# DESIGN AND SYNTHESIS OF A NOVEL CLASS OF RNA POLYMERASE I INHIBITORS 

by
Tony E. Dorado

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

October 2023
© 2023 Tony E. Dorado
All Rights Reserved


#### Abstract

RNA Polymerase I (Pol I) is one of three DNA-dependent RNA polymerases and is responsible for transcription of the 47S ribosomal RNA (rRNA) precursor. The 47S rRNA transcript is subsequently processed to release the 18S, 5.8 S , and 28 rRNAs which are assembled into ribosomes. Pol I transcription serves as the rate-limiting step in ribosome biogenesis, accounting for up to $60 \%$ of active transcription in eukaryotic cells, directly influencing protein accumulation, cell growth, and cell division. Many types of cancers exhibit dysregulated rates of Pol I transcription, reflecting a need for increased ribosome synthesis to generate proteins to sustain heightened growth rates. Cancer cells may be selectively vulnerable to agents that inhibit Pol I transcription, providing an attractive therapeutic strategy for cancer treatment. BMH-21 is the first specific and selective Pol I inhibitor and does so by intercalating into GC-rich rDNA and creating a transcription block, leading to the ubiquitination and proteasomal degradation of the large catalytic subunit, RPA194. Notably, it accomplishes this independently of p53 and without eliciting a DNA damage response. BMH-21 is the first of only a small number of compounds to exhibit the RPA194 degradation phenotype, and a quantitative cell-based assay has been developed to measure the extent of RPA194 degradation caused by compound treatment. The primary goal of this work is to design and synthesize small molecule inhibitors of Pol I, with a focus on determining key pharmacophores and generating structure-activity relationship (SAR) data. SAR studies revealed key pharmacophores, but also showed that activity was limited within narrow chemical space. Further SAR efforts,


summarized by this work, have produced additional scaffolds as well as addressed some off-target activity while maintaining desired RPA194 degradation potency. In collaboration with Evotec, the RPA194 degradation assay was translated from 96well plate format to 384 -well plate format to facilitate high-throughput screening (HTS) efforts, providing the opportunity to discover new Pol I inhibitors and to generate new SARs. Efforts were made to transform BMH-21 into a chemical probe to gain structural insight about its binding interactions and to identify its molecular target, providing rationale for the improved design of future compounds.

Primary Reader and Thesis Advisor: Professor James C. Barrow

Additional Readers: Professor Craig A. Townsend and Professor Gregory V. Carr

## Acknowledgements

I am grateful to many for their help and guidance that has made it possible to complete the body of work presented here.

To my advisor, Jim Barrow, I could not have chosen a better mentor. You are patient and understanding, while remaining firm and fair. I appreciate the autonomy you allow to be an independent researcher while always making time when I needed guidance. Our conversations always sparked inspiration and it was a pleasure to work with you.

To the members of the Drug Discovery team at the Lieber Institute, I will always cherish the advice you have given me and the "go to the board" moments in lab. You all have taught me to think deeply about the science and how to be an effective communicator. I want to thank Daming Chen for his help with maintaining the cells and performing assays to evaluate my compounds. I also wish to thank our collaborators in the Laiho Lab for their expertise and support.

The CBI program has been a great asset to my development as a scientist. Thank you to Dr. Steve Rokita for always striving to improve the program and welcoming feedback. Thank you also to Lauren McGhee, John Kidwell, and Jasmine Harris for keeping all administrative operations running smoothly.

Finally, I want to thank my family for their love and support. To Mom and Dad, I appreciate all you did to provide for me and the work ethic you instilled in me. To Grandma and Grandpa, without your support, I never would have made it this far, and I thank you. To my fiancée, Jazmin, I cannot thank you enough for
providing the stability and motivation to keep going. In the most challenging times, you were always there with a shoulder to lean on to get me through.

## Table of Contents

Abstract ..... ii
Acknowledgements ..... iv
List of Tables ..... viii
List of Figures ..... x
List of Schemes ..... xii
Chapter 1. Introduction ..... 1
RNA Polymerase I Transcription Cycle ..... 1
RNA Polymerase I and Cancer ..... 7
Small Molecule Inhibitors of RNA Polymerase I ..... 12
References ..... 27
Chapter 2. Discovery and Evaluation of Novel Angular Fused
Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors ..... 42
Introduction ..... 42
Results and Discussion ..... 45
Chemistry ..... 55
Experimental ..... 63
References ..... 130
Chapter. 3 High-Throughput Screening Efforts Towards the Discovery of New RNA Polymerase I Inhibitors ..... 133
Introduction ..... 133
Results and Discussion ..... 134
Chemistry ..... 147
Experimental ..... 154
References ..... 199
Chapter. 4 Progress Towards Generation of a Photoaffinity Labeling Chemical Probe to Determine the Molecular Target of BMH-21 ..... 202
Introduction ..... 202
Results and Discussion ..... 202
Chemistry ..... 223
Experimental ..... 236
References ..... 273

## List of Tables

Chapter 1. Introduction ..... 1
Chapter 2. Discovery and Evaluation of Novel Angular Fused
Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors ..... 42
Table 2-1. Compound 1 Sidechain Modifications ..... 46
Table 2-2. Modification of the Tetracyclic Core ..... 48
Table 2-3. Compound 31 analogs ..... 50
Table 2-4. Modifications to the tricycle core at the three- and four- positions.. 52
Chapter. 3 High-Throughput Screening Efforts Towards the Discovery of New RNA Polymerase I Inhibitors ..... 133
Table 3-1. Analogs of 1 substituted at the 2 - or 4-position ..... 138
Table 3-2. Analogs of 2 substituted at the 2 - or 4 -position ..... 142
Table 3-3. Amide side-chain at the incorrect position of the tetracycle core and
addition of the amide side chain to the 8-position of the quinazoline core ..... 143
Table 3-4. Aryl quinazolinone series ..... 145
Chapter. 4 Progress Towards Generation of a Photoaffinity Labeling Chemical Probe to Determine the Molecular Target of BMH-21 ..... 202
Table 4-1. Modified cores with alkyne handle ..... 209
Table 4-2. Spirocyclic diazirine analogs ..... 211
Table 4-3. Freely rotatable diazirine analogs ..... 212
Table 4-4. Non-diazirine amide analogs ..... 214
Table 4-5. Bifunctional amide analogs ..... 216
Table 4-6. Cell viability of selected amide analogs ..... 218Table 4-7. Top proteins identified by mass spectrometry proteomics afterphotoaffinity pulldown with photoprobe 32 in A375 cells ............................... 222

## List of Figures

Chapter 1. Introduction ..... 1
Figure 1-1. The mammalian rDNA repeat and the 47S rRNA promoter ..... 2
Figure 1-2. The RNA polymerase I (Pol I) transcription cycle ..... 4
Figure 1-3. Transcriptional and post-translation regulation of rRNA transcription
by oncogenic pathways ..... 8
Chapter 2. Discovery and Evaluation of Novel Angular Fused
Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors ..... 42
Figure 2-1. Comparison of key data for previously reported compounds 1 and 244
Figure 2-2. Cell viability analysis of 31 ..... 53
Figure 2-3. qPCR analysis of 1 and 31 ..... 54
Figure 2-4. Intravenous (IV), Oral (PO), and Intraperitoneal (IP)
Pharmacokinetic Parameters for 31 in CD-1 mice ..... 55
Chapter. 3 High-Throughput Screening Efforts Towards the Discovery of New
RNA Polymerase I Inhibitors ..... 133
Figure 3-1. Fluorescent microscopy images of RPA194 degradation assay . ..... 135
Figure 3-2. High-throughput screening cascade of the Evotec library ..... 136
Figure 3-3. Profile of hits 1 and 2 ..... 137

# Chapter. 4 Progress Towards Generation of a Photoaffinity Labeling Chemical Probe to Determine the Molecular Target of BMH-21 <br> 202 

Figure 4-1. Chemical structures of BMH-21 and 9-amino DACA................... 203

Figure 4-2. BMH-21 (1) and previously reported potent analogs (2-4) .......... 207

Figure 4-3. Comparison of parent compound 26 and photoprobe 32............ 217

Figure 4-4. General application of photoaffinity probes ................................. 220

## List of Schemes

Chapter 1. Introduction ..... 1
Scheme 1-1. Chemical structure of Actinomycin D (ActD) ..... 12
Scheme 1-2. Chemical structure of Doxorubicin and Mitoxantrone ..... 13
Scheme 1-3. Chemical structures of Amsacrine, Quinacrine, and 9- aminoacridine. ..... 14
Scheme 1-4. Chemical structures of Camptothecin, Topotecan, and Irinotecan.Scheme 1-5. Chemical structure of Mitomycin C17
Scheme 1-6. Chemical structures of Cisplatin and Oxaliplatin ..... 18
Scheme 1-7. Chemical structures of QQ58, CX-3543 (Quarfloxin), CX-5461 (Pidnarulex), and PMR-116. ..... 20
Scheme 1-8. Chemical structures of Amodiaquine, Amopyroquine, Hernandonine, and Sempervirine. ..... 23
Scheme 1-9. Chemical structure of BMH-21. ..... 25
Chapter 2. Discovery and Evaluation of Novel Angular Fused
Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors ..... 42
Scheme 2-1. General scheme for the synthesis to provide compounds 1-21. 56
Scheme 2-2. General scheme for the synthesis to provide compounds 22-24,
26-27 ..... 56
Scheme 2-3. Synthesis of compound 25 ..... 57
Scheme 2-4. Synthesis of compound 28 ..... 58
Scheme 2-5. Synthesis of compounds 31, 34-46 ..... 58
Scheme 2-6. Synthesis of compounds 29, 30 ..... 59
Scheme 2-7. Synthesis of compound 32 ..... 60
Scheme 2-8. Synthesis of compound 33 ..... 61
Scheme 2-9. Synthesis of compounds 47-52 ..... 62
Chapter. 3 High-Throughput Screening Efforts Towards the Discovery of New
RNA Polymerase I Inhibitors ..... 133
Scheme 3-1. General scheme for the synthesis to provide compounds 1, 3-12148
Scheme 3-2. General scheme for the synthesis to provide compounds 2, 13-18149
Scheme 3-3. Synthesis of compounds 23 and 24 ..... 150
Scheme 3-4. Synthesis of compounds 25 and 26 ..... 151
Scheme 3-5. Synthesis of compounds 27-29 ..... 153
Chapter. 4 Progress Towards Generation of a Photoaffinity Labeling Chemical Probe to Determine the Molecular Target of BMH-21 ..... 202
Scheme 4-1. General synthetic route for various brominated tricycles and attempts to brominate the $\mathrm{BMH}-21$ tetracycle ..... 209
Scheme 4-2. General scheme for the synthesis to provide compounds 1-4 . 224
Scheme 4-3. Synthesis of compound 5 ..... 224
Scheme 4-4. Synthesis of compound 6 ..... 226
Scheme 4-5. General scheme for the synthesis to provide compounds 7-12226
Scheme 4-6. Synthesis of compounds 13-14 ..... 227
Scheme 4-7. General scheme for the synthesis to provide compounds 15-18227
Scheme 4-8. General scheme for the synthesis to provide compounds 19-25228
Scheme 4-9. Synthesis of compounds 26-27 ..... 229
Scheme 4-10. Synthesis of compound 28 ..... 230
Scheme 4-11. Synthesis of compounds 29-30 ..... 231
Scheme 4-12. Synthesis of compounds 31-32 ..... 233
Scheme 4-13. Synthesis of compounds 33-34 ..... 234

## Chapter 1. Introduction

## RNA Polymerase I Transcription Cycle

RNA polymerase I (Pol I) is one of three DNA-dependent RNA polymerases and is responsible for transcribing ribosomal DNA into ribosomal RNA. Ribosomal RNAs (rRNAs) from Pol I are combined with the 5S rRNA from Pol III and several ribosomal proteins (translated from mRNAs produced by Pol II) in the nucleolus to assemble ribosomes. Pol I is responsible for up to $60 \%$ of active transcription in eukaryotic cells and is rate-limiting with respect to ribosome biogenesis, directly influencing protein accumulation, cell growth, and cell division. ${ }^{1}$

Approximately 400 rDNA repeats are found in the human genome, but only about half of these genes are actively transcribed at any given time. ${ }^{1-3}$ These rDNA repeats are contained within nucleolar organizer regions (NORs) and arranged in a head-to-tail fashion located on the short arms of the five acrocentric chromosomes $13,14,15,21$, and $22 .{ }^{4}$ NORs can be classified as active or inactive, depending on epigenetic characteristics of the rDNA. ${ }^{1,5}$ Transcriptionally active NORs typically exhibit an open chromatin structure, hypomethylation of DNA, acetylation of histone H 4 , and dimethylation of histone H 3 at lysine 4, while transcriptionally inactive NORs are characterized by hypermethylation of CpG, hypoacetylation of histone H 4 , and methylation of histone H 3 at lysine 9 and histone H 4 at lysine $20 .{ }^{5}$ Pictured in Figure 1-1, human rDNA repeats are made up of approximately 43 kb and contain a $\sim 13 \mathrm{~kb}$ transcribed region that encodes the 47S pre-rRNA interspaced with a $\sim 30$ kb intergenic spacer (IGS) that contains


Figure 1-1. The mammalian rDNA repeat and the 47S rRNA promoter. The top panel illustrates key elements and the general organization of a mammalian rDNA repeat. The lower panel illustrates the layout of the 47 S rRNA promoter. This figure was adapted from Goodfellow and Zomerdijk, 2013, ${ }^{1}$ reproduced with permission from Springer Nature.
regulatory elements such as gene promoters, spacer promoters, repetitive enhancer elements, and transcription terminators. ${ }^{1,6,7}$
rDNA promoters contain two regulatory elements necessary for transcription initiation: the core promoter and the upstream control element (UCE). ${ }^{1}$ Generally, RNA polymerases have little affinity for promoter sequence elements, requiring formation of a pre-initiation complex (PIC) prior to transcription initiation. ${ }^{1}$ Upstream binding factor (UBF) binds as a dimer to the core promoter and the UCE, causing a bending of the DNA to produce a 140 bp loop of DNA in a single turn, creating a nucleosome-like structure called the enhanceosome. ${ }^{5,8}$ Binding of UBF recruits selectivity factor 1 (SL1), which is a complex of a TATA-binding-protein (TBP) and a combination of TBP-associated factors (TAFs): TAFı110, TAFı63, TAFı48, TAFı41, and TAFı12. ${ }^{1}$ SL1 recognizes and binds in a sequence-specific manner to the core promoter as well as to UBF. ${ }^{1,9}$ In the
absence of SL1, UBF binds rDNA loosely, rapidly associating and dissociating from the rDNA. ${ }^{8}$ However, SL1 recruitment causes a decrease in the UBF off-rate, resulting in a stable UBF - SL1 complex that is capable of recruiting an initiationcompetent Pol I. ${ }^{8-10}$

Human Pol I is a 13 -subunit complex made up of a 10 -subunit horseshoeshaped core formed around the two Pol l-specific large subunits, RPA1 and RPA2, and bound by a peripheral stalk composed solely of the RPA43 subunit. ${ }^{11,12}$ Pol I exists in at least two functionally distinct complexes (Pol la and Pol Iß), with the initiation-competent Pol I $\beta$ distinguished by RRN3 (also known as TIF-1A ${ }^{10}$ ) being tethered to RPA43. ${ }^{1,8,11}$ The Pol I transcription cycle is shown in Figure 1-2. Pol I is recruited to the PIC by interaction between RRN3 and SL1. ${ }^{1,7,8,10}$ Transcription initiates upon promoter opening, however Pol I stutters during the synthesis of the first few nucleotides due to inhibitory interactions between the enzyme complex and the transcription factors at the promoter. ${ }^{13}$ Pol I escapes the promoter by releasing RRN3, converting into the elongating form of the polymerase, leaving behind UBF and SL1 still bound to the promoter, able to recruit another Pol I complex to reinitiate transcription from the same promoter. ${ }^{1,7,13}$

After promoter escape, the elongation complex extends the transcribed rRNA at a rate of 95 nucleotides/second in humans. ${ }^{14} \mathrm{Poll}$ is able to sustain baseline levels of transcription by itself, but is aided by transcription elongation factors and formation of RNA structures. In addition, co-transcriptional RNA processing occurs in yeast but not higher eukaryotes. ${ }^{7}$ DISF (5,6-dichloro-1- $\beta$-d-


Figure 1-2. The RNA polymerase I (Pol I) transcription cycle. Pre-initiation complex (PIC) formation, transcription initiation, promoter escape and clearance, elongation, termination, and reinitiation. This figure was adapted from Russell and Zomerdijk, 2005, ${ }^{13}$ reproduced with permission from Elsevier.
ribofuranosylbenzimidazole sensitivity inducing factor) is a human analog of the yeast Spt4 - Spt5 complex transcription elongation factor, which is thought to induce pausing in early elongation and to promote elongation and processivity during later transcription. ${ }^{7}$ UBF binds throughout the rDNA and ensures an openchromatin orientation through phosphorylation-dependent remodeling of the rDNA chromatin to allow transcription ${ }^{1,7}$ As the transcribing Pol I complex proceeds, torsional strain develops in the rDNA in the form of positive supercoils ahead of the polymerase and negative supercoils behind, requiring topoisomerase activity to relieve the strain. ${ }^{13} \mathrm{~A}$ wider exit tunnel found in human Pol I relative to yeast Pol I allows for nascent RNA folding and accommodation of double-stranded RNA structures. ${ }^{11}$ The elongation rate is positively affected by these rRNA structures because they prevent rRNA re-entry into the active site prohibiting backtracking. ${ }^{11,12}$ Elongating Pol I complexes are densely packed on the rDNA,
with multiple elongation complexes transcribing the same active gene, forming structures called Miller trees. ${ }^{1,7}$ In the event that Pol I needs to pause and backtrack, due to DNA damage or a mismatched nucleotide incorporation, the cleft and funnel are opened to permit entry of RPA12. The gating tyrosine is pushed aside and left in an open position, allowing backtracking to the mismatched RNA. Finally, the phosphodiester bond is cleaved, likely by the negatively-charged tip of RPA12, facilitated by nucleotide rearrangement in the catalytic center. ${ }^{12}$ Pol I addition rate constants are faster than those of Pol II, however, Pol I elongation complexes are less stable and are more error prone than Pol II. ${ }^{15}$

As the elongating Pol I complex proceeds through the 3' end of the rDNA coding region and reaches the IGS, it encounters repeated transcription terminator DNA elements. In humans, there are eleven repeated transcription terminator elements in the IGS, abbreviated with $\mathrm{T}_{(n)}$ where $n$ indicates the position of the individual element. ${ }^{16}$ The terminator element is a conserved 11 bp sequence motif in humans and contains the GTCGAC Sall restriction site, also known as "Sal box." ${ }^{16}$ Each terminator element is a specific binding site for transcription termination factor (TTF1). Although the majority of transcription termination occurs at $T_{1}, T_{2}$ acts as a 'fail-safe' by creating a replication fork barrier, preventing readthrough and prohibiting collision of Pol I with the DNA replication machinery. ${ }^{7,16}$ TTF1 binds to terminator sequences, induces DNA bending, and pauses Pol I transcription. ${ }^{7}$ At this point, the transcription termination mechanism diverges between yeast and mammalian Pol I. In yeast, the ternary Pol I transcription complex dissociates through a 'torpedo' mechanism, similar to Pol II transcription
termination. ${ }^{1,16}$ In mammals, the Pol I and transcript release factor (PTRF) assists in dissociating Pol I and the nascent rRNA transcript from the DNA. ${ }^{7,13,16}$ The DNA bending caused by TTF1 binding leads to formation of DNA loops that juxtapose the terminator and promoter elements in 3D-space, possibly increasing the speed of transcription re-initiation through Pol I recycling. ${ }^{1,7}$

Pol I transcribes the 47S rRNA precursor that contains the 5' external transcribed spacer (5'ETS), 18S, internal transcribed spacer 1 (ITS1), 5.8 S , ITS2, 28S, and 3'ETS. ${ }^{17}$ The 47S transcript undergoes a complex series of endo- and exonucleolytic cleavage events that involve several possible maturation pathways depending on the relative kinetics of the processing steps. ${ }^{18}$ The released $18 S$, 5.8S, and 28 S rRNAs are chemically modified by small nucleolar ribonucleoprotein complexes (snoRNPs). ${ }^{19}$ Small nucleolar RNA (snoRNA) base pairs with the rRNA and coordinates the snoRNP to site-selectively modify the target residue. ${ }^{19,20}$ The majority of chemical modifications involve 2'-O-methylation of the ribose and isomerization of uridine to pseudouridine, carried out by either the methyltransferase fibrillarin (C/D box snoRNPs) or the psuedouridyl synthase dyskerin (H/ACA box snoRNPs), respectively. ${ }^{18,19}$ Cleavage events and nucleotide modifications occur simultaneously with the folding of pre-rRNAs and assembly with 79 ribosomal proteins (RPs) and numerous ribosomal association factors (RAFs). ${ }^{18}$ In human ribosomes, the 40S small ribosomal subunit is composed of the 18 S rRNA from Pol I and 33 RPs from Pol II, while the 60S large ribosomal subunit assembled from the 5.8 S and 28 S rRNAs from Pol I, 47 RPs from Pol II, and the 5S rRNA from Pol III. ${ }^{18}$

## RNA Polymerase I and Cancer

Pol I transcription is frequently dysregulated in cancer, supplementing a need for increased ribosome biogenesis and protein synthesis to facilitate heightened growth rates. ${ }^{21}$ Often, nucleolar size proportionally tracks with Pol I transcription rate, ${ }^{22}$ and the association between nucleolar size and cancer has been known for over a century. ${ }^{23}$ Since gain of function mutations in Pol I subunits are rare, the increase in Pol I transcription is mainly driven by post-translational modifications of transcription factors, activation of oncogenic pathways, and inactivation of tumor suppressor pathways, ${ }^{10}$ summarized in Figure 1-3.

