotted as a function of time. Because the inactivation process became nonlinear after the first time point, the observed inactivation rate (k_{obs}) was estimated using the one-minute time point. The k_{obs} values were re-plotted against inhibitor concentration and fitted to the equation, $k_{obs} = k_{inact}\,[I]/(K_I+[I])$, to obtain k_{inact} and K_I in GraphPad Prism 7.03. Experiments were performed three times.

Thermodynamic Solubility and Microsomal Stability.

Thermodynamic solubility for compounds was determined by Analiza Inc. (Cleveland OH, analiza.com) using a miniaturized shake-flask solubility assay. Microsomal stability determined by the Pharmacokinetics and Mass Spectrometry Core at the University of Michigan. Compounds **9c**, **9e** and **9j** were dissolved in DMSO (1 mM), and then further diluted to 100 μM with 0.1 M phosphate buffer (with 3.3 mM $MgCl_2$). Microsomes (20 mg/mL) in 0.1 M phosphate buffer (with 3.3 mM MgCl) were dosed with 20 μL of NADPH (4 mg in 240 μL of 0.1 M phosphate buffer) and incubated at 37 °C for 3 minutes. Microsomes were then dosed with 4 μL of 100 μM of **9c**, **9e** and **9j** respectively. At the following time points, 0, 5, 10, 15, 30, 45 and 60 minutes, the reaction solutions were then stopped with cold acetonitrile containing 25 nM CE302 as an internal standard. The incubation solution was centrifuged at 3500 rpm for 10 minutes to precipitate protein. The supernatant was used for LC/MS/MS analysis.

Chemical Synthesis and Validation.

(R)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (3).—To a round bottom flask was added 297.7 mg (1.69 mmol) of 2-oxoindoline-5-carboxylic acid, dissolved in 7 mL of dry DMF. To this dark red solution were added 0.244 mL (1.88 mmol) of (S)-1-phenylethan-1-amine, 0.300 mL (1.69 mmol) of DIPEA and 746.1 mg (1.95 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 401.9 mg, 84% Molecular Formula: $C_{17}H_{16}N_2O_2$ ESI-MS calc: 280.12 ESI-MS found: 281.1283 [M+1] HPLC: 5.198 1H NMR (400 MHz, DMSO-d6) δ 10.62 (s, 1H), 8.63 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.0 Hz, 2H), 7.39 – 7.35 (m, 2H), 7.31 (dd, J = 8.4, 6.8 Hz, 2H), 7.23 – 7.18 (m, 1H), 6.88 – 6.83 (m, 1H), 5.15 (p, J = 7.2 Hz, 1H), 3.53 (s, 2H), 1.46 (d, J = 7.1 Hz, 3H). 13C NMR (100 MHz, DMSO) δ 177.17, 165.76, 146.85, 145.65, 129.29, 128.67, 126.52, 126.06, 108.90, 48.85, 36.09, 22.84.

(S)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (3a).—To a round bottom flask was added 201.8 mg (1.13 mmol) of 2-oxoindoline-5-carboxylic acid dissolved in 7 mL of dry DMF. To this dark red solution were added 0.160 mL (1.24 mmol) of (S)-1-phenylethan-1-amine, 0.200 mL (1.13 mmol) of DIPEA and 492.4 mg (1.30 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 280 mg, 84% Molecular Formula: $C_{17}H_{16}N_2O_2$ ESI-MS calc: 280.12 ESI-MS found: 281.0903 [M+1] HPLC: 5.249 1H NMR (500 MHz, DMSO-d6) δ 10.67 (s, 1H), 8.66 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.2 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 7.6 Hz, 2H), 7.20 (t, J = 7.3 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 5.15 (p, J = 7.2 Hz, 1H), 3.53 (s, 2H), 1.46 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 176.83, 165.50, 146.49, 145.22, 128.27, 127.77, 127.54, 126.60, 126.14, 125.64, 123.69, 108.62, 48.52, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 35.70, 22.40.

(R)-2-oxo-N-(1-(m-tolyl)ethyl)indoline-5-carboxamide (3b).—To a round bottom flask was added 236.1 mg (1.33 mmol) of 2-oxoindoline-5-carboxylic acid, dissolved in 7 mL of dry DMF. To this dark red solution were added 0.200 mL (1.47 mmol) of (R)-1-(m-tolyl)ethan-1-amine, 0.300 mL (1.73 mmol) of DIPEA and 510.2 mg (1.33 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 125.6 mg, 31% Molecular Formula: $C_{18}H_{18}N_2O_2$ ESI-MS calc: 294.14 ESI-MS found: 295.0167 [M+1] HPLC: 5.395 1H NMR (700 MHz, DMSO-d6) δ 10.61 (s, 1H), 8.58 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.18 (p, J = 7.6 Hz, 4H), 7.02 (d, J = 7.3 Hz, 1H), 6.85 (d, J = 7.9 Hz, 1H), 5.11 (p, J = 7.3 Hz, 1H), 2.28 (s, 3H), 1.44 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 176.62, 165.16, 146.32, 145.07, 137.11, 128.06, 127.65, 127.48, 127.10, 126.66, 125.53, 123.51, 123.09, 108.36, 48.29, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 38.22, 35.57, 22.34, 21.11.

(R)-N-(1-(4-fluorophenyl)ethyl)-2-oxoindoline-5-carboxamide (3c).—To a round bottom flask was added 246.6 mg (1.41 mmol) of 2-oxoindoline-5-carboxylic acid, dissolved in 7 mL of dry DMF. To this dark red solution were added 0.200 mL (1.55 mmol) of (R)-1-(4-fluorophenyl)ethan-1-amine, 0.25 mL (1.41 mmol) of DIPEA and 667.1 mg (1.61 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 315 mg, 71% Molecular Formula: $C_{17}H_{15}FN_2O_2$ ESI-MS calc: 298.11 ESI-MS found: 299.1216 [M+1] HPLC: 5.414 1H NMR (500 MHz, DMSO-d6) δ 10.61 (s, 1H), 8.62 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.43 – 7.38 (m, 2H), 7.16 – 7.08 (m, 2H), 6.87 – 6.82 (m, 1H), 5.14 (p, J = 7.2 Hz, 1H), 3.53 (s, 2H), 1.45 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 176.84, 165.47,

160.34, 146.46, 141.34, 128.06, 128.01, 127.77, 127.49, 125.69, 123.63, 114.99, 114.87, 108.55, 47.90, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 38.33, 35.67, 22.35.

(R)-2-oxo-N-(1-(pyridin-4-yl)ethyl)indoline-5-carboxamide (3d).—To a round bottom flask was added 248.1 mg (1.41 mmol) of 2-oxoindoline-5-carboxylic acid dissolved in 7 mL of dry DMF. To this dark red solution were added 0.200 mL (1.55 mmol) of (R)-1-(pyridin-4-yl)ethan-1-amine, 0.25 mL (1.41 mmol) of DIPEA and 652.4 mg (1.61 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 200 mg, 48% Molecular Formula: $C_{16}H_{15}N_3O_2$ ESI-MS calc: 281.12 ESI-MS found: 282.1236 [M+1] HPLC: 2.378 1H NMR (700 MHz, DMSO-d6) δ 10.64 (s, 1H), 8.74 (dd, J = 7.6, 2.1 Hz, 1H), 8.55 – 8.52 (m, 2H), 7.79 (dd, J = 7.5, 2.2 Hz, 2H), 7.45 – 7.42 (m, 2H), 7.08 (d, J = 2.3 Hz, 1H), 7.01 (s, 1H), 6.89 – 6.85 (m, 1H), 5.14 (td, J = 7.3, 2.2 Hz, 1H), 3.55 (s, 2H), 3.46 – 3.41 (m, 3H), 1.47 (dd, J = 7.2, 2.3 Hz, 3H), 1.07 – 1.03 (m, 3H). 13C NMR (176 MHz, DMSO) δ 176.63, 165.65, 155.16, 148.63, 146.56, 127.76, 127.03, 125.62, 123.57, 121.58, 108.43, 56.00, 47.92, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 35.56, 21.45, 18.54.

(R)-2-oxo-N-(1-(pyridin-2-yl)ethyl)indoline-5-carboxamide (3e).—To a round bottom flask was added 251.1 mg (1.41 mmol) of 2-oxoindoline-5-carboxylic acid dissolved in 7 mL of dry DMF. To this dark red solution were added 0.200 mL (1.55 mmol) of (R)-1-(pyridin-2-yl)ethan-1-amine, 0.25 mL (1.41 mmol) of DIPEA and 625.1 mg (1.62 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 165 mg, 39% Molecular Formula: $C_{16}H_{15}N_3O_2$ ESI-MS calc: 281.12 ESI-MS found: 282.1236 [M+1] HPLC: 2.111 1H NMR (700 MHz, DMSO-d6) δ 10.62 (d, J = 5.2 Hz, 1H), 8.63 (t, J = 6.6 Hz, 1H), 8.51 (t, J = 5.3 Hz, 1H), 7.80 (d, J = 5.6 Hz, 2H), 7.74 (q, J = 7.0 Hz, 1H), 7.38 (t, J = 6.8 Hz, 1H), 7.24 (q, J = 6.0 Hz, 1H), 6.86 (t, J = 6.7 Hz, 1H), 5.17 (p, J = 7.2 Hz, 1H), 3.54 (d, J = 5.2 Hz, 3H), 1.49 (t, J = 6.4 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 177.27, 166.16, 163.50, 149.12, 137.26, 128.18, 127.79, 126.11, 124.08, 122.46, 120.59, 108.98, 50.76, 40.22, 40.10, 39.98, 39.86, 39.74, 39.62, 39.50, 36.07, 21.45.

(R)-N-(1-(3-chlorophenyl)ethyl)-2-oxoindoline-5-carboxamide (3f).—To a round bottom flask was added 251.1 mg (1.41 mmol) of 2-oxoindoline-5-carboxylic acid dissolved in 7 mL of dry DMF. To this dark red solution were added 0.200 mL (1.55 mmol) of (R)-1-(3-chlorophenyl)ethan-1-amine, 0.25 mL (1.41 mmol) of DIPEA and 717.7 mg (1.91 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a

strawberry pink solid. Yield: 423.3 mg, 91% Molecular Formula: $C_{17}H_{15}ClN_2O_2$ ESI-MS calc: 314.08 ESI-MS found: 315.0438 [M+1] HPLC: 5.730 1H NMR (700 MHz, DMSO-d6) δ 10.64 – 10.57 (m, 1H), 8.65 (dd, J = 7.5, 2.2 Hz, 1H), 7.78 – 7.74 (m, 2H), 7.41 (t, J = 2.1 Hz, 1H), 7.33 (dt, J = 6.7, 1.8 Hz, 2H), 7.26 (dp, J = 6.4, 2.2 Hz, 1H), 6.85 (dd, J = 8.1, 2.5 Hz, 1H), 5.15 – 5.06 (m, 1H), 3.53 (s, 2H), 1.45 – 1.42 (m, 3H). 13C NMR (176 MHz, DMSO) δ 176.91, 165.61, 148.09, 146.73, 133.15, 130.39, 127.97, 126.74, 126.14, 125.88, 125.13, 123.78, 108.70, 56.27, 48.40, 40.12, 40.00, 39.88, 39.76, 39.64, 39.52, 39.40, 35.83, 22.43, 18.80.

(R)-2-oxo-N-(1-(p-tolyl)ethyl)indoline-5-carboxamide (3g).—To a round bottom flask was added 244.2 mg (1.41 mmol) of 2-oxoindoline-5-carboxylic acid dissolved in 7 mL of dry DMF. To this dark red solution were added 0.220 mL (1.55 mmol) of (R)-1-(3-chlorophenyl)ethan-1-amine, 0.25 mL (1.41 mmol) of DIPEA and 622.1 mg (1.62 mmol) of HATU. The resultant dark red solution was allowed to stir at room temperature for 12 hours, and then added 200 mL of sat. Na_2CO_3 and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (2 × 50 mL) and then dried over $MgSO_4$. Purified by column chromatography (0–15% DCM/MeOH) to give the desired product as a strawberry pink solid. Yield: 300.8 mg, 69% Molecular Formula: $C_{18}H_{18}N_2O_2$ ESI-MS calc: 294.14 ESI-MS found: 317 [M+Na] HPLC: 5.556 1H NMR (700 MHz, DMSO-d6) δ 10.61 (s, 1H), 8.57 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 7.8 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 6.85 (d, J = 8.0 Hz, 1H), 5.12 (p, J = 7.3 Hz, 1H), 3.53 (s, 2H), 2.26 (s, 3H), 1.44 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 176.63, 165.17, 146.30, 142.10, 135.44, 128.66, 127.64, 127.54, 125.93, 125.51, 123.51, 108.36, 48.03, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 35.57, 22.29, 20.59.

(R)-N-(1-(4-chlorophenyl)ethyl)-2-oxoindoline-5-carboxamide (3h).—Prepared using the protocol described for **3**. Yields a strawberry pink solid, 471.0 mg, quantitative yield. Molecular Formula: $C_{17}H_{15}ClN_2O_2$ ESI-MS calc: 314.08 ESI-MS found: 337.0717 [M+Na] HPLC: 5.682 1H NMR (500 MHz, DMSO-d6) δ 10.61 (s, 1H), 8.64 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.42 – 7.34 (m, 4H), 6.85 (d, J = 8.1 Hz, 1H), 5.13 (p, J = 7.2 Hz, 1H), 3.53 (s, 2H), 1.45 (d, J = 7.0 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 176.61, 165.29, 146.40, 144.17, 130.98, 128.09, 127.92, 127.67, 127.28, 125.55, 123.49, 108.37, 47.87, 40.00, 39.83, 39.67, 39.50, 39.33, 39.17, 39.00, 35.55, 22.09.

N-benzyl-2-oxoindoline-5-carboxamide (3i).—Prepared with protocol described for **3**. Yields a strawberry pink solid, 174.5 mg, 56% Molecular Formula: $C_{16}H_{14}N_2O_2$ ESI-MS calc: 266.11 ESI-MS found: 267.2180 [M+1] HPLC: 4.822 1H NMR (700 MHz, DMSO-d6) δ 10.61 (s, 1H), 8.87 (t, J = 6.1 Hz, 1H), 7.79 – 7.75 (m, 2H), 7.34 – 7.28 (m, 5H), 7.23 (dt, J = 7.2, 4.3 Hz, 2H), 6.86 (d, J = 8.0 Hz, 1H), 4.46 (d, J = 5.9 Hz, 2H), 3.53 (s, 2H). 13C NMR (176 MHz, DMSO) δ 176.60, 165.95, 146.42, 139.88, 128.65, 128.19, 128.12, 127.70, 127.59, 127.47, 127.29, 127.11, 126.61, 125.66, 123.45, 108.44, 47.80, 45.00, 42.52, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 38.22, 35.57.

2-oxo-N-(2-phenylpropan-2-yl)indoline-5-carboxamide (3j).—Prepared using the protocol described for **3**. Yields a strawberry pink solid, 210.8 mg, 48%. Molecular Formula:

$C_{18}H_{18}N_2O_2$ ESI-MS calc: 294.14 ESI-MS found: 295.1456 [M+1], 317.1275 [M+Na] HPLC: 5.199 1H NMR (700 MHz, DMSO-d6) δ 10.60 (s, 1H), 8.23 (s, 1H), 7.74 (s, 1H), 7.74 – 7.71 (m, 1H), 7.35 (d, J = 7.8 Hz, 2H), 7.26 (t, J = 7.7 Hz, 2H), 7.15 (t, J = 7.2 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 3.53 (s, 2H), 1.65 (s, 6H). 13C NMR (176 MHz, DMSO) δ 176.64, 165.50, 148.19, 146.15, 128.51, 128.35, 127.82, 127.67, 125.59, 125.37, 124.81, 124.61, 123.65, 108.26, 55.22, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 35.59, 29.66.

(R)-N-(2-methyl-1-phenylpropyl)-2-oxoindoline-5-carboxamide (3k).—Prepared using the protocol described for **3**. Yields a strawberry pink solid, 389.9 mg, quantitative yield. Molecular Formula: $C_{19}H_{20}N_2O_2$ ESI-MS calc: 308.15 ESI-MS found: 309.2172 HPLC: 5.793 1H NMR (700 MHz, DMSO-d6) δ 10.44 (s, 1H), 8.37 (d, J = 8.9 Hz, 1H), 7.58 (dd, J = 10.7, 2.9 Hz, 2H), 7.22 (d, J = 7.6 Hz, 2H), 7.12 (t, J = 7.5 Hz, 2H), 7.03 (t, J = 7.3 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 4.48 (t, J = 9.2 Hz, 1H), 3.36 (s, 2H), 1.06 (d, J = 6.4 Hz, 2H), 0.83 (d, J = 6.5 Hz, 3H), 0.53 (d, J = 6.7 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 176.69, 165.65, 164.62, 162.32, 146.32, 143.33, 128.02, 127.75, 127.70, 127.41, 126.61, 125.54, 123.53, 108.43, 59.92, 56.08, 41.67, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 38.23, 35.78, 35.62, 32.55, 30.77, 20.09, 19.90, 18.56.

2-oxo-N-(3-phenyloxetan-3-yl)indoline-5-carboxamide (3l).—Prepared using the protocol described for **3**. Yields a strawberry pink solid, 62.1 mg, 31% Molecular Formula: $C_{18}H_{16}N_2O_3$ ESI-MS calc: 308.12 ESI-MS found: 309.1235 HPLC: 4.601 1H NMR (700 MHz, DMSO-d6) δ 10.49 (d, J = 4.6 Hz, 1H), 9.16 (d, J = 4.6 Hz, 1H), 7.65 – 7.60 (m, 2H), 7.38 – 7.34 (m, 2H), 7.22 – 7.18 (m, 2H), 7.10 (tdd, J = 7.3, 4.9, 2.2 Hz, 1H), 6.74 – 6.70 (m, 1H), 4.82 (dd, J = 6.7, 4.8 Hz, 2H), 4.59 (dd, J = 6.7, 4.8 Hz, 2H), 3.38 (d, J = 4.7 Hz, 2H). 13C NMR (176 MHz, DMSO) δ 176.66, 165.18, 146.78, 143.10, 129.97, 128.28, 128.17, 127.72, 126.91, 126.72, 126.06, 125.80, 125.41, 124.81, 123.53, 120.11, 108.72, 108.56, 81.89, 58.27, 53.49, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 38.23, 35.58, 35.52, 30.66, 14.07.