Post-translational modifications of UBF, SL1, and RRN3 contribute to the increase in Pol I transcription typically by promoting PIC formation. UBF is phosphorylated by cyclin/cell dependent kinase (CDK) complexes, extracellular signal-regulated kinase (ERK), the mammalian target of rapamycin (mTOR) through S6 kinase (S6K1) signalling, ${ }^{25,26}$ and casein kinase 2 (CK2) resulting in improved binding of UBF to the rDNA and to SL1. ${ }^{5}$ UBF is also acetylated by CREB-binding protein (CBP) and histone acetyl-transferase (hALP), which prevents UBF inhibition by the tumor suppressor pRB and improves the interaction between UBF and PAF53, respectively. ${ }^{5,25}$ Similarly, SL1 is phosphorylated by CK2 ${ }^{5}$ and acetylated at TAF,68 (mouse homolog of TAF। $63^{13}$ ) by the p300/CBPassociated factor (PCAF) resulting in enhanced binding at the rDNA promoter. ${ }^{25,27}$ RRN3 phosphorylation by CK2 causes its release from Pol I, allowing the complex to convert to its elongating form. ${ }^{5}$ mTOR-S6 kinase and ERK also provide


Figure 1-3. Transcriptional and post-translation regulation of rRNA transcription by oncogenic pathways. (A) Post-translational modifications induced by oncogenes or tumor suppressor genes. (B) Convergent regulatory pathways that boost rRNA transcription upon oncogene activation or repress it through tumor suppressor genes. This figure was adapted from Gaviraghi et al., 2019. ${ }^{24}$
phosphorylations that are required for RRN3 activity. ${ }^{5,24}$ In addition to posttranslational modifications, several oncogenes upregulate Pol I transcription either by directly affecting Pol I machinery activity or by increasing the abundance of PIC components and even Pol I subunits. Perhaps the most well-understood and influential example is the oncoprotein c-MYC. c-MYC can stimulate transcription in all three polymerases by binding as a heterodimer with MYC associated factor X
(MAX) to site-specific Enhancer Box sequences ( E boxes) at the DNA promoter. ${ }^{10,25}$ Specifically relevant to Pol I, c-MYC directly and indirectly upregulates transcription. By binding directly to rDNA, c-MYC recruits the transformation/transcription domain-associated protein (TRRAP), a histone acetyltransferase cofactor that increases the histone acetylation levels on rDNA chromatin, a modification associated with transcriptional activation. ${ }^{24,25,28}$ c-MYC also binds SL1, stabilizing the SL1/UBF complex and increasing UBF recruitment to the rDNA promoter. ${ }^{25,29}$ Indirectly, c-MYC upregulates Pol I transcription by increasing the abundance of Pol I transcription components, such as UBF, RRN3, and Pol I subunits through increased expression of Pol II genes of Pol I factors. ${ }^{5,10,25,28}$

Other oncogenes include nucleophosmin (NPM or B23) and the fusion protein acute myeloid leukemia 1 Eight-Twenty-One (AML1-ETO). Oddly, NPM has been observed to exhibit both oncogenic and tumor suppressive behavior dependent on cell type and abundance, but can stimulate rDNA transcription through increased recruitment of TAF/48 to the rDNA promoter. ${ }^{5}$ Overexpression of NPM can also promote c-MYC-driven Pol I transcription by increasing the localization of nucleolar c-MYC. ${ }^{25}$ Finally, AML1-ETO promotes Pol I transcription by binding to transcriptionally active rDNA repeats and associating with UBF. ${ }^{25}$

Under normal conditions, several tumor suppressor proteins regulate Pol I transcription to control ribosome biogenesis. However, many tumor suppressors are often inactivated by loss-of-function mutations in cancer, contributing to
malignancy through unchecked Pol I transcription. p53 prevents PIC formation by interacting with TBP and TAF 110 of SL1, interfering with SL1/UBF complexation. ${ }^{5}$ Similarly phosphatase and tensin homolog deleted on chromosome ten (PTEN) also prevents PIC formation by disrupting the SL1 complex and is found associated with another potential tumor suppressor, glycogen synthase kinase (GSK) $3 \beta$ at the rDNA promoter in Ras-transformed cells. ${ }^{5,29}$ Retinoblastoma-associated protein ( pRB ) and p130 bind to UBF and prevent its association with the rDNA, disrupting PIC formation. ${ }^{5}$ Finally p14 ${ }^{\text {ARF }}$ impairs PIC formation by interfering with UBF phosphorylation as well as preventing nucleolar import of TTF1. ${ }^{25}$

Upon disruption to ribosome biogenesis, the nucleolus triggers a "nucleolar stress response" that results in downstream effects including cell cycle arrest and apoptosis. ${ }^{30}$ Rubbi and Milner ${ }^{31}$ observed that p 53 could be activated through induction of nucleolar stress both in the presence and absence of DNA damage. Targeting ribosome biogenesis through activation of p53 has become an attractive therapeutic strategy against cancer. Indeed, many classical chemotherapeutics have been observed to involve nucleolar stress induction/ribosome biogenesis inhibition and p53 activation as part of their efficacy. ${ }^{32,33}$ Typically, p 53 levels are kept low by interaction with human double minute 2 (HDM2), an E3 ubiquitin ligase that binds to and ubiquitinates p53, leading to its proteasomal degradation. ${ }^{34}$ Upon induction of nucleolar stress, for example by Pol I transcription inhibition, free RPL5, RPL11, and 5S rRNA assemble to produce the 5 S ribonucleoprotein particle (RNP), which binds and sequesters HDM2, releasing and activating p53. 35,36

Although p53 activation is a rational therapeutic strategy that is currently being pursued, unfortunately $\sim 50 \%$ of cancers lack functional p53. ${ }^{37-39}$ However, induction of nucleolar stress can also trigger p53 independent responses that result in cell cycle arrest and/or apoptosis. ${ }^{37,40}$ When Pol I transcription is inhibited, the resulting free RPL11 can bind to HDM2, disrupting the stabilizing interaction between HDM2 and E2F-1, a transcription factor, resulting in proteasomal degradation of E2F-1 and cell cycle arrest. ${ }^{37,40,41}$ Free RPL11 can also regulate cMYC activity and mRNA turnover. ${ }^{40}$ RPL11 prevents the recruitment of the coactivator TRRAP to c-MYC by binding to the Myc box II (MB II), inhibiting c-MYCinduced transcription and cell proliferation. ${ }^{40,42}$ RPL11 also binds to c-MYC mRNA at its 3 ' untranslated region (3'-UTR), repressing c-MYC expression through miR24/miRISC recruitment during nucleolar stress. ${ }^{40,43}$ Another ribosomal protein, RPL3 can also trigger downstream cell cycle arrest or apoptosis during ribosome biogenesis disruption. ${ }^{37,44}$ Free RPL3 can form a multi-protein complex with Sp1 and NPM at the p21 gene promoter, activating p21 expression and subsequent cell cycle arrest or apoptosis. ${ }^{37,44}$ Another effect of nucleolar stress is the destabilization and degradation of PIM1, a constitutively active serine/threonine kinase that normally phosphorylates and marks for degradation the cell cycle inhibitor, p27 ${ }^{\text {Kip1 }}$. ${ }^{37,45}$ Nucleolar stress lowers levels of PIM1 through proteasomal degradation, leading to stabilization of $\mathrm{p} 27^{\mathrm{Kip} 1}$ resulting in cell cycle arrest and apoptosis. ${ }^{37,40,45}$

## Small Molecule Inhibitors of RNA Polymerase I

The remainder of this review will summarize several small molecules that have been observed to affect ribosome biogenesis through Pol I transcription inhibition. In order to prevent substantial overlap with previous recent Pol I medicinal chemistry reviews, ${ }^{30,36,46,47}$ a comparison between in vitro potencies and in vivo exposures will be highlighted where available.

DNA intercalating agents are one class of chemotherapeutic agent observed to affect Pol I transcription. One of the earliest observed Pol I transcription inhibitors is the antibiotic Actinomycin D (ActD), shown in Scheme 11. ActD is comprised of a phenoxazone ring system that contains two pentapeptide lactone rings attached at opposite ends of the ring system. ActD can inhibit all three RNA polymerases at high enough doses, ${ }^{47}$ however, at low doses ${ }^{48}$ it can specifically inhibit Pol I by intercalating in GC-rich rDNA with the phenoxazone end


Actinomycin D (ActD)
Scheme 1-1. Chemical structure of Actinomycin D (ActD).
of the molecule. ActD has been used for several decades as a clinically approved treatment for several types of cancers, however toxicity has limited its clinical use. ${ }^{36}$ Part of the toxicity associated with its use could possibly be due to DNA damage caused by the increase in covalent intermediates formed between topoisomerase I (TOP1) and DNA. ${ }^{49}$ ActD has also been observed to bind to Gquadruplex (G4) structures. ${ }^{50-52} \mathrm{G} 4$ binding stabilizes the quadruplex structure and prevents gene expression, providing a small molecule target within the promoter region of several proto-oncogenes, such as c-MYC, that have the potential to form G4 structures. ${ }^{53}$

Doxorubicin and mitoxantrone, shown in Scheme 1-2, are examples of a class of molecules known as anthracyclines. Anthracyclines intercalate into DNA and selectively trap topoisomerase II (TOP2) cleavage complexes at preferential cleavage sites. ${ }^{54}$ The compounds "poison" TOP2 by preventing re-ligation of the

Doxorubicin


Mitoxantrone

Scheme 1-2. Chemical structure of Doxorubicin and Mitoxantrone.

DNA strand breaks, trapping the enzyme on the DNA, and creating protein-linked DNA breaks (PDB). ${ }^{55,56}$ Along with DNA damage, anthracyclines can generate reactive oxygen species (ROS) leading to cardiac toxicity. ${ }^{57}$ Poisoning of the TOP2 $\beta$ isoform has also been implicated in inducing cardiotoxicity. ${ }^{58}$ Via the TOP2 $\beta$ inhibition mechanism, doxorubicin and mitoxantrone inhibit production of the 47S rRNA precursor just below clinically relevant concentrations ( $\mathrm{IC}_{50}$ of 0.3 $\mu \mathrm{M}$ and $0.65 \mu \mathrm{M}$ compared to clinically relevant ranges of 2.2-7.7 $\mu \mathrm{M}$ and $0.55-$ $0.77 \mu \mathrm{M}$, respectively) ${ }^{32}$ by preventing the formation of the PIC. ${ }^{59}$ However, the associated cardiotoxicity and bone marrow suppression limit their therapeutic benefits. ${ }^{60}$

Acridines are another class of planar aromatic intercalators. Specifically, 9substituted acridine derivatives (examples depicted in Scheme 1-3) have been studied for a variety of therapeutic applications such as cancer, malaria, and bacterial infections. ${ }^{61}$ Amsacrine, the first clinically approved topoisomerase inhibitor, functions as a topoisomerase II poison. However, further development of


Scheme 1-3. Chemical structures of Amsacrine, Quinacrine, and 9-aminoacridine.
the acridine scaffold has yielded new derivatives that act as topoisomerase I inhibitors or topoisomerase II catalytic inhibitors, reducing their overall toxicity. ${ }^{61}$ Among the variety of biological effects 9 -substituted acridines exert, the antimalarial drug quinacrine has gone through drug repurposing efforts after showing anticancer activity in a variety of cancers ${ }^{62,63}$ and even participating in several clinical trials. ${ }^{64}$ Unfortunately, it has not advanced past phase II, even when in combination with other therapies, although it is generally well tolerated. ${ }^{64}$ This possibly reflects a mismatch of in vivo exposures (0.32 $\mu \mathrm{M}$ maximal plasma concentration documented on a standard malaria regimen ${ }^{63,65}$ ) compared to observed in vitro efficacy (often used in excess of $1 \mu \mathrm{M}$ ). Quinacrine and a structurally simpler analog, 9-aminoacridine, were observed to induce activation of p53 by inhibiting NF-кB, without eliciting a DNA damage response nor directly poisoning TOP2. ${ }^{66}$ Furthermore, it was observed that 9-aminoacridine could inhibit Pol I transcription, reducing levels of 47 S rRNA, albeit at relatively high concentrations. In one study, treatment of 3 T 3 cells at $10 \mu \mathrm{M}$ provided the same amount of reduction in the 47S rRNA transcript as ActD at $5 \mathrm{nM} .{ }^{67}$ Similar RNA synthesis inhibition potencies have been observed for several acridine derivatives. ${ }^{68}$ Of potentially more interest is 9 -aminoacridine's inhibition of early 32S pre-rRNA processing that occurs as low as $3 \mu \mathrm{M}$ and that it readily binds to RNA. ${ }^{67}$

Camptothecin and its derivatives topotecan and irinotecan, shown in Scheme 1-4, are alkaloids that intercalate into the DNA and prevent elongation by poisoning TOP1, trapping TOP1 cleavage complexes and creating PDBs. ${ }^{69,70}$ In


Camptothecin


Topotecan


Irinotecan

Scheme 1-4. Chemical structures of Camptothecin, Topotecan, and Irinotecan. one study, a reconstituted Pol I transcription experiment was performed in the absence of TOP1. While a $1 \mu \mathrm{M}$ treatment with camptothecin resulted in a roughly $60 \%$ reduction in transcription activity, there was a relatively small difference in activity when the treatment concentration was increased to $10 \mu \mathrm{M} .{ }^{71}$ Camptothecin inhibits 475 rRNA synthesis ${ }^{32}$ through a proposed blocking of the Pol I complex recruitment to the PIC, independent of its TOP1 activity. ${ }^{71}$ However, this activity is quickly reversible after removal of the drug, ${ }^{72}$ as opposed to ActD, because camptothecin does not affect the catalytic activity of the enzyme. ${ }^{71}$ Camptothecin also inhibits a specific rRNA processing step, preventing conversion of 32S rRNA to 28 S rRNA. ${ }^{32,72}$ Camptothecin is no longer pursued clinically due to intolerable adverse effects and low therapeutic index. ${ }^{73}$ Likewise, topotecan and irinotecan have experienced limited clinical use due to dose-limiting toxicity and chemical instability involving opening of lactone E-ring. ${ }^{73}$

Alkylating agents are another class of compounds observed to have inhibitory effects on rRNA transcription. ${ }^{32}$ Alkylating agents can be monofunctional or bifunctional, reacting with only one DNA strand or two DNA strands resulting in a covalent crosslink, respectively. ${ }^{74}$ The antibiotic mitomycin C, shown in Scheme

1-5, has been shown to affect rRNA transcription, ${ }^{32}$ presumably through sitespecific crosslinking of CpG sequences, ${ }^{75}$ preventing separation of the DNA strands for transcription. ${ }^{74}$ Mitomycin C, a quinone, undergoes enzymatic reduction, triggering a cascade of spontaneous transformations resulting in the opening of the aziridine ring and production of a reactive electrophilic vinylogous quinone methide intermediate. ${ }^{75}$ The short-lived intermediate can undergo two DNA alkylation reactions with the N-7 guanine residues to produce covalent crosslinks. ${ }^{74,75}$ However, there has been some controversy regarding nuclear DNA damage being the primary mechanism of action. Mitomycin C reduction likely occurs in the cytosol and translocation to the nucleus is speculative and improbable. ${ }^{76}$ Indeed, to observe 47S transcription inhibition, concentrations well in excess of $10 \mu \mathrm{M}$ were required, ${ }^{32}$ and nonbiologically relevant conditions have been required to observe mitomycin C - DNA adducts. ${ }^{76}$ Instead, it has been proposed that mitomycin C primarily affects ribosome biogenesis by binding to 18 S rRNA in the cytosol and causing its degradation at more physiologically relevant concentrations. ${ }^{76}$


Mitomycin C

Scheme 1-5. Chemical structure of Mitomycin C.

The platinum-based drugs cisplatin and oxaliplatin, depicted in Scheme 16 , are alkylating agents that have been observed to inhibit rDNA transcription. ${ }^{32}$ Cisplatin reacts with the N7-site of purine DNA bases to form primarily 1,2intrastrand GpG and ApG crosslinks, resulting in DNA damage-mediated activation of apoptosis. ${ }^{77}$ Although cisplatin cytotoxicity has been primarily attributed to DNA damage, the notion of Pol I inhibition playing a part in its mechanism of action has been questioned. This stems from observations that UBF binds to cisplatin $\mathrm{d}(\mathrm{GpG})$ intrastrand crosslinks with similar affinity compared to its affinity for the rDNA promoter. ${ }^{78,79}$ There is some uncertainty about specifically how UBF-binding contributes to the cytotoxicity. The two main hypotheses are: (1) that high mobility group (HMG) box proteins (HMG-BPs), such as UBF, bind to cisplatin adducts and shield the damaged DNA sites from DNA-repair enzymes, resulting in inefficient DNA lesion repair, and (2) that UBF binding to cisplatin adducts prevents its binding to the rDNA promoter, reducing SL1 recruitment and PIC formation, resulting in Pol I inhibition. ${ }^{77-81}$ However, there is some contention that these observations may not be clinically relevant because the dose of cisplatin needed to inhibit Pol I



Cisplatin
Oxaliplatin

Scheme 1-6. Chemical structures of Cisplatin and Oxaliplatin.
transcription $\left(\mathrm{IC}_{50} 30 \mu \mathrm{M}\right)$ is far higher than physiologically relevant doses (1.4-19 $\mu \mathrm{M}) .{ }^{32,82}$ Cisplatin and oxaliplatin differ in this aspect. Oxaliplatin exhibited similar or greater cytotoxicity, compared with cisplatin, in several human tumor cell lines despite forming significantly fewer DNA adducts ${ }^{83}$ and reduced UBF binding to the DNA adduct. ${ }^{79}$ Paradoxically, oxaliplatin is a better Pol I inhibitor than cisplatin even though oxaliplatin produces less DNA damage and UBF decoy recruitment. ${ }^{32,82}$ Instead, oxaliplatin is proposed to induce cytotoxicity by ribosome biogenesis stress at clinically relevant and lower concentrations, whereas cisplatin cytotoxicity is primarily driven by DNA damage. ${ }^{82,84,85}$ Although the platin drugs are widely used to treat solid tumors, cisplatin treatment has been associated with toxic side effects such as nephrotoxicity, hepatotoxicity, and cardiotoxicity, as well as several resistance mechanisms. ${ }^{77,86}$ Cross-resistance is not common between cisplatin and oxaliplatin, ${ }^{84,87}$ however, oxaliplatin is still dose-limited by peripheral neuropathy-induced neurotoxicity. ${ }^{88}$

Recently, there has been a shift towards discovering molecules that inhibit ribosome biogenesis as a primary mode of action, rather than as a secondary effect. Cylene Pharmaceuticals has studied a series of G4-binders that have anticancer properties. The series begins with QQ58, a fluoroquinolone dual G4binder and TOP2 catalytic inhibitor, ${ }^{89}$ shown in Scheme 1-7. From this scaffold, CX-3543 (Quarfloxin) and CX-5461 (Pidnarulex) were derived ${ }^{90}$ (Scheme 1-7). Quarfloxin was proposed to inhibit Pol I transcription by binding to rDNA G4s, disrupting the interaction between the G4 and nucleolin, ${ }^{91}$ a nucleolar protein necessary for Pol I transcription. ${ }^{92-94}$ By stabilizing the G4 and dislocating nucleolin
from the nucleolus to the nucleoplasm, a transcription block occurs and prevents the Pol I elongation step. ${ }^{91}$ Pol I rRNA transcription is preferentially inhibited over Pol II transcription as well as over DNA and protein synthesis. ${ }^{91}$ Interestingly, the TOP2 inhibitory activity from QQ58 was lost in Quarfloxin, as no TOP1/2 inhibition was observed. ${ }^{91}$ Quarfloxin has completed two separate phase I clinical trials. ${ }^{95,96}$ Although Quarfloxin exposure levels were achieved within the range of observed in vitro potency, ${ }^{91}$ and it was advanced to a phase II clinical trial for neuroendocrine and carcinoid tumors (NTC00780663), it lacked sufficient efficacy to justify further clinical development. ${ }^{90}$


QQ58


CX-5461 (Pidnarulex)


CX-3543 (Quarfloxin)


PMR-116

Scheme 1-7. Chemical structures of QQ58, CX-3543 (Quarfloxin), CX-5461 (Pidnarulex), and PMR-116.

CX-5461 (Pidnarulex) was discovered in a cell-based screening assay identifying preferential Pol I transcription inhibitors compared to Pol II inhibitors. ${ }^{97}$ Pidnarulex exhibited ~200-fold higher sensitivity for Pol I inhibition over Pol II, and it was initially proposed that transcription inhibition was the result of prevention of PIC formation by disruption of SL1 binding to the rDNA promoter. ${ }^{97}$ A later study concluded that instead of blocking PIC formation, Pol I inhibition was achieved by blocking the promoter escape step of the conversion from initiation-competent Pol I to the elongation complex. ${ }^{98}$ It was then discovered that Pidnarulex was able to kill BRCA-deficient cancer cells with an $\mathrm{IC}_{50}$ of 4.8 nM , a lower drug concentration than that needed to observe Pol I inhibition. ${ }^{99}$ Like its predecessors, Pidnarulex also binds and stabilizes G4 structures and is accompanied by a dramatic increase of DNA damage foci in cells. ${ }^{99}$ BRCA-deficient cells are particularly sensitive to this mechanism because of less efficient repair of G4-associated DNA damage and inability to bypass the drug-stabilized G4 structures during DNA replication leading to DNA damage accumulation and apoptosis. ${ }^{99,100}$ Soon after, it was proposed that the actual target of Pidnarulex is TOP2 instead of Pol I. ${ }^{101-103}$ Pidnarulex-induced TOP2 $\beta$ poisoning killed neuroblastoma cell lines CHP-134 and IMR-5 at low enough concentrations ( $0.2 \mu \mathrm{M}$ and $0.05 \mu \mathrm{M}$, respectively) that Pol I inhibition was not observed. ${ }^{102}$ Increased radiosensitization in solid tumor cell lines was also observed at concentrations that did not involve Pol I inhibition. ${ }^{104}$ Pidnarulex has completed phase I clinical trials in advanced hematologic cancers ${ }^{105}$ and advanced solid tumors with DNA-repair deficiencies. ${ }^{106}$ Unfortunately, TOP2 $\beta$ poisoning may pose a major roadblock to further clinical development. In addition to associated
cardiotoxicity, ${ }^{58}$ TOP2 $\beta$ is highly expressed in the brain, necessitating a blood-brain barrier penetration assessment. ${ }^{102}$ A new generation of Pol I inhibitor is being developed by Pimera Inc., represented by PMR-116 ${ }^{107,108}$ (Scheme 1-7). Although details are scarce, the proposed mechanism of action seems to be dual targeting of Pol I and 4E-BP1 phosphorylation, and the compound is currently in a phase I dose escalation trial in patients with solid tumors (ACTRN12620001146987). ${ }^{107,108}$

Non-genotoxic induction of nucleolar stress is an attractive therapeutic approach, benefiting from Pol I transcriptional inhibition yet limiting toxicity from DNA-damage. One strategy that has recently gained popularity is Pol I inhibition via proteasomal degradation of the Pol I large catalytic subunit, RPA194. This mechanism is exemplified by BMH-21 (described in detail later in the review), but recently additional compounds such as amodiaquine, ${ }^{109}$ hernandonine, ${ }^{110}$ and sempervirine, ${ }^{111}$ shown in Scheme 1-8, have been observed to cause proteasomal degradation of RPA194.

The antimalarial drug amodiaquine was observed to inhibit autophagy and trigger proteasomal degradation of RPA194, albeit at likely clinically irrelevant doses $(10-20 \mu \mathrm{M}) .{ }^{109}$ To put this into perspective, previous pharmacokinetic profiling of amodiaquine at a normal malarial treatment dose of $10 \mathrm{mg} / \mathrm{kg} / \mathrm{day}$ resulted in an average $\mathrm{C}_{\text {max }}$ of roughly $0.08 \mu \mathrm{M}$, an exposure far below observed


Amodiaquine


Hernandonine


Amopyroquine


Sempervirine

Scheme 1-8. Chemical structures of Amodiaquine, Amopyroquine, Hernandonine, and Sempervirine.
in vitro efficacious doses. ${ }^{112}$ Although this phenotype was observed without a subsequent DNA damage response, measured by quantification of $\gamma-\mathrm{H} 2 \mathrm{AX}$ positive foci, amodiaquine antimalarial use is limited by hepatotoxicity. ${ }^{113}$ Amodiaquine contains a $p$-aminophenol moiety that is susceptible to metabolic oxidation to produce a reactive quinone imine intermediate that is able to covalently bind to liver proteins. ${ }^{109,113,114}$ Amopyroquine, a pyrrolidone side chain analog of amodiaquine, showed a slight improvement in RPA194 degradation potency, ${ }^{109}$ however, in vivo exposures ${ }^{115,116}$ are still far below doses required to observe any degradation of RPA194.

Hernandonine, an oxoaporphine alkaloid, was also observed to inhibit Pol I transcription via RPA194 proteasomal degradation in a variety of both cancer cell lines (HeLa and SAS) and normal cell lines (NOK and Beas-2B). ${ }^{110}$ Cytotoxicity
$\mathrm{IC}_{50}$ values in cancer cell lines ranged from $5-15 \mu \mathrm{~g} / \mathrm{mL}$ with high doses (>10 $\mu \mathrm{g} / \mathrm{mL}$ ) causing cellular apoptosis in normal cell lines. A slim therapeutic window may be caused by overlapping mechanisms. Interestingly, other oxoaporphine compounds have been observed to inhibit TOP1, leading to cytotoxicity. ${ }^{117}$ Although the authors did not explicitly perform an orthogonal TOP1 inhibition study, it was stated that TOP1 inhibitory activity could not be ruled out. ${ }^{110}$ Coincidentally, statistically significant $\gamma$-H2AX staining was observed in HeLa, SAS, and NOK cell lines at $10 \mu \mathrm{~g} / \mathrm{mL}$ doses, revealing that TOP1 inhibition or other DNA-damaging mechanisms partially contribute to the observed cytotoxicity.

Sempervirine, a pentacyclic anhydronium indole alkaloid, was recently reported to inhibit Pol I transcription and induce RPA194 proteasomal degradation. ${ }^{111}$ Sempervirine intercalates into both DNA and RNA, with a preference for RNA, without inducing DNA damage, causing a transcriptional block, leading to RPA194 degradation in both p53-wt and p53-null testicular germ cell tumor (TGCT) cell lines. However, it is unclear as to whether sempervirine's RPA194 degradation ability directly or indirectly contributes to its overall efficacy since colony formation inhibition $\mathrm{IC}_{50}$ values ranged from $0.46-0.67 \mu \mathrm{M}$ while RPA194 degradation was observed at a single $5 \mu \mathrm{M}$ dose rather than in a dosetitration method. ${ }^{111}$ Regardless, there may be clinical benefit in TGCT cells in combination with cisplatin. Sempervirine synergized with cisplatin to restore cisplatin sensitivity in cisplatin-resistant cells and increased sensitivity in cisplatinsensitive cells. ${ }^{111}$

BMH-21, the first specific and selective Pol I transcription inhibitor, was initially discovered in a cell-based high-throughput screen as a non-DNA damagesignaling p53 activator. ${ }^{118}$ However, potent growth repression was also observed in a variety of TP53 null and mutant cell lines, indicating that the compound acts in both a p53-dependent and p53-independent manner. ${ }^{119}$ The tetracyclic pyridoquinazolinone fused ring system of $\mathrm{BMH}-21$, pictured in Scheme 1-9, intercalates into rDNA, with a preference for GC-rich DNA, without eliciting a DNA damage response. ${ }^{119,120}$ Intercalation results in a transcription block that prevents elongation of the Pol I complex and causes a nucleolar stress response, ultimately leading to ubiquitination of RPA194 by the E3 ligase SCFFBXL14 and its proteasomemediated degradation. ${ }^{119,121,122}$ Intriguingly, Pol I transcription inhibition and reduced occupancy of the Pol I complex on the rDNA are observed temporally prior to the observed degradation of RPA194, reflecting that the transcriptional blocks directly affect transcription stalling. ${ }^{121}$ Importantly, BMH-21 specifically affects only Pol I transcription ${ }^{119,123}$ and does not exhibit any topoisomerase inhibitory


BMH-21

Scheme 1-9. Chemical structure of BMH-21.
activity. ${ }^{118} \mathrm{~A}$ recent study discovered that BMH-21 affects several steps of the Pol I transcription process, namely initiation, promoter escape, and elongation, with elongation being the most vulnerable to BMH-21 treatment. ${ }^{124}$ This study also confirmed the previously observed GC-rich intercalation preference, with paused Pol I complexes being observed upstream of G-rich DNA regions in BMH-21 treated samples. ${ }^{124}$ The discovery of BMH-21 is an important contribution to the cancer therapeutics field - a nongenotoxic activator of p53dependent/independent nucleolar stress that directly regulates RPA194 through proteasome-mediated control leading to cancer cell death without negatively affecting normal cells and is devoid of off-target effects observed in other chemotherapeutics. Structure-activity relationship studies ${ }^{120,125}$ have been conducted, and preclinical studies are ongoing on BMH-21 analogs. Current efforts center around identifying key pharmacophores and addressing off-targets to improve the therapeutic window. BMH-21 provides the starting point upon which this work aims to further understand from a medicinal chemistry and chemical biology perspective.

Pol I transcription of the 47S rRNA precursor serves as the rate-limiting step in ribosome biogenesis, and consequently, is frequently deregulated in many cancers. Recently, there has been more appreciation and attention dedicated to targeting the nucleolus as a cancer therapy, with nongenotoxic nucleolar stress induction as a particularly attractive therapeutic approach. Pol I inhibitors offer a promising approach to disproportionally affecting cancer cells while limiting toxicity to normal cells. While many classical chemotherapeutic agents exhibit secondary
effects on Pol I, a new generation of selective Pol I inhibitors will provide new cancer therapies and novel tools to aid a further mechanistic understanding of Pol I and ribosome biogenesis.

## References

(1) Goodfellow, S. J.; Zomerdijk, J. C. B. M. Basic Mechanisms in RNA Polymerase I Transcription of the Ribosomal RNA Genes. Subcell. Biochem. 2013, 61, 211-236. https://doi.org/10.1007/978-94-007-45254_10/TABLES/2.
(2) Birch, J. L.; Zomerdijk, J. C. B. M. Structure and Function of Ribosomal RNA Gene Chromatin. Biochem. Soc. Trans. 2008, 36 (4), 619-624. https://doi.org/10.1042/BST0360619.
(3) Nurk, S.; Koren, S.; Rhie, A.; Rautiainen, M.; Bzikadze, A. V.; Mikheenko, A.; Vollger, M. R.; Altemose, N.; Uralsky, L.; Gershman, A.; Aganezov, S.; Hoyt, S. J.; Diekhans, M.; Logsdon, G. A.; Alonge, M.; Antonarakis, S. E.; Borchers, M.; Bouffard, G. G.; Brooks, S. Y.; Caldas, G. V.; Chen, N.-C.; Cheng, H.; Chin, C.-S.; Chow, W.; de Lima, L. G.; Dishuck, P. C.; Durbin, R.; Dvorkina, T.; Fiddes, I. T.; Formenti, G.; Fulton, R. S.; Fungtammasan, A.; Garrison, E.; Grady, P. G. S.; Graves-Lindsay, T. A.; Hall, I. M.; Hansen, N. F.; Hartley, G. A.; Haukness, M.; Howe, K.; Hunkapiller, M. W.; Jain, C.; Jain, M.; Jarvis, E. D.; Kerpedjiev, P.; Kirsche, M.; Kolmogorov, M.; Korlach, J.; Kremitzki, M.; Li, H.; Maduro, V. V.; Marschall, T.; McCartney, A. M.; McDaniel, J.; Miller, D. E.; Mullikin, J. C.; Myers, E. W.; Olson, N. D.; Paten, B.; Peluso, P.; Pevzner, P. A.; Porubsky, D.; Potapova, T.; Rogaev, E. I.; Rosenfeld, J. A.; Salzberg, S. L.; Schneider, V. A.; Sedlazeck, F. J.; Shafin, K.; Shew, C. J.; Shumate, A.; Sims, Y.; Smit, A. F. A.; Soto, D. C.; Sović, I.; Storer, J. M.; Streets, A.; Sullivan, B. A.; Thibaud-Nissen, F.; Torrance, J.; Wagner, J.; Walenz, B. P.; Wenger, A.; Wood, J. M. D.; Xiao, C.; Yan, S. M.; Young, A. C.; Zarate, S.; Surti, U.; McCoy, R. C.; Dennis, M. Y.; Alexandrov, I. A.; Gerton, J. L.; O’Neill, R. J.; Timp, W.; Zook, J. M.; Schatz, M. C.; Eichler, E. E.; Miga, K. H.; Phillippy, A. M. The Complete Sequence of a Human Genome. Science 2022, 376 (6588), 44-53. https://doi.org/10.1126/science.abj6987.
(4) McStay, B. Nucleolar Organizer Regions: Genomic ‘Dark Matter’ Requiring Illumination. Genes Dev. 2016, 30 (14), 1598-1610. https://doi.org/10.1101/gad.283838.116.
(5) Drygin, D.; Rice, W. G.; Grummt, I. The RNA Polymerase I Transcription Machinery: An Emerging Target for the Treatment of Cancer. Annu. Rev.

Pharmacol. Toxicol. 2010, 50 (1), 131-156.
https://doi.org/10.1146/annurev.pharmtox.010909.105844.
(6) McStay, B.; Grummt, I. The Epigenetics of RRNA Genes: From Molecular to Chromosome Biology. Annu. Rev. Cell Dev. Biol. 2008, 24 (1), 131-157. https://doi.org/10.1146/annurev.cellbio.24.110707.175259.
(7) Girbig, M.; Misiaszek, A. D.; Müller, C. W. Structural Insights into Nuclear Transcription by Eukaryotic DNA-Dependent RNA Polymerases. Nat. Rev. Mol. Cell Biol. 2022, 23 (9), 603-622. https://doi.org/10.1038/s41580-022-00476-9.
(8) Russell, J.; Zomerdijk, J. C. B. M. The RNA Polymerase I Transcription Machinery. Biochem. Soc. Symp. 2006, 73, 203-216.
https://doi.org/10.1042/bss0730203.
(9) Moss, T.; Langlois, F.; Gagnon-Kugler, T.; Stefanovsky, V. A Housekeeper with Power of Attorney: The RRNA Genes in Ribosome Biogenesis. Cell. Mol. Life Sci. 2007, 64 (1), 29-49. https://doi.org/10.1007/s00018-006-6278-1.
(10) Bywater, M. J.; Pearson, R. B.; McArthur, G. A.; Hannan, R. D. Dysregulation of the Basal RNA Polymerase Transcription Apparatus in Cancer. Nat. Rev. Cancer 2013, 13 (5), 299-314. https://doi.org/10.1038/nrc3496.
(11) Misiaszek, A. D.; Girbig, M.; Grötsch, H.; Baudin, F.; Murciano, B.; Lafita, A.; Müller, C. W. Cryo-EM Structures of Human RNA Polymerase I. Nat. Struct. Mol. Biol. 2021, 28 (12), 997-1008. https://doi.org/10.1038/s41594-021-00693-4.
(12) Zhao, D.; Liu, W.; Chen, K.; Wu, Z.; Yang, H.; Xu, Y. Structure of the Human RNA Polymerase I Elongation Complex. Cell Discov. 2021, 7 (97), 1-12. https://doi.org/10.1038/s41421-021-00335-5.
(13) Russell, J.; Zomerdijk, J. C. B. M. RNA-Polymerase-I-Directed RDNA Transcription, Life and Works. Trends Biochem. Sci. 2005, 30 (2), 87-96. https://doi.org/10.1016/j.tibs.2004.12.008.
(14) Dundr, M.; Hoffmann-Rohrer, U.; Hu, Q.; Grummt, I.; Rothblum, L. I.; Phair, R. D.; Misteli, T. A Kinetic Framework for a Mammalian RNA Polymerase in Vivo. Science 2002, 298 (5598), 1623-1626. https://doi.org/10.1126/science.1076164.
(15) Jacobs, R. Q.; Ingram, Z. M.; Lucius, A. L.; Schneider, D. A. Defining the Divergent Enzymatic Properties of RNA Polymerases I and II. J. Biol. Chem. 2021, 296. https://doi.org/10.1074/jbc.RA120.015904.
(16) Németh, A.; Perez-Fernandez, J.; Merkl, P.; Hamperl, S.; Gerber, J.; Griesenbeck, J.; Tschochner, H. RNA Polymerase I Termination: Where Is the End? Biochim. Biophys. Acta - Gene Regul. Mech. 2013, 1829 (3-4), 306-317. https://doi.org/10.1016/j.bbagrm.2012.10.007.
(17) Henras, A. K.; Plisson-Chastang, C.; O'Donohue, M. F.; Chakraborty, A.; Gleizes, P. E. An Overview of Pre-Ribosomal RNA Processing in Eukaryotes. Wiley Interdiscip. Rev. RNA 2015, 6 (2), 225-242. https://doi.org/10.1002/wrna.1269.
(18) Aubert, M.; O'Donohue, M.-F.; Lebaron, S.; Gleizes, P.-E. Pre-Ribosomal RNA Processing in Human Cells: From Mechanisms to Congenital Diseases. Biomolecules 2018, 8 (4), 123. https://doi.org/10.3390/biom8040123.
(19) Sloan, K. E.; Warda, A. S.; Sharma, S.; Entian, K.-D.; Lafontaine, D. L. J.; Bohnsack, M. T. Tuning the Ribosome: The Influence of RRNA Modification on Eukaryotic Ribosome Biogenesis and Function. RNA Biol. 2017, 14 (9), 1138-1152. https://doi.org/10.1080/15476286.2016.1259781.
(20) Watkins, N. J.; Bohnsack, M. T. The Box C/D and H/ACA SnoRNPs: Key Players in the Modification, Processing and the Dynamic Folding of Ribosomal RNA. WIREs RNA 2012, 3 (3), 397-414. https://doi.org/10.1002/wrna.117.
(21) Montanaro, L.; Treré, D.; Derenzini, M. Nucleolus, Ribosomes, and Cancer. Am. J. Pathol. 2008, 173 (2), 301-310. https://doi.org/10.2353/ajpath.2008.070752.
(22) Derenzini, M.; Montanaro, L.; Treré, D. What the Nucleolus Says to a Tumour Pathologist. Histopathology 2009, 54 (6), 753-762. https://doi.org/10.1111/j.1365-2559.2008.03168.x.
(23) Pianese, G. Beitrag zur Histologie und Aetiologie des Carcinoms; G. Fischer, 1896.
(24) Gaviraghi, M.; Vivori, C.; Tonon, G. How Cancer Exploits Ribosomal RNA Biogenesis: A Journey beyond the Boundaries of RRNA Transcription. Cells 2019, 8 (9), 1098. https://doi.org/10.3390/cells8091098.
(25) Hannan, K. M.; Sanij, E.; Rothblum, L. I.; Hannan, R. D.; Pearson, R. B. Dysregulation of RNA Polymerase I Transcription during Disease. Biochim. Biophys. Acta BBA - Gene Regul. Mech. 2013, 1829 (3-4), 342-360. https://doi.org/10.1016/J.BBAGRM.2012.10.014.
(26) Hannan, K. M.; Brandenburger, Y.; Jenkins, A.; Sharkey, K.; Cavanaugh, A.; Rothblum, L.; Moss, T.; Poortinga, G.; McArthur, G. A.; Pearson, R. B.;

Hannan, R. D. MTOR-Dependent Regulation of Ribosomal Gene Transcription Requires S6K1 and Is Mediated by Phosphorylation of the Carboxy-Terminal Activation Domain of the Nucleolar Transcription Factor UBF†. Mol. Cell. Biol. 2003, 23 (23), 8862-8877.
https://doi.org/10.1128/MCB.23.23.8862-8877.2003.
(27) Muth, V.; Nadaud, S.; Grummt, I.; Voit, R. Acetylation of TAFI68, a Subunit of TIF-IB/SL1, Activates RNA Polymerase I Transcription. EMBO J. 2001, 20 (6), 1353-1362. https://doi.org/10.1093/emboj/20.6.1353.
(28) Hein, N.; Hannan, K. M.; George, A. J.; Sanij, E.; Hannan, R. D. The Nucleolus: An Emerging Target for Cancer Therapy. Trends Mol. Med. 2013, 19 (11), 643-654. https://doi.org/10.1016/j.molmed.2013.07.005.
(29) Grummt, I. Wisely Chosen Paths - Regulation of RRNA Synthesis: Delivered on 30 June 2010 at the 35th FEBS Congress in Gothenburg, Sweden. FEBS J. 2010, 277 (22), 4626-4639. https://doi.org/10.1111/j.1742-4658.2010.07892.x.
(30) Ferreira, R.; Schneekloth, J. S.; Panov, K. I.; Hannan, K. M.; Hannan, R. D. Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age. Cells 2020 Vol 9 Page 266 2020, 9 (2), 266. https://doi.org/10.3390/CELLS9020266.
(31) Rubbi, C. P.; Milner, J. Disruption of the Nucleolus Mediates Stabilization of P53 in Response to DNA Damage and Other Stresses. EMBO J. 2003, 22 (22), 6068-6077. https://doi.org/10.1093/emboj/cdg579.
(32) Burger, K.; Mühl, B.; Harasim, T.; Rohrmoser, M.; Malamoussi, A.; Orban, M.; Kellner, M.; Gruber-Eber, A.; Kremmer, E.; Hölzel, M.; Eick, D. Chemotherapeutic Drugs Inhibit Ribosome Biogenesis at Various Levels. J. Biol. Chem. 2010, 285 (16), 12416-12425.
https://doi.org/10.1074/jbc.M109.074211.
(33) Ladds, M. J. G. W.; Laín, S. Small Molecule Activators of the P53 Response. J. Mol. Cell Biol. 2019, 11 (3), 245-254.
https://doi.org/10.1093/jmcb/mjz006.
(34) Catez, F.; Dalla Venezia, N.; Marcel, V.; Zorbas, C.; Lafontaine, D. L. J.; Diaz, J. J. Ribosome Biogenesis: An Emerging Druggable Pathway for Cancer Therapeutics. Biochem. Pharmacol. 2019, 159, 74-81. https://doi.org/10.1016/j.bcp.2018.11.014.
(35) Sloan, K. E.; Bohnsack, M. T.; Watkins, N. J. The 5S RNP Couples P53 Homeostasis to Ribosome Biogenesis and Nucleolar Stress. Cell Rep. 2013, 5 (1), 237-247. https://doi.org/10.1016/j.celrep.2013.08.049.
(36) Zisi, A.; Bartek, J.; Lindström, M. S. Targeting Ribosome Biogenesis in Cancer: Lessons Learned and Way Forward. Cancers 2022 Vol 14 Page 2126 2022, 14 (9), 2126. https://doi.org/10.3390/CANCERS14092126.
(37) James, A.; Wang, Y.; Raje, H.; Rosby, R.; DiMario, P. Nucleolar Stress with and without P53. Nucleus 2014, 5 (5), 402-426.
https://doi.org/10.4161/nucl.32235.
(38) Soussi, T.; Dehouche, K.; Béroud, C. P53 Website and Analysis of P53 Gene Mutations in Human Cancer: Forging a Link between Epidemiology and Carcinogenesis. Hum. Mutat. 2000, 15 (1), 105-113.
https://doi.org/10.1002/(SICI)1098-1004(200001)15:1<105::AID-HUMU19>3.0.CO;2-G.
(39) Greenblatt, M. S.; Bennett, W. P.; Hollstein, M.; Harris, C. C. Mutations in the P53 Tumor Suppressor Gene: Clues to Cancer Etiology and Molecular Pathogenesis1. Cancer Res. 1994, 54 (18), 4855-4878.
(40) Olausson, K. H.; Nistér, M.; Lindström, M. S. P53 -Dependent and Independent Nucleolar Stress Responses. Cells 2012, 1 (4), 774-798. https://doi.org/10.3390/cells1040774.
(41) Donati, G.; Brighenti, E.; Vici, M.; Mazzini, G.; Treré, D.; Montanaro, L.; Derenzini, M. Selective Inhibition of RRNA Transcription Downregulates E2F-1: A New P53-Independent Mechanism Linking Cell Growth to Cell Proliferation. J. Cell Sci. 2011, 124 (17), 3017-3028. https://doi.org/10.1242/jcs.086074.
(42) Dai, M.-S.; Arnold, H.; Sun, X.-X.; Sears, R.; Lu, H. Inhibition of C-Myc Activity by Ribosomal Protein L11. EMBO J. 2007, 26 (14), 3332-3345. https://doi.org/10.1038/sj.emboj.7601776.
(43) Challagundla, K. B.; Sun, X.-X.; Zhang, X.; DeVine, T.; Zhang, Q.; Sears, R. C.; Dai, M.-S. Ribosomal Protein L11 Recruits MiR-24/MiRISC To Repress c-Myc Expression in Response to Ribosomal Stress. Mol. Cell. Biol. 2011, 31 (19), 4007-4021. https://doi.org/10.1128/MCB.05810-11.
(44) Russo, A.; Esposito, D.; Catillo, M.; Pietropaolo, C.; Crescenzi, E.; Russo, G. Human RpL3 Induces $G_{1} / S$ Arrest or Apoptosis by Modulating P21waf1/Cip1 Levels in a P53-Independent Manner. Cell Cycle 2013, 12 (1), 76-87. https://doi.org/10.4161/cc.22963.
(45) Iadevaia, V.; Caldarola, S.; Biondini, L.; Gismondi, A.; Karlsson, S.; Dianzani, I.; Loreni, F. PIM1 Kinase Is Destabilized by Ribosomal Stress Causing Inhibition of Cell Cycle Progression. Oncogene 2010, 29 (40), 5490-5499. https://doi.org/10.1038/onc.2010.279.
(46) Pitts, S.; Laiho, M. Regulation of RNA Polymerase I Stability and Function. Cancers 2022 Vol 14 Page 5776 2022, 14 (23), 5776. https://doi.org/10.3390/CANCERS14235776.
(47) Carotenuto, P.; Pecoraro, A.; Palma, G.; Russo, G.; Russo, A. Therapeutic Approaches Targeting Nucleolus in Cancer. Cells 2019 Vol 8 Page 1090 2019, 8 (9), 1090. https://doi.org/10.3390/CELLS8091090.
(48) Perry, R. P.; Kelley, D. E. Inhibition of RNA Synthesis by Actinomycin D: Characteristic Dose-Response of Different RNA Species. J. Cell. Physiol. 1970, 76 (2), 127-139. https://doi.org/10.1002/jcp. 1040760202.
(49) Trask, D. K.; Muller, M. T. Stabilization of Type I Topoisomerase-DNA Covalent Complexes by Actinomycin D. Proc. Natl. Acad. Sci. 1988, 85 (5), 1417-1421. https://doi.org/10.1073/pnas.85.5.1417.
(50) Hudson, J. S.; Brooks, S. C.; Graves, D. E. Interactions of Actinomycin D with Human Telomeric G-Quadruplex DNA. Biochemistry 2009, 48 (21), 4440-4447. https://doi.org/10.1021/bi900203z.
(51) Kang, H.-J.; Park, H.-J. Novel Molecular Mechanism for Actinomycin D Activity as an Oncogenic Promoter G-Quadruplex Binder. Biochemistry 2009, 48 (31), 7392-7398. https://doi.org/10.1021/bi9006836.
(52) Niknezhad, Z.; Hassani, L.; Norouzi, D. Investigating Actinomycin D Binding to G-Quadruplex, i-Motif and Double-Stranded DNA in 27-Nt Segment of c-MYC Gene Promoter. Mater. Sci. Eng. C 2016, 58, 11881193. https://doi.org/10.1016/j.msec.2015.09.072.
(53) Maizels, N. Dynamic Roles for G4 DNA in the Biology of Eukaryotic Cells. Nat. Struct. Mol. Biol. 2006, 13 (12), 1055-1059. https://doi.org/10.1038/nsmb1171.
(54) Pommier, Y.; Marchand, C. Interfacial Inhibitors: Targeting Macromolecular Complexes. Nat. Rev. Drug Discov. 2012, 11 (1), 25-36. https://doi.org/10.1038/nrd3404.
(55) Ashour, M. E.; Atteya, R.; El-Khamisy, S. F. Topoisomerase-Mediated Chromosomal Break Repair: An Emerging Player in Many Games. Nat. Rev. Cancer 2015, 15 (3), 137-151. https://doi.org/10.1038/nrc3892.
(56) Nitiss, J. L. Targeting DNA Topoisomerase II in Cancer Chemotherapy. Nat. Rev. Cancer 2009, 9 (5), 338-350. https://doi.org/10.1038/nrc2607.
(57) Damiani, R. M.; Moura, D. J.; Viau, C. M.; Caceres, R. A.; Henriques, J. A. P.; Saffi, J. Pathways of Cardiac Toxicity: Comparison between