N-(2-(diethylamino)ethyl)-5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxamide (4a).—To a round bottom flask were added 200.9 mg (1.20 mmol) of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid, 365.8 mg (1.79 mmol) of EDCI, 277.7 mg (1.79 mmol) of HOBt, 7 mL of dry DMF, 0.200 mL (1.44 mmol) of N1,N1-diethylethane-1,2-diamine and 0.34 mL (2.39 mmol) of TEA. The dark red solution was allowed to stir at room temperature for 12 hours before being quenched with water and extracted with DCM (3 × 30 mL). The combined organic layer was washed with brine (2 × 20 mL) and then washed with 10% citric acid (3 × 50 mL), drawing the desired product into the water layer. The aqueous layer was basified with Na_2CO_3, bringing the pH up to 10, and then extracted with DCM (4 × 50 mL). The combined organic layers were dried over $MgSO_4$ and then evaporated onto silica gel and purified by column chromatography (5–15% MeOH/DCM). The solvent was removed under pressure to give a yellow solid. Result: light yellow solid, 90 mg, 27% Molecular Formula: $C_{14}H_{23}N_3O_2$, ESI-MS Calc: 265.18 ESI-MS found: 266.1749 HPLC: 2.681 1H NMR (400 MHz, DMSO-d6) δ 11.85 (s, 1H), 9.54 (s, 1H), 7.95 (s, 2H), 2.34 (d, J = 20.8 Hz, 7H), 0.99 (s, 6H).

5-formyl-2,4-dimethyl-N-(prop-2-yn-1-yl)-1H-pyrrole-3-carboxamide (4b).—To a round bottom flask were added 199.4 mg (1.20 mmol) of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid, 688.0 mg (1.79 mmol) of HATU, 6 mL of dry DMF, 0.100 mL (1.44 mmol) of propargylamine and 0.34 mL (2.39 mmol) of TEA. The dark red solution was allowed to stir at room temperature for 12 hours before being quenched with water and extracted with DCM (3 × 30 mL). Took the combined organic layers and washed with brine (2 × 20 mL) and then washed with 10% citric acid (3 × 50 mL), taking the desired product into the aqueous layer. The aqueous layer was basified with Na_2CO_3, bringing the pH up to 10, and then extracted with DCM (4 × 50 mL). The combined organic layer was dried over $MgSO_4$ and then removed solvent to give the final product as a light orange oil. Result: light orange oil, 61 mg, 25% Molecular Formula: $C_{11}H_{12}N_2O_2$ ESI-MS calc: 204.09 ESI-MS found: 205.0873 [M+1], 242.1167 [M+K] HPLC: 3.625 1H NMR (700 MHz, DMSO-d6) δ 11.87 (s, 1H), 9.55 (s, 1H), 7.95 (s, 16H), 3.97 (ddd, J = 12.7, 5.7, 2.5 Hz, 3H), 3.08 (t, J = 2.4 Hz, 1H), 2.36 (s, 3H), 2.31 (s, 3H). 13C NMR (176 MHz, DMSO) δ 177.82, 164.77, 139.11, 138.51, 128.26, 119.20, 89.92, 82.16, 72.95, 55.38, 40.24, 38.71, 28.42, 12.90, 10.00.

N-allyl-5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxamide (4c).—To a round bottom flask were added 204.4 mg (1.20 mmol) of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid, 350.3 mg of EDCI (1.79 mmol), 275.3 mg (1.79 mmol) of HOBt, 6 mL of dry DMF, 0.110 mL (1.44 mmol) of allylamine and 0.34 mL (2.39 mmol) of TEA. The dark red solution was allowed to stir at room temperature for 12 hours before being quenched with water and extracted with DCM (3 × 30 mL). Took combined organic layers and washed with LiCl 3 × 20 mL) and then washed with 10% Citric acid (3 × 50 mL), drawing the desired product into the aqueous layer. The aqueous layer was basified with Na_2CO_3, bringing the pH up to 8, and then extracted with DCM (4 × 50 mL) The combined organic layer was dried over $MgSO_4$ and then removed solvent to give a yellow solid. Result: light yellow solid, 64 mg, 25% Molecular Formula: $C_{11}H_{14}N_2O_2$ ESI-MS calc: 206.11 ESI-MS: 246.1621 [M+ MeCN] HPLC: 3.885 ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.38 (s, 1H), 9.54 (s, 1H),8.17 (s, 1H), 6.01 (tt, J = 10.8, 5.4 Hz, 1H), 5.87 (ddt, J = 16.4, 10.6, 5.3 Hz, 2H), 5.18 (t, J = 15.7 Hz, 3H), 5.08 (dd, J = 16.5, 10.2 Hz, 3H), 4.14 (d, J = 5.5 Hz, 2H), 3.82 (t, J = 5.7 Hz, 3H), 2.31 (s, 5H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 161.78, 150.29, 135.45, 135.21, 115.01, 114.35, 114.20, 40.54, 40.50, 39.50, 39.33, 39.17, 39.00, 38.83, 38.67, 38.50, 35.26, 30.25, 11.95, 9.27.

N-(but-3-yn-1-yl)-5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxamide (4d).—To a round bottom flask were added 199.6 mg (1.20 mmol) of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid, 684.3 mg (1.79 mmol) of HATU, 6 mL of dry DMF, 0.100 mL (1.44 mmol) of 1-amino-3-butyne and 0.30 mL (2.39 mmol) of DIPEA. The dark red solution was allowed to stir at room temperature for 12 hours before being quenched with water and extracted with DCM (3 × 30 mL). The combined organic layers were washed with LiCl (2 × 20 mL) and then washed with 10% citric acid (3 × 50 mL), drawing the desired product into the water layer. The aqueous layer was basified with Na_2CO_3, bringing the pH up to 10, and then extracted with DCM (4 × 50 mL). The combined organic layer was dried over $MgSO_4$ and then removed solvent under pressure to give the final product as a yellow

solid. Result: light yellow solid, 99.6 mg, 38.1% Molecular Formula: $C_{12}H_{14}N_2O_2$ ESI-MS calc: 218.11 ESI-MS found: 219.1129 HPLC: 3.997 1H NMR (700 MHz, DMSO-d6) δ 11.83 (s, 1H), 2.83 (s, 1H), 2.69 (d, J = 1.0 Hz, 1H), 2.60 (d, J = 2.4 Hz, 3H), 2.37 (s, 4H), 2.32 (s, 3H). 13C NMR (176 MHz, DMSO) δ 178.94, 164.54, 160.23, 152.56, 147.04, 146.58, 126.55, 115.31, 88.48, 82.43, 72.02, 55.77, 37.85, 18.85, 13.97, 10.55.

N-(but-3-en-1-yl)-5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxamide (4e).—To a round bottom flask were added 197.8 mg (1.20 mmol) of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid, 685.8 mg (1.79 mmol) of HATU, 6 mL of dry DMF, 107.1 mg (1.44 mmol) of but-3-en-1-amine and 0.45 mL (2.40 mmol) of DIPEA. The dark red solution was allowed to stir at room temperature for 12 hours before being quenched with water and extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine (2 × 20 mL) and then washed with 10% citric acid (3 × 50 mL), drawing the desired material in to the aqueous layer. The aqueous layer was basified with Na_2CO_3, bringing the pH up to 10, and then extracted with DCM (4 × 50 mL). The combined organic layer was dried over $MgSO_4$. Result: light orange oil, 106.1 mg, 37.2% Molecular Formula: $C_{12}H_{16}N_2O_2$ ESI-MS calc: 220.12 MS: 221.1304 HPLC: 5.554 1H NMR (500 MHz, DMSO-d6) δ 9.77 (s, 1H), 8.84 (d, J = 4.5 Hz, 1H), 8.73 (d, J = 8.4 Hz, 1H), 7.95 (s, 4H), 7.66 (dd, J = 8.7, 4.6 Hz, 1H), 5.12 – 4.99 (m, 1H), 2.59 (s, 7H). 13C NMR (126 MHz, DMSO) δ 179.37, 165.02, 162.73, 152.99, 146.27, 140.66, 134.88, 130.23, 122.09, 36.21, 31.20, 14.39, 10.98.

(R,Z)-3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (5a).—To a dried sealed tube were added 97.9 mg (0.371 mmol) of (3), 107.0 mg (0.357 mmol) of (**4a**) all of which were dissolved in abs. EtOH (3.5 mL). To this solution were added 2 drops of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as an orange solid. Yield: orange solid, 38.3 mg, 22% Molecular Formula: $C_{31}H_{37}N_5O_3$ ESI-MS calc: 527.29 ESI-MS found: 528.2955 HPLC: 5.621 1HNMR (500 MHz, DMSO-d6) δ 13.59 (s, 1H), 11.13 (s, 1H), 8.59 (d, J = 8.1 Hz, 1H), 8.25 (s, 1H), 7.71 (d, J = 6.8 Hz, 2H), 7.44 (d, J = 6.0 Hz, 1H), 7.41 (d, J = 7.9 Hz, 2H), 7.33 (t, J = 7.7 Hz, 2H), 7.22 (t, J = 7.7 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 5.19 (t, J = 7.5 Hz, 1H), 3.28 (d, J = 7.0 Hz, 2H), 2.44 (d, J = 6.7 Hz, 6H), 1.51 (d, J = 7.1 Hz, 3H), 0.97 (t, J = 7.1 Hz, 7H). 13CNMR (176 MHz, DMSO) δ 169.90, 165.95, 164.71, 145.11, 140.64, 136.48, 129.93, 128.30, 127.78, 126.64, 126.37, 126.18, 125.86, 125.28, 124.09, 120.76, 117.69, 114.37, 108.94, 51.72, 48.55, 46.59, 45.44, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 37.06, 22.35, 13.40, 11.92, 10.79.

(S,Z)-3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (5b).—To a dried sealed tube were added 91.2 mg (0.325 mmol) of (**3a**), 79.9 mg (0.299 mmol) of (**4a**) all of which were dissolved in abs. EtOH (2.5 mL). To this solution were added 2 drops of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as an orange solid. Yield: orange solid, 38.3 mg, 22% Molecular Formula: $C_{31}H_{37}N_5O_3$ ESI-MS calc: 527.29 ESI MS found: 528.2043

HPLC: 5.753 1H NMR (500 MHz, DMSO-d6) δ 13.59 (s, 1H), 11.16 (s, 1H), 8.67 (s, 1H), 8.32 (d, J = 5.5 Hz, 1H), 7.75 – 7.68 (m, 2H), 7.48 (s, 1H), 7.42 (d, J = 7.7 Hz, 3H), 7.32 (t, J = 7.6 Hz, 3H), 7.22 (t, J = 7.3 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 5.19 (p, J = 7.2 Hz, 1H), 2.45 (d, J = 3.4 Hz, 8H), 2.37 (s, 1H), 2.32 (s, 1H), 2.08 (s, 1H), 1.51 (d, J = 7.1 Hz, 4H), 0.99 (t, J = 7.5 Hz, 8H). 13C NMR (126 MHz, DMSO) δ 170.28, 147.82, 141.01, 130.32, 128.66, 127.23, 126.98, 126.61, 126.26, 125.66, 124.60, 118.30, 106.84, 102.36, 48.92, 47.02, 22.80, 17.46, 13.83, 11.24, 9.18.

(R,Z)-3-((3,5-dimethyl-4-(prop-2-yn-1-ylcarbamoyl)-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (5c).—To a dried sealed tube were added 75.1 mg (0.27 mmol) of (**3**), 50 mg (0.28 mmol) of (**4b**) all of which were dissolved in abs. EtOH (1.8 mL). To this solution were added 0.05 mL of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and filtered off an orange solid. The solid was rinsed with cold EtOH to give the final product. Yield: bright orange solid, 58.6 mg, 46% Molecular Formula: $C_{28}H_{26}N_4O_3$ ESI-MS calc: 466.20 ESI-MS found: 467.2037 HPLC: 6.360 1H NMR (700 MHz, DMSO-d6) δ 13.61 (s, 1H), 11.15 (s, 1H), 8.61 (d, J = 8.0 Hz, 1H), 8.26 (s, 1H), 8.06 (t, J = 5.5 Hz, 1H), 7.71 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 7.8 Hz, 2H), 7.33 (q, J = 5.8, 3.9 Hz, 2H), 7.23 (t, J = 7.2 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 5.20 (t, J = 7.7 Hz, 1H), 4.02 (d, J = 5.5 Hz, 2H), 3.14 – 3.08 (m, 1H), 2.44 (d, J = 9.2 Hz, 7H), 1.51 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 170.30, 166.26, 145.52, 141.06, 136.92, 130.46, 128.67, 128.29, 127.00, 126.82, 126.58, 126.28, 124.49, 120.45, 118.23, 115.03, 109.28, 82.17, 73.04, 48.87, 28.49, 22.77, 13.76, 11.13.

(R,Z)-3-((4-(allylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (5d).—To a dried sealed tube were added 53.6 mg (0.27 mmol) of (**3**), 50 mg (0.28 mmol) of (**4c**) all of which were dissolved in abs. EtOH (3 mL). To this solution were added 2 drops (0.05 mL) of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, the reaction was cooled to room temperature and then an orange solid was filtered off. The solid was washed with cold EtOH to give the final product. Result: orange solid, 79.5 mg, 63% Molecular Formula: $C_{28}H_{28}N_4O_3$ ESI-MS calc: 468.22 ESI-MS found: 469.2224 HPLC: 6.450 1H NMR (700 MHz, DMSO-d6) δ 13.60 (s, 1H), 11.14 (s, 1H), 8.60 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 1.6 Hz, 1H), 7.83 (t, J = 5.8 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.41 (d, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.22 (t, J = 7.3 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 5.90 (ddt, J = 15.7, 10.3, 5.2 Hz, 1H), 5.22 – 5.18 (m, 2H), 5.10 (dd, J = 10.3, 1.8 Hz, 1H), 3.87 (t, J = 5.6 Hz, 2H), 2.44 (d, J = 7.8 Hz, 6H), 1.51 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 170.50, 145.29, 135.97, 130.84, 129.65, 128.44, 128.02, 126.34, 115.20, 108.42, 103.80, 91.16, 59.93, 22.53, 13.55, 10.93.

(R,Z)-3-((4-(but-3-yn-1-ylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (5e).—To a dried sealed tube were added 99.9 mg (0.357 mmol) of (**3**), 82 mg of (**4d**) all of which were dissolved in abs. EtOH (3.5 mL). To this solution were added 2 drops (0.05 mL) of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, the solution was cooled to room temperature and then an orange solid was filtered off. The solid was washed with cold EtOH to give the final product. Yield: orange solid, 19 mg, 10% Molecular Formula: $C_{29}H_{28}N_4O_3$ ESI-MS calc:

480.22 ESI-MS found: 481.2226 HPLC: 6.444 1H NMR (700 MHz, DMSO-d6) δ 13.60 (s, 1H), 11.15 (s, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.25 (s, 1H), 7.79 (t, J = 5.8 Hz, 1H), 7.71 (d, J = 6.3 Hz, 2H), 7.41 (d, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 7.3 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 5.19 (p, J = 7.3 Hz, 1H), 2.86 (t, J = 2.5 Hz, 1H), 2.45 (d, J = 12.0 Hz, 6H), 2.42 (td, J = 7.1, 2.4 Hz, 2H), 1.51 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.83, 165.81, 164.71, 145.08, 140.56, 136.34, 130.00, 128.23, 127.80, 126.55, 126.12, 125.79, 125.18, 124.08, 120.61, 117.76, 82.50, 72.12, 48.42, 37.95, 22.33, 13.33, 10.70.