Chemotherapeutic Drugs Doxorubicin and Mitoxantrone. Arch. Toxicol. 2016, 90 (9), 2063-2076. https://doi.org/10.1007/s00204-016-1759-y.
(58) Zhang, S.; Liu, X.; Bawa-Khalfe, T.; Lu, L.-S.; Lyu, Y. L.; Liu, L. F.; Yeh, E. T. H. Identification of the Molecular Basis of Doxorubicin-Induced Cardiotoxicity. Nat. Med. 2012, 18 (11), 1639-1642. https://doi.org/10.1038/nm.2919.
(59) Ray, S.; Panova, T.; Miller, G.; Volkov, A.; Porter, A. C. G.; Russell, J.; Panov, K. I.; Zomerdijk, J. C. B. M. Topoisomerase Ila Promotes Activation of RNA Polymerase i Transcription by Facilitating Pre-Initiation Complex Formation. Nat. Commun. 2013, 4. https://doi.org/10.1038/ncomms2599.
(60) Pommier, Y. Drugging Topoisomerases: Lessons and Challenges. ACS Chem. Biol. 2013, 8 (1), 82-95. https://doi.org/10.1021/cb300648v.
(61) Kozurkova, M.; Sabolova, D.; Kristian, P. A New Look at 9-Substituted Acridines with Various Biological Activities. J. Appl. Toxicol. 2021, 41 (1), 175-189. https://doi.org/10.1002/jat.4072.
(62) Oien, D. B.; Ray, U.; Pathoulas, C. L.; Jin, L.; Thirusangu, P.; Jung, D.; Kumka, J. E.; Xiao, Y.; Sarkar Bhattacharya, S.; Montoya, D.; Chien, J.; Shridhar, V. Quinacrine Induces Nucleolar Stress in Treatment-Refractory Ovarian Cancer Cell Lines. Cancers 2021, 13 (18), 4645.
https://doi.org/10.3390/cancers13184645.
(63) Eriksson, A.; Österroos, A.; Hassan, S.; Gullbo, J.; Rickardson, L.; Jarvius, M.; Nygren, P.; Fryknäs, M.; Höglund, M.; Larsson, R. Drug Screen in Patient Cells Suggests Quinacrine to Be Repositioned for Treatment of Acute Myeloid Leukemia. Blood Cancer J. 2015, 5 (4), e307-e307. https://doi.org/10.1038/bcj.2015.31.
(64) Oien, D. B.; Pathoulas, C. L.; Ray, U.; Thirusangu, P.; Kalogera, E.; Shridhar, V. Repurposing Quinacrine for Treatment-Refractory Cancer. Semin. Cancer Biol. 2021, 68, 21-30. https://doi.org/10.1016/j.semcancer.2019.09.021.
(65) Ehsanian, R.; Van Waes, C.; Feller, S. M. Beyond DNA Binding - a Review of the Potential Mechanisms Mediating Quinacrine’s Therapeutic Activities in Parasitic Infections, Inflammation, and Cancers. Cell Commun. Signal. 2011, 9 (1), 13. https://doi.org/10.1186/1478-811X-9-13.
(66) Gurova, K. V.; Hill, J. E.; Guo, C.; Prokvolit, A.; Burdelya, L. G.; Samoylova, E.; Khodyakova, A. V.; Ganapathi, R.; Ganapathi, M.; Tararova, N. D.; Bosykh, D.; Lvovskiy, D.; Webb, T. R.; Stark, G. R.; Gudkov, A. V. Small Molecules That Reactivate P53 in Renal Cell Carcinoma Reveal a NF-KBDependent Mechanism of P53 Suppression in Tumors. Proc. Natl. Acad.

Sci. 2005, 102 (48), 17448-17453.
https://doi.org/10.1073/pnas.0508888102.
(67) Anikin, L.; Pestov, D. G. 9-Aminoacridine Inhibits Ribosome Biogenesis by Targeting Both Transcription and Processing of Ribosomal RNA. Int. J. Mol. Sci. 2022, 23 (3). https://doi.org/10.3390/ijms23031260.
(68) Piestrzeniewicz, M. K.; Wilmańska, D.; Studzian, K.; Szemraj, J.; Czyż, M.; Denny, W. A.; Gniazdowski, M. Inhibition of RNA Synthesis in Vitro by Acridines - Relation between Structure and Activity. Z. Für Naturforschung C 1998, 53 (5-6), 359-368. https://doi.org/10.1515/znc-1998-5-610.
(69) Zhang, H.; Wang, J. C.; Liu, L. F. Involvement of DNA Topoisomerase I in Transcription of Human Ribosomal RNA Genes. Proc. Natl. Acad. Sci. 1988, 85 (4), 1060-1064. https://doi.org/10.1073/pnas.85.4.1060.
(70) Hsiang, Y.-H.; Liu, L. F. Identification of Mammalian DNA Topoisomerase I as an Intracellular Target of the Anticancer Drug Camptothecin1. Cancer Res. 1988, 48 (7), 1722-1726.
(71) Andrews, W. J.; Ray, S.; Panova, T.; Engel, C.; Panov, K. I. DNA Intercalators Inhibit Eukaryotic Ribosomal RNA Synthesis by Impairing the Initiation of Transcription. Genes 2021, 12 (9), 1412.
https://doi.org/10.3390/genes12091412.
(72) Wu, R. S.; Kumar, A.; Warner, J. R. Ribosome Formation Is Blocked by Camptothecin, a Reversible Inhibitor of RNA Synthesis. Proc. Natl. Acad. Sci. 1971, 68 (12), 3009-3014. https://doi.org/10.1073/pnas.68.12.3009.
(73) Delgado, J. L.; Hsieh, C.-M.; Chan, N.-L.; Hiasa, H. Topoisomerases as Anticancer Targets. Biochem. J. 2018, 475 (2), 373-398. https://doi.org/10.1042/BCJ20160583.
(74) Ralhan, R.; Kaur, J. Alkylating Agents and Cancer Therapy. Expert Opin. Ther. Pat. 2007, 17 (9), 1061-1075. https://doi.org/10.1517/13543776.17.9.1061.
(75) Tomasz, M. Mitomycin C: Small, Fast and Deadly (but Very Selective). Chem. Biol. 1995, 2 (9), 575-579. https://doi.org/10.1016/1074-5521(95)90120-5.
(76) Snodgrass, R. G.; Collier, A. C.; Coon, A. E.; Pritsos, C. A. Mitomycin C Inhibits Ribosomal RNA: A NOVEL CYTOTOXIC MECHANISM FOR BIOREDUCTIVE DRUGS*. J. Biol. Chem. 2010, 285 (25), 19068-19075. https://doi.org/10.1074/jbc.M109.040477.
(77) Siddik, Z. H. Cisplatin: Mode of Cytotoxic Action and Molecular Basis of Resistance. Oncogene 2003, 22 (47), 7265-7279. https://doi.org/10.1038/sj.onc. 1206933.
(78) Treiber, D. K.; Zhai, X.; Jantzen, H. M.; Essigmann, J. M. Cisplatin-DNA Adducts Are Molecular Decoys for the Ribosomal RNA Transcription Factor HUBF (Human Upstream Binding Factor). Proc. Natl. Acad. Sci. 1994, 91 (12), 5672-5676. https://doi.org/10.1073/pnas.91.12.5672.
(79) Zhai, X.; Beckmann, H.; Jantzen, H.-M.; Essigmann, J. M. Cisplatin-DNA Adducts Inhibit Ribosomal RNA Synthesis by Hijacking the Transcription Factor Human Upstream Binding Factor. Biochemistry 1998, 37 (46), 16307-16315. https://doi.org/10.1021/bi981708h.
(80) Hamdane, N.; Herdman, C.; Mars, J.-C.; Stefanovsky, V.; Tremblay, M. G.; Moss, T. Depletion of the Cisplatin Targeted HMGB-Box Factor UBF Selectively Induces P53-Independent Apoptotic Death in Transformed Cells. Oncotarget 2015, 6 (29), 27519-27536.
https://doi.org/10.18632/oncotarget.4823.
(81) Jordan, P.; Carmo-Fonseca, M. Cisplatin Inhibits Synthesis of Ribosomal RNA in Vivo. Nucleic Acids Res. 1998, 26 (12), 2831-2836. https://doi.org/10.1093/nar/26.12.2831.
(82) Sutton, E. C.; DeRose, V. J. Early Nucleolar Responses Differentiate Mechanisms of Cell Death Induced by Oxaliplatin and Cisplatin. J. Biol. Chem. 2021, 296. https://doi.org/10.1016/j.jbc.2021.100633.
(83) Woynarowski, J. M.; Faivre, S.; Herzig, M. C. S.; Arnett, B.; Chapman, W. G.; Trevino, A. V.; Raymond, E.; Chaney, S. G.; Vaisman, A.; Varchenko, M.; Juniewicz, P. E. Oxaliplatin-Induced Damage of Cellular DNA. Mol. Pharmacol. 2000, 58 (5), 920-927. https://doi.org/10.1124/mol.58.5.920.
(84) Bruno, P. M.; Liu, Y.; Park, G. Y.; Murai, J.; Koch, C. E.; Eisen, T. J.; Pritchard, J. R.; Pommier, Y.; Lippard, S. J.; Hemann, M. T. A Subset of Platinum-Containing Chemotherapeutic Agents Kills Cells by Inducing Ribosome Biogenesis Stress. Nat. Med. 2017, 23 (4), 461-471. https://doi.org/10.1038/NM.4291.
(85) Sutton, E. C.; E. McDevitt, C.; Y. Prochnau, J.; V. Yglesias, M.; M. Mroz, A.; Chieh Yang, M.; M. Cunningham, R.; H. Hendon, C.; J. DeRose, V. Nucleolar Stress Induction by Oxaliplatin and Derivatives. J. Am. Chem. Soc. 2019, 141 (46), 18411-18415. https://doi.org/10.1021/jacs.9b10319.
(86) Dasari, S.; Bernard Tchounwou, P. Cisplatin in Cancer Therapy: Molecular Mechanisms of Action. Eur. J. Pharmacol. 2014, 740, 364-378. https://doi.org/10.1016/j.ejphar.2014.07.025.
(87) Rixe, O.; Ortuzar, W.; Alvarez, M.; Parker, R.; Reed, E.; Paull, K.; Fojo, T. Oxaliplatin, Tetraplatin, Cisplatin, and Carboplatin: Spectrum of Activity in Drug-Resistant Cell Lines and in the Cell Lines of the National Cancer Institute's Anticancer Drug Screen Panel. Biochem. Pharmacol. 1996, 52 (12), 1855-1865. https://doi.org/10.1016/S0006-2952(97)81490-6.
(88) McKeage, M. J.; Hsu, T.; Screnci, D.; Haddad, G.; Baguley, B. C. Nucleolar Damage Correlates with Neurotoxicity Induced by Different Platinum Drugs. Br. J. Cancer 2001, 85 (8), 1219-1225. https://doi.org/10.1054/bjoc.2001.2024.
(89) Duan, W.; Rangan, A.; Vankayalapati, H.; Kim, M.-Y.; Zeng, Q.; Sun, D.; Han, H.; Fedoroff, O. Yu.; Nishioka, D.; Rha, S. Y.; Izbicka, E.; Von Hoff, D. D.; Hurley, L. H. Design and Synthesis of Fluoroquinophenoxazines That Interact with Human Telomeric G-Quadruplexes and Their Biological Effects. Mol. Cancer Ther. 2001, 1 (2), 103-120.
(90) Xu, H.; Hurley, L. H. A First-in-Class Clinical G-Quadruplex-Targeting Drug. The Bench-to-Bedside Translation of the Fluoroquinolone QQ58 to CX5461 (Pidnarulex). Bioorg. Med. Chem. Lett. 2022, 77, 129016. https://doi.org/10.1016/j.bmcl.2022.129016.
(91) Drygin, D.; Siddiqui-Jain, A.; O’Brien, S.; Schwaebe, M.; Lin, A.; Bliesath, J.; Ho, C. B.; Proffitt, C.; Trent, K.; Whitten, J. P.; Lim, J. K. C.; Von Hoff, D.; Anderes, K.; Rice, W. G. Anticancer Activity of CX-3543: A Direct Inhibitor of RRNA Biogenesis. Cancer Res. 2009, 69 (19), 7653-7661.
https://doi.org/10.1158/0008-5472.CAN-09-1304/655359/P/ANTICANCER-ACTIVITY-OF-CX-3543-A-DIRECT-INHIBITOR.
(92) Rickards, B.; Flint, S. J.; Cole, M. D.; LeRoy, G. Nucleolin Is Required for RNA Polymerase I Transcription In Vivo. Mol. Cell. Biol. 2007, 27 (3), 937948. https://doi.org/10.1128/MCB.01584-06.
(93) Cong, R.; Das, S.; Ugrinova, I.; Kumar, S.; Mongelard, F.; Wong, J.; Bouvet, P. Interaction of Nucleolin with Ribosomal RNA Genes and Its Role in RNA Polymerase I Transcription. Nucleic Acids Res. 2012, 40 (19), 9441-9454. https://doi.org/10.1093/NAR/GKS720.
(94) Durut, N.; Sáez-Vásquez, J. Nucleolin: Dual Roles in RDNA Chromatin Transcription. Gene 2015, 556 (1), 7-12. https://doi.org/10.1016/j.gene.2014.09.023.
(95) Papadopoulos, K.; Mita, A.; Ricart, A.; Hufnagel, D.; Northfelt, D.; Von Hoff, D.; Darjania, L.; Lim, J.; Padgett, C.; Marschke, R. Pharmacokinetic Findings from the Phase I Study of Quarfloxin (CX-3543): A Protein-RDNA Quadruplex Inhibitor, in Patients with Advanced Solid Tumors. Mol. Cancer Ther. 2007, 6 (11_Supplement), B93.
(96) Lim, J.; Padgett, C.; Von Hoff, D.; Rice, W.; Darjania, L.; Phung, J.; Stansfield, R.; Anderes, K.; Marschke, R. Abstract \#3599: Quarfloxin Phase I Clinical Data and Scientific Findings Supporting the Selection of Carcinoid/Neuroendocrine Tumors as the Phase II Indication. Cancer Res. 2009, 69 (9_Supplement), 3599.
(97) Drygin, D.; Lin, A.; Bliesath, J.; Ho, C. B.; O’Brien, S. E.; Proffitt, C.; Omori, M.; Haddach, M.; Schwaebe, M. K.; Siddiqui-Jain, A.; Streiner, N.; Quin, J. E.; Sanij, E.; Bywater, M. J.; Hannan, R. D.; Ryckman, D.; Anderes, K.; Rice, W. G. Targeting RNA Polymerase I with an Oral Small Molecule CX5461 Inhibits Ribosomal RNA Synthesis and Solid Tumor Growth. Cancer Res. 2011, 71 (4), 1418-1430. https://doi.org/10.1158/0008-5472.CAN-10-1728/649391/AM/TARGETING-RNA-POLYMERASE-I-WITH-AN-ORALSMALL.
(98) Mars, J. C.; Tremblay, M. G.; Valere, M.; Sibai, D. S.; Sabourin-Felix, M.; Lessard, F.; Moss, T. The Chemotherapeutic Agent CX-5461 Irreversibly Blocks RNA Polymerase I Initiation and Promoter Release to Cause Nucleolar Disruption, DNA Damage and Cell Inviability. NAR Cancer 2021, 2 (4). https://doi.org/10.1093/NARCAN/ZCAA032.
(99) Xu, H.; Di Antonio, M.; McKinney, S.; Mathew, V.; Ho, B.; O’Neil, N. J.; Santos, N. D.; Silvester, J.; Wei, V.; Garcia, J.; Kabeer, F.; Lai, D.; Soriano, P.; Banáth, J.; Chiu, D. S.; Yap, D.; Le, D. D.; Ye, F. B.; Zhang, A.; Thu, K.; Soong, J.; Lin, S. C.; Tsai, A. H. C.; Osako, T.; Algara, T.; Saunders, D. N.; Wong, J.; Xian, J.; Bally, M. B.; Brenton, J. D.; Brown, G. W.; Shah, S. P.; Cescon, D.; Mak, T. W.; Caldas, C.; Stirling, P. C.; Hieter, P.; Balasubramanian, S.; Aparicio, S. CX-5461 Is a DNA G-Quadruplex Stabilizer with Selective Lethality in BRCA1/2 Deficient Tumours. Nat. Commun. 2017, 8. https://doi.org/10.1038/ncomms14432.
(100) Zimmer, J.; Tacconi, E. M. C.; Folio, C.; Badie, S.; Porru, M.; Klare, K.; Tumiati, M.; Markkanen, E.; Halder, S.; Ryan, A.; Jackson, S. P.; Ramadan, K.; Kuznetsov, S. G.; Biroccio, A.; Sale, J. E.; Tarsounas, M. Targeting BRCA1 and BRCA2 Deficiencies with G-Quadruplex-Interacting Compounds. Mol. Cell 2016, 61 (3), 449-460. https://doi.org/10.1016/j.molcel.2015.12.004.
(101) Bruno, P. M.; Lu, M.; Dennis, K. A.; Inam, H.; Moore, C. J.; Sheehe, J.; Elledge, S. J.; Hemann, M. T.; Pritchard, J. R. The Primary Mechanism of Cytotoxicity of the Chemotherapeutic Agent CX-5461 Is Topoisomerase II Poisoning. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (8), 4053-4060. https://doi.org/10.1073/PNAS.1921649117/-/DCSUPPLEMENTAL.
(102) Pan, M.; Wright, W. C.; Chapple, R. H.; Zubair, A.; Sandhu, M.; Batchelder, J. E.; Huddle, B. C.; Low, J.; Blankenship, K. B.; Wang, Y.; Gordon, B.;