(R,Z)-3-((4-(but-3-en-1-ylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (5f).—To a dried sealed tube were added 94.8 mg (0.27 mmol) of (**3**), 87.2 mg (0.28 mmol) of (**4e**) all of which were dissolved in abs. EtOH (2.2 mL). To this solution were added 2 drops (0.07 mL) of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, solution was cooled to room temperature and then purified by column chromatography. Fractions were collected and solvent was removed under pressure to give the desired product, as an orange solid. Yield: orange solid, 103 mg, 59% Molecular Formula: $C_{29}H_{30}N_4O_3$ ESI-MS calc: 482.23 ESI-MS found: 483.2382 [M+1] HPLC: 6.720 1H NMR (700 MHz, DMSO-d6) δ 13.54 (s, 1H), 11.13 (s, 1H), 8.61 (d, J = 8.2 Hz, 2H), 8.27 – 8.18 (m, 2H), 7.73 – 7.71 (m, 1H), 7.68 (s, 1H), 7.42 (d, J = 8.3 Hz, 3H), 7.35 – 7.31 (m, 4H), 7.25 – 7.21 (m, 2H), 6.94 (d, J = 8.1 Hz, 1H), 5.21 (p, J = 7.3 Hz, 2H), 2.43 (d, J = 9.0 Hz, 2H), 2.29 (d, J = 11.4 Hz, 7H), 1.63 – 1.59 (m, 3H), 1.51 (d, J = 7.1 Hz, 6H). 13C NMR (176 MHz, DMSO) δ 169.79, 165.81, 165.04, 145.07, 140.57, 133.90, 128.74, 128.19, 127.78, 126.52, 126.11, 125.22, 123.96, 120.71, 117.70, 116.21, 114.09, 108.74, 48.41, 35.77, 33.74, 22.26, 12.40, 10.15.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2oxo-N-(1-phenylethyl)indoline-5-carboxamide (7a).—To a dried sealed tube were added 91.2 mg (0.892 mmol) of (**3**), 79.9 mg (1.39 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (2.5 mL). To this solution were added 2 drops (0.05 mL) of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, the reaction was cooled to room temperature and an orange solid was filtered off. The solid was washed with cold EtOH to give the desired product. Result: orange solid, 269.3 mg, 69% Molecular Formula: $C_{24}H_{22}N_4O_4$ ESI-MS calc: 430.16 ESI-MS found: 431.1715 HPLC: 7.400 1H NMR (700 MHz, DMSO-d6) δ 8.63 (d, J = 8.0 Hz, 1H), 8.36 (d, J = 1.6 Hz, 1H), 7.83 (s, 1H), 7.76 (dd, J = 8.1, 1.7 Hz, 1H), 7.41 (d, J = 7.5 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.22 (t, J = 7.3 Hz, 1H), 6.97 (d, J = 8.1 Hz, 1H), 5.19 (p, J = 7.2 Hz, 1H), 2.08 (d, J = 1.0 Hz, 1H), 1.50 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.90, 165.69, 144.98, 142.47, 136.80, 132.12, 130.48, 129.11, 128.20, 127.74, 126.10, 125.56, 123.33, 121.76, 115.38, 53.66, 22.28, 18.23, 10.50.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(m-tolyl)ethyl)indoline-5-carboxamide (7b).—To a dried sealed tube were added 200 mg (0.679 mmol) of (**3b**), 139.6 mg (0.815 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (6.0 mL). To this solution were added 0.05 ml of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled

to room temperature and then filtered off the product as a lemon yellow solid. Yield: lemon yellow solid, 100 mg, 29.8 % Molecular Formula: $C_{25}H_{24}N_4O_4$ ESI-MS calc: 444.18 ESI-MS found: 445.1862 [M+1] HPLC: 7.732 1H NMR (700 MHz, DMSO-d6) δ 11.34 (s, 1H), 8.58 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 1.6 Hz, 1H), 7.78 (s, 1H), 7.76 (dd, J = 8.2, 1.6 Hz, 1H), 7.23 – 7.18 (m, 3H), 7.03 (d, J = 6.1 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 5.16 (p, J = 7.2 Hz, 1H), 2.62 (s, 3H), 2.57 (s, 3H), 2.30 (s, 3H), 1.49 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.83, 165.49, 144.90, 141.26, 137.14, 136.75, 133.66, 128.34, 128.09, 127.51, 127.15, 126.74, 125.07, 124.71, 124.39, 123.59, 123.17, 119.39, 118.96, 109.09, 48.36, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 22.26, 21.11, 14.76, 11.21.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-N-(1-(4-fluorophenyl)ethyl)-2-oxoindoline-5-carboxamide (7c).—To a dried sealed tube were added 128 mg (0.429 mmol) of (**3c**), 101.3 mg (0.60 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (3.0 mL). To this solution were added 0.05 ml of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as an orange solid. Yield: orange solid, 96.3 mg, 50 % Molecular Formula: $C_{24}H_{21}FN_4O_4$ ESI-MS calc: 448.15 ESI-MS found: 449.1619 [M+1] HPLC: 7.422 1H NMR (700 MHz, DMSO-d6) δ 11.37 (s, 1H), 8.64 (d, J = 7.8 Hz, 1H), 8.35 (d, J = 1.7 Hz, 1H), 7.83 (s, 1H), 7.76 (dd, J = 8.1, 1.7 Hz, 1H), 7.47 – 7.44 (m, 2H), 7.18 – 7.14 (m, 2H), 6.97 (d, J = 8.1 Hz, 1H), 5.19 (p, J = 7.2 Hz, 1H), 2.65 (s, 3H), 2.60 (s, 3H), 1.51 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.85, 165.62, 136.79, 133.69, 128.31, 128.02, 127.97, 124.71, 123.71, 119.03, 114.88, 114.76, 50.22, 47.86, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 22.26, 14.78, 11.22.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(pyridin-4-yl)ethyl)indoline-5-carboxamide (7d).—To a dried sealed tube were added 100 mg (0.455 mmol) of (**3d**), 77.1 mg (0.459 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (3.0 mL). To this solution were added 0.05 ml of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as a yellow solid. Yield: yellow solid, 48.0 mg, 31.3 % Molecular Formula: $C_{23}H_{21}N_5O_4$ ESI-MS calc: 431.16 ESI-MS found: 432.1656 [M+1] HPLC: 5.496 1H NMR (700 MHz, DMSO-d6) δ 11.36 (s, 1H), 8.73 (d, J = 7.6 Hz, 1H), 8.52 – 8.50 (m, 2H), 8.36 (d, J = 1.6 Hz, 1H), 7.82 (s, 1H), 7.77 (dd, J = 8.1, 1.6 Hz, 1H), 7.40 (d, J = 5.2 Hz, 2H), 6.97 (d, J = 8.0 Hz, 1H), 5.15 (p, J = 7.3 Hz, 1H), 2.64 (s, 3H), 2.59 (s, 3H), 1.51 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 170.11, 166.24, 153.97, 149.80, 137.07, 135.63, 128.31, 127.80, 125.45, 124.98, 124.02, 121.55, 119.37, 109.42, 48.18, 40.12, 40.00, 39.88, 39.76, 39.64, 39.52, 39.40, 21.78, 15.04, 11.48.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2oxo-N-(1-(pyridin-2-yl)ethyl)indoline-5-carboxamide (7e).—To a dried sealed tube were added 165 mg (0.587 mmol) of (**3e**), 122.8 mg (0.762 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (3.0 mL). To this solution were added 0.05 ml of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as a yellow solid. Yield: yellow solid, 116 mg, 34 % Molecular Formula: $C_{23}H_{21}N_5O_4$ ESI-MS calc: 431.16 ESI-MS found:

432.0558 [M+1] HPLC: 5.49 1H NMR (700 MHz, DMSO-d6) δ 11.36 (d, J = 7.2 Hz, 1H), 8.65 – 8.58 (m, 1H), 8.55 – 8.47 (m, 1H), 8.39 (t, J = 7.7 Hz, 1H), 7.78 (ddd, J = 33.0, 15.8, 7.2 Hz, 3H), 7.42 (q, J = 10.5, 9.0 Hz, 1H), 7.25 (dd, J = 12.7, 6.8 Hz, 1H), 6.95 (d, J = 7.0 Hz, 1H), 5.22 (q, J = 8.8, 8.0 Hz, 1H), 2.66 – 2.60 (m, 3H), 2.59 (s, 3H), 1.56 – 1.49 (m, 3H). 13C NMR (176 MHz, DMSO) δ 169.21, 165.90, 148.79, 136.83, 128.23, 124.86, 123.81, 120.44, 119.09, 109.32, 58.91, 50.44, 40.00, 39.88, 39.76, 39.74, 39.64, 39.62, 39.52, 39.40, 39.28, 21.09, 11.38.

(R,Z)-N-(1-(3-chlorophenyl)ethyl)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxoindoline-5-carboxamide (7f).—To a dried sealed tube were added 242.2 mg (0.769 mmol) of (**3f**), 150.1 mg (0.892 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (6.0 mL). To this solution were added 0.05 ml of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as an orange solid. Yield: orange solid, 46 mg, 16% Molecular Formula: $C_{24}H_{21}ClN_4O_4$ ESI-MS calc: 464.13 ESI-MS found: 465.1318 HPLC: 7.699 1H NMR (700 MHz, DMSO-d6) δ 11.38 (s, 1H), 8.68 (d, J = 7.8 Hz, 1H), 8.36 (d, J = 1.6 Hz, 1H), 7.84 (s, 1H), 7.76 (dd, J = 8.2, 1.7 Hz, 1H), 7.46 (d, J = 2.1 Hz, 1H), 7.37 (d, J = 6.6 Hz, 2H), 7.29 (dt, J = 6.7, 2.3 Hz, 1H), 6.97 (d, J = 8.1 Hz, 1H), 5.17 (p, J = 7.2 Hz, 1H), 2.65 (s, 3H), 2.60 (s, 3H), 1.50 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 165.88, 147.85, 144.31, 138.20, 130.27, 126.64, 126.10, 125.34, 125.04, 123.98, 119.28, 109.31, 102.54, 48.41, 40.00, 39.88, 39.76, 39.64, 39.52, 39.40, 39.28, 22.27, 14.93.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(p-tolyl)ethyl)indoline-5-carboxamide (7g).—To a dried sealed tube were added 253.9 mg (0.862 mmol) of (**3g**), 140.9 mg (0.837 mmol) of 3,5-dimethyl-4-nitro-1H-pyrrole-2-carbaldehyde, all of which were dissolved in abs. EtOH (3.0 mL). To this solution was added 0.05 ml of piperidine and heated to reflux (95 °C) for 4 hours. Once complete, cooled to room temperature and then filtered off the product as an orange solid. Yield: orange solid, 76.1 mg, 25% Molecular Formula: $C_{25}H_{24}N_4O_4$ ESI-MS calc: 444.18 ESI-MS found: 445.1864 [M+1] HPLC: 7.662 1H NMR (700 MHz, DMSO-d6) δ 9.73 (s, 1H), 8.57 (d, J = 8.2 Hz, 2H), 8.35 (d, J = 1.7 Hz, 1H), 7.83 (s, 1H), 7.76 (dd, J = 8.1, 1.7 Hz, 2H), 7.30 (d, J = 7.8 Hz, 3H), 7.14 (d, J = 7.9 Hz, 3H), 6.96 (d, J = 8.0 Hz, 2H), 5.17 (p, J = 7.2 Hz, 1H), 2.55 (d, J = 8.7 Hz, 6H), 2.28 (s, 5H). 13C NMR (176 MHz, DMSO) δ 170.11, 165.77, 155.70, 142.17, 141.51, 137.04, 135.76, 135.76, 128.94, 126.27, 125.36, 119.23, 109.39, 52.91, 40.12, 40.00, 39.88, 39.76, 39.64, 39.52, 39.40, 22.49, 20.86, 15.04, 11.49, 10.19.

(Z)-N-benzyl-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxoindoline-5-carboxamide (7i).—Prepared using the protocol described for **7a**. Yields an orange solid, 150 mg, 55% Molecular Formula: $C_{23}H_{20}N_4O_4$ ESI-MS calc: 416.15 ESI-MS found: 417.2934, HPLC: 7.158, 1H NMR (700 MHz, DMSO-d6) δ 11.44 – 11.06 (m, 1H), 8.84 (t, J = 6.1 Hz, 1H), 8.34 (s, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.69 (s, 1H), 7.34 (d, J = 5.6 Hz, 4H), 7.25 (d, J = 6.6 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 4.52 (d, J = 5.7 Hz, 2H). 13C NMR (176 MHz, DMSO) δ 169.75, 166.10, 141.33, 139.82, 136.69, 133.57, 128.23, 127.89,

127.37, 127.18, 126.67, 124.95, 124.69, 124.44, 123.25, 119.29, 118.57, 109.16, 42.62, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 14.73, 11.11.

(Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxo-N-(2-phenylpropan-2-yl)indoline-5-carboxamide (7j).—Prepared using the protocol described for **7a**. Yields an orange solid, 76.1 mg, 32.1%. Molecular Formula: $C_{25}H_{24}N_4O_4$ ESI-MS calc: 444.18 ESI-MS found:445.2680 [M+1] HPLC: 7.713 1H NMR (700 MHz, DMSO-d6) δ 11.32 (s, 1H), 8.31 (d, J = 1.7 Hz, 1H), 8.25 (s, 1H), 7.80 (s, 1H), 7.72 (dd, J = 8.0, 1.7 Hz, 1H), 7.42 – 7.39 (m, 2H), 7.28 (t, J = 7.8 Hz, 2H), 7.17 (t, J = 7.3 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 2.61 (s, 3H), 2.57 (s, 3H), 1.70 (s, 6H). 13C NMR (176 MHz, DMSO) δ 169.84, 165.70, 148.16, 141.11, 136.71, 133.63, 129.19, 127.83, 127.58, 125.62, 125.05, 124.73, 124.69, 124.29, 123.61, 119.44, 119.02, 108.96, 55.31, 39.86, 39.74, 39.62, 39.50, 39.38, 39.26, 39.14, 29.65, 14.76, 11.21.

(R,Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-N-(2-methyl-1-phenylpropyl)-2-oxoindoline-5-carboxamide (7k).—Prepared using the protocol described for **7a**. Yields an orange solid, 144.6 mg, 64.8% Molecular Formula: $C_{26}H_{26}N_4O_4$ ESI-MS calc: 458.20 ESI-MS found: 459.2109 HPLC: 7.896 1H NMR (700 MHz, DMSO-d6) δ 11.32 (s, 1H), 8.56 (p, J = 9.3, 8.3 Hz, 1H), 8.29 (dq, J = 13.4, 7.5 Hz, 1H), 7.88 – 7.63 (m, 2H), 7.48 – 7.28 (m, 4H), 7.22 (qd, J = 14.5, 9.4, 7.5 Hz, 1H), 6.94 (tt, J = 14.2, 7.5 Hz, 1H), 4.79 – 4.64 (m, 1H), 2.64 – 2.58 (m, 3H), 2.58 (s, 3H), 1.05 (tt, J = 13.4, 8.0 Hz, 3H), 0.75 (dp, J = 25.8, 6.7 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.69, 165.86, 143.12, 141.05, 127.89, 127.29, 127.17, 126.47, 125.00, 124.61, 124.32, 123.62, 119.24, 119.05, 108.85, 59.80, 39.74, 39.62, 39.50, 39.38, 39.26, 39.15, 39.03, 32.40, 19.97, 19.82, 14.64, 11.05.

(Z)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxo-N-(3-phenyloxetan-3-yl)indoline-5-carboxamide (7l).—Prepared using the protocol described for **7a**. Yields an orange solid, 47 mg, 42%. Molecular Formula: $C_{25}H_{22}N_4O_5$ ESI-MS calc: 458.16 ESI-MS found: 459.1656 HPLC: 6.923, 1H NMR (700 MHz, DMSO-d6) δ 11.39 (s, 1H), 9.36 (s, 1H), 8.40 (d, J = 2.0 Hz, 1H), 7.83 (d, J = 2.2 Hz, 1H), 7.81 – 7.77 (m, 1H), 7.59 – 7.55 (m, 2H), 7.40 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.4 Hz, 1H), 6.99 (dd, J = 8.4, 2.0 Hz, 1H), 5.04 (d, J = 6.7 Hz, 2H), 4.81 (d, J = 6.7 Hz, 2H), 2.63 (d, J = 2.0 Hz, 3H), 2.58 (d, J = 2.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.85, 165.50, 142.99, 141.58, 136.84, 133.69, 127.65, 127.44, 126.92, 125.26, 124.89, 124.72, 124.58, 123.78, 119.24, 109.26, 81.88, 58.33, 14.77, 11.15.

(R,Z)-N-(1-(4-chlorophenyl)ethyl)-3-((3,5-dimethyl-4-nitro-1H-pyrrol-2-yl)methylene)-2-oxoindoline-5-carboxamide (7k).—Prepared using the protocol described for **7a**. Yields an orange solid, 213.3 mg, 96%. Molecular Formula: $C_{24}H_{21}ClN_4O_4$ ESI-MS calc: 464.13 ESI-MS found: 465.1318 [M+1] HPLC: 7.795 1H NMR (500 MHz, DMSO-d6) δ 11.34 (s, 1H), 8.65 (d, J = 7.8 Hz, 1H), 8.35 – 8.30 (m, 1H), 7.79 (s, 1H), 7.75 (dd, J = 8.1, 1.7 Hz, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.5 Hz, 3H), 6.94 (d, J = 8.1 Hz, 1H), 5.17 (p, J = 7.2 Hz, 1H), 2.62 (s, 3H), 2.57 (s, 3H), 1.49 (d, J = 7.1 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 169.83, 165.67, 144.05, 141.32, 136.76,

133.66, 131.02, 128.18, 128.10, 128.00, 127.49, 125.11, 124.70, 124.41, 123.63, 119.35, 118.98, 109.10, 48.00, 40.00, 39.83, 39.67, 39.50, 39.34, 39.17, 39.00, 22.07, 14.76, 11.21.

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)-methylene)2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (8a).—To a flask were added 90.7 mg of (**7a**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 226.8 mg (14 equivalents) of Zn powder and 2 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then add EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under pressure to give the desired product as a red solid. Because the free amine is very reactive, it was moved forward without further characterization. Result: orange solid, 66.7 mg, 79.0% Molecular Formula: $C_{24}H_{24}N_4O_2$ ESI-MS calc: 400.19 ESI-MS found: 401.1955 HPLC: 5.177 1H NMR (700 MHz, DMSO-d6) δ 13.45 (s, 1H), 10.88 (s, 1H), 8.56 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 1.6 Hz, 1H), 7.62 (dd, J = 8.1, 1.6 Hz, 1H), 7.46 (s, 1H), 7.41 (d, J = 7.6 Hz, 3H), 7.33 (t, J = 7.6 Hz, 3H), 7.22 (t, J = 7.3 Hz, 1H), 6.89 (d, J = 8.1 Hz, 1H), 5.20 (q, J = 7.4 Hz, 1H), 4.02 (d, J = 14.5 Hz, 2H), 2.26 (s, 3H), 2.17 (s, 3H), 1.51 (d, J = 7.1 Hz, 3H).