Archer, P.; Brady, S. W.; Natarajan, S.; Posgai, M. J.; Schuetz, J.; Miller, D.; Kalathur, R.; Chen, S.; Connelly, J. P.; Babu, M. M.; Dyer, M. A.; PruettMiller, S. M.; Freeman, B. B.; Chen, T.; Godley, L. A.; Blanchard, S. C.; Stewart, E.; Easton, J.; Geeleher, P. The Chemotherapeutic CX-5461 Primarily Targets TOP2B and Exhibits Selective Activity in High-Risk Neuroblastoma. Nat. Commun. 2021, 12 (1), 6468.
https://doi.org/10.1038/s41467-021-26640-x.
(103) Bossaert, M.; Pipier, A.; Riou, J.-F.; Noirot, C.; Nguyên, L.-T.; Serre, R.-F.; Bouchez, O.; Defrancq, E.; Calsou, P.; Britton, S.; Gomez, D. TranscriptionAssociated Topoisomerase 2a (TOP2A) Activity Is a Major Effector of Cytotoxicity Induced by G-Quadruplex Ligands. eLife 2021, 10, e65184. https://doi.org/10.7554/eLife.65184.
(104) Lehman, S. L.; Schwartz, K. R.; Maheshwari, S.; Camphausen, K.; Tofilon, P. J. CX-5461 Induces Radiosensitization through Modification of the DNA Damage Response and Not Inhibition of RNA Polymerase I. Sci. Rep. 2022, 12 (1). https://doi.org/10.1038/s41598-022-07928-4.
(105) Khot, A.; Brajanovski, N.; Cameron, D. P.; Hein, N.; Maclachlan, K. H.; Sanij, E.; Lim, J.; Soong, J.; Link, E.; Blombery, P.; Thompson, E. R.; Fellowes, A.; Sheppard, K. E.; McArthur, G. A.; Pearson, R. B.; Hannan, R. D.; Poortinga, G.; Harrison, S. J. First-in-Human RNA Polymerase I Transcription Inhibitor CX-5461 in Patients with Advanced Hematologic Cancers: Results of a Phase I Dose-Escalation Study. Cancer Discov. 2019, 9 (8), 1036-1049. https://doi.org/10.1158/2159-8290.CD-18-1455/333397/AM/FIRST-IN-HUMAN-RNA-POLYMERASE-ITRANSCRIPTION.
(106) Hilton, J.; Gelmon, K.; Bedard, P. L.; Tu, D.; Xu, H.; Tinker, A. V.; Goodwin, R.; Laurie, S. A.; Jonker, D.; Hansen, A. R.; Veitch, Z. W.; Renouf, D. J.; Hagerman, L.; Lui, H.; Chen, B.; Kellar, D.; Li, I.; Lee, S. E.; Kono, T.; Cheng, B. Y. C.; Yap, D.; Lai, D.; Beatty, S.; Soong, J.; Pritchard, K. I.; Soria-Bretones, I.; Chen, E.; Feilotter, H.; Rushton, M.; Seymour, L.; Aparicio, S.; Cescon, D. W. Results of the Phase I CCTG IND. 231 Trial of CX-5461 in Patients with Advanced Solid Tumors Enriched for DNA-Repair Deficiencies. Nat. Commun. 2022, 13 (1). https://doi.org/10.1038/s41467-022-31199-2.
(107) Huglo, A.; Rebello, R.; Lawrence, M.; Risbridger, G.; Drygin, D.; Haddach, M.; Hannan, K.; Hannan, R.; Furic, L. Abstract 2155: PMR-116, a Novel Inhibitor of Ribosome Biogenesis with Antitumor Activity in Preclinical Models of Prostate Cancer. Cancer Res. 2022, 82 (12_Supplement), 2155. https://doi.org/10.1158/1538-7445.AM2022-2155.
(108) Huglo, A.; Hedwards, S.; Lawrence, M.; Rebello, R.; Clark, A.; Risbridger, G.; Taylor, R.; Drygin, D.; Haddach, M.; Hannan, K. M.; Hannan, R. D.; Furic, L. Abstract PR006: PMR-116, a Novel Inhibitor of Ribosome Biogenesis with Antitumor Activity in Preclinical Models of Prostate Cancer. Cancer Res. 2023, 83 (11_Supplement), PR006. https://doi.org/10.1158/1538-7445.PRCA2023-PR006.
(109) Espinoza, J. A.; Zisi, A.; Kanellis, D. C.; Carreras-Puigvert, J.; Henriksson, M.; Hühn, D.; Watanabe, K.; Helleday, T.; Lindström, M. S.; Bartek, J. The Antimalarial Drug Amodiaquine Stabilizes P53 through Ribosome Biogenesis Stress, Independently of Its Autophagy-Inhibitory Activity. Cell Death Differ. 2020, 27 (2), 773-789. https://doi.org/10.1038/s41418-019-0387-5.
(110) Chen, Y. T.; Chen, J. J.; Wang, H. T. Targeting RNA Polymerase i with Hernandonine Inhibits Ribosomal RNA Synthesis and Tumor Cell Growth. Mol. Cancer Res. 2019, 17 (11), 2294-2305. https://doi.org/10.1158/1541-7786.MCR-19-0402/82067/AM/TARGETING-RNA-POLYMERASE-I-WITHHERNANDONINE.
(111) Caggiano, C.; Guida, E.; Todaro, F.; Bielli, P.; Mori, M.; Ghirga, F.; Quaglio, D.; Botta, B.; Moretti, F.; Grimaldi, P.; Rossi, P.; Jannini, E. A.; Barchi, M.; Dolci, S. Sempervirine Inhibits RNA Polymerase I Transcription Independently from P53 in Tumor Cells. Cell Death Discov. 2020, 6 (1), 115. https://doi.org/10.1038/s41420-020-00345-4.
(112) Orrell, C.; Little, F.; Smith, P.; Folb, P.; Taylor, W.; Olliaro, P.; Barnes, K. I. Pharmacokinetics and Tolerability of Artesunate and Amodiaquine Alone and in Combination in Healthy Volunteers. Eur. J. Clin. Pharmacol. 2008, 64 (7), 683-690. https://doi.org/10.1007/S00228-007-0452-8/TABLES/2.
(113) Shimizu, S.; Atsumi, R.; Itokawa, K.; Iwasaki, M.; Aoki, T.; Ono, C.; Izumi, T.; Sudo, K.; Okazaki, O. Metabolism-Dependent Hepatotoxicity of Amodiaquine in Glutathione-Depleted Mice. Arch. Toxicol. 2009, 83 (7), 701-707. https://doi.org/10.1007/s00204-009-0436-9.
(114) Maggs, J. L.; Tingle, M. D.; Kitteringham, N. R.; Park, B. K. Drug-Protein Conjugates-XIV: Mechanisms of Formation of Protein-Arylating Intermediates from Amodiaquine, a Myelotoxin and Hepatotoxin in Man. Biochem. Pharmacol. 1988, 37 (2), 303-311. https://doi.org/10.1016/0006-2952(88)90733-2.
(115) Verdier, F.; Pussard, E.; Clavier, F.; Bras, J. L.; Gaudebout, C.; Claude Bernard, pital; Cedex, P. Pharmacokinetics of Intramuscular Amopyroquin in Healthy Subjects and Determination of a Therapeutic Regimen for Plasmodium Falciparum Malaria; 1989; Vol. 33, pp 316-321.
(116) Pussard', E.; Chassard, D.; Clavier, F.; Bry<, P.; Verdier, F. Pharmacokinetics and Metabolism of Amopyroquin after Administration of Two Doses of 6 Mg/Kg Im 24 h Apart to Healthy Volunteers; 1994; Vol. 34, pp 803-808. https://academic.oup.com/jac/article/34/5/803/776102.
(117) Wei, Y.-B.; Li, Y.-X.; Song, H.; Feng, X.-J. Design, Synthesis and Anticancer Activity of Oxoaporphine Alkaloid Derivatives. J. Enzyme Inhib. Med. Chem. 2014, 29 (5), 722-727. https://doi.org/10.3109/14756366.2013.845818.
(118) Peltonen, K.; Colis, L.; Liu, H.; Jäämaa, S.; Moore, H. M.; Enbäck, J.; Laakkonen, P.; Vaahtokari, A.; Jones, R. J.; af Hällström, T. M.; Laiho, M. Identification of Novel P53 Pathway Activating Small-Molecule Compounds Reveals Unexpected Similarities with Known Therapeutic Agents. PLoS ONE 2010, 5 (9), e12996. https://doi.org/10.1371/journal.pone.0012996.
(119) Peltonen, K.; Colis, L.; Liu, H.; Trivedi, R.; Moubarek, M. S.; Moore, H. M.; Bai, B.; Rudek, M. A.; Bieberich, C. J.; Laiho, M. A Targeting Modality for Destruction of RNA Polymerase I That Possesses Anticancer Activity. Cancer Cell 2014, 25 (1), 77-90. https://doi.org/10.1016/j.ccr.2013.12.009.
(120) Colis, L.; Peltonen, K.; Sirajuddin, P.; Liu, H.; Sanders, S.; Ernst, G.; Barrow, J. C.; Laiho, M. DNA Intercalator BMH-21 Inhibits RNA Polymerase I Independent of DNA Damage Response. Oncotarget 2014, 5 (12), 43614369. https://doi.org/10.18632/oncotarget. 2020.
(121) Wei, T.; Najmi, S. M.; Liu, H.; Peltonen, K.; Kucerova, A.; Schneider, D. A.; Laiho, M. Small-Molecule Targeting of RNA Polymerase I Activates a Conserved Transcription Elongation Checkpoint. Cell Rep. 2018, 23 (2), 404-414. https://doi.org/10.1016/J.CELREP.2018.03.066.
(122) Pitts, S.; Liu, H.; Ibrahim, A.; Garg, A.; Felgueira, C. M.; Begum, A.; Fan, W.; Teh, S.; Low, J. Y.; Ford, B.; Schneider, D. A.; Hay, R.; Laiho, M. Identification of an E3 Ligase That Targets the Catalytic Subunit of RNA Polymerase I upon Transcription Stress. J. Biol. Chem. 2022, 298 (12). https://doi.org/10.1016/j.jbc.2022.102690.
(123) Jacobs, R. Q.; Fuller, K. B.; Cooper, S. L.; Carter, Z. I.; Laiho, M.; Lucius, A. L.; Schneider, D. A. RNA Polymerase I Is Uniquely Vulnerable to the SmallMolecule Inhibitor BMH-21. Cancers 2022, 14 (22), 5544. https://doi.org/10.3390/cancers14225544.
(124) Jacobs, R. Q.; Huffines, A. K.; Laiho, M.; Schneider, D. A. The Small Molecule BMH-21 Directly Inhibits Transcription Elongation and DNA Occupancy of RNA Polymerase I in Vivo and in Vitro. J. Biol. Chem. 2022, 298 (1). https://doi.org/10.1016/j.jbc.2021.101450.
(125) Dorado, T. E.; de León, P.; Begum, A.; Liu, H.; Chen, D.; Rajeshkumar, N. V.; Rey-Rodriguez, R.; Hoareau-Aveilla, C.; Alcouffe, C.; Laiho, M.; Barrow, J. C. Discovery and Evaluation of Novel Angular Fused Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors. ACS Med. Chem. Lett. 2022, 13 (4), 608-614.
https://doi.org/10.1021/acsmedchemlett.1c00660.

## Chapter 2. Discovery and Evaluation of Novel Angular Fused Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors

This chapter was adapted with permission from Dorado, T. E.; de León, P.; Begum, A.; Liu, H.; Chen, D.; Rajeshkumar, N. V.; Rey-Rodriguez, R.; HoareauAveilla, C.; Alcouffe, C.; Laiho, M.; Barrow, J. C. Discovery and Evaluation of Novel Angular Fused Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors. ACS Med. Chem. Lett. 2022, 13 (4), 608-614. (DOI:
10.1021/acsmedchemlett.1c00660). Copyright 2022 American Chemical Society.

## Introduction

RNA polymerase $I(\operatorname{Pol} I)$ is a DNA-dependent RNA polymerase that is responsible for transcription of the 47S ribosomal RNA (rRNA) precursor in the nucleolus. This 47S pre-rRNA is further processed into the mature $28 \mathrm{~S}, 5.8 \mathrm{~S}$, and 18 S rRNAs that are assembled into ribosomes. Pol I transcriptional activity is frequently deregulated in cancers, reflecting a need for increased ribosome biogenesis and protein synthesis. Increased Pol I activity is generally not attributed to gain-of-function mutations or amplification in the core Pol I transcription apparatus. Rather, increased rDNA transcription can be accomplished by the activation of oncogenes and upstream signaling pathways that promote preinitiation complex assembly or loss-of-function mutations in tumor suppressors that repress Pol I transcription. Although there is currently no evidence that suggests that increased Pol I transcription is a causative factor of cancer formation, it is certainly possible that cancer cells can become reliant on the process and subsequently become selectively vulnerable to therapeutics that inhibit Pol I. ${ }^{1}$ Instead of targeting specific features of certain cancers, inhibiting a process that is critical for a wide range of cancers can provide a therapeutic benefit. ${ }^{2}$

The morphology of the nucleolus in tumors has been appreciated by pathologists for over a century, and prognostic markers staining the nucleolar components have been developed. ${ }^{3}$ As our understanding of the roles of Pol I and the nucleolus in malignancy has evolved, ${ }^{4}$ interest in developing compounds that specifically target Pol I is increasing. ${ }^{5}$ During the normal function of Pol I transcription, the tumor suppressor, p53, is sequestered by the E3 ubiquitin ligase, Mdm2. ${ }^{6}$ This interaction keeps p53 levels low as it is constantly ubiquitinated and degraded. Induction of nucleolar stress activates the ribosomal surveillance pathway, resulting in the accumulation of $\mathrm{p} 53^{7}$ and potentially leading to outcomes such as apoptosis and cell cycle arrest in cancer cells. Furthermore, as demonstrated by our previous work ${ }^{8}$ and others, ${ }^{9}$ normal cells are able to recover from this treatment. Thus, the inhibition of Pol I transcription in the nucleolus and the induction of nucleolar stress on target cancer cells is an attractive therapeutic strategy. Whereas several chemotherapeutics such as Actinomycin $D^{10}$ and CX$5461{ }^{11}$ have Pol I inhibition as part of their multimodal mode of action, no specific Pol I inhibitor is in clinical use. ${ }^{2}$

A proof of principle has been established for lead compound BMH-21 (1), shown in Figure 2-1. Compound 1 was found to inhibit Pol I transcription in a p53independent manner by inducing the proteasome-mediated degradation of RPA194, the large catalytic subunit of Pol I, as a result of intercalating into GC-rich rDNA without eliciting a DNA damage response. ${ }^{8}$ Additionally, the destruction of RPA194 has been correlated with cancer cell killing. ${ }^{8}$ This offers a novel mechanism of action of inhibition of Pol I transcription, and the basis for




Figure 2-1. Comparison of key data for previously reported compounds 1 and 2. aRPA194 degradation measured in A375 cells. $\mathrm{IC}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate. ${ }^{\mathrm{b}} \mathrm{CYP} 1 \mathrm{~A} 2$ inhibition analysis performed using human hepatic CYP450s (baculovirus-insect-cell expression system) expressing the isoform 1A2. chERG inhibition analysis performed using HEK293 cells stably transfected with hERG cDNA and measured by QPatch. IC50 value represents the mean of $n=3$.
determining the on-target potency of compounds. The key components of this mechanism include the absence of eliciting a DNA damage response and independence of p53 activity. Compound $\mathbf{1}$ induces nucleolar stress, ${ }^{12}$ but is still efficacious in the absence of p53. This highlights the importance that RPA194 has in rDNA transcription, and a quantitative assay measuring the extent of RPA194 degradation after compound treatment in A375 (human malignant melanoma) cells has been developed and is used to determine potency of compounds. ${ }^{8}$ A375 cells are treated with compounds in an eight-point titration for 3 h . After fixing, permeabilizing, and blocking, cells are immunostained for RPA194 and observed by fluorescence microscopy. Using this assay, previous findings indicated that a four-ring tetracycle intercalator, secondary amide, and two-carbon linker between amide and terminal basic amine were optimal for potency (Figure 2-1). ${ }^{12}$ Whereas the presence of a basic amine is important for both potency and solubility, it engenders some hERG inhibitory activity ( $4.8 \mu \mathrm{M}$ ), an undesirable off-target. The human ether-a-go-go related gene (hERG) protein is involved in cardiac repolarization. The inhibition of hERG can cause QT prolongation and result in
torsades de pointes and cardiac arrhythmia. ${ }^{13}$ Further, CYP1A2 inhibition has been seen as a consistent feature of 1 and similar compounds. CYP1A2 is part of the cytochrome P450 enzyme superfamily and is responsible for metabolism of numerous commonly used drugs and endogenous compounds. ${ }^{14}$ The inhibition of CYP1A2 could cause drug-drug interactions, especially in an oncology setting where combination therapy is common. Herein, we describe efforts to improve this class of RNA Pol I inhibitor, especially in regards to unwanted hERG and CYP1A2 activity.

## Results and Discussion

Initial structure-activity relationships (SARs) suggested that although the dimethylamino side chain present in compound 1 was optimal, similar RPA194 potency could be achieved when the methyl groups were wrapped into rings, demonstrated by compounds 2 and 3 (Table 2-1). The pyrrolidine side chain in 2 was found to greatly reduce CYP1A2 inhibition while maintaining adequate potency (Figure 2-1). As shown in Table 2-1, additional conservative changes in these structures were explored to modulate physical properties such as basicity and lipophilicity that may eventually help address potential hERG ${ }^{13}$ and CYP1A2 ${ }^{14}$ inhibition liabilities.

Offsetting the ring by incorporating one carbon of the linker into the ring allowed compounds 4,5 , and 6 to retain some potency (IC50 of $0.78,0.59$, and $0.92 \mu \mathrm{M}$, respectively). Conversion of the piperidine to an offset capped

|  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Ex. | R | $\begin{aligned} & \text { RPA194 IC50 } \\ & (\mu \mathrm{M})^{\mathrm{a}} \end{aligned}$ | Ex. | R | $\begin{aligned} & \text { RPA194 IC }_{50} \\ & (\mu \mathrm{M})^{\mathrm{a}} \end{aligned}$ |
| 1 |  | 0.33 | 12 |  | 5.8 |
| 2 |  | 0.37 | 13 |  | >10 |
| 3 |  | 0.18 | 14 |  | >10 |
| 4 | $\langle\text { HN }$ | 0.78 | 15 |  | >10 |
| 5 |  | 0.59 | 16 |  | >10 |
| 6 |  | 0.92 | 17 |  | 0.78 |
| 7 | $\mathrm{T}_{\mathrm{H}_{3} \mathrm{C}^{\mathrm{N}} \mathrm{O}}$ | 1.0 | 18 |  | 0.60 |
| 8 |  | 0.65 | 19 |  | 0.74 |
| 9 | $\left\langle\underset{\mathrm{H}_{3} \mathrm{C}^{\mathrm{N}}}{ }\right.$ | 3.9 | 20 |  | 10 |
| 10 |  | 0.99 | 21 |  | >10 |
| 11 |  | 10 |  |  |  |

Table 2-1. Compound 1 Sidechain Modifications. aRPA194 degradation measured in A375 cells. $\mathrm{IC}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
morpholine 7 resulted in reduced potency. Installation of hydroxymethyl 8 was moderately tolerated, allowing a potential avenue for reduction of lipophilicity, but no constraint resulted in improved potency. Furthermore, capping the secondary amine generated from these constraints led to decreases in activity (compounds 9, 10, and $11 \mathrm{IC}_{50}$ of $3.9,0.99$, and $10 \mu \mathrm{M}$, respectively). Attempting to modulate basicity of the amine by replacing a hydrogen atom with a fluorine led to a substantial loss of potency or inactivity (compounds 12-16). Interestingly, the fourposition monofluoro analog 17 was moderately tolerated in addition to other four-position-substituted analogs (compounds 18 and $19 \mathrm{IC}_{50}$ of 0.60 and $0.74 \mu \mathrm{M}$, respectively). However, replacing the piperidine ring with thiomorpholine $\mathbf{2 0}$ or the corresponding sulphone 21 led to loss of activity. With SAR tolerability of the side chain being seemingly more sensitive than originally predicted, another effort was made to modify the central tetracyclic core.

A prior SAR suggested the four fused ring tetracycle of 1 was optimal. ${ }^{12}$ Importantly, this ring structure is somewhat basic, which imparts improved solubility of the compounds at slightly acidic pH . Truncating the core to a tricycle has previously been shown to decrease RPA194 potency, ${ }^{12}$ so new heterocycles, depicted in Table 2-2, were designed to further probe the sensitivity of RPA194 potency to changes in the core structure. Replacing the "D" ring (Figure 2-1) with various aliphatic rings in the case of compounds 22, 23, 24, and 25 resulted in lower potency ( $\mathrm{IC}_{50}$ of $1.0,1.4,0.54$, and $0.42 \mu \mathrm{M}$, respectively). Similar to previous observations, substituted tricyclic cores 26 and 27 showed decreased potency (IC50 of 1.2 and $6.9 \mu \mathrm{M}$, respectively). An attempt was made to introduce
Ex.

Table 2-2. Modification of the Tetracyclic Core. aRPA194 degradation measured in A375 cells. IC50 represents the mean of duplicate independent biological experiments performed in triplicate.
a conservative change in the tetracycle core to affect the electronics of the ring system, but this was also not tolerated (Compound 28, IC50 of $5.5 \mu \mathrm{M}$ ). Removing the carbonyl of the tetracycle was also not tolerated and possibly disrupted an essential hydrogen-bonding interaction with rDNA (exemplified by compounds 29 and 30; IC 50 of 1.7 and $6.0 \mu \mathrm{M}$, respectively). Introducing a turn in the tetracycle between the " $C$ " and " $D$ " rings resulted in similar potency to that of reference compound 1 (Compound 31, IC50 of $0.21 \mu \mathrm{M}$ ). Remarkably, introducing a turn in the opposite direction was not tolerated as exemplified by compounds 32 and 33 ( $\mathrm{IC}_{50}$ of $>10$ and $4.2 \mu \mathrm{M}$ ). With the discovery of compound 31 , a further SAR was conducted to determine optimal side chains to pair with the new core as summarized in Table 2-3.

As previously seen, a basic amine was required for activity. Replacing the amine with a hydroxyl essentially resulted in inactivity (compound 34, $\mathrm{IC}_{50}$ of 9.5 $\mu \mathrm{M})$. The preference for the amine to exist as a secondary or tertiary amine for activity is also worth noting (compounds 35,36 , and 37 ; $\mathrm{IC}_{50}$ of $>10,0.47,0.60 \mu \mathrm{M}$, respectively). Tying back the methyl groups into rings such as pyrrolidines and piperidines was tolerated, but this was not the case with piperazine (compounds 31, 39, and 40; $\mathrm{IC}_{50}$ of $0.21,0.64$ and $>10 \mu \mathrm{M}$, respectively). Compound 31 was also observed to notably decrease the CYP1A2 inhibition, a desirable quality to reduce potential drug-drug interactions. Further derivatization of the pyrrolidine side chain showed that some potency could be retained while introducing another vector for an additional SAR, and that this activity was dependent on the stereochemistry of the substituted pyrrolidine (compounds 41 and 42, $\mathrm{IC}_{50}$ of $>10$

| Ex. | R | RPA194 IC $50(\mu \mathrm{M})^{\text {a }}$ | CYP1A2 IC $50(\mu \mathrm{M})^{\text {b }}$ | hERG $\mathrm{IC}_{50}(\mu \mathrm{M})^{\text {c }}$ |
| :---: | :---: | :---: | :---: | :---: |
| 31 | $\vdash^{-N}$ | 0.21 | >50 | 3.6 |
| 34 | $\chi^{\text {OH }}$ | 9.5 | 0.1 | >10 |
| 35 | $\chi^{\mathrm{NH}+}$ | >10 | >10 | >10 |
| 36 |  | 0.47 | 0.3 | >10 |
| 37 | $\psi_{\mathrm{NCH}_{3}}^{\mathrm{CH}_{3}}$ | 0.16 | 13 | 4.5 |
| 38 | $\psi_{\mathrm{H}} \mathrm{CO} \mathrm{~N}^{2} \mathrm{CH}_{3}$ | 0.60 | 0.5 | 3.8 |
| 39 | $\vdash^{-n}$ | 0.64 | >10 | 3.7 |
| 40 | $1 \sim^{-2}{ }^{\text {N-CH3 }}$ | >10 | >10 | >10 |
| 41 | $\left.\vdash^{N}\right]^{F}$ | >10 | 4.5 | >10 |
| 42 | $\Gamma^{N}$ | 1.9 | 2.1 | 8.3 |
| 43 |  | >10 | >10 | 5.6 |
| 44 |  | >10 | >10 | 4.4 |
| 45 | $\mathrm{H}^{\text {NHt}}$ | >10 | 0.2 | 4.0 |
| 46 | $\mathrm{H}^{\mathrm{N}-\mathrm{CH}_{3}}$ | >10 | >10 | 7.4 |

Table 2-3. Compound 31 analogs. aRPA194 degradation measured in A375 cells. IC 50 represents the mean of duplicate independent biological experiments performed in triplicate. ${ }^{\mathrm{b}} \mathrm{CYP} 1 \mathrm{~A} 2$ inhibition analysis performed using human hepatic CYP450s (baculovirus-insect-cell expression system) expressing the isoform 1A2. chERG inhibition analysis performed using HEK293 cells stably transfected with hERG cDNA and measured by QPatch. IC $\mathrm{C}_{50}$ value represents the mean of $\mathrm{n}=3$.
and $1.9 \mu \mathrm{M}$, respectively). Finally, the two-carbon linker was again observed to be optimal (compounds 31 and 37 vs. 43 and 44 ; $\mathrm{IC}_{50}$ of 0.21 and $0.16 \mu \mathrm{M}$ vs. $>10$ and $>10 \mu \mathrm{M}$, respectively). However, incorporating the linker into a ring was not tolerated (compounds 45 and $46, \mathrm{IC}_{50}$ of $>10 \mu \mathrm{M}$ ).

After discovery of 31 , we questioned again whether the " $D$ " ring of the fused tetracycle could be substituted with other groups at the three- and four-positions of the tricycle. Coupled with the optimal pyrrolidine amide side chain, we evaluated a new series of modified tricycle cores, shown in Table 2-4. Replacing the fused aromatic ring with a methyl group at the three-position 47 retained decent potency (RPA194 IC $500.81 \mu \mathrm{M}$ ), while allowing the phenyl ring to have freedom of rotation at the three-position 48 resulted in a larger loss in potency (RPA194 IC50 $3.2 \mu \mathrm{M}$ ). Substitution at the four-position was less tolerated with methyl 49 and phenyl 50 having comparable activity (RPA194 IC $503.0 \mu \mathrm{M}$ and $3.2 \mu \mathrm{M}$, respectively). Interestingly, we observed a synergistic response in potency when installing methyl groups at both the three- and four-position 51 (RPA194 IC $500.28 \mu \mathrm{M}$ ) compared to only substituting at one position or the other. Finally, we evaluated the difluorodioxolane " $D$ " ring 52, similar to 25 . Unfortunately, this resulted in a complete loss of activity (RPA194 $\mathrm{IC}_{50}>10 \mu \mathrm{M}$ ) while simultaneously reverting the improvement to CYP1A2 inhibition afforded by the pyrrolidine side chain (CYP1A2 $\left.I_{50} 0.5 \mu \mathrm{M}\right)$. Although CYP1A2 inhibition was generally resolved with the pyrrolidine side chain, except for 52 , this series overall exhibited a robust increase in hERG inhibition. Consequently, allowing the terminal phenyl ring to have

|  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Ex. | R1 | R2 | $\begin{gathered} \text { RPA194 } \\ \text { IC50 }(\mu \mathrm{M})^{\mathrm{a}} \\ \hline \end{gathered}$ | $\begin{aligned} & \text { CYP1A2 } \\ & \text { IC50 }(\mu \mathrm{M})^{\mathrm{b}} \end{aligned}$ | hERG IC 50 $(\mu \mathrm{M})^{\mathrm{c}}$ |
| 47 | $\mathrm{CH}_{3}$ | H | 0.81 | - | 0.83 |
| 48 | Ph | H | 3.2 | - | 0.31 |
| 49 | H | $\mathrm{CH}_{3}$ | 3.0 | >10 | 5.3 |
| 50 | H | Ph | 3.2 | $>10$ | 0.36 |
| 51 | $\mathrm{CH}_{3}$ | $\mathrm{CH}_{3}$ | 0.28 | >10 | 2.0 |
| 52 |  |  | >10 | 0.5 | 1.4 |

Table 2-4. Modifications to the tricycle core at the three- and four- positions. aRPA194 degradation measured in A375 cells. IC 50 represents the mean of duplicate independent biological experiments performed in triplicate. ${ }^{\text {b }}$ CYP1A2 inhibition analysis performed using human hepatic CYP450s (baculovirus-insect-cell expression system) expressing the isoform 1A2. chERG inhibition analysis performed using HEK293 cells stably transfected with hERG cDNA and measured by QPatch. IC $\mathrm{C}_{50}$ value represents the mean of $\mathrm{n}=3$.
freedom of rotation at positions three 48 and four 50 resulted in the greatest increase in hERG inhibition ( $\mathrm{IC}_{50} 0.31 \mu \mathrm{M}$ and $0.36 \mu \mathrm{M}$, respectively).

With these results, compound 31 was identified as the optimal analog, exhibiting similar RPA194 potency to that of 1 but a more than 100-fold improvement in CYP1A2 inhibition. In addition, compound 31 did not invoke a DNA damage response, as measured by immunofluorescence of $\gamma-\mathrm{H} 2 \mathrm{AX}$, a commonly used biomarker for DNA damage. ${ }^{15}$ Compound 31 was also a potent inhibitor of A375 cell viability, with an $\mathrm{IC}_{50}$ of 38 nM (Figure 2-2).

As seen in Table 2-3, changes to the side chain that are consistent with potency in the RPA194 assay did not substantially change hERG activity,


Figure 2-2. Cell viability analysis of 31. Determined using CellTiter-Glo ${ }^{\circledR}$ Luminescent Cell Viability Assay. Data shown represents the mean of 4 experiments.
suggesting an overlap of the SAR between the two assays in this region of the molecule. More remarkable was the difference in hERG potency between 31 (3.6 $\mu \mathrm{M})$ and $32(0.07 \mu \mathrm{M})$ just by changing the direction of the turn introduced to the tetracycle core.

To determine selectivity for Pol I inhibition versus Pol II and Pol III, we performed quantitative polymerase chain reaction (qPCR), as shown in Figure 23. A375 cells were treated with compounds $(1 \mu \mathrm{M})$ for 6 hours. RNA was isolated using the Qiagen RNeasy kit, reverse transcribed, and used to perform qPCR with SYBR GREEN master mix. ${ }^{16}$ Both 1 and 31 showed the selective, robust transcription inhibition of Pol I rRNA transcript 5' ETS851 whereas Pol II gene DHFR and Pol III gene tRNA-Valine were unaffected.

A pharmacokinetic (PK) profile in CD-1 mice for compound 31 was obtained by administering a single intravenous (IV) dose of $1 \mathrm{mg} / \mathrm{kg}$, an oral (PO) dose of $30 \mathrm{mg} / \mathrm{kg}$, or an intraperitoneal (IP) dose of $30 \mathrm{mg} / \mathrm{kg}$. Relevant PK parameters are summarized in Table 2-5.


Figure 2-3. qPCR analysis of 1 and 31. Performed in A375 cells with SYBR GREEN master mix. Data shown represents the mean of triplicate independent biological experiments performed in triplicate. P values calculated using unpaired two-tailed $t$ test in GraphPad Prism 9.3.1.

After IV administration, the calculated maximum plasma concentration $\left(C_{\max }\right)$ and area under the curve (AUC) values were $46 \mathrm{ng} / \mathrm{mL}$ and $31 \mathrm{~h} \cdot \mathrm{ng} / \mathrm{mL}$, respectively. A short half-life ( $\mathrm{t}_{1 / 2}$ ) of 1.2 hours was observed along with an extremely high clearance (Clobs) value of $508 \mathrm{~mL} / \mathrm{min} / \mathrm{kg}$, possibly influenced by the high volume of distribution. Following PO administration, the bioavailability was low; however, IP administration saw improved exposure, which suggests a route for examining this class of compounds for in vivo efficacy. The metabolic stability for compound 31 was also measured in mouse liver microsomes and rat liver hepatocytes. Compound 31 exhibited intrinsic clearance values of $132 \mu \mathrm{~L} / \mathrm{min} / \mathrm{mg}$ and $1058 \mathrm{~mL} / \mathrm{min} / \mathrm{kg}$, respectively. Taken together, these values serve as a starting point for further PK property optimization.

## Mean Plasma Concentration vs Time Profile for Compound 31 in CD1 Mouse <br>  <br> $\rightarrow$ IV $1 \mathrm{mg} / \mathrm{kg}$ <br> - PO $30 \mathrm{mg} / \mathrm{kg}$ <br> $\rightarrow \mathrm{IP} 30 \mathrm{mg} / \mathrm{kg}$

| Route | $\begin{gathered} \mathrm{C}_{\text {max }} \\ (\mathrm{ng} / \mathrm{mL}) \end{gathered}$ | AUC(0-t) <br> (h•ng/mL) | $\mathrm{t}_{1 / 2}(\mathrm{~h})$ | $\begin{gathered} \text { Clobs } \\ (\mathrm{mL} / \mathrm{min} / \mathrm{kg}) \end{gathered}$ | $\mathbf{V}_{\text {ss obs }}$ (L/kg) | $\begin{gathered} \hline \text { F } \\ (\%) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { IV } 1 \\ \mathrm{mg} / \mathrm{kg} \end{gathered}$ |  | $31 \pm 2$ | $1.2 \pm 0.1$ | $508 \pm 27$ | $33 \pm 4$ |  |
| PO 30 mg/kg | $14 \pm 6$ | $122 \pm 40$ |  |  |  | $13 \pm 4$ |
| $\begin{aligned} & \text { IP } 30 \\ & \mathrm{mg} / \mathrm{kg} \end{aligned}$ | $83 \pm 6$ | $518 \pm 96$ | $7.5 \pm 1.7$ |  |  |  |

Figure 2-4. Intravenous (IV), Oral (PO), and Intraperitoneal (IP) Pharmacokinetic Parameters for 31 in CD-1 mice. Pharmacokinetic parameters were determined after a single dose administered intravenously ( $1 \mathrm{mg} / \mathrm{kg}, \mathrm{n}=3$ ), orally ( $30 \mathrm{mg} / \mathrm{kg}, \mathrm{n}=3$ ), or intraperitoneally ( $30 \mathrm{mg} / \mathrm{kg}$, $n=3$ ). IV compound was formulated in 30\% dimethylacetamide (DMA) + 10\% polyethylene glycol 200 (PEG200) $+5 \%$ Kolliphor ELP in Milli-Q water. PO and IP compounds were formulated in 0.2 M phosphate buffer, pH 6.8 .

## Chemistry

Compound 1 was synthesized as previously reported ${ }^{12}$ by the acidpromoted cyclocondensation of 2-chloronicotinic acid and 3-amino-2-napthoic acid followed by TBTU-mediated amide coupling. Commercially available diamines were coupled in this fashion to furnish amide analogs (compounds 2-11). Diamines that were not commercially available were prepared, as represented in Scheme 2-

1. Boc-protected diamine was produced by nucleophilic substitution of 2-(Bocamino)ethyl bromide (53) with various secondary amines (54a-j). Following Boc deprotection, TBTU amide coupling with 56 allowed the production of the corresponding amide analogs (compounds 12-21). Several alternate cores were synthesized in a similar fashion as the 1 tetracycle. Summarized in Scheme 2-2, an acid-promoted cyclocondensation of 2-chloronicotinic acid (57) and various


Scheme 2-1. General scheme for the synthesis to provide compounds 1-21. Reagents and conditions: (a) DIPEA, MeCN, rt. (b) 4 M HCl in 1,4-dioxane, 1,4-dioxane, rt or TFA, DCM, rt. (c) amine, TBTU, DIPEA, DMF, rt.


Scheme 2-2. General scheme for the synthesis to provide compounds 22-24, 26-27. Reagents and conditions: (a) $\mathrm{HCl}, \mathrm{EtOH}$, reflux. (b) $\mathrm{HCl}, 100{ }^{\circ} \mathrm{C}$. (c) 2-pyrrolidin-1-ylethanamine, TBTU, DIPEA, DMF/THF, rt.

60
61
62
63

25

Scheme 2-3. Synthesis of compound 25. Reagents and conditions: (a) $\mathrm{Pd}_{2}(\mathrm{dba})_{3}$ ( $5 \mathrm{~mol} \%$ ), Xantphos (10 mol \%), NaOtBu, 1,4-dioxane, $90^{\circ} \mathrm{C}$. (b) [ $\mathrm{NBnMe}_{3} \cdot \mathrm{Br}_{3}$ ], $2: 1 \mathrm{DCM} / \mathrm{MeOH}$. (c) 2-pyrrolidin-1-ylethanamine, TBTU, DIPEA, DMF, rt. (d) Pd(OAc) 2 ( $5 \mathrm{~mol} \%$ ), Xantphos (15 mol \%), Xantphos Pd G3 (5 mol \%), $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{CO}(\mathrm{g})$ (saturating), toluene, $100{ }^{\circ} \mathrm{C}$.
anthranilic acids (58a-e) followed by hydrolysis of the ester and TBTU-mediated amide coupling produced the corresponding amides with modifications to the tetracycle (compounds 22-24, 26-27).

However, the cyclocondensation chemistry was not amenable to all substrates. An alternative route based on a cyclocarbonylation report by Xu and Alper ${ }^{17}$ was envisioned to produce tetracyclic cores that could not be furnished by the acid-promoted cyclocondensation method. Scheme 2-3 summarizes the synthesis of 25 . Following the initial palladium-catalyzed cross coupling and selective bromination to produce the key bromoanilinopyridine intermediate, subsequent amide coupling and palladium-catalyzed cyclocarbonylation were performed to give 25. Summarized in Scheme 2-4, 28 was synthesized in a similar fashion.

The synthesis of 31 and subsequent analogs is summarized in Scheme 25. Buchwald-Hartwig cross-coupling produced the desired key intermediate 56. Successful palladium-catalyzed cyclocarbonylation allowed closing of the ring and insertion of the carbonyl. Hydrolysis of the ester followed by amide coupling with the corresponding amine led to formation of compounds 31, 34-46.

In the case of 29 and 30 , unexpected results led to additional unique structures. Scheme 2-6 shows the overall transformation to produce 29. During the initial palladium-catalyzed cross coupling, a second cross coupling event between


Scheme 2-4. Synthesis of compound 28. Reagents and conditions: (a) $\mathrm{Pd}(\mathrm{OAc}) 2$ (10 mol \%), Xantphos ( $15 \mathrm{~mol} \%$ ), $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, toluene, $130{ }^{\circ} \mathrm{C}$. (b) $1 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (c) 2-pyrrolidin-1ylethanamine, TBTU, DIPEA, DMF, rt. (d) Pd(OAc)2 (10 mol \%), Xantphos (15 mol \%), Xantphos Pd G3 (5 mol \%), $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{CO}(\mathrm{g})$ (saturating), toluene, $115{ }^{\circ} \mathrm{C}$.


Scheme 2-5. Synthesis of compounds 31, 34-46. Reagents and conditions: (a) $\mathrm{Pd}(\mathrm{OAc})_{2}(4 \mathrm{~mol}$ $\%$ ), rac-BINAP ( $6 \mathrm{~mol} \%$ ), $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, toluene, $130^{\circ} \mathrm{C}$. (b) $\mathrm{Pd}(\mathrm{OAc})_{2}(10 \mathrm{~mol} \%$ ), Xantphos ( 15 mol $\%$ ), Xantphos Pd G3 (5 mol \%), $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Mo}(\mathrm{CO})_{6}$, toluene, $100{ }^{\circ} \mathrm{C}$. (c) $2 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (d) amine, TBTU, DIPEA, DMF, rt.


Scheme 2-6. Synthesis of compounds 29, 30. Reagents and conditions: (a) $\mathrm{Pd}(\mathrm{OAc})_{2}(5 \mathrm{~mol} \%)$, Xantphos (10 mol\%), $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, toluene, $130{ }^{\circ} \mathrm{C}$. (b) $1 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (c) 2-pyrrolidin-1ylethanamine, TBTU, DIPEA, DMF, rt. (d) NBS, DCM, rt.
the pyridyl nitrogen and the aryl bromide resulted in a ring closing producing the 6-5-6-6 ring system and notably lacking the carbonyl seen in many of the other tetracycles. A similar result occurred with 30. After initial cross coupling, a bromination attempt to give the desired key intermediate instead resulted in the $S_{N} A R$ product, giving another 6-5-6-6 ring system lacking the carbonyl.

Scheme 2-7 summarizes the production of 32. After preparation of the two cross-coupling partners and subsequent coupling, palladium-catalyzed cyclocarbonylation successfully closed the ring and inserted the carbonyl, as desired. Ester hydrolysis followed by amide coupling with the corresponding amine furnished compound 32.

Compound 33 was synthesized as summarized in Scheme 2-8. Following the initial cross coupling, a selective bromination was attempted. Bromination was achieved but at a different position than originally predicted. This intermediate was carried forward and successfully underwent palladium-catalyzed


78



79



80 b

81


82


83



32

Scheme 2-7. Synthesis of compound 32. Reagents and conditions: (a) DEAD, $\mathrm{PPh}_{3}, \mathrm{MeOH}$, diethyl ether, rt. (b) NBS, DMF, rt. (c) rac-BINAP-Pd-G3 (10 mol \%), 1,4-dioxane, $100{ }^{\circ} \mathrm{C}$. (d) $\mathrm{Pd}(\mathrm{OAc}) 2$ ( 0.5 eq ), Xantphos (1 eq.), $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{CO}(\mathrm{g})$ (saturating), 1,4-dioxane, $100^{\circ} \mathrm{C} .(\mathrm{e}) 2 \mathrm{M} \mathrm{NaOH}$, MeOH , rt. (f) 2-pyrrolidin-1-ylethanamine, TBTU, DIPEA, DMF, rt.
cyclocarbonylation to ultimately afford 33 after ester hydrolysis and amide coupling.

Scheme 2-9 summarizes the synthesis of 47-52. Acid-promoted cyclocondensation followed by TBTU-mediated amide coupling was sufficient to produce 47, 49-51. For 48, a Suzuki-Miyaura coupling between the aryl bromide and phenylboronic acid was carried out prior to acid-promoted cyclocondensation and TBTU-mediated amide coupling. $N$-bromosuccinimide bromination of 93 resulted in two mono-brominated regioisomers at the five- and seven-position. Fortunately, the desired five-position regioisomer was produced in $\sim 3: 1$ excess


60


84


85
86

87

Scheme 2-8. Synthesis of compound 33. Reagents and conditions: (a) $\operatorname{Pd}(\mathrm{OAc})_{2}(10 \mathrm{~mol} \%)$, Xantphos (15 mol \%), $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, toluene, $130^{\circ} \mathrm{C}$. (b) $\left[\mathrm{BnNMe}_{3} \cdot \mathrm{Br}_{3}\right], 2: 1 \mathrm{DCM}: \mathrm{MeOH}$, rt. (c) $\mathrm{Pd}(\mathrm{OAc})_{2}$ (10 mol \%), Xantphos (15 mol \%), Xantphos Pd G3 (5 mol \%), $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Mo}(\mathrm{CO})_{6}$, toluene, $115{ }^{\circ} \mathrm{C}$. (d) $1 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (e) 2-pyrrolidin-1-ylethanamine, TBTU, DIPEA, DMF, rt. compared to the undesired seven-position regioisomer and could be isolated by column chromatography. Following Buchwald-Hartwig coupling and palladiumcatalyzed cyclocarbonylation, ester hydrolysis and amide coupling were carried out to provide 52.

In summary, optimization of 1 to reduce the hERG and CYP activity while maintaining the potency demonstrated that only modest changes to the basic sidechain were tolerated. The requirement for a basic amine for RPA194 potency has thus far prevented the complete elimination of hERG activity. Undesired CYP1A2 inhibition was successfully removed, while potency was maintained by utilizing cyclic amines. Whereas a tetracyclic aromatic core structure is required for robust activity, subtle changes such as changing the orientation of the terminal ring (compound 32) can have a remarkable effect on the potency. From this work, compound 31 emerged as an important lead compound, retaining substantial
potency while reducing CYP1A2 inhibition by over 100-fold as compared with compound 1 without a substantial increase in the hERG activity.


Scheme 2-9. Synthesis of compounds 47-52. Reagents and conditions: (a) $\mathrm{H}_{2} \mathrm{SO}_{4}, 1: 1$ DMF/H2O, $110-130{ }^{\circ} \mathrm{C}$ or $\mathrm{HCl}, \mathrm{EtOH}$, reflux or $1 \mathrm{M} \mathrm{HCl}, 105^{\circ} \mathrm{C}$. (b) 2-pyrrolidin-1-ylethanamine, TBTU, DIPEA, DMF, rt. (c) phenylboronic acid, $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(3 \mathrm{~mol} \%), \mathrm{K}_{3} \mathrm{PO}_{4}, 6: 1 \mathrm{DMF} / \mathrm{H}_{2} \mathrm{O}, 150{ }^{\circ} \mathrm{C}$. (d) N -bromosuccinimide, DCM, $0{ }^{\circ} \mathrm{C}$-rt. (e) $\mathrm{Pd}(\mathrm{OAc})_{2}(4 \mathrm{~mol} \%)$, DPEphos (6 mol \%), KOtBu,
toluene, $100^{\circ} \mathrm{C}$. (f) $\mathrm{Pd}(\mathrm{OAc})_{2}\left(10 \mathrm{~mol} \%\right.$ ), Xantphos ( $15 \mathrm{~mol} \%$ ), Xantphos Pd G3 ( $5 \mathrm{~mol} \%$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}$, $\mathrm{Mo}(\mathrm{CO})_{\text {e }}$, toluene, $115^{\circ} \mathrm{C}$. (g) $1 \mathrm{M} \mathrm{HCl}, \mathrm{MeOH}, 100^{\circ} \mathrm{C}$.

PK profiling provided a starting point for further optimization. Future experiments will focus on improving pharmacokinetics, namely, improving the clearance and obtaining a structural understanding of how the compounds interact with the DNAPol I complex assembly.

## Experimental

Synthesis. General Methods. All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on an ISCO CombiFlash Rf or Biotage Isolera using Biotage Flash cartridges with peak detection at 254 nm . Reverse phase purification was accomplished using a Gilson 215 liquid handler equipped with a Phenomenex C18 column (150 mm x 20 mm i.d., $5 \mu \mathrm{~m}$ ). Peak collection was triggered by UV detection at 214 or $254 \mathrm{~nm} .{ }^{1} \mathrm{H}$ NMR spectra were recorded on a Bruker 400 instrument operating at 400 MHz with tetramethylsilane or residual protonated solvent used as a reference. Analytical LC/MS was performed using an Agilent 1260 equipped with autosampler (Agilent Poroshell 120 C18 column (50 $\mathrm{mm} \times 4.6 \mathrm{~mm}$ i.d., $3.5 \mu \mathrm{~m}$ ); $0.05 \%$ TFA in water/acetonitrile gradient; UV detection at 215 and 254 nm ) and electrospray ionization. All final compounds showed purity greater than $95 \%$ at 215 and 254 nm using this method. Compounds 22-24 and 26-27 were synthesized at WuXi AppTec. Compound 25 was synthesized by Pablo de Leon. Compounds 28-36, and 38-52 were synthesized at Evotec.

12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (56). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961. N-(2-(dimethylamino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide (1). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961.

12-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-12H-benzo[g]pyrido[2, 1-b]quinazoline-4carboxamide (2). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961.

12-oxo-N-(2-(piperidin-1-yl)ethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide (3). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961.

12-oxo-N-(pyrrolidin-2-ylmethyl)-12H-benzo[g]pyrido[2, 1-b]quinazoline-4carboxamide trifluoroacetate (4). In a 20 mL vial charged with a magnetic stir bar was added 56 ( $100 \mathrm{mg}, 0.34 \mathrm{mmol}$ ), TBTU ( $123.41 \mathrm{mg}, 0.38 \mathrm{mmol}$ ), DMF ( 1.5 mL ), and DIPEA ( $0.19 \mathrm{~mL}, 1.05 \mathrm{mmol}$ ). The resulting mixture was stirred for 15 minutes at room temperature before adding pyrrolidin-2-ylmethanamine ( $52.76 \mathrm{mg}, 0.53$ $\mathrm{mmol})$. The reaction mixture was stirred for 1.5 hours at room temperature, poured into 100 mL of cold water with stirring, and the solid was collected by vacuum filtration. The solid was taken up in a solution of 4M HCI in 1,4-dioxane, stirred for 1 hour at room temperature, and concentrated in vacuo. The solid was dissolved in a minimal amount of DMSO and purified via automated reverse phase liquid chromatography on a $50 \times 21.2 \mathrm{~mm}$ Luna $10 \mu \mathrm{~m}$ C18(2) $100 \AA$ column with $10-$
$50 \%$ ACN/water ( $0.05 \%$ TFA buffer) to give 12-oxo- N -(pyrrolidin-2-ylmethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide trifluoroacetate (7 mg, 0.014 $\mathrm{mmol}, 4 \%$ yield) as a red solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 11.36-11.28$ (m, 1H), $9.13(\mathrm{~s}, 1 \mathrm{H}), 8.96$ (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.83-8.64(\mathrm{~m}, 1 \mathrm{H}), 8.60-8.51$ (m, 1H), $8.32(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.12(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.77-7.69(\mathrm{~m}, 1 \mathrm{H}), 7.65-$ $7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.42$ (obs.), $3.82(\mathrm{~d}, J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 3.36-$ $3.16(\mathrm{~m}, 2 \mathrm{H}), 2.27-2.10(\mathrm{~m}, 1 \mathrm{H}), 2.06-1.87(\mathrm{~m}, 1 \mathrm{H}), 1.86-1.72(\mathrm{~m}, 1 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{22} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}_{2}, 372.2$; found $373.0[\mathrm{M}+\mathrm{H}]^{+}$.