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(m-tolyl)ethyl)indoline-5-carboxamide (8b).—To a flask were added 86.4 mg of (**7b**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 210.9 mg (14 equivalents) of Zn powder and 2 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then added EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under pressure to give the desired product as a red solid. *Note: the free amine is very reactive, so it was moved forward without further characterization* Result: red solid, 86.6 mg, 94% Molecular Formula: $C_{25}H_{26}N_4O_2$ ESI-MS calc: 414.21 ESI-MS found: 415.2120 [M+1] HPLC: 5.627

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(4-fluorophenyl)ethyl)-2-oxoindoline-5-carboxamide (8c).—To a flask were added 96.3 mg of (**7c**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 295 mg (14 equivalents) of Zn powder and 2 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then added EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under pressure to give the desired product as a red solid. The free amine is very reactive, so it was moved forward without further characterization. Result: red solid, 96 mg, 100% Molecular Formula: $C_{24}H_{23}FN_4O_2$ ESI-MS calc: 418.18 ESI-MS found: 419.1938 [M+1] HPLC: 5.713

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(pyridin-4-yl)ethyl)indoline-5-carboxamide (8d).—To a flask were added 48.0 mg of (**7d**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 208 mg (14 equivalents) of Zn powder and 2 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then add EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under pressure to give the desired product as a red solid. The free amine is very reactive, so it was moved forward without further characterization. Result: red solid, 41.5 mg, 89% Molecular Formula: $C_{23}H_{23}N_5O_2$ ESI-MS calc: 401.19 ESI-MS found: 402.1920 [M+1] HPLC: 3.928

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(pyridin-2-yl)ethyl)indoline-5-carboxamide (8e).—To a flask were added 73.9 mg of (**7e**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 289 mg (14 equivalents) of Zn powder and 2 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then added EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under pressure to give the desired product as a red solid. The free amine is very reactive, so it was moved forward without further characterization. Result: red solid, 73.8 mg, 100% Molecular Formula: $C_{23}H_{23}N_5O_2$ ESI-MS calc: 401.19 ESI-MS found: 402.1450 [M+1] HPLC: 3.920

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(3-chlorophenyl)ethyl)-2-oxoindoline-5-carboxamide (8f).—To a flask were added 46.0 mg of (**7f**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 170.1 mg (14 equivalents) of Zn powder and 1.4 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then add EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under pressure to give the desired product as a red solid. The free amine is very reactive, so it was moved forward without further characterization. Result: red solid, 40 mg, 95% Molecular Formula: $C_{24}H_{23}ClN_4O_2$ ESI-MS calc: 434.15 ESI-MS found: 435.1569 [M +1] HPLC: 5.556

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(p-tolyl)ethyl)indoline-5-carboxamide (8g).—To a flask were added 76.1 mg of (**7g**) and 5 mL of 2:1 EtOH/EtOAc. To this slurry were added 314.4 mg (14 equivalents) of Zn powder and 2.0 mL (150 equivalents) of AcOH. The turbid orange solution was allowed to stir at 50 °C for 2 hours. Once complete, the reaction was cooled to room temperature and then added EtOAc before basifying with sat. Na_2CO_3. The basified aqueous layer was extracted with EtOAc (3 × 30 mL) and then washed with water and brine (1 × 30 mL), respectively. The organic layer was dried over $MgSO_4$ and then solvent was removed under

pressure to give the desired product as a red solid. The free amine is very reactive, so it was moved forward without further characterization. Result: red solid, 220 mg, 100% Molecular Formula: $C_{25}H_{26}N_4O_2$ ESI-MS calc: 414.21 ESI-MS found: 415.2119 [M+1] HPLC: 5.573

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(4-chlorophenyl)ethyl)-2-oxoindoline-5-carboxamide (8h).—Synthesized using protocol described for **8a**. Yields a red solid, 36.1 mg, 48%. Molecular Formula: $C_{24}H_{23}ClN_4O_2$ ESI-MS calc: 434.15 ESI-MS found: 435.15612 HPLC: 5.398

(Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-benzyl-2-oxoindoline-5-carboxamide (8i).—Synthesized using protocol described for **8a**. Yields a red solid, 210 mg, quantitative yield. Molecular Formula: $C_{23}H_{22}N_4O_2$ ESI-MS calc: 386.17 ESI-MS found: 387.1810 HPLC: 5.129.

(Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(2-phenylpropan-2-yl)indoline-5-carboxamide (8j).—Synthesized using protocol described for **8a**. Yields a red solid, 307 mg, quantitative yield. Molecular Formula: $C_{25}H_{26}N_4O_2$ ESI-MS calc: 414.21 ESI-MS found: 415.2119 [M+1] HPLC: 5.245

(R,Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)-methylene)-N-(2-methyl-1-phenylpropyl)-2-oxoindoline-5-carboxamide (8k).—Synthesized using protocol described for **8a**. Yields a red solid, 220 mg, quantitative yield. Molecular Formula: $C_{26}H_{28}N_4O_2$ ESI-MS calc: 428.22 ESI-MS found: 429.2201 HPLC: 5.497

(Z)-3-((4-amino-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(3-phenyloxetan-3-yl)indoline-5-carboxamide (8l).—Synthesized using protocol described for **8a**. Yields a red solid, 20.6 mg, 47%. Molecular Formula: $C_{25}H_{24}N_4O_3$ ESI-MS calc: 428.18 ESI-MS found: 429.1494 HPLC: 4.710

(R,Z)-3-((4-(but-2-ynamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9a).—To a flask were added 22.3 mg (0.37 mmol) of butynoic acid, 157.5 mg (0.37 mmol) of HATU, cat. DMAP and 50 mg (0.19 mmol) of 8a dissolved in 1 mL of DMF. To this bright red solution was added 0.25 mL (1.3 mmol) of DIPEA, and the solution was allowed to stir at room temperature for 2 hours. Once complete, the reaction mixture was diluted with EtOAc and washed with sat. LiCl and then dried over $MgSO_4$. The crude material was purified by preparatory TLC with 50% acetone/hexanes. The desired band was collected, and the material was rinsed off the silica gel with acetone and then solvent was removed under pressure to give the desired product. Result: yellow solid, 17.5 mg, 20% Molecular Formula: $C_{28}H_{26}N_4O_3$ ESI-MS calc: 466.20 ESI-MS found 467.2071 HPLC: 6.336 ^{1}H NMR (700 MHz, DMSO-d_6) δ 13.53 (s, 1H), 11.07 (s, 1H), 9.78 (s, 1H), 8.86 (d, J = 4.5 Hz, 2H), 8.68 (d, J = 8.8 Hz, 2H), 8.60 (d, J = 7.7 Hz, 2H), 8.21 (s, 1H), 7.69 (d, J = 8.9 Hz, 2H), 7.65 – 7.62 (m, 3H), 7.41 (d, J = 7.9 Hz, 5H), 7.33 (t, J = 7.3 Hz, 7H), 7.23 (d, J = 7.0 Hz, 3H), 6.93 (d, J = 8.1 Hz, 1H), 6.59 (s, 3H), 5.21 – 5.17 (m, 1H), 2.19 (s, 4H), 2.17 (s, 4H), 2.03 (s, 3H), 1.52 (s, 2H). ^{13}C NMR (176 MHz, DMSO) δ 170.28, 167.22, 165.79, 149.50, 148.74, 145.19, 139.73, 135.08, 130.24, 128.98, 128.19,

127.41, 126.55, 125.97, 125.02, 122.58, 120.62, 117.41, 112.97, 55.89, 22.67, 12.09, 9.59, 3.60.

(R,Z)-3-((4-acrylamido-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9b).—To a round bottom flask that contained **8a** (50 mg, 0.12 mmol) were added 0.01 mL (0.12 mmol) of acrylic acid, 58.8 mg (0.12 mmol) of HATU, cat. DMAP and 0.50 mL (0.87 mmol) of TEA. All materials were dissolved in 2 mL of DMF and sonicated to give a homogenous solution. The solution was allowed to stir at 56 °C for 2 hours. Once complete, quenched with sat. LiCl and then extracted with EtOAc (3 × 30 mL). The combined organic layers were then dried over $MgSO_4$ and the material was purified by preparatory TLC (50% Acetone/hexanes) collecting the baseline product, which was washed off the silica gel with acetone. The solvent was removed to give the final product as an orange solid, 13.9 mg, 24%. Molecular Formula: $C_{27}H_{26}N_4O_3$ ESI-MS calc: 454.20 ESI-MS found: 455.1632 [M+1] HPLC: 6.147 ^{1}H NMR (700 MHz, DMSO-d_6) δ 13.53 (s, 1H), 11.07 (s, 1H), 9.41 (s, 1H), 8.60 (d, J = 8.1 Hz, 2H), 8.21 (d, J = 9.5 Hz, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.66 (s, 1H), 7.41 (d, J = 7.8 Hz, 5H), 7.33 (t, J = 7.5 Hz, 5H), 7.22 (t, J = 7.4 Hz, 3H), 6.93 (dd, J = 8.1, 3.6 Hz, 1H), 6.45 (dd, J = 17.1, 10.3 Hz, 1H), 6.23 – 6.19 (m, 1H), 5.72 (dd, J = 10.7, 1.9 Hz, 1H), 5.19 (t, J = 7.3 Hz, 2H), 2.21 (s, 3H), 2.20 (s, 3H), 1.50 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 180.81, 169.69, 169.00, 167.87, 165.86, 153.99, 145.07, 141.22, 140.27, 136.23, 131.78, 129.29, 128.18, 127.56, 126.86, 126.49, 126.09, 124.16, 120.41, 109.40, 108.64, 53.84, 22.28.

(R,Z)-3-((3,5-dimethyl-4-(vinylsulfonamido)-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9c).—Step 1: To a dried flask were added 0.03 mL (0.12 mmol) of vinyl sulfonic acid, 2 drops of DMF and 2 mL of DCM. The solution was cooled to 0 °C and then 0.02 mL (0.14 mmol) of oxalyl chloride was added in one portion. The solution was warmed to room temperature and allowed to stir until complete (1 hour). Once complete, then solvent was removed, and the resultant clear oil was rinsed with DCM (3 × 15 mL) and dried under high pressure until ready for step 2. Step 2: To the flask holding the acid chloride was added 8a (50 mg, 0.12 mmol) dissolved in 3 mL of THF. To this murky orange solution was added 0.50 mL (7 equivalents) of TEA, and the solution was allowed to stir at room temperature for 5 hours. Once complete, removed solvent under pressure and brought yellow residue back up in EtOAc. The organic layer was washed with sat. Na_2CO_3 and then brine (1 × 30 mL) respectively. Purified by preparatory TLC plate (40% acetone/hexanes), collecting secondary spot (Rf = 0.2–0.3). The material was rinsed off silica with acetone and the solvent was removed to give a dark orange solid as the desired product. Result: orange solid,16 mg, 27% Molecular Formula: $C_{26}H_{26}N_4O_4S$ ESI-MS calc: 490.17 ESI-MS found: 491.1249 HPLC: 6.43 ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.50 (s, 1H), 11.38 (s, 1H), 11.09 (s, 1H), 8.98 (s, 1H), 8.64 – 8.57 (m, 2H), 8.36 (s, 1H), 8.20 (s, 1H), 7.84 (s, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.63 (s, 1H), 7.41 (d, J = 7.8 Hz, 5H), 7.33 (t, J = 7.6 Hz, 5H), 7.21 (d, J = 7.3 Hz, 2H), 6.98 (s, 1H), 6.93 (d, J = 8.1 Hz, 1H), 6.84 (dd, J = 16.5, 9.8 Hz, 1H), 5.93 (d, J = 8.0 Hz, 1H), 5.19 (t, J = 7.4 Hz, 2H), 2.29 (s, 4H), 2.26 (s, 3H), 1.50 (d, J = 7.1 Hz, 6H). 13C NMR (176 MHz, DMSO) δ 169.90, 165.56, 145.07, 141.33, 136.84, 133.74, 128.32, 128.19, 127.65, 126.53, 126.15,

125.20, 124.79, 124.46, 123.83, 119.49, 119.12, 109.21, 48.52, 45.44, 39.88, 39.76, 39.64, 39.52, 39.40, 39.28, 39.16, 22.33, 14.83, 11.29, 8.63.

(R,Z)-3-((4-(2-cyanoacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9d).—To a round bottom flask were added 20.2 mg of **8a** (0.05 mmol), 6.7 mg (0.075 mmol) of cyanoacetic acid and 24.6 mg (0.1 mmol) of DMTMM. All materials were brought up in 2 mL of DMF, and the resulting solution was allowed to stir at room temperature for 12 hours. Once complete, the reaction was quenched with sat. NaCl, and extracting with EtOAc (3 × 50 mL). The organic layer was washed with sat. Na_2CO_3 and brine (1 × 50 mL) respectively and then dried over $MgSO_4$. The solvent was removed under pressure, evaporating the material onto silica gel. Purified by column chromatography 5–100% Acetone/hexanes. Flushed the column with 7 N NH_3 in MeOH to yield the final product as a bright orange solid. Result: orange solid, 10 mg, 43% Molecular Formula: $C_{27}H_{25}N_5O_3$ ESI-MS calc: 467.20 ESI-MS found: 468.1297 HPLC: 6.100. 1H NMR (700 MHz, DMSO-d6) δ 13.51 (s, 1H), 11.08 (s, 1H), 9.52 (s, 1H), 8.60 (d, J = 8.2 Hz, 1H), 8.21 (d, J = 1.7 Hz, 1H), 7.69 (dd, J = 8.1, 1.7 Hz, 1H), 7.65 (s, 1H), 7.41 (d, J = 7.7 Hz, 2H), 7.33 (t, J = 7.6 Hz, 3H), 7.22 (t, J = 7.3 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 5.19 (p, J = 7.2 Hz, 1H), 3.88 (s, 2H), 2.20 (dd, J = 12.9, 10.8 Hz, 6H), 1.50 (d, J = 7.1 Hz, H).

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9e).—To a dried round bottom flask was added **8a** (60 mg, 0.15 mmol) dissolved in 3 mL of THF. The yellow solution was cooled to 0 °C and 0.01 mL (0.18 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, quenched with water and extracted with EtOAc. The solvent was removed, and the crude material was purified by prep plate (50% acetone/hexanes) collecting the major product. The desired product was rinsed off of silica gel and then the solvent was removed to give a bright yellow solid. The yellow solid was washed with DCM and sonicated to give a red solid. The red solid was then pulped in water:acetone (30:1) to give the final product. Result: red solid, 25.1 mg, 35% Molecular Formula: $C_{26}H_{25}ClN_4O_3$ ESI-MS calc: 476.16 ESI-MS found: 477.0999 HPLC: 6.388 ^{1}H NMR (700 MHz, DMSO-d_6) δ 11.08 (s, 1H), 9.53 (s, 1H), 8.60 (d, J = 8.0 Hz, 1H), 8.22 (s, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.65 (s, 1H), 7.41 (d, J = 7.8 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.22 (t, J = 7.4 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 5.19 (t, J = 7.5 Hz, −1H), 2.20 (d, J = 12.1 Hz, 7H), 2.08 (s, 3H), 1.50 (d, J = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.67, 168.51, 156.78, 145.12, 140.30, 131.64, 129.98, 128.13, 127.50, 126.89, 126.44, 126.11, 124.45, 122.62, 121.01, 117.42, 116.93, 112.94, 48.39, 42.71, 22.29, 11.67, 9.24.

(Z)-3-((4-((E)-4-(dimethylamino)but-2-enamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-((R)-1-phenylethyl)indoline-5-carboxamide (9f).—To a round bottom flask were added **8a** (50 mg, 0.12 mmol), 20.2 mg (0.12 mmol) of N,N-dimethylaminobutenoic acid and 36 mg (0.12 mmol) of DMTMM and 0.20 mL (0.24 mmol) of TEA. All materials were brought up in 2 mL of DMF, and the resulting solution was allowed to stir at room temperature for 12 hours. Once complete, the reaction was quenched with Sat. NaCl, and extracting with EtOAc (3 × 50 mL). The organic layer was washed with

sat. Na_2CO_3 and brine (1 × 50 mL) respectively and then dried over $MgSO_4$. The solvent was removed under pressure, evaporating the material onto silica gel. Purified by column chromatography 5–100% acetone/hexanes. Flushed the column with 7 N NH_3 in MeOH to yield the final product as a bright yellow oil. Result: yellow oil, 13 mg, 20% Molecular Formula: $C_{30}H_{33}N_5O_3$ ESI-MS calc: 511.26 ESI-MS found: 512.2110 [M+1], 534.1905 [M +Na] HPLC: 5.424 ^{1}H NMR (700 MHz, DMSOd_6) δ 13.56 (s, 1H), 11.06 (s, 2H), 9.29 (s, 2H), 8.59 (d, *J* = 7.9 Hz, 2H), 8.21 (d, *J* = 1.5 Hz, 2H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.65 (s, 2H), 7.41 (d, *J* = 7.6 Hz, 4H), 7.33 (t, *J* = 7.6 Hz, 4H), 7.22 (t, *J* = 7.5 Hz, 2H), 6.93 (d, *J* = 8.1 Hz, 2H), 6.71 – 6.64 (m, 1H), 6.27 (d, *J* = 15.5 Hz, 1H), 5.21 – 5.17 (m, 1H), 3.05 (d, *J* = 5.6 Hz, 2H), 2.21 (s, 6H), 2.19 (s, 3H), 2.18 (s, 3H), 1.50 (d, *J* = 7.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 170.09, 166.24, 163.98, 145.47, 141.00, 140.66, 132.25, 128.68, 128.55, 127.91, 127.30, 126.87, 126.48, 126.08, 125.81, 125.74, 125.61, 124.86, 124.52, 122.19, 117.61, 112.99, 109.03, 62.41, 48.96, 25.82, 22.64, 14.44, 12.32, 9.77.

(R,Z)-3-((4-(2-bromoacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9g).—To a round bottom flask were added 83.7 mg of **8a** (0.21 mmol), 60.1 mg (0.41 mmol) of bromoacetic acid and 100 mg (0.25 mmol) of DMTMM. All materials were brought up in 2 mL of DMF, and the resulting solution was allowed to stir at room temperature for 1.5 hours. Once complete, the reaction was quenched with brine and then extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and then solvent was removed under pressure to give a dark orange solid. The solid was then dissolved in DCM, and the resultant solution was sonicated to give a dark red precipitate. The solid was filtered off and rinsed with excess cold DCM to give the final product. Result: red solid, 42.9 mg, 39%. Molecular Formula: $C_{26}H_{25}BrN_4O_3$ ESI-MS calc: 520.11 ESI-MS found: 523.1164 HPLC: 6.397 1H NMR (700 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.08 (s, 1H), 9.60 (s, 1H), 8.60 (d, J = 7.9 Hz, 1H), 8.21 (d, J = 1.6 Hz, 1H), 7.69 (dd, J = 8.1, 1.7 Hz, 1H), 7.65 (s, 1H), 7.41 (d, J = 7.7 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.22 (t, J = 7.3 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 5.20 (p, J = 7.2 Hz, 1H), 4.03 (s, 2H), 2.20 (d, J = 10.4 Hz, 6H), 1.51 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 169.68, 165.82, 165.54, 145.05, 140.30, 131.64, 128.15, 127.59, 126.78, 126.47, 126.07, 125.76, 125.33, 124.44, 124.13, 121.01, 117.35, 112.95, 108.62, 48.34, 29.33, 22.26, 11.61, 9.14.