12-oxo-N-(piperidin-2-ylmethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride (5). In a 20 mL vial charged with a magnetic stir bar was added $56(100 \mathrm{mg}, 0.34 \mathrm{mmol})$, TBTU ( $123.41 \mathrm{mg}, 0.38 \mathrm{mmol}$ ), DMF ( 1.5 mL ), and DIPEA ( $0.19 \mathrm{~mL}, 1.05 \mathrm{mmol})$. The resulting mixture was stirred for 15 minutes at room temperature before adding piperidin-2-ylmethanamine $(60.15 \mathrm{mg}, 0.53$ $\mathrm{mmol})$. The reaction was stirred for 1 hour at room temperature, poured into 100 mL of cold water with stirring, and the solid was collected by vacuum filtration. The solid was dissolved in $\sim 1 \mathrm{~mL}$ of 1 M HCl and stirred for 3 hours at room temperature then concentrated to dryness to give 12-oxo- N -(piperidin-2-ylmethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (102 mg, 0.24 mmol, $70 \%$ yield) as a yellow-orange solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ $11.29(\mathrm{t}, J=6.2 \mathrm{~Hz}, 1 \mathrm{H}), 9.15(\mathrm{~s}, 1 \mathrm{H}), 8.97(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.78-8.68(\mathrm{~m}$, $1 \mathrm{H}), 8.62(\mathrm{~d}, J=11.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.57(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.54(\mathrm{~s}, 1 \mathrm{H}), 8.33(\mathrm{~d}$, $J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.13(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.70(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.58(\mathrm{~m}, 1 \mathrm{H})$, $7.10(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.87-3.75(\mathrm{~m}, 1 \mathrm{H}), 3.75-3.65(\mathrm{~m}, 1 \mathrm{H}), 3.27(\mathrm{~d}, J=12.4$
$\mathrm{Hz}, 1 \mathrm{H}), 2.96-2.83(\mathrm{~m}, 1 \mathrm{H}), 2.01(\mathrm{~d}, \mathrm{~J}=11.5 \mathrm{~Hz}, 1 \mathrm{H}), 1.89-1.79(\mathrm{~m}, 1 \mathrm{H}), 1.74(\mathrm{~d}$, $J=13.1 \mathrm{~Hz}, 1 \mathrm{H}$ ), 1.67-1.48 (m, 2H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 386.2$; found $387.0[\mathrm{M}+\mathrm{H}]^{+}$.

## 12-oxo-N-(1-(piperidin-2-yl)ethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-

 carboxamide hydrochloride (6). To a 4 mL vial charged with a magnetic stir bar was added 56 ( $75 \mathrm{mg}, 0.26 \mathrm{mmol}$ ), TBTU ( $92.56 \mathrm{mg}, 0.29 \mathrm{mmol}$ ), DMF ( 0.92 mL ), and DIPEA ( $0.14 \mathrm{~mL}, 0.79 \mathrm{mmol})$. The resulting mixture was stirred for 15 minutes at room temperature before adding (1-piperidin-2-ylethyl)amine ( $50.66 \mathrm{mg}, 0.40$ $\mathrm{mmol})$. The reaction mixture was stirred for 3 hours at room temperature then poured into 100 mL of cold water and stirred overnight at room temperature. The solid was removed by vacuum filtration. The filtrate was transferred to a separatory funnel and extracted with DCM ( $4 \times 5 \mathrm{~mL}$ ). Combined organic layers were dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo to give an orange liquid. This material was taken up in a solution of 4 M HCl in 1,4-dioxane, stirred overnight at room temperature, and concentrated in vacuo to give 12-oxo- N -(1-(piperidin-2-yl)ethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $25.3 \mathrm{mg}, 0.058 \mathrm{mmol}, 22 \%$ yield, mixture of diastereomers) as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d 6 ) $\delta$ ppm $11.34(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 11.22(\mathrm{~d}, J=8.4$ $\mathrm{Hz}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 9.04-8.93(\mathrm{~m}, 2 \mathrm{H}), 8.71(\mathrm{~s}, 2 \mathrm{H}), 8.58(\mathrm{dd}, J=6.9,1.8 \mathrm{~Hz}$, 1H), 8.55 (dd, $J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.47$ (s, 1H), 8.37 (s, 1H), 8.32 (d, J = 8.4 Hz , 2 H ), 8.22-8.15 (m, 2H), 7.78-7.71 (m, 2H), 7.67-7.57 (m, 2H), 7.11 (td, J = 7.1, $2.6 \mathrm{~Hz}, 2 \mathrm{H}$ ), 4.55 (d, J=10.2 Hz, 1H), 4.42-4.33 (obs.), 3.34 (s, 2H), 3.26 (d, $J=$ $12.4 \mathrm{~Hz}, 2 \mathrm{H}$ ), 2.91 (s, 2H), $2.68(\mathrm{~s}, 1 \mathrm{H}), 2.21(\mathrm{~d}, J=2.1 \mathrm{~Hz}, 1 \mathrm{H}), 2.02(\mathrm{~d}, J=12.3$$\mathrm{Hz}, 1 \mathrm{H}), 1.87(\mathrm{~s}, 2 \mathrm{H}), 1.72(\mathrm{~s}, 2 \mathrm{H}), 1.56(\mathrm{~s}, 1 \mathrm{H}), 1.46(\mathrm{dd}, \mathrm{J}=9.3,6.9 \mathrm{~Hz}, 6 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{24} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}$, 400.2; found $401.0[\mathrm{M}+\mathrm{H}]^{+}$.

Method A: Synthesis of amide analogs with commercially available amine and formation of the hydrochloride salt. N-((4-methylmorpholin-3-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (7). In a 4 mL vial charged with a magnetic stir bar was added 56 ( $25 \mathrm{mg}, 0.086 \mathrm{mmol}$ ), TBTU ( $30.85 \mathrm{mg}, 0.096 \mathrm{mmol}$ ), DMF ( 0.5 mL ), and DIPEA ( $0.05 \mathrm{~mL}, 0.28 \mathrm{mmol}$ ). The resulting mixture was stirred for 15 minutes at room temperature before adding (4-methylmorpholin-3-yl)methanamine ( $17.14 \mathrm{mg}, 0.13 \mathrm{mmol}$ ). The reaction mixture was stirred overnight at room temperature, then poured into 50 mL of cold water with stirring. The solid was collected by filtration and dried under vacuum. The solid was taken up in a solution of 4 M HCl in 1,4-dioxane, stirred overnight at room temperature, and concentrated in vacuo to give $N$-((4-methylmorpholin-3-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $32.9 \mathrm{mg}, 0.075 \mathrm{mmol}, 87 \%$ yield) as a light orange solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.26-11.16 (m, 1H), 9.15 (s, 1H), 8.97 (dd, J = 7.3, $1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.57-8.50(\mathrm{~m}, 2 \mathrm{H}), 8.34(\mathrm{~d}, \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.14(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H})$, $7.81-7.72(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.30(\mathrm{~d}, \mathrm{~J}=12.5$ $\mathrm{Hz}, 1 \mathrm{H}$ ), 3.95-3.86 (m, 1H), 3.74-3.62 (m, 1H), 3.47-3.44 (obs.) 3.09 (d, $J=4.2$ $\mathrm{Hz}, 2 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{3}, 402.2$; found $403.0[\mathrm{M}+\mathrm{H}]^{+}$. N-((6-(hydroxymethyl)piperidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (8). This compound was synthesized
from $56(25 \mathrm{mg}, 0.086 \mathrm{mmol})$ and [6-(aminomethyl)piperidin-2-yl]methanol (18.99 $\mathrm{mg}, 0.13 \mathrm{mmol})$ according to method A to give N -((6-(hydroxymethyl)piperidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $28.5 \mathrm{mg}, 0.063 \mathrm{mmol}, 73 \%$ yield, mixture of diastereomers) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta \mathrm{ppm} 11.14(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 1 \mathrm{H}), 9.14$ $(\mathrm{s}, 1 \mathrm{H}), 8.96(\mathrm{dd}, \mathrm{J}=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.75(\mathrm{~d}, \mathrm{~J}=10.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.54(\mathrm{dd}, J=6.9$, $1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.48(\mathrm{~s}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.13(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-$ $7.71(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.97-3.84(\mathrm{~m}, 1 \mathrm{H}), 3.80-$ $3.68(\mathrm{~m}, 1 \mathrm{H}), 3.64(\mathrm{dd}, J=11.4,4.4 \mathrm{~Hz}, 1 \mathrm{H}), 3.42(\mathrm{~s}, 1 \mathrm{H}), 3.17(\mathrm{~s}, 1 \mathrm{H}), 2.05(\mathrm{~d}, \mathrm{~J}$ $=9.7 \mathrm{~Hz}, 1 \mathrm{H}), 1.88(\mathrm{~d}, \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 1.82-1.73(\mathrm{~m}, 1 \mathrm{H}), 1.66-1.46(\mathrm{~m}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{3}$, 416.2; found $417.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-((1-methylpyrrolidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (9). This compound was synthesized from 56 (25 $\mathrm{mg}, 0.086 \mathrm{mmol}$ ) and (1-methylpyrrolidin-2-yl)methanamine ( $15.04 \mathrm{mg}, 0.13$ $\mathrm{mmol})$ according to method A to give N -((1-methylpyrrolidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride $(28.7 \mathrm{mg}$, $0.068 \mathrm{mmol}, 79 \%$ yield) as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ 11.34-11.28 (m, 1H), $10.26(\mathrm{~s}, 1 \mathrm{H}), 9.13(\mathrm{~s}, 1 \mathrm{H}), 8.98-8.93(\mathrm{~m}, 1 \mathrm{H}), 8.69(\mathrm{~s}, 1 \mathrm{H})$, $8.55(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.12(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H})$, $7.78-7.70(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.94(\mathrm{q}, J=6.1 \mathrm{~Hz}$, 2H), 3.77-3.69 (obs.), 3.49-3.06 (obs.), 3.18-3.06 (m, 1H), 2.96 (d, $J=4.9 \mathrm{~Hz}$, $3 H), 2.34-2.30(\mathrm{~m}, 1 \mathrm{H}), 2.05(\mathrm{~s}, 1 \mathrm{H}), 2.00-1.88(\mathrm{~m}, 1 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 386.2$; found $387.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-((1-ethylpiperidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride (10). This compound was synthesized from 56 (33.38 $\mathrm{mg}, 0.12 \mathrm{mmol}$ ) and (1-ethylpiperidin-2-yl)methanamine ( $25 \mathrm{mg}, 0.18 \mathrm{mmol}$ ) according to method A to give N -((1-ethylpiperidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride $(29.1 \mathrm{mg}, 0.064$ mmol, $56 \%$ yield) as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.27-$ $11.20(\mathrm{~m}, 1 \mathrm{H}), 9.77(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.54$ (dd, $J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.48(\mathrm{~d}, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.15(\mathrm{~d}, J$ $=8.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.67-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.13-7.04(\mathrm{~m}, 1 \mathrm{H}), 3.97-$ 3.90 (obs.), 3.69-3.64 (obs.), 3.53-3.34 (m, 3H), 3.34-3.21 (m, 1H), 3.08 (d, J = $11.9 \mathrm{~Hz}, 1 \mathrm{H}), 2.21(\mathrm{~d}, \mathrm{~J}=13.9 \mathrm{~Hz}, 1 \mathrm{H}), 1.89-1.70(\mathrm{~m}, 3 \mathrm{H}), 1.31(\mathrm{t}, \mathrm{J}=7.2 \mathrm{~Hz}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{25} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}, 414.2$; found $415.0[\mathrm{M}+\mathrm{H}]^{+}$.

## N-((1-isopropylpiperidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-

 b]quinazoline-4-carboxamide hydrochloride (11). This compound was synthesized from 56 ( $25 \mathrm{mg}, 0.086 \mathrm{mmol}$ ) and (1-propan-2-ylpiperidin-2-yl)methanamine (20.58 mg, 0.13 mmol$)$ according to method A to give N -((1-isopropylpiperidin-2-yl)methyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $29.7 \mathrm{mg}, 0.064 \mathrm{mmol}, 74 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.24-11.18 (m, 1H), $9.62(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.97-8.94$ (m, 1H), 8.53 (dd, $J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.46(\mathrm{~s}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.15$ (d, J = 8.6 Hz, 1H), 7.78-7.71 (m, 1H), 7.66-7.59(m, 1H), 7.08 (t, J=7.1 Hz, 1H), 4.27-4.15 (m, 1H), 4.07-3.98 (obs.), 3.81-3.74 (obs.), 2.90 (d, J = $11.5 \mathrm{~Hz}, 1 \mathrm{H}$ ), $2.21(\mathrm{~d}, J=14.1 \mathrm{~Hz}, 1 \mathrm{H}), 1.91(\mathrm{~d}, J=13.2 \mathrm{~Hz}, 1 \mathrm{H}), 1.86-1.77(\mathrm{~m}, 3 \mathrm{H}), 1.38(\mathrm{~d}, J$$=6.5 \mathrm{~Hz}, 3 \mathrm{H}), 1.27(\mathrm{~d}, \mathrm{~J}=6.6 \mathrm{~Hz}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{26} \mathrm{H}_{28} \mathrm{~N}_{4} \mathrm{O}_{2}$, 428.2; found $429.0[\mathrm{M}+\mathrm{H}]^{+}$.

Method B: Synthesis of amide analogs with synthesized amines and formation of the hydrochloride salt. N -(2-(3-fluoropyrrolidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (12). To a 20 mL vial charged with a magnetic stir bar was added 3-fluoropyrrolidine hydrochloride (54a) $\quad(280.17 \mathrm{mg}, \quad 2.23 \mathrm{mmol}), \quad \mathrm{ACN}(5.6 \mathrm{~mL})$, tert-butyl N -(2bromoethyl)carbamate (53) ( $250 \mathrm{mg}, 1.12 \mathrm{mmol}$ ), and DIPEA ( $0.6 \mathrm{~mL}, 3.35 \mathrm{mmol}$ ). The resulting mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo, the residue was taken up in DCM, and was washed 1 x with water. The aqueous layer was extracted with DCM (3x20 mL). Combined organic layers were dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. The residue was taken up in 1,4-dioxane ( 2.6 mL ) and a solution of 4 M HCl in 1,4-dioxane ( 0.78 $\mathrm{mL}, 3.10 \mathrm{mmol}$ ) was added. The resulting mixture was stirred overnight at room temperature then concentrated in vacuo. A portion of this material, 2-(3-fluoropyrrolidin-1-yl)ethanamine hydrochloride (55a) (100.22 $\mathrm{mg}, 0.59 \mathrm{mmol})$ was dissolved in DMF ( 4 mL ) and DIPEA ( $0.21 \mathrm{~mL}, 1.19 \mathrm{mmol}$ ) was added. The mixture was stirred at room temperature until all solid dissolved and then was added dropwise to a 20 mL vial containing $56(115 \mathrm{mg}, 0.40 \mathrm{mmol})$, TBTU (190.81, 0.59 $\mathrm{mmol})$, DMF ( 4 mL ), and DIPEA ( $0.21 \mathrm{~mL}, 1.19 \mathrm{mmol}$ ). The resulting mixture was stirred overnight at room temperature then poured into 100 mL of cold water with stirring. The solid was vacuum filtered and washed with cold water. The crude material was purified via automated normal phase liquid chromatography using a

12 g silica cartridge with $1-10 \% \mathrm{MeOH} / \mathrm{DCM}$. This material was taken up in a solution of 4 M HCl in 1,4-dioxane, stirred for 4.5 hours at room temperature, and concentrated in vacuo to give N -(2-(3-fluoropyrrolidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $59.9 \mathrm{mg}, 0.14$ mmol, $34 \%$ yield) as a yellow-brown solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm}$ 11.25-11.07 (m, 1H), $10.80(\mathrm{~s}, 1 \mathrm{H}), 9.12(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{~d}, \mathrm{~J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.65(\mathrm{~d}$, $J=12.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.58-8.54(\mathrm{~m}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~d}, J=8.5 \mathrm{~Hz}$, $1 \mathrm{H}), 7.74(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.65-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 5.47(\mathrm{~d}, J$ $=56.9 \mathrm{~Hz}, 1 \mathrm{H}), 3.97-3.77(\mathrm{~m}, 2 \mathrm{H}), 3.33(\mathrm{~s}, 1 \mathrm{H}), 2.24-2.04(\mathrm{~m}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{21} \mathrm{FN}_{4} \mathrm{O}_{2}$, 404.2; found $405.0[\mathrm{M}+\mathrm{H}]^{+}$.

Method C: Alternate synthesis of amide analogs with synthesized amines and formation of the hydrochloride salt. N -(2-(3,3-difluoropyrrolidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (13). In a 20 mL vial charged with a magnetic stir bar was added tert-butyl N -(2bromoethyl)carbamate (53) ( $836.98 \mathrm{mg}, 3.73 \mathrm{mmol}$ ), ACN ( 9.3 mL ), 3,3difluoropyrrolidine (54b) ( $200 \mathrm{mg}, 1.87 \mathrm{mmol}$ ), and DIPEA ( $1 \mathrm{~mL}, 5.6 \mathrm{mmol}$ ). The resulting mixture was stirred for 2 days at room temperature. The reaction mixture was poured into water and extracted with DCM ( $3 \times 20 \mathrm{~mL}$ ). Combined organic layers were dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. The resulting residue was taken up in DCM ( 13.6 mL ) and TFA ( $0.57 \mathrm{~mL}, 7.47 \mathrm{mmol}$ ) was added. The resulting mixture was stirred for 2 days at room temperature then concentrated in vacuo. A portion of this material, 2-(3,3-difluoropyrrolidin-1-yl)ethanamine trifluoroacetate (55b) (493.35 mg, 1.87 mmol ) was dissolved in DMF ( 3 mL ) and

DIPEA ( $0.65 \mathrm{~mL}, 3.66 \mathrm{mmol}$ ) was added. This solution was added dropwise to a 20 mL vial containing 56 ( $354.51 \mathrm{mg}, 1.22 \mathrm{mmol}$ ), TBTU ( $437.49 \mathrm{mg}, 1.36 \mathrm{mmol}$ ), DMF ( 3 mL ), and DIPEA ( $0.65 \mathrm{~mL}, 3.66 \mathrm{mmol}$ ). The resulting mixture was stirred overnight at room temperature then poured into 100 mL of cold water with stirring. The solid was vacuum filtered and washed with cold water. The crude material was purified via automated normal phase liquid chromatography using a 12 g silica cartridge with $0-4 \% \mathrm{MeOH} / \mathrm{DCM}$. Mixed fractions were subjected to another purification via automated normal phase liquid chromatography using a 12 g silica cartridge with $1-10 \%$, hold $10 \%$ for $\sim 3$ column volumes, then $10-25 \%$ acetone/DCM. This material was taken up in a solution of 4 M HCl in 1,4-dioxane, stirred overnight at room temperature, and concentrated in vacuo to give N -(2-(3,3-difluoropyrrolidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride ( $27.7 \mathrm{mg}, 0.060 \mathrm{mmol}, 4 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (500 MHz, MeOD) $\delta$ ppm 9.33-9.25 (m, 1H), 9.18 (s, 1H), 8.95-8.87 (m, 1H), $8.43(\mathrm{~s}, 1 \mathrm{H}), 8.25(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.13(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.80(\mathrm{t}, J=7.6 \mathrm{~Hz}$, $1 \mathrm{H}), 7.69(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.46(\mathrm{~s}, 1 \mathrm{H}), 3.97(\mathrm{t}, J=5.5 \mathrm{~Hz}, 2 \mathrm{H}), 3.72(\mathrm{t}, J=5.6$ $\mathrm{Hz}, 2 \mathrm{H})$, 2.83-2.69 (m, 2H). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{20} \mathrm{~F}_{2} \mathrm{~N}_{4} \mathrm{O}_{2}, 422.2$; found $423.2[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(3-fluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2, 1-b]quinazoline-4-carboxamide hydrochloride (14). This compound was synthesized using 3fluoropiperidine (54c) (498 mg, 3.57 mmol ) according to method B. A portion of deprotected diamine, 2-(3-fluoro-1-piperidyl)ethanaminechydrochloride (55c) ( $94.4 \mathrm{mg}, 0.52 \mathrm{mmol}$ ) was coupled with $56(100 \mathrm{mg}, 0.34 \mathrm{mmol})$ to give N -(2-(3-
fluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride ( $60.5 \mathrm{mg}, 0.13 \mathrm{mmol}, 39 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.27-11.12 (m, 1H), $9.87(\mathrm{~s}, 1 \mathrm{H}), 9.13(\mathrm{~d}, \mathrm{~J}=$ $2.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.96(\mathrm{dd}, J=7.2,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.58(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{dd}, J=6.9,1.8 \mathrm{~Hz}$, $1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-$ $7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 5.17(\mathrm{~d}, J=45.3 \mathrm{~Hz}, 1 \mathrm{H}), 4.01-3.86(\mathrm{~m}, 2 \mathrm{H})$, 3.66-3.59 (m, 1H), 3.48-3.38 (m, 1H), 3.18-3.04 (m, 1H), 2.53-2.51 (obs.), 2.462.43 (obs.), 2.06-1.93 (m, 1H), 1.86-1.73 (m, 1H). LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{23} \mathrm{FN}_{4} \mathrm{O}_{2}, 418.2$; found $419.0[\mathrm{M}+\mathrm{H}]^{+}$.