(Z)-3-((4-(3-chloro-2-hydroxypropanamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-((R)-1-phenylethyl)indoline-5-carboxamide (9h).—To a round bottom flask were added 80 mg of **8a** (0.20 mmol), 31.1 mg (0.26 mmol) of 3-chloro-2hydroxypropionic acid and 81.8 mg (0.32 mmol) of DMTMM. All materials were brought up in 2 mL of DMF, and the resulting solution was allowed to stir at room temperature for 12 hours. Once complete, the reaction was quenched with sat. NaCl, and extracting with EtOAc (3 × 50 mL). The organic layer was washed with sat. Na_2CO_3 and brine (1 × 50 mL) respectively and then dried over $MgSO_4$. The solvent was removed under pressure, evaporating the material onto silica gel. Purified by column chromatography 5–100% acetone/hexanes. Result: orange solid, 72.8 mg, 58% Molecular Formula: $C_{27}H_{27}ClN_4O_4$ ESI-MS calc: 506.17 ESI-MS found: 507.1809 [M+1], 540.2377 [M+H +MeOH] HPLC: 6.043 1H NMR (700 MHz, DMSO-d6) δ 13.58 – 13.42 (m, 1H), 11.07 (d, J = 4.9 Hz, 1H), 8.60 (d, J = 8.1 Hz, 1H), 8.23 – 8.19 (m, 1H), 7.95 (s, 1H), 7.69 – 7.67 (m,

1H), 7.64 (d, J = 5.9 Hz, 1H), 7.41 (d, J = 7.2 Hz, 2H), 7.33 (t, J = 7.1 Hz, 3H), 7.22 (t, J = 7.2 Hz, 1H), 6.93 (dt, J = 8.2, 2.1 Hz, 1H), 5.19 (p, J = 7.3 Hz, 1H), 3.96 (d, J = 7.9 Hz, 1H), 3.74 (s, 1H), 2.21 – 2.18 (m, 4H), 2.16 (s, 2H), 1.50 (d, J = 7.0 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 172.44, 166.31, 162.74, 145.53, 128.63, 127.95, 126.94, 126.54, 125.88, 124.64, 121.80, 117.71, 115.92, 109.07, 107.36, 55.41, 48.82, 22.73, 21.51, 9.74.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(m-tolyl)ethyl)indoline-5-carboxamide (9i).—To a dried round bottom flask was added 86.6 mg of **8b**, (0.209 mmol) dissolved in 3 mL of THF. The yellow solution was cooled to 0 °C and 0.04 mL (0.30 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, quenched with water and extracted with EtOAc. The solvent was removed, and the crude material was purified by prep plate (50% acetone/hexanes) collecting the major product. The desired product was rinsed off of silica gel and then the solvent was removed to give a bright yellow solid. The yellow solid was washed with DCM and sonicated to give a red solid. The red solid was then pulped in water:acetone (30:1) to give the final product. Result: red solid, 57.2 mg, 55% Molecular Formula: $C_{27}H_{27}ClN_4O_3$ ESI-MS calc: 490.18 ESI-MS found: 491.1133 HPLC: 6.68 1H NMR (500 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.07 (s, 1H), 9.53 (s, 1H), 8.56 (d, J = 8.1 Hz, 1H), 8.21 (s, 1H), 7.74 – 7.62 (m, 2H), 7.24 – 7.15 (m, 4H), 7.03 (s, 1H), 6.93 (d, J = 8.0 Hz, 1H), 5.15 (s, 1H), 4.26 (s, 2H), 2.30 (s, 3H), 2.20 (d, J = 8.9 Hz, 6H), 1.49 (d, J = 7.0 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 170.18, 169.06, 166.24, 165.84, 145.51, 140.80, 137.63, 132.18, 128.59, 128.10, 127.62, 127.24, 126.26, 125.83, 124.93, 123.68, 121.47, 117.93, 113.45, 109.13, 48.80, 43.22, 22.79, 21.62, 12.15, 9.70.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(4-fluorophenyl)ethyl)-2-oxoindoline-5carboxamide (9j).—To a dried round bottom flask was added 96.0 mg of **8c**, (0.23 mmol) dissolved in 4 mL of THF. The yellow solution was cooled to 0 °C and 0.02 mL (0.25 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, quenched with water and extracted with EtOAc. The solvent was removed, and the crude material was purified by prep plate (50% acetone/hexanes) collecting the major product. The desired product was rinsed off of silica gel and then the solvent was removed to give a bright yellow solid. The yellow solid was washed with DCM and sonicated to give a red solid. The red solid was then pulped in water:acetone (30:1) to give the final product. Result: red solid, 46.5 mg, 41% Molecular Formula: $C_{26}H_{24}FClN_4O_3$ ESI-MS calc: 494.15 ESI-MS found: 495.0480 [M+1] HPLC: 6.439 1H NMR (700 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.08 (s, 1H), 9.54 (s, 1H), 8.63 (d, J = 7.9 Hz, 1H), 8.23 (d, J = 1.7 Hz, 1H), 7.68 (dd, J = 8.1, 1.8 Hz, 1H), 7.66 (s, 1H), 7.46 – 7.43 (m, 2H), 7.15 (tt, J = 9.9, 3.2 Hz, 3H), 6.93 (d, J = 8.2 Hz, 1H), 5.19 (p, J = 7.2 Hz, 1H), 4.28 – 4.26 (m, 4H), 2.20 (d, J = 10.1 Hz, 6H), 1.50 (d, J = 6.9 Hz, 4H). 13C NMR (176 MHz, DMSO) δ 170.20, 169.07, 166.36, 165.86, 162.12, 160.75, 141.78, 141.76, 140.85, 132.22, 128.54, 128.49, 128.41, 128.02, 127.42, 126.27, 125.87, 124.96, 124.70, 121.50, 117.90, 115.38, 115.26, 113.44, 109.15, 48.33, 43.23, 41.97, 40.37, 40.25, 40.13, 40.01, 39.89, 39.77, 39.65, 36.07, 29.51, 22.78, 12.16, 9.72.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(pyridin-4-yl)ethyl)indoline-5-carboxamide (9k).—To a dried round bottom flask was added 41.5 mg of 8d, (0.103 mmol) dissolved in 2 mL of THF. The yellow solution was cooled to 0 °C and 0.01 mL (0.103 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, quenched with water and extracted with EtOAc. The solvent was removed, and the crude material was purified by prep plate (50% acetone/hexanes) collecting the major product as the desired product. Result: red solid, 6.3 mg, 13% Molecular Formula: $C_{25}H_{24}ClN_5O_3$ ESI-MS calc: 477.16 ESI-MS found: 478.1734 [M+H] HPLC: 4.363 1H NMR (700 MHz, DMSO-d6) δ 13.51 (s, 1H), 11.15 (s, 1H), 10.56 (s, 1H), 9.86 (s, 1H), 8.73 (s, 1H), 8.48 (s, 2H), 7.75 (s, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.34 (d, J = 7.6 Hz, 2H), 7.27 (s, 1H), 6.96 (d, J = 7.9 Hz, 1H), 5.33 (t, J = 7.0 Hz, 1H), 4.27 (s, 2H), 2.21 (s, 6H), 1.50 (d, J = 7.8 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 168.53, 166.49, 165.34, 154.01, 141.60, 140.65, 130.38, 129.30, 129.05, 127.97, 124.37, 123.04, 122.68, 121.09, 119.53, 118.36, 108.73, 54.90, 42.72, 21.66, 10.38, 8.77.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(pyridin-2-yl)ethyl)indoline-5-carboxamide (9l).—To a dried round bottom flask was added 73.8 mg of **8e**, (0.18 mmol) dissolved in 4 mL of THF. The yellow solution was cooled to 0 °C and 0.04 mL (0.50 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, removed solvent under pressure and then added DCM to the dark red residue. The DCM solution was sonicated to give a dark red precipitate. The solid was filtered off and rinsed with THF and DCM to give the desired product as a dark red solid. Result: red solid, 37.1 mg, 42% Molecular Formula: $C_{25}H_{24}ClN_5O_3$ ESI-MS calc: 477.16 ESI-MS found: 478.1547 [M+1] HPLC: 4.743 1H NMR (700 MHz, DMSO-d6) δ 13.28 (t, J = 13.6 Hz, 1H), 11.02 (q, J = 7.5, 5.9 Hz, 1H), 9.86 (s, 1H), 8.98 (d, J = 52.4 Hz, 1H), 8.57 (d, J = 9.4 Hz, 1H), 8.42 (d, J = 9.4 Hz, 1H), 8.26 (q, J = 9.9, 9.4 Hz, 2H), 7.86 (d, J = 11.1 Hz, 1H), 7.64 (s, 2H), 7.58 (d, J = 9.2 Hz, 1H), 7.50 (dt, J = 27.4, 8.6 Hz, 1H), 6.72 (p, J = 8.1 Hz, 1H), 5.23 – 5.16 (m, 1H), 4.03 (t, J = 8.6 Hz, 1H), 2.92 (d, J = 16.9 Hz, 14H), 2.26 (d, J = 10.2 Hz, 7H), 2.17 (dt, J = 18.1, 8.8 Hz, 4H), 1.97 (q, J = 8.9 Hz, 2H), 1.48 – 1.40 (m, 3H). 13C NMR (176 MHz, DMSO) δ 169.75, 166.56, 165.36, 159.41, 145.67, 142.08, 141.13, 140.72, 131.91, 128.62, 127.17, 126.78, 126.44, 125.11, 124.85, 124.26, 123.99, 121.10, 118.54, 52.68, 42.73, 20.48, 11.23, 8.81.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(3-chlorophenyl)ethyl)-2-oxoindoline-5-carboxamide (9m).—To a dried round bottom flask was added 40 mg of **8f**, (0.16 mmol) dissolved in 4 mL of THF. The yellow solution was cooled to 0 °C and 0.01 mL (0.18 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, removed solvent under pressure and then added DCM to the dark red residue. The DCM solution was sonicated to give a dark red precipitate. The solid was filtered off and rinsed with THF and DCM to give the desired product as a dark red solid. Result: red solid, 23.4 mg, 28% Molecular Formula: $C_{26}H_{24}Cl_2N_4O_3$ ESI-MS calc: 510.12 ESI-MS found: 511.1579 [M+1] HPLC: 6.742 1H NMR (500 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.09 (s, 1H), 9.53 (s, 1H),

8.66 (d, J = 7.9 Hz, 1H), 8.21 (d, J = 1.7 Hz, 1H), 7.68 (dd, J = 8.2, 1.7 Hz, 1H), 7.66 (s, 1H), 7.47 – 7.45 (m, 1H), 7.38 – 7.36 (m, 2H), 7.29 (dq, J = 6.2, 2.2 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 5.17 (p, J = 7.2 Hz, 1H), 4.26 (s, 2H), 2.20 (d, J = 8.6 Hz, 6H), 1.50 (d, J = 7.1 Hz, 4H). 13C NMR (126 MHz, DMSO) δ 169.51, 165.17, 147.64, 140.23, 132.71, 131.57, 129.96, 127.20, 126.77, 126.30, 125.79, 125.55, 125.23, 124.73, 124.28, 120.82, 117.27, 112.71, 48.03, 42.55, 39.83, 39.67, 39.50, 39.33, 39.17, 39.00, 38.83, 21.96, 11.48, 9.02.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(1-(p-tolyl)ethyl)indoline-5-carboxamide (9n).—To a dried round bottom flask was added 93 mg of **8g**, (0.19 mmol) dissolved in 5 mL of THF. The yellow solution was cooled to 0 °C and 0.1 mL (1.3 mmol) of chloroacetylchloride was added dropwise. The solution was allowed to stir at 0 °C for 30 minutes. Once complete by TLC, removed solvent under pressure and then added DCM to the dark red residue. The DCM solution was sonicated to give a dark red precipitate. The solid was filtered off and rinsed with THF and DCM to give the desired product as a dark red solid. Result: red solid, 73.6 mg, 77% Molecular Formula: $C_{27}H_{27}ClN_4O_3$ ESI-MS calc: 490.18 ESI-MS found 473.05 [M-Cl] HPLC: 6.625 1H NMR (500 MHz, DMSO-d6) δ 13.51 (s, 1H), 11.06 (s, 1H), 9.52 (s, 1H), 8.53 (d, J = 8.0 Hz, 1H), 8.20 (s, 1H), 7.71 – 7.65 (m, 1H), 7.64 (s, 1H), 7.27 (t, J = 10.7 Hz, 4H), 7.12 (d, J = 7.8 Hz, 6H), 6.92 (d, J = 8.1 Hz, 1H), 5.18 – 5.12 (m, 2H), 2.26 (d, J = 3.9 Hz, 8H), 1.48 (d, J = 7.1 Hz, 5H).

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(4-chlorophenyl)ethyl)-2-oxoindoline-5carboxamide (9o).—Synthesized using the protocol described in **8h**. Yields a brown solid, 43.5 mg, 100%. Molecular Formula: $C_{26}H_{24}Cl_2N_4O_3$ ESI-MS calc: 510.12 ESI-MS found: 511.12917 [M+1] HPLC: 6.723. 1H NMR (499 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.08 (d, J = 7.8 Hz, 1H), 9.53 (s, 1H), 8.64 (dd, J = 7.9, 5.3 Hz, 2H), 8.20 (d, J = 1.5 Hz, 1H), 7.67 (dt, J = 8.0, 2.1 Hz, 2H), 7.65 (s, 1H), 7.44 – 7.40 (m, 4H), 7.40 – 7.37 (m, 5H), 6.93 (d, J = 8.1 Hz, 1H), 5.16 (dq, J = 13.7, 7.0 Hz, 1H), 4.26 (d, J = 2.8 Hz, 2H), 2.20 (d, J = 8.4 Hz, 6H), 1.49 (d, J = 7.1 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 170.31, 169.68, 165.92, 165.35, 144.15, 140.36, 131.73, 130.99, 128.11, 128.01, 127.44, 126.92, 126.09, 125.76, 125.35, 124.44, 123.36, 122.09, 121.43, 120.99, 120.43, 117.37, 108.64, 55.81, 42.72, 22.10, 11.65, 9.21.

(R,Z)-3-((4-(2-bromoacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(1-(4-fluorophenyl)ethyl)-2-oxoindoline-5-carboxamide (9p).—This material was prepared using the protocol described for **8c**. Yields a bright orange solid, 13.7 mg, 23%. HRMS: 541.1106 [M+1, Br^{81}], HPLC: 6.449. 1H NMR (700 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.08 (s, 1H), 9.60 (s, 1H), 8.61 (d, J = 8.1 Hz, 1H), 8.20 (s, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.65 (s, 1H), 7.44 (t, J = 4.8 Hz, 2H), 7.17 – 7.13 (m, 2H), 6.93 (dd, J = 8.2, 2.5 Hz, 1H), 5.19 (q, J = 7.4 Hz, 1H), 4.26 (d, J = 2.4 Hz, 1H), 4.03 (d, J = 2.4 Hz, 1H), 2.22 – 2.18 (m, 6H), 1.51 – 1.48 (m, 3H). 13C NMR (176 MHz, DMSO) δ 170.07, 167.64, 166.24, 162.00, 155.00, 144.70, 140.72, 132.05, 129.71, 128.41, 127.92, 124.56, 122.76, 121.42, 117.77, 115.96, 115.26, 109.01, 48.19, 29.73, 22.66, 12.01, 9.54.

(Z)-N-benzyl-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxoindoline-5-carboxamide (9q).—Synthesized using protocol described for **9i.** Yields an orange solid, 18.2 mg, 21%. Molecular Formula: $C_{25}H_{23}ClN_4O_3$ ESI-MS calc: 462.15 ESI-MS found: 463.1529 [M+1] HPLC: 6.177. 1H NMR (700 MHz, DMSO-d6) δ 13.49 (s, 1H), 11.08 (s, 1H), 9.52 (s, 1H), 8.85 (dt, J = 12.4, 6.1 Hz, 1H), 8.26 (d, J = 1.7 Hz, 1H), 7.78 (d, J = 9.4 Hz, 1H), 7.71 (dd, J = 8.2, 1.7 Hz, 1H), 7.64 (s, 1H), 7.35 – 7.32 (m, 4H), 7.24 (tt, J = 6.2, 3.0 Hz, 2H), 6.94 (d, J = 8.1 Hz, 1H), 4.51 (d, J = 6.0 Hz, 2H), 4.26 (s, 2H), 2.21 (s, 3H), 2.18 (s, 3H). 13C NMR (176 MHz, DMSO) δ 170.18, 166.91, 165.85, 140.89, 140.42, 132.22, 128.74, 128.72, 128.70, 127.98, 127.78, 127.68, 127.67, 127.62, 127.38, 127.15, 126.21, 125.93, 124.95, 124.54, 123.95, 121.48, 117.63, 113.41, 109.28, 68.98, 56.32, 43.22, 43.09, 43.03, 40.37, 40.25, 40.13, 40.01, 39.89, 39.77, 39.65, 36.08, 32.59, 30.08, 12.14, 11.67, 9.92, 9.65.

(Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(2-phenylpropan-2-yl)indoline-5-carboxamide (9r).—Synthesized using the protocol described for **9i**. Yields a brown solid, 14.2 mg, 15%. Molecular Formula: $C_{27}H_{27}ClN_4O_3$ ESI-MS calc: 490.18 ESI-MS found: 491.1832 [M+1], 513.1656 [M+Na] HPLC: 6.63. 1H NMR (500 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.06 (s, 1H), 9.53 (s, 1H), 8.24 (s, 1H), 8.19 (d, J = 1.8 Hz, 1H), 7.68 (s, 1H), 7.64 (dd, J = 8.1, 1.7 Hz, 1H), 7.40 (dd, J = 7.9, 1.8 Hz, 3H), 7.28 (t, J = 7.8 Hz, 2H), 7.16 (dd, J = 8.3, 6.3 Hz, 1H), 6.91 (d, J = 8.1 Hz, 1H), 4.26 (s, 2H), 2.20 (d, J = 8.2 Hz, 6H), 1.69 (s, 6H). 13C NMR (126 MHz, DMSO) δ 170.12, 166.37, 165.75, 148.67, 143.56, 140.59, 132.03, 128.86, 128.24, 127.26, 126.00, 125.65, 125.11, 124.86, 124.66, 121.37, 117.90, 108.94, 55.69, 43.14, 30.13, 12.07, 9.61.

(R,Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(2-methyl-1-phenylpropyl)-2-oxoindoline-5-carboxamide (9s).—Synthesized using the protocol described for **9i**. Yields an orange solid, 5.5 mg, 5.8%. Molecular Formula: $C_{28}H_{29}ClN_4O_3$ ESI-MS calc: 504.19 ESI-MS found: 505.1997 HPLC: 6.803 1H NMR (700 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.06 (s, 1H), 9.52 (s, 1H), 8.53 (d, J = 8.9 Hz, 1H), 8.14 (d, J = 1.6 Hz, 1H), 7.67 – 7.62 (m, 2H), 7.43 – 7.40 (m, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.22 (t, J = 7.3 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 4.69 (t, J = 9.2 Hz, 1H), 4.26 (s, 2H), 2.20 (d, J = 10.3 Hz, 6H), 1.04 (d, J = 6.6 Hz, 4H), 0.73 (d, J = 6.7 Hz, 4H). 13C NMR (126 MHz, DMSO) δ 170.14, 166.69, 165.80, 151.43, 143.78, 140.68, 132.14, 128.47, 128.45, 127.89, 127.78, 127.38, 127.05, 125.84, 124.90, 124.74, 121.43, 118.01, 108.97, 60.35, 43.19, 32.97, 20.45, 12.12, 9.62.

(Z)-3-((4-(2-chloroacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-(3-phenyloxetan-3-yl)indoline-5-carboxamide (9t).—Synthesized using the protocol described for **9i**. Yields a red solid, 5.0 mg, 21%. Molecular Formula: $C_{27}H_{25}ClN_4O_4$ ESI-MS calc: 504.16 ESI-MS found: 505.1619 HPLC: 5.332 1H NMR (700 MHz, DMSO-d6) δ 13.52 (s, 1H), 11.28 (s, 1H), 9.54 (d, J = 13.2 Hz, 1H), 8.43 (s, 1H), 7.84 (s, 2H), 7.77 (d, J = 13.4 Hz, 2H), 7.52 (t, J = 9.1 Hz, 4H), 7.39 (dt, J = 14.7, 7.8 Hz, 5H), 7.31 (dd, J = 16.7, 7.7 Hz, 3H), 7.19 (s, 1H), 7.11 (s, 1H), 7.04 (s, 2H), 4.85 (d, J = 9.0 Hz, 2H), 4.45 (t, J = 9.9 Hz, 2H), 4.27 (d, J = 3.1 Hz, 2H), 2.22 (d, J = 4.8 Hz, 6H). 13C NMR (126 MHz, DMSO) δ 170.16, 167.57, 165.90, 154.45, 143.80, 130.21, 129.17, 129.09,

129.04, 128.64, 126.55, 126.07, 125.78, 125.47, 125.31, 122.08, 121.39, 120.35, 115.60, 110.34, 78.59, 72.40, 43.21, 11.78, 9.23.

(Z)-3-((4-((E)-2-cyano-4,4-dimethylpent-2-enamido)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxo-N-((R)-1-phenylethyl)indoline-5-carboxamide (10a).—To a sealed tube were added 30 mg of **9g** (0.064 mmol), 0.05 mL of pivaldehyde (0.45 mmol), and 0.10 mL of piperidine (1.0 mmol). All materials were then dissolved in 3 mL of abs. EtOH. The solution was then heated to 75 °C for 12 hours. Once complete, the reaction was cooled to room temperature and then solvent was removed under pressure, evaporating the crude material onto silica gel. The material was purified by column chromatography (50% acetone/hexanes) to give the desired product. Yields a yellow solid, 14 mg, 41%. MS: 536.1375 [M+1], 558 [M+Na] HPLC: 7.69 1H NMR (700 MHz, DMSO-d6) δ 13.54 (s, 1H), 11.09 (s, 1H), 9.57 (d, J = 11.5 Hz, 1H), 8.60 (s, 2H), 8.22 (s, 1H), 7.95 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 9.8 Hz, 1H), 7.41 (d, J = 7.7 Hz, 3H), 7.33 (t, J = 7.5 Hz, 3H), 7.23 (d, J = 7.9 Hz, 2H), 6.93 (d, J = 8.4 Hz, 1H), 5.19 (s, 1H), 2.22 – 2.17 (m, 6H), 1.50 (d, J = 7.2 Hz, 4H), 1.15 (d, J = 9.5 Hz, 9H), 1.04 (d, J = 6.1 Hz, 3H). 13C NMR (176 MHz, DMSO) δ 170.08, 167.90, 166.20, 160.95, 153.74, 145.51, 141.02, 140.65, 135.87, 132.53, 128.55, 128.02, 126.86, 126.46, 124.51, 123.07, 120.43, 117.75, 115.67, 98.56, 56.19, 28.98, 25.24, 22.64, 12.00, 9.70.

(Z)-3-((3,5-dimethyl-4-(oxirane-2-carboxamido)-1H-pyrrol-2-yl)methylene)-2-oxo-N-((R)-1-phenylethyl)indoline-5-carboxamide (10b).—To a flask were added 36.4 mg of **9h**, and 47.2 mg of K_2CO_3. All materials were brought up in 3.0 mL of acetone and 1 mL of MeCN and then the reaction was refluxed (60 °C) for 30 minutes. After 30 minutes, the reaction was heated to 70 °C for 1 h, with cat. KI to push the reaction to completion. Once complete, the reaction was quenched with water (1 × 30 mL) and then extracted with EtOAc (2 × 25 mL). The combined organic layers were dried over Na_2SO_4 and then purified by column chromatography (25–100% acetone/hexanes). The desired fractions were collected, and the solvent was removed *in vacuo* giving the product as a yellow solid. Result: yellow solid, 3.0 mg, 6% Molecular Formula: $C_{27}H_{26}ClN_4O_4$ ESI-MS calc: 470.20 ESI-MS found: 471.2158 [M+1] HPLC: 6.591 1H NMR (700 MHz, acetone-d6) δ 13.62 (s, 1H), 10.01 (s, 1H), 8.24 – 8.22 (m, 2H), 7.83 (d, J = 8.3 Hz, 1H), 7.75 (dt, J = 8.1, 2.2 Hz, 1H), 7.69 (s, 1H), 7.46 (d, J = 8.0 Hz, 3H), 7.33 (t, J = 7.7 Hz, 3H), 7.23 (t, J = 7.3 Hz, 1H), 6.99 (dd, J = 8.3, 5.5 Hz, 1H), 5.33 (q, J = 7.3 Hz, 2H), 3.91 (s, 2H), 3.79 (s, 2H), 2.30 (s, 2H), 2.25 (d, J = 2.7 Hz, 3H), 2.19 (d, J = 5.5 Hz, 2H), 2.14 (d, J = 5.1 Hz, 3H), 1.56 (dd, J = 7.1, 1.3 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 171.84, 169.70, 167.77, 152.56, 145.07, 140.26, 132.32, 128.18, 127.58, 127.45, 126.50, 126.09, 124.43, 121.76, 120.08, 118.18, 108.61, 54.27, 54.08, 48.37, 22.28, 11.87, 9.44.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AST	active site tether
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
P-loop	phosphate-binding loop
MS	mass spectrometry
RTK	receptor tyrosine kinase
SAR	structure-activity relationship

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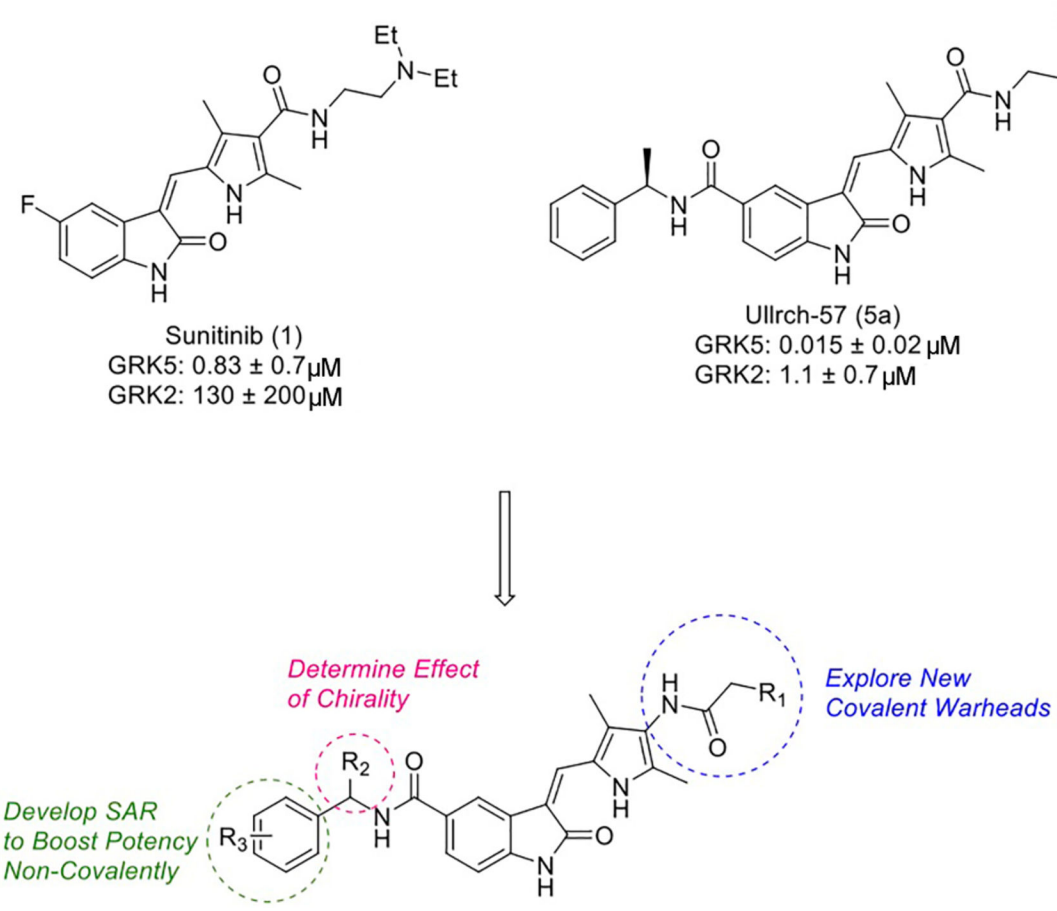


Figure 1.
Lead compounds **1** and **5a** with design strategy.

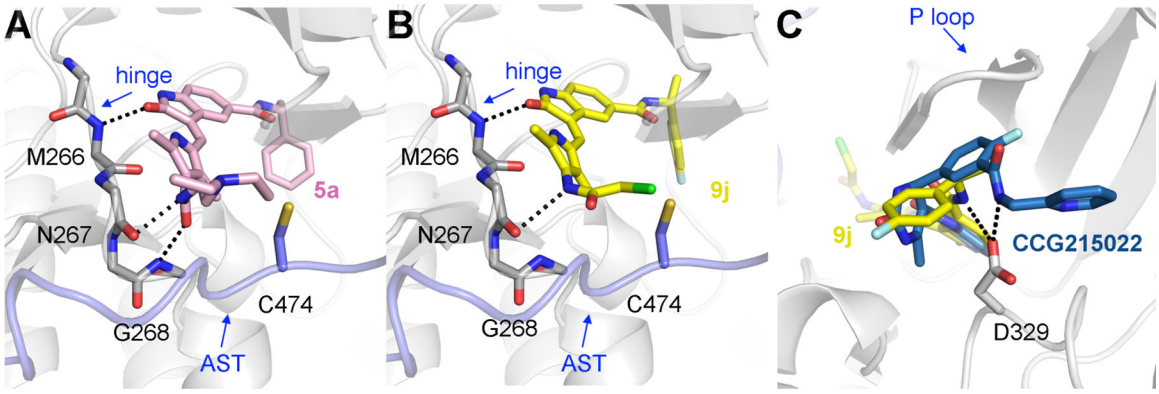


Figure 2.

Docking models of lead compound **5a** and most potent derivative **9j**, and their comparison with the GRK5·CCG215022 complex. (A) **5a**, with pink carbons, red oxygens, and blue nitrogens docked using the program MOE into the structure of GRK5 (grey, PDB ID 4WNK), with the addition of the AST loop from GRK6 (purple, PDB ID 3NYN) because this element was disordered in the 4WNK structure and contains the target Cys474 residue. The diamine moiety extends towards the AST loop where Cys474 is located. Three hydrogen bonds, shown as dashed lines, are formed with the hinge of the kinase domain. None of the compounds in the **5a-f** series were able to form adducts despite some that demonstrated high potency. (B) **9j** (yellow carbons) wherein the amide functionality in the covalent warhead was flipped relative to **5a**, and therefore forms one less hydrogen bond with the hinge. Being less constrained, we hypothesize that this modification allows the warhead to leave the hinge region along a different vector that leads to less steric collisions with the AST and to closer proximity with Cys474. Consequently, compounds in the **9a** series show adduct formation. Bromine is colored green. (C) Comparison of the fluorophenyl groups of **9j** and CCG215022 (from 4WNK). The fluorine atom is colored cyan. Both ligands are modeled to form a hydrogen bond with Asp329, an invariant catalytic residue in protein kinases. However, it is unclear whether this region of the scaffold is docked correctly, because the conformation of the GRK5 kinase domain, and in particular its P loop and AST, is not known. The SAR in Table 2 is best explained with the fluorophenyl packing under the P loop as it does in the CCG215022 complex.

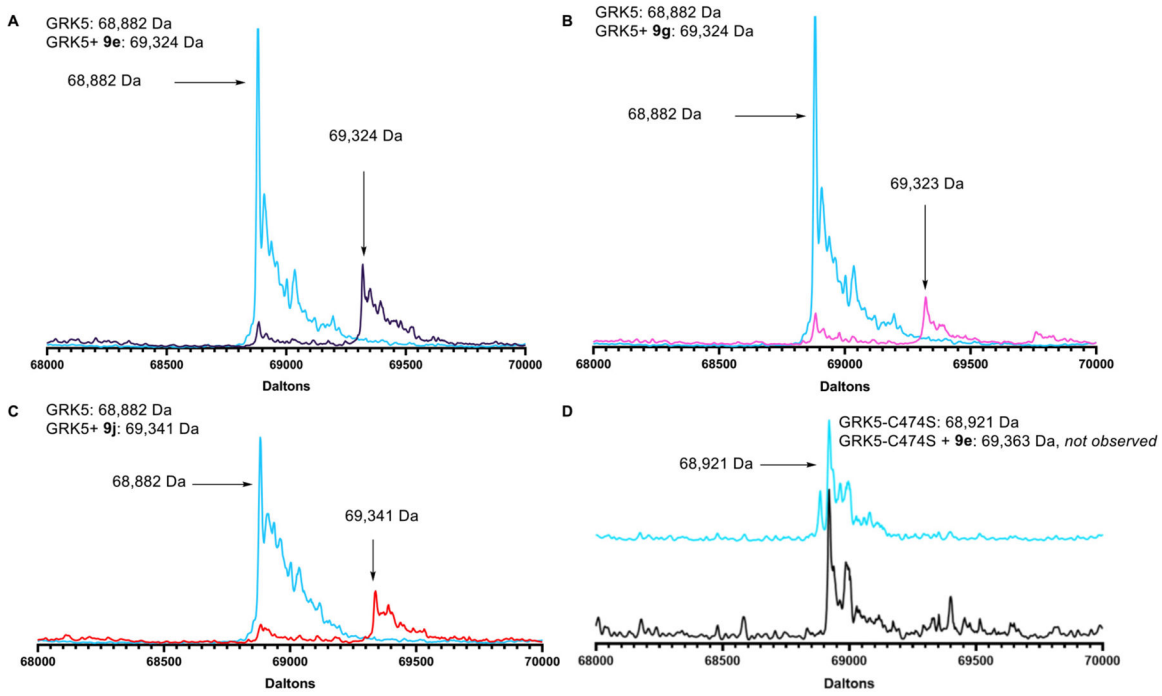


Figure 3.

Intact protein MS for compounds **9e**, **9g**, and **9j**. (A) GRK5 (blue) and GRK5+**9e** (black) incubated for 30 min. **9e** demonstrated full labelling of GRK5. (B) GRK5 and GRK5+**9g** (pink) incubated for 30 min. **9g** only labeled 50% of GRK5. (C) GRK5 and GRK5+**9j** (red) incubated for 30 min. **9j** fully labeled GRK5 in this timeframe. (D) GRK5-C474S mutant (purple) and GRK5-C474S+**9e** (teal) incubated for 30 min. **9e** did not label GRK5-C474S, indicating that **9e** and related compounds are engaging Cys474.

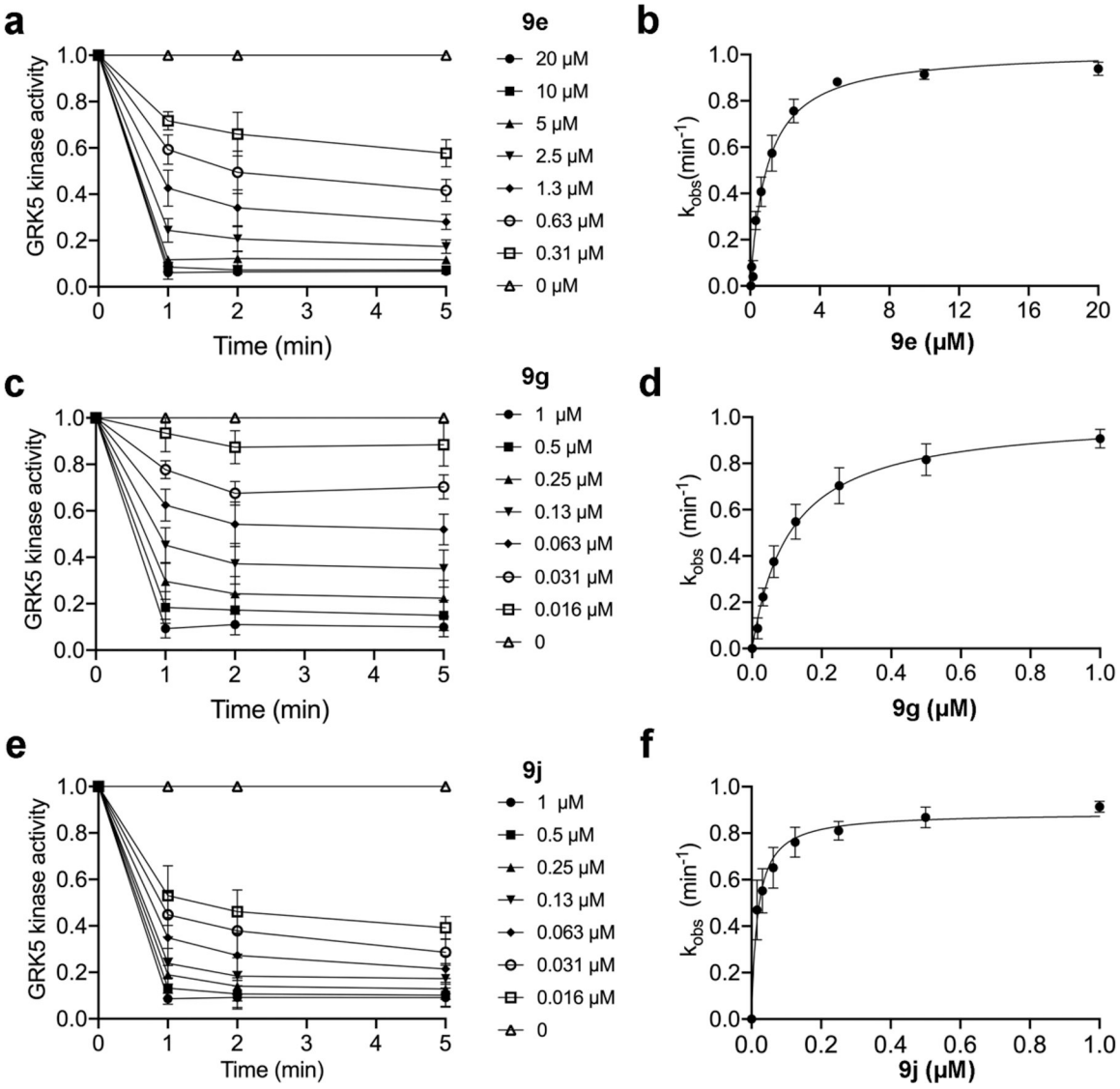


Figure 4.

Kinetic analysis for (A, B) **9e**, (C, D) **9g**, and (E, F) **9j**. Extracted values for K_I and k_{inact} from fitting the plots on the right are given in Table 4. Data points represent three measurements with error bars indicating standard deviation. Because the time courses (left panels) were not linear, only the 1 minute time point was used to estimate k_{obs}.

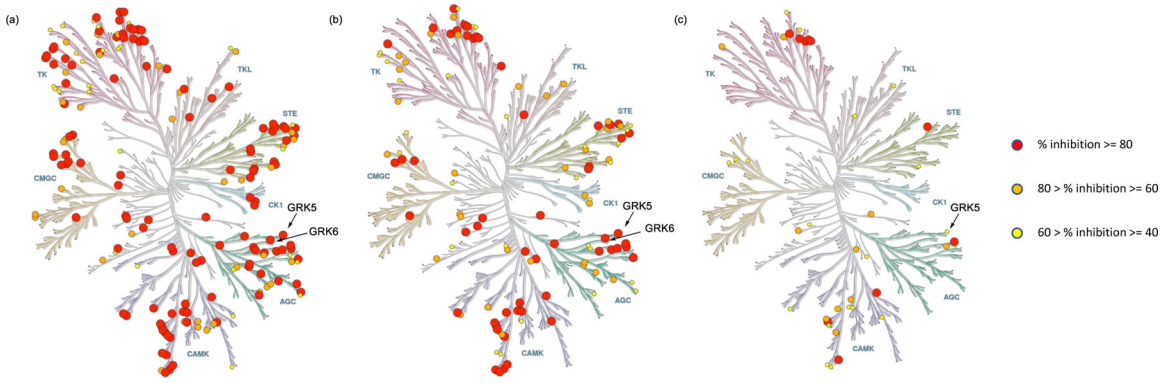
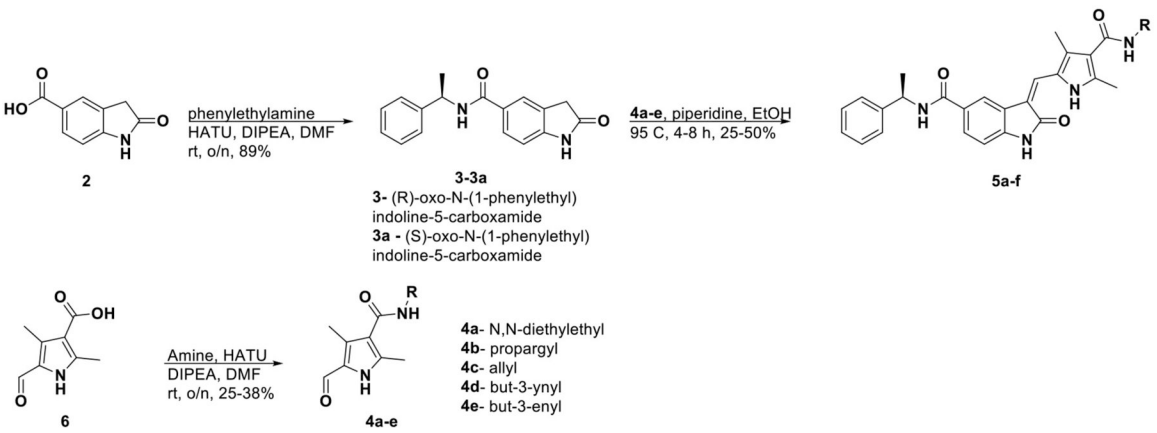


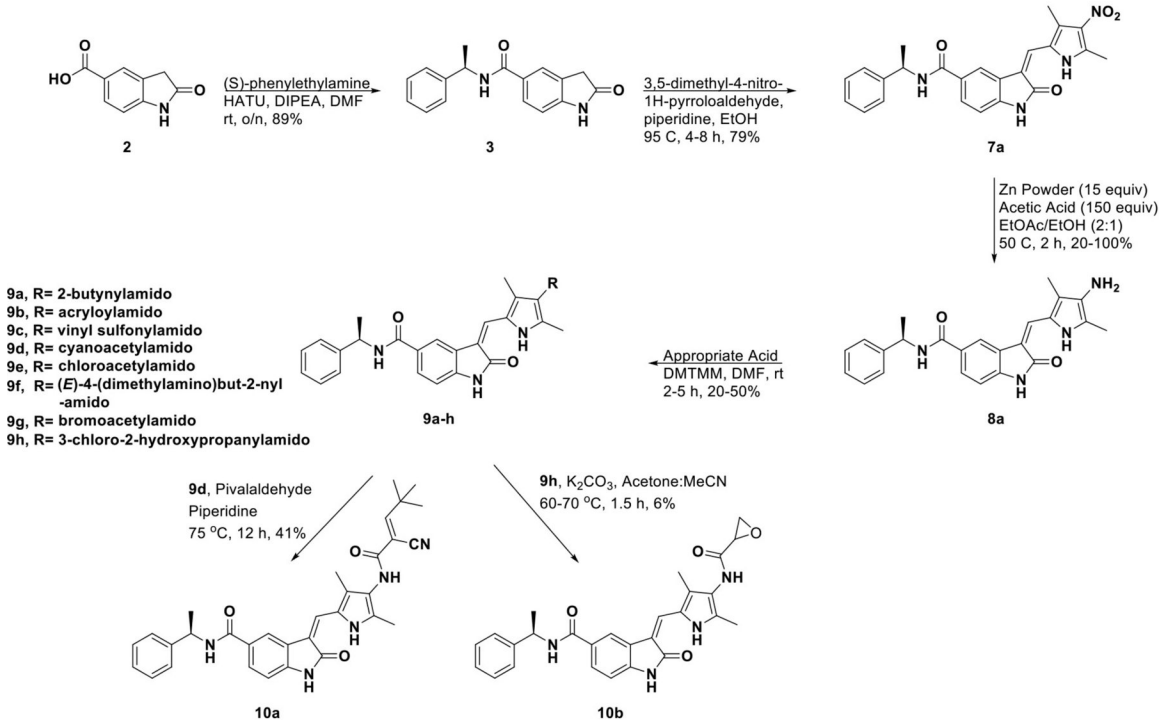
Figure 5.

Kinome-wide selectivity panel for **9g** at concentrations of (a) 1 μM, (b) 0.1 μM, and (c) 0.01 μM. Locations of GRK5 and GRK6 are denoted by arrows. A clear dose response is evident, with fewer kinases inhibited at the lowest concentration of **9g**. Illustration reproduced courtesy of Cell Signaling Technology, Inc (www.cellsignal.com).



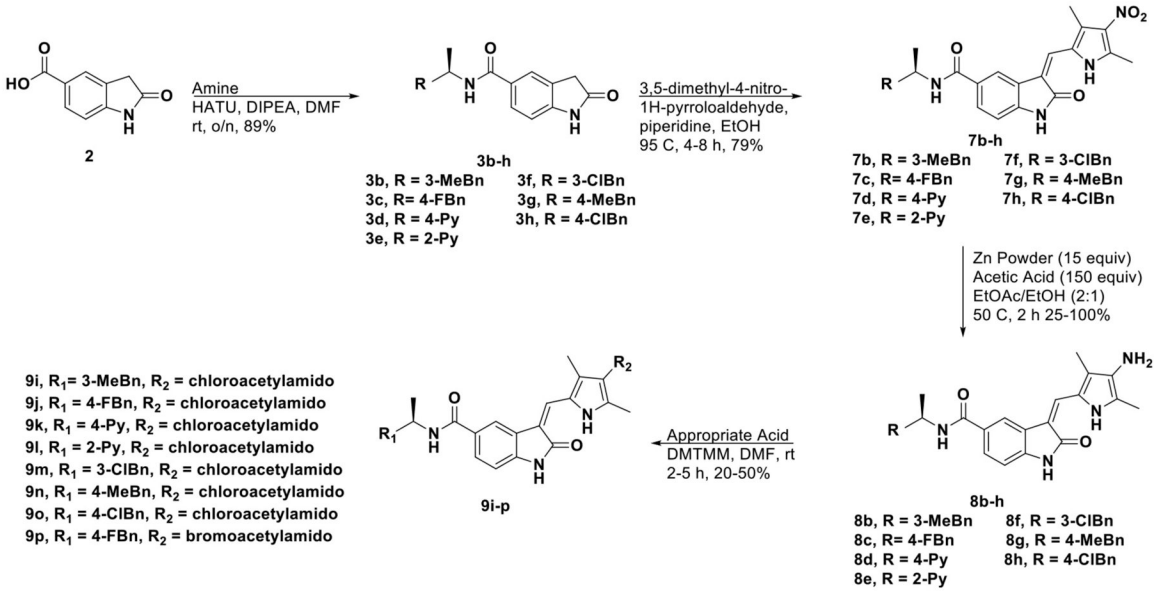
Scheme 1.

Convergent route to **5a-f**. **5a** and **5b** are enantiomers derived from **3** and **3a**, respectively



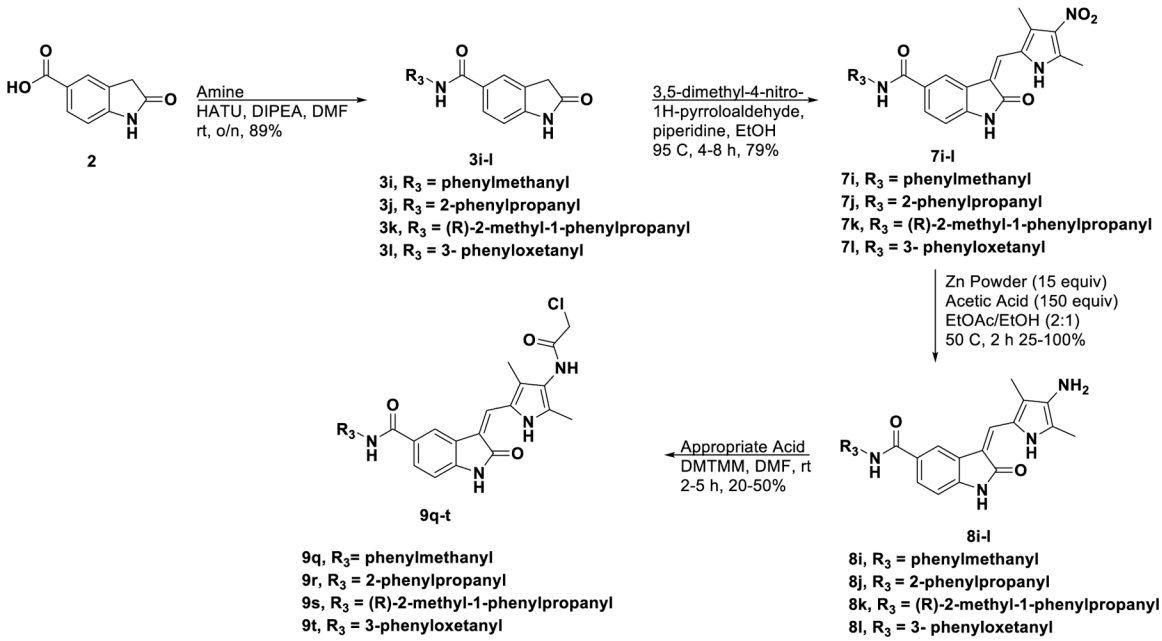
Scheme 2.

General synthetic route to **9a-h** and **10a-b**.



Scheme 3.

General synthetic route to **9i-p**.



Scheme 4.

General synthetic route to **9q-t**.

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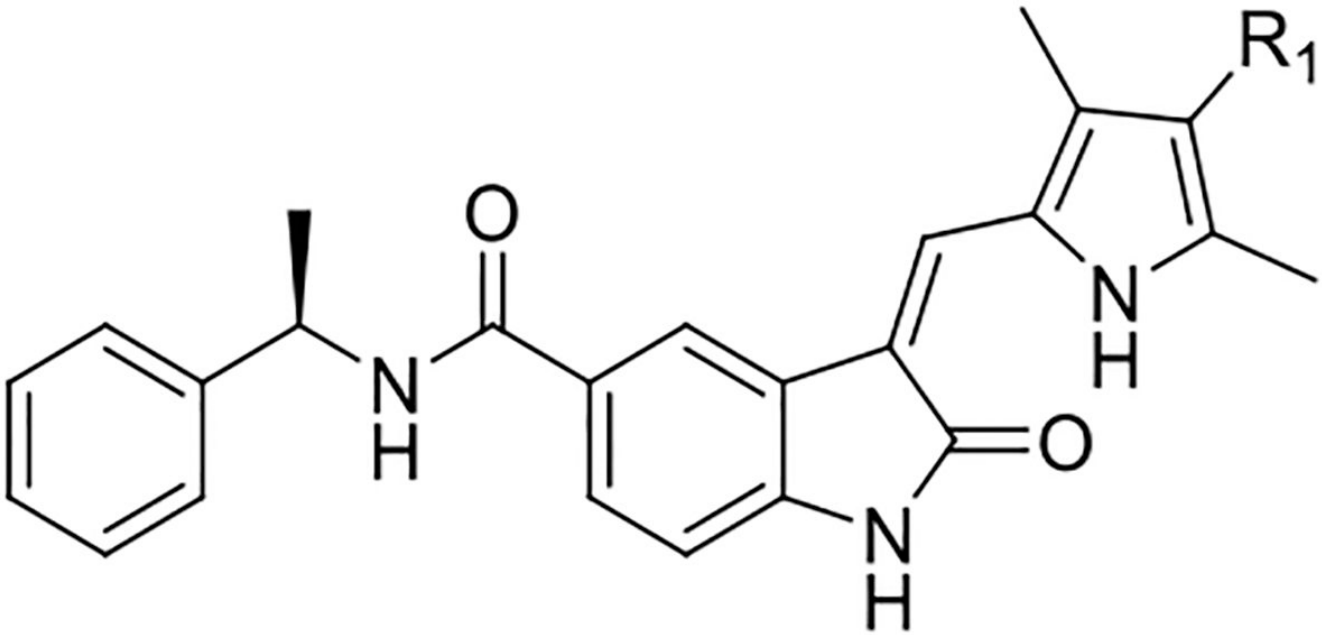
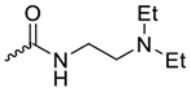
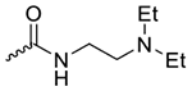
Table 1:

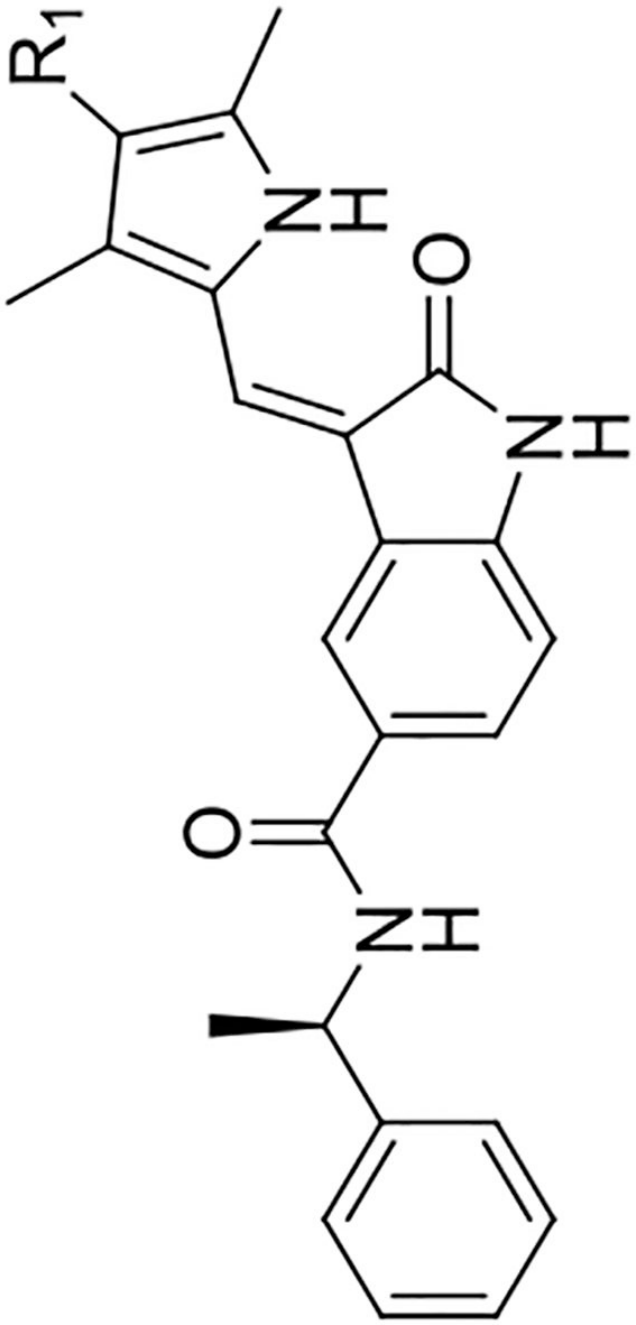
IC_{50} Values (μM ± SD) and Reactivity of Indolinone Compounds

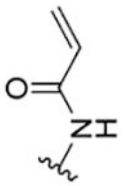
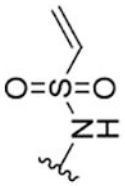


Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
Sunitinib	**1**		0.83 ± 0.7 (3)	130 ± 200 (3)	150	ND	NA
CCG 271421	**5a**†		0.015 ± 0.02 (7)	1.1 ± 0.7 (4)	74	>250 (2)	NA

Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
CCG 273262	**5b** †		1.30 ± 0.1 (3)	44 ± 18 (3)	32	ND	NA
CCG 271423	**5c**		0.021 ± 0.01 (7)	44 ± 40 * (6)	2100	ND	>30 min
CCG 271424	**5d**		0.048 ± 0.008 (3)	22 ± 10 (3)	460	>250 (2)	>30 min

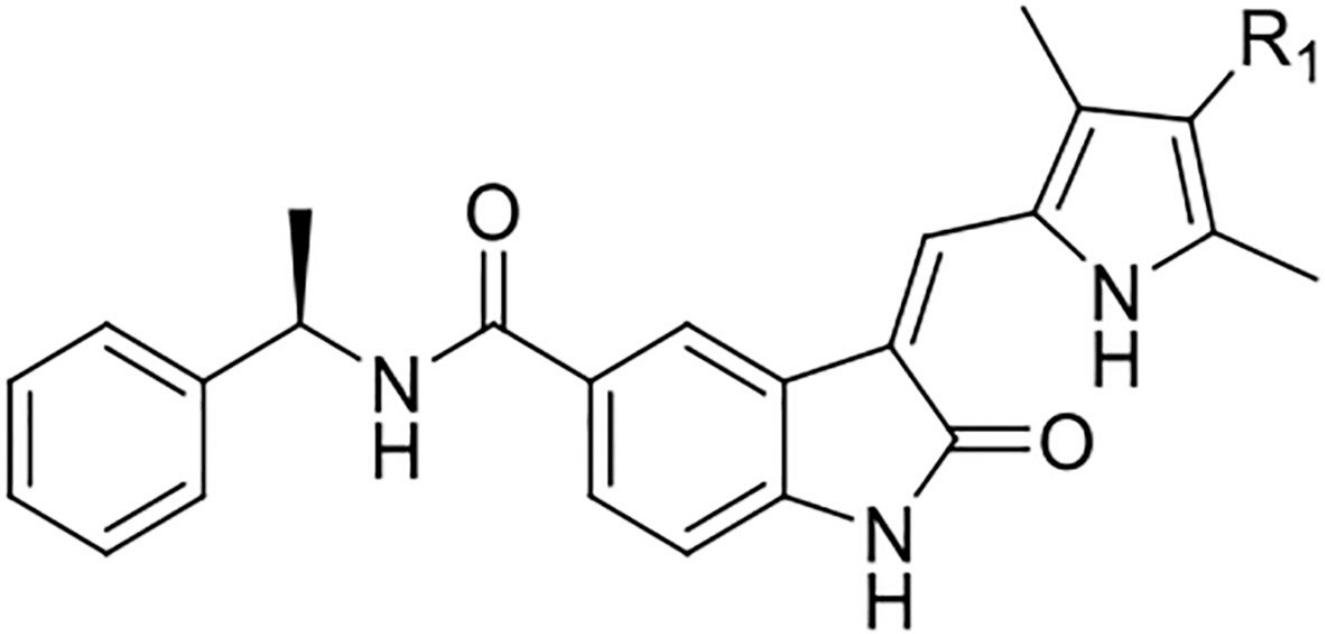
Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
CCG 271441	**5e**		0.091 ± 0.04 (3)	130 ± 50 (3)	1400	>250 (2)	>30 min
CCG 271442	**5f**		1.94 ± 0.05 (2)	630 ± 200 (2)	330	ND	>30 min
CCG 273183	**7a**	NO_2	0.73 ± 0.5 (3)	7.2 ± 3 (3)	10.	ND	ND
CCG 273180	**9a**		2.5 ± 0.8 (3)	150 ± 30 (3)	61	ND	>3 hr

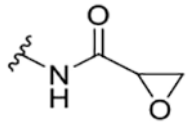


Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
CCG 273181	**9b**		0.81 ± 0.7 (4)	87 ± 30 (3)	110	ND	>3 hr
CCG 273182	**9c**		0.74 ± 0.6 (5)	280 ± 110 (3)	370	ND	100% 3 hr

Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
CCG 273220	**9e**		0.22 ± 0.04 (3)	350 ± 100 (2)	1500	>250 (2)	90% 30 min
CCG 273221	**9f**		0.36 ± 0.2 (3)	17 ± 10 (2)	47	ND	>30 min
CCG 273463	**9g**		0.0086 ± 0.003 (7)	12 ± 20 (3)	1400	>250 (2)	70% 30 min

Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
CCG 273464	**9h**		0.08 ± 0.03 (3)	6.7 ± 5 (3)	83	>250 (2)	>30 min
CCG 273240	**10a**		0.28 ± 0.1 (3)	120 ± 80 (2)	430	>250 (2)	>30 min



Compound		R_1	GRK5	GRK2	GRK2/GRK5	PKA	Adduct by MS*
CCG 273462	**10b**		0.74 ± 0.2 (3)	>250 (5)	>340	ND	>30 min
CCG 215022		-	0.28 ± 0.1 (6)	ND	-	>250 (2)	NA
paroxetine		-	ND	0.78 ± 0.3 (3)	-	850 ± 400 (4)	NA

All data were fit to a log([inhibitor]) versus response model with variable slope and automatic outlier rejection in GraphPad Prism. Curves that had R squared values less than 0.8 after fitting were omitted. ND, not determined. NA, not applicable Values in parentheses indicate the number of independent experimental curves. CCG215022 and paroxetine are positive controls for GRK5 and GRK2, respectively.

*Incubation time needed to observe adduct formation with GRK5 by intact mass MS.

†**5a** and **5b** are the (R) and (S) enantiomers of Ullrich-57, respectively.

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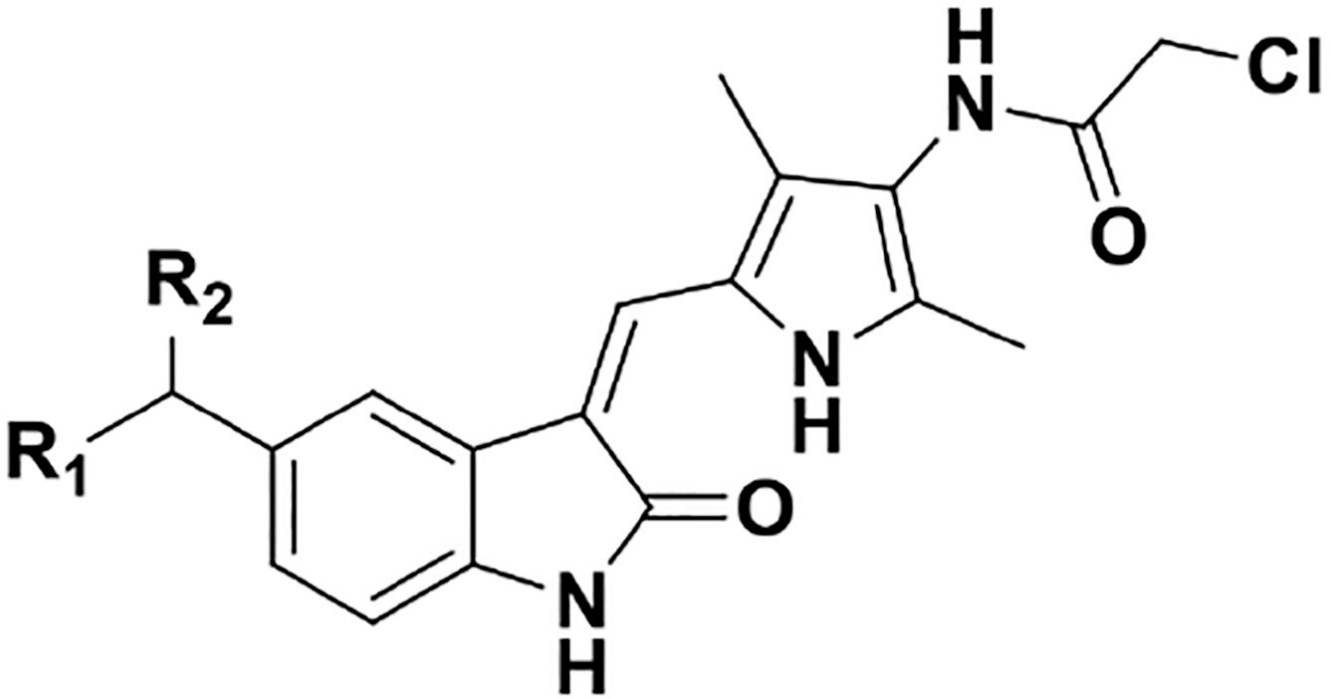
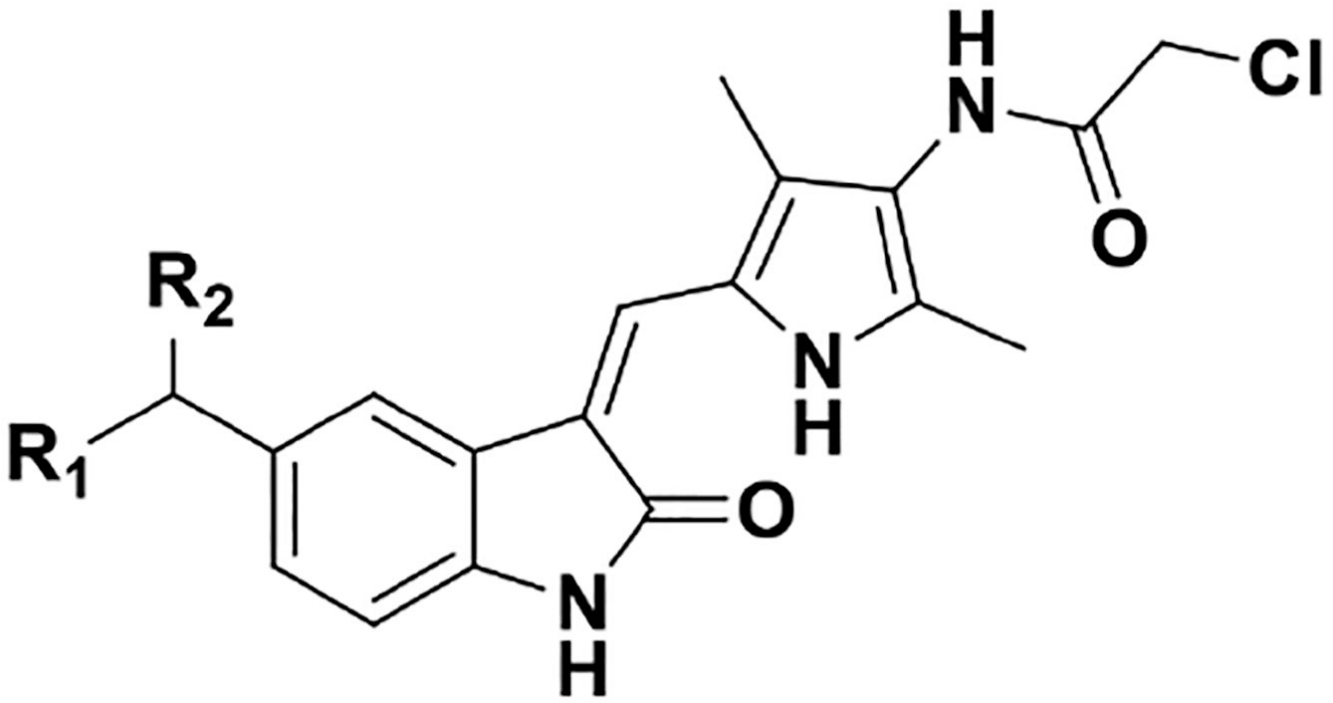
Table 2:

IC_{50} Values (μM ± SD) and Reactivity of Indolinone Variants with a Haloacetylamido Warhead



Compound		R_1	R_2	GRK5	GRK2	GRK2/GRK5[§]	PKA	Adduct by MS[*]
CCG 273261	**9i**	3-MeBn	(R)-CH_3	0.029 ± 0.03 (5)	11 ± 6 (3)	360	>250 (6)	>30 min
CCG 273441	**9j**	4-FBn	(R)-CH_3	0.0038 ± 0.001 (7)	4.8 ± 3 (3)	1300	>250 (6)	90% 30 min
CCG 273442	**9k**	4-Py	(R)-CH_3	0.13 ± 0.05 (3)	1.7 ± 2 (3)	13	ND	>30 min
CCG 273443	**91**	2-Py	(R)-CH_3	0.45 ± 0.1 (3)	9.6 ± 5 (3)	21	ND	>30 min
CCG 273444	**9m**	3-ClBn	(R)-CH_3	0.14 ± 0.04 (3)	19 ± 10 (3)	140	>250 (6)	>30 min

Compound		R_1	R_2	GRK5	GRK2	GRK2/GRK5[§]	PKA	Adduct by MS[*]
CCG 273445	**9n**	4-MeBn	(R)-CH_3	0.78 ± 03 (3)	2.1 ± 1 (5)	3	ND	>30 min
CCG 273583	**9o**	4-ClBn	(R)-CH_3	0.11 ± 0.05 (3)	0.70 ± 0.2 (3)	7	ND	50% 8 hr
CCG 359090[†]	**9p**	4-FBn	(R)-CH_3	0.015 ± 0.007 (3)	3.6 ± 2 (3)	230	ND	ND
CCG 273561	**9q**	Bn	H	0.13 ± 0.09 (4)	13 ± 3 (3)	99	>250 (6)	50% 8 hr
CCG 273562[‡]	**9r**	Bn	Gem-$(CH_3)_2$	12 ± 5 (3)	190 ± 70 (2)	15	ND	50% 8 hr
CCG 273564	**9s**	Bn	(R)-iPr	0.087 ± 0.02 (3)	23 ± 20 (4)	260	>250 (6)	90% 8 hr



Compound		R_1	R_2	GRK5	GRK2	GRK2/GRK5[§]	PKA	Adduct by MS[*]
CCG 273563	**9t**	Bn		0.095 ± 0.03 (3)	15 ± 7 (3)	160	69±10 (6)	>8 hr

Data were fit to a log([inhibitor]) versus response model with variable slope and automatic outlier rejection in GraphPad Prism. Curves with R^2 values <0.8 after fitting were omitted. ND, not determined. Numbers in parentheses indicate the number of independent experimental curves.

[*] Incubation time needed to observe adduct formation with GRK5 by intact mass MS.

[†] Same as **9j** but with a 2-bromoacteylamido warhead.

‡ IC_{50} estimate for this compound for GRK5 and GRK2 are likely high due to its poor solubility in our assay system.

Table 3:

Impact and Kinetics of Covalent Modification at GRK5-Cys474

Compound		GRK5 IC_{50} (μM)	GRK5-C474S IC_{50} (μM)	GRK5-C474S/GRK5	K_I (μM)	k_{inact} (min^{-1})
CCG 273220	**9e**	0.22 ± 0.04 (3)	0.60 ± 0.2 (5)	3	1.0 ± 0.3 (3)	>1.0 ± 0.05 (3)
CCG 273463	**9g**	0.0086 ± 0.003 (7)	0.044 ± 0.02 (5)	5	0.11 ± 0.03 (3)	>1.0 ± 0.03 (3)
CCG 273441	**9j**	0.0038 ± 0.001 (7)	0.019 ± 0.007 (5)	5	0.019 ± 0.01 (3)	>0.9 ± 0.02 (3)
CCG 215022		0.28 ± 0.01 (6)	0.22 ± 0.05 (5)	0.8	n/a	n/a

Errors correspond to standard deviation, with number of replicates given in parentheses. n/a, not applicable.