## N-(2-(3,3-difluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-

b]quinazoline-4-carboxamide hydrochloride (15). This compound was synthesized using 3,3-difluoropiperidine hydrochloride (54d) ( $506.31 \mathrm{mg}, 3.21 \mathrm{mmol}$ ) according to method B. A portion of deprotected diamine, 2-(3,3-difluoro-1piperidyl)ethanamine hydrochloride (55d) (103.69 mg, 0.52 mmol$)$ was coupled with 56 (100 mg, 0.34 mmol$)$ to give $N$-(2-(3,3-difluoropiperidin-1-yl)ethyl)-12-oxo$12 H$-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride $(23.7 \mathrm{mg}$, $0.050 \mathrm{mmol}, 14 \%$ yield) as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{MeOD}$ ) $\delta \mathrm{ppm}$ 9.10-9.01 (m, 1H), 8.71-8.62 (m, 1H), $8.39(\mathrm{~s}, 1 \mathrm{H}), 8.17(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.05$ $(\mathrm{d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.71(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.60(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.16-7.04(\mathrm{~m}$, $1 \mathrm{H}), 4.05(\mathrm{t}, J=5.7 \mathrm{~Hz}, 2 \mathrm{H}), 4.00-3.83(\mathrm{~m}, 5 \mathrm{H}), 3.66(\mathrm{t}, J=5.8 \mathrm{~Hz}, 2 \mathrm{H}), 3.62-3.47$ $(\mathrm{m}, 2 \mathrm{H}), 2.32-2.19(\mathrm{~m}, 3 \mathrm{H}), 2.19-2.10(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{22} \mathrm{~F}_{2} \mathrm{~N}_{4} \mathrm{O}_{2}, 436.2$; found $437.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4,4-difluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxamide hydrochloride (16). This compound was synthesized using 4,4-difluoropiperidine ( $270 \mathrm{mg}, 2.23 \mathrm{mmol}$ ) (54e) according to method C. Deprotected diamine, 2-(4,4-difluoropiperidin-1-yl)ethan-1-amine trifluoroacetate (55e) (109.1 mg, 0.39 mmol$)$ was coupled with $56(75 \mathrm{mg}, 0.26 \mathrm{mmol})$ to give N -(2-(4,4-difluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $59.7 \mathrm{mg}, 0.126 \mathrm{mmol}, 49 \%$ yield). ${ }^{1} \mathrm{H}$ NMR (400 $\left.\mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}\right) \delta \mathrm{ppm} 11.23(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.97(\mathrm{~s}, 1 \mathrm{H}), 9.12(\mathrm{~s}, 1 \mathrm{H}), 8.95$ (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.66(\mathrm{~s}, 1 \mathrm{H}), 8.55(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=$ $8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08$ $(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.03-3.86(\mathrm{~m}, 8 \mathrm{H}), 3.86-3.75(\mathrm{~m}, 2 \mathrm{H}), 3.55-3.47(\mathrm{~m}, 2 \mathrm{H}), 3.39-$ 3.23 (m, 2H), 2.44-2.33 (m, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{22} \mathrm{~F}_{2} \mathrm{~N}_{4} \mathrm{O}_{2}, 436.2$; found $437.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-fluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2, 1-b]quinazoline-4-carboxamide hydrochloride (17). This compound was synthesized using 4fluoropiperidine hydrochloride (54f) ( $311.47 \mathrm{mg}, 2.23 \mathrm{mmol}$ ) according to method B. Deprotected diamine, 2-(4-fluoro-1-piperidyl)ethanamine hydrochloride (55f) ( $70.8 \mathrm{mg}, 0.39 \mathrm{mmol}$ ) was coupled with $56(75 \mathrm{mg}, 0.26 \mathrm{mmol})$ to give N -(2-(4-fluoropiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride ( $64.3 \mathrm{mg}, 0.14 \mathrm{mmol}, 54 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.24-11.14 (m, 1H), 10.53 (s, 1H), 9.12 (s, $1 \mathrm{H}), 8.96(\mathrm{~d}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.65(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{~d}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.4$ $\mathrm{Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.62(\mathrm{t}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H})$,
$7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 5.01(\mathrm{~d}, J=47.8 \mathrm{~Hz}, 1 \mathrm{H}), 4.0-3.90(\mathrm{~m}, 2 \mathrm{H}), 3.75-3.65(\mathrm{~m}$, $1 \mathrm{H}), 3.49-3.36(\mathrm{~m}, 2 \mathrm{H}), 3.31-3.10(\mathrm{~m}, 2 \mathrm{H}), 2.29-2.16(\mathrm{~m}, 1 \mathrm{H}), 2.16-2.05(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{24} \mathrm{H}_{23} \mathrm{FN}_{4} \mathrm{O}_{2}$, 418.2; found $419.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-bJquinazoline-4-carboxamide hydrochloride (18). This compound was synthesized using piperidine-4,4-diol hydrochloride (54g) ( $200 \mathrm{mg}, 1.30 \mathrm{mmol}$ ) according to method B. A portion of deprotected diamine, 1-(2-aminoethyl)piperidine-4,4-diol hydrochloride $(55 \mathrm{~g})(101.64 \mathrm{mg}, 0.52 \mathrm{mmol})$ was coupled with $56(100 \mathrm{mg}, 0.34$ mmol ) to give N -(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $48.7 \mathrm{mg}, 0.10$ mmol, $30 \%$ yield) as a yellow-orange solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d 6 ) $\delta \mathrm{ppm}$ $11.27(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.63(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H})$, 8.63 (s, 1H), 8.57 (dd, $J=7.0,1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=$ $8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.72(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.02-$ $3.96(\mathrm{~m}, 1 \mathrm{H}), 3.94-3.87(\mathrm{~m}, 1 \mathrm{H}), 2.93-2.76(\mathrm{~m}, 1 \mathrm{H}), 2.57(\mathrm{~d}, J=14.7 \mathrm{~Hz}, 2 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{24} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{4}, 432.2$; found $433.2[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-cyanopiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (19). This compound was synthesized using piperidine-4-carbonitrile (54h) ( $0.20 \mathrm{~mL}, 1.82 \mathrm{mmol}$ ) according to method C. A portion of deprotected diamine, 1-(2-aminoethyl)piperidine-4-carbonitrile trifluoroacetate (55h) (138.1 $\mathrm{mg}, 0.52 \mathrm{mmol}$ ) was coupled with $\mathbf{5 6}(100 \mathrm{mg}, 0.34$ mmol ) and purified via automated normal phase liquid chromatography using a 12
g silica cartridge with $1-10 \% \mathrm{MeOH} / \mathrm{DCM}$ to give N -(2-(4-cyanopiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $109.9 \mathrm{mg}, 0.24 \mathrm{mmol}, 69 \%$ yield) as an orange solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.27-11.15 (m, 1H), $10.59(\mathrm{~s}, 1 \mathrm{H}), 9.13(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{~d}$, $J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.65(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{~d}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H})$, $8.11(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.66-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, J=7.0$ $\mathrm{Hz}, 1 \mathrm{H}), 4.00-3.86(\mathrm{~m}, 2 \mathrm{H}), 3.78-3.64(\mathrm{~m}, 3 \mathrm{H}), 3.55-3.45(\mathrm{~m}, 1 \mathrm{H}), 3.43-3.33(\mathrm{~m}$, 1H), 3.13-3.01 (m, 2H), 2.32-2.22 (m, 1H), 2.20-2.05 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{25} \mathrm{H}_{23} \mathrm{~N}_{5} \mathrm{O}_{2}$, 425.2; found $426.2[\mathrm{M}+\mathrm{H}]^{+}$.

## 12-oxo-N-(2-thiomorpholinoethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-

carboxamide hydrochloride (20). This compound was synthesized using thiomorpholine (54i) ( $0.19 \mathrm{~mL}, 1.94 \mathrm{mmol}$ ) according to method B. A portion of deprotected diamine, 2-thiomorpholinoethanamine hydrochloride (55i) (94.42 mg, $0.52 \mathrm{mmol})$ was coupled with $56(100 \mathrm{mg}, 0.34 \mathrm{mmol})$ to give $12-\mathrm{oxo}-\mathrm{N}$-(2-thiomorpholinoethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $90.7 \mathrm{mg}, 0.20 \mathrm{mmol}, 58 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.23 (t, $J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.29(\mathrm{~s}, 1 \mathrm{H}), 9.13(\mathrm{~s}, 1 \mathrm{H}), 8.96$ (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.63(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=$ $8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08$ (t, J = 7.1 Hz, 1H), 4.03-3.80 (m, 3H), 3.50-3.42 (m, 2H), $3.30(\mathrm{q}, \mathrm{J}=11.0,10.6$ $\mathrm{Hz}, 2 \mathrm{H}), 3.13(\mathrm{t}, J=13.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.89(\mathrm{~d}, J=14.5 \mathrm{~Hz}, 2 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2} \mathrm{~S}, 418.2$; found $419.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(1,1-dioxidothiomorpholino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxamide hydrochloride (21). This compound was synthesized using thiomorpholine-1,1-dioxide (54j) (200 mg, 1.48 mmol$)$ according to method B. A portion of deprotected diamine, 2-(1,1-dioxo-1,4-thiazinan-4-yl)ethanamine hydrochloride (55j) (110.95 mg, 0.52 mmol$)$ was coupled with $56(100 \mathrm{mg}, 0.34$ mmol ) and purified via automated normal phase liquid chromatography using a 12 g silica cartridge with 1-10\% MeOH/DCM followed by a second column using a 12 $g$ silica cartridge with 6-50\% Acetone/DCM to give $N$-(2-(1,1-dioxidothiomorpholino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride ( $44 \mathrm{mg}, 0.090 \mathrm{mmol}, 26 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.25-11.18 (m, 1H), 9.13 (s, 1H), 8.95 (dd, J $=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.62(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{dd}, J=6.9,1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.4 \mathrm{~Hz}$, $1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.70(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.57(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, \mathrm{J}=$ $7.1 \mathrm{~Hz}, 1 \mathrm{H}$ ), 3.96-3.87 (obs.), 3.71-3.59 (obs.), 3.54-3.44 (obs.), 2.53-2.52 (obs.). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{4} \mathrm{~S}, 450.1$; found $451.0[\mathrm{M}+\mathrm{H}]^{+}$.

Method D: Synthesis of alternate cores. 12-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-2,3-dihydro-12H-[1,4]dioxino[2, 3-g]pyrido[2, 1-b]quinazoline-7-carboxamide hydrochloride (22). To a solution of 7-amino-2,3-dihydrobenzo[b][1,4]dioxine-6carboxylic acid (58a) (1.00 g, 5.12 mmol$)$ and 2-chloronicotinic acid (57) (807 mg, $5.12 \mathrm{mmol})$ in EtOH $(20 \mathrm{~mL})$ was added hydrochloric acid $(37 \%, 505 \mathrm{mg}, 5.12$ $\mathrm{mmol})$. The mixture was stirred for 12 hours at $90{ }^{\circ} \mathrm{C}$. Upon completion, the reaction mixture was concentrated under reduced pressure to give a residue and was purified by prep-HPLC (TFA condition; column: Phenomenex luna C18
$250 * 50 \mathrm{~mm} * 10 \mu \mathrm{~m}$;mobile phase: [water ( $0.1 \%$ TFA)- ACN]; B\%: 10\%-35\%, 18min.) to give ethyl 12-oxo-2,3-dihydro-12H-[1,4]dioxino[2,3-g]pyrido[2,1-b]quinazoline-7-carboxylate (59a) ( $1.25 \mathrm{~g}, 3.83 \mathrm{mmol}, 75 \%$ yield) as yellow oil. Ethyl 12-oxo-2,3-dihydro-12H-[1,4]dioxino[2,3-g]pyrido[2,1-b]quinazoline-7carboxylate (59a) ( $500 \mathrm{mg}, 1.53 \mathrm{mmol}$ ) was added to HCl aq. ( $2 \mathrm{M}, 20 \mathrm{~mL}$ ) in $\mathrm{H}_{2} \mathrm{O}$ $(20 \mathrm{~mL})$. The mixture was stirred for 12 hours at $100^{\circ} \mathrm{C}$. Upon completion, the reaction mixture was concentrated under reduced pressure to give 12-oxo-2,3-dihydro-12H-[1,4]dioxino[2,3-g]pyrido[2,1-b]quinazoline-7-carboxylic acid ( 0.31 g , crude) as a yellow solid, used without further purification. To a solution of 12-oxo-2,3-dihydro-12H-[1,4]dioxino[2,3-g]pyrido[2,1-b]quinazoline-7-carboxylic acid $(70.0 \mathrm{mg}, 0.24 \mathrm{mmol})$ in DMF ( 3.0 mL ) was added TBTU ( $90 \mathrm{mg}, 0.28 \mathrm{mmol}$ ) and DIPEA ( $91.0 \mathrm{mg}, 0.70 \mathrm{mmol}$ ). The mixture was added to 2-pyrrolidin-1ylethanamine ( $40 \mathrm{mg}, 0.35 \mathrm{mmol}$ ) in THF ( 1.0 mL ). The mixture was stirred for 12 hours at $25^{\circ} \mathrm{C}$. Upon completion, the reaction mixture was concentrated under reduced pressure and purified by prep-HPLC (HCI condition; column: Luna C18 $100 * 305$;-mobile phase: [water ( $0.04 \% \mathrm{HCl}$ )-ACN]; B\%: $1 \%-30 \%, 15 \mathrm{~min}$ ) to give 12-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-2,3-dihydro-12H-[1,4]dioxino[2,3-g]pyrido[2,1-b]quinazoline-7-carboxamide hydrochloride ( $62 \mathrm{mg}, 0.16 \mathrm{mmol}, 67 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{MeOD}$ ) $\delta \mathrm{ppm} 9.38(\mathrm{~d}, J=6.4 \mathrm{~Hz}, 1 \mathrm{H}), 9.03(\mathrm{~d}, J$ $=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.89(\mathrm{~s}, 1 \mathrm{H}), 7.67(\mathrm{t}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.49(\mathrm{~s}, 1 \mathrm{H}), 4.55-4.45(\mathrm{~m}$, $4 \mathrm{H}), 3.95-3.90(\mathrm{~m}, 4 \mathrm{H}), 3.59(\mathrm{t}, \mathrm{J}=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 3.25-3.21(\mathrm{~m}, 2 \mathrm{H}), 2.23-2.09(\mathrm{~m}$, 4 H ). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{4}, 394.2$; found $395.1[\mathrm{M}+\mathrm{H}]^{+}$.

12-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-7,9,10,12-tetrahydro-8H-benzo[g]pyrido[2,1-bJquinazoline-4-carboxamide hydrochloride (23). This compound was synthesized using 3 -amino-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid (58b), 2chloronicotinic acid (57), and 2-(pyrrolidine-1-yl)ethan-1-amine according to method D to give $14.6 \mathrm{mg}, 0.034 \mathrm{mmol} .{ }^{1} \mathrm{H}$ NMR ( 400 MHz , MeOD) $\delta \mathrm{ppm} 9.30$ (d, J = $6.4 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.90 (d, $J=7.2 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.20 (s, 1H), 7.70 (s, 1H), 7.48 (b.s., 1 H ), 3.97-3.88 (m, 4H), $3.58(\mathrm{t}, \mathrm{J}=6 \mathrm{~Hz}, 2 \mathrm{H}), 3.24$ (b.s, 2H) 3.08-3.04 (m, 4H), 2.22-2.11 ( $\mathrm{m}, 4 \mathrm{H}$ ), 1.94-1.93 ( $\mathrm{m}, 4 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}$, 390.2; found $391.1[\mathrm{M}+\mathrm{H}]^{+}$.

11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-[1,3]dioxolo[4,5-g]pyrido[2,1-
bJquinazoline-6-carboxamide hydrochloride (24). This compound was synthesized using 6 -aminobenzo[d][1,3]dioxole-5-carboxylic acid (58c), 2-chloronicotinic acid (57), and 2-pyrrolidin-1-ylethan-1-amine according to method D to give 1.3 mg , $0.0031 \mathrm{mmol} .{ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.31$ (s, 1H), 8.96 (m, 1H), 8.58 (m, 1H), 7.70-7.56 (m, 2H) 7.20 (s, 1H), 6.28 (s, 2H), 3.54 (s, 2H), 2.70-2.50 (m, 6H), 1.81 (b.s., 4H). LCMS (ESI): m/z calcd for $\mathrm{C}_{2} \mathrm{OH}_{2} \mathrm{~N}_{4} \mathrm{O}_{4}, 380.2$; found 381.1 $[\mathrm{M}+\mathrm{H}]^{+}$.

2,2-difluoro-11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-[1,3]dioxolo[4,5-g]pyrido[2,1-b]quinazoline-6-carboxamide hydrochloride (25). To a $10-20 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar, 5-bromo-2,2-difluoro-1,3-benzodioxole (61) (500 $\mathrm{mg}, 2.1 \mathrm{mmol}$ ), methyl 2-aminopyridine-3-carboxylate ( $\mathbf{6 0}$ ) ( $320 \mathrm{mg}, 2.1 \mathrm{mmol}$ ), tris(dibenzylideneacetone)dipalladium(0) ( $97 \mathrm{mg}, 0.10 \mathrm{mmol}$ ), and Xantphos (120
$\mathrm{mg}, 0.21 \mathrm{mmol}$ ) was added 1,4-dioxane ( 11 mL ) [deoxygenated by bubbling nitrogen for 5 min prior to initiation]. To this reaction mixture was added sodium tbutoxide ( $510 \mathrm{mg}, 5.3 \mathrm{mmol}$ ). Sealed reaction with teflon cap. Heated for 2 hours at $90^{\circ} \mathrm{C}$ using microwaves. Upon completion, desired mass of hydrolyzed product $[\mathrm{M}+1=295]$ is observed. Poured into brine, extracted with EtOAc, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered off solids, concentrated under reduced pressure. Purified by automated normal phase chromatography ( $0-100 \%$ EtOAC/heptane) to give 2 -((2,2-difluorobenzo[d][1,3]dioxol-5-yl)amino)nicotinic acid (62) (350 mg, 1.19 mmol, $56 \%$ yield). ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 6.91$ (dd, $J=7.71,4.80$ $\mathrm{Hz}, 1 \mathrm{H}) 7.28-7.37(\mathrm{~m}, 2 \mathrm{H}) 8.04(\mathrm{~d}, J=1.96 \mathrm{~Hz}, 1 \mathrm{H}) 8.26(\mathrm{dd}, J=7.74,1.99 \mathrm{~Hz}$, $1 \mathrm{H}) 8.39$ (dd, J = 4.77, $1.99 \mathrm{~Hz}, 1 \mathrm{H}) 10.47$ (s, 1 H ).

Benzyltrimethylammonium tribromide ( $230 \mathrm{mg}, 0.59 \mathrm{mmol}, 0.5$ equivalents) was added to a solution of 2-((2,2-difluorobenzo[d][1,3]dioxol-5-yl)amino)nicotinic acid (62) (350 mg, 1.2 mmol ) in $2: 1 \mathrm{DCM}(8 \mathrm{~mL}) / \mathrm{MeOH}(4 \mathrm{~mL})$ at room temperature. Stirred for 5 minutes. The reaction was monitored by LCMS. The expected mass for mono-brominated product is observed $[M+1]=373$, unreacted starting material remains. Added another 0.45 equivalents for a total of 0.95 equivalents. Upon completion, the crude reaction was poured into water, acidified to pH 1 using 1 N HCl aq., extracted with EtOAc, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered off solids, and concentrated under reduced pressure. Purified by automated reverse phase liquid chromatography (5-95\% ACN/water; $0.05 \%$ TFA buffer). The productcontaining fractions were combined. Concentrated under reduced pressure to give 2-((6-bromo-2,2-difluorobenzo[d][1,3]dioxol-5-yl) amino)nicotinic acid (63) (320
$\mathrm{mg}, 0.86 \mathrm{mmol}, 72 \%$ yield). ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 6.99$ (dd, $J=$ $7.74,4.77 \mathrm{~Hz}, 1 \mathrm{H}) 7.89(\mathrm{~s}, 1 \mathrm{H}) 8.31$ (dd, $J=7.77,1.96 \mathrm{~Hz}, 1 \mathrm{H}) 8.42$ (dd, $J=$ $4.83,2.05 \mathrm{~Hz}, 1 \mathrm{H}) 8.58(\mathrm{~s}, 1 \mathrm{H}) 10.68$ (s, 1 H$)$.

To a 20 mL scintillated vial charged with a magnetic stir bar, 2-((6-bromo-2,2-difluorobenzo[d][1,3]dioxol-5-yl) amino)nicotinic acid (63) (50 mg, 0.13 mmol ) was taken up in DMF ( 1.0 mL ). To this solution was added DIPEA (72 $\mu \mathrm{L}, 0.40$ mmol ), followed by TBTU ( $65 \mathrm{mg}, 0.20 \mathrm{mmol}$ ) and 2-pyrrolidin-1-ylethanamine (28 $\mu \mathrm{L}, 0.20 \mathrm{mmol})$. The reaction was stirred for 24 hours at room temperature and monitored by LCMS. Upon completion, the crude reaction was diluted with EtOAc, poured into water. The organic phase was washed with water, followed by brine, dried over sodium sulfate, filtered off solids, and concentrated under reduced pressure. Purified by automated reverse phase chromatography (5-95\% ACN/water; 0.05 \% TFA buffer). The product-containing fractions were combined. Concentrated under reduced pressure. To a $10-20 \mathrm{~mL}$ microwave vial with a septum were added of 2-[(6-bromo-2,2-difluoro-1,3-benzodioxol-5-yl)amino]-N-(2-pyrrolidin-1-ylethyl)pyridine-3-carboxamide (64) (135 mg, 0.29 mmol ) and degassed toluene ( 6 mL ). palladium(II) acetate ( $3.2 \mathrm{mg}, 0.01 \mathrm{mmol}$ ), Xantphos ( 25 $\mathrm{mg}, 0.04 \mathrm{mmol})$, Xantphos Pd G3 (13.6 mg, 0.01 mmol$)$ and $\mathrm{K}_{3} \mathrm{PO}_{4}(177 \mathrm{mg}, 0.83$ mmol ) were added while the solution was degassed with $\mathrm{N}_{2}$. The septum was replaced by the seal, and the mixture was degassed with CO for 5 min . The mixture was heated for 18 hours at $100^{\circ} \mathrm{C}$, then cooled to room temperature. The mixture was concentrated under reduced pressure. The material was purified by prepHPLC using ACN and 10 mM AmBic pH 10. The product-containing fractions were
combined. The material was taken in $\mathrm{DCM}(5 \mathrm{~mL})$ and $\mathrm{HCl}(4 \mathrm{~N}$ in dioxane, 0.15 $\mathrm{mL}, 0.58 \mathrm{mmol})$ was added. The mixture was concentrated under reduced pressure and lyophilized to provide title compound ( $28 \mathrm{mg}, 0.067 \mathrm{mmol}, 22 \%$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $\left.400 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}\right) \delta \mathrm{ppm} 9.14(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H})$, 8.78 (dd, $J=7.1,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~s}, 1 \mathrm{H}), 7.86(\mathrm{~s}, 1 \mathrm{H}), 7.24(\mathrm{t}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H})$, $3.97(\mathrm{t}, J=5.9 \mathrm{~Hz}, 2 \mathrm{H}), 3.88-3.81(\mathrm{~m}, 2 \mathrm{H}), 3.57(\mathrm{t}, J=5.9 \mathrm{~Hz}, 2 \mathrm{H}), 3.25-3.17(\mathrm{~m}$, $2 H), 2.26-2.15(\mathrm{~m}, 2 \mathrm{H}), 2.10-2.00(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{20} \mathrm{H}_{18} \mathrm{~F}_{2} \mathrm{~N}_{4} \mathrm{O}_{4}, 416.1$; found $416.2[\mathrm{M}+\mathrm{H}]^{+}$.

2,3-dimethyl-11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6carboxamide hydrochloride (26). This compound was synthesized using 2-amino-4,5-dimethylbenzoic acid (58d), 2-chloronicotinic acid (57), and 2-(pyrrolidine-1-yl)ethan-1-amine according to method D to give $23.9 \mathrm{mg}, 0.060 \mathrm{mmol} .{ }^{1} \mathrm{H}$ NMR (400 MHz, MeOD) $\delta$ ppm $8.95(\mathrm{~d}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.58(\mathrm{~d}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.28$ (b.s., 1H), $7.97(\mathrm{~s}, 1 \mathrm{H}), 7.55(\mathrm{~s}, 1 \mathrm{H}), 7.06(\mathrm{t}, \mathrm{J}=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 3.95(\mathrm{~m}, 2 \mathrm{H}), 3.94-$ $3.52(\mathrm{~m}, 6 \mathrm{H}), 2.42(\mathrm{~d}, 6 \mathrm{H}), 2.12(\mathrm{~s}, 4 \mathrm{H}) . \mathrm{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}$, 364.2; found $365.2[\mathrm{M}+\mathrm{H}]^{+}$.

2,3-dimethoxy-11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6carboxamide hydrochloride (27). This compound was synthesized using 2-amino-4,5-dimethoxybenzoic acid (58e), 2-chloronicotinic acid (57), and 2-(pyrrolidine-1-yl)ethan-1-amine according to method D to give $4.7 \mathrm{mg}, 0.011 \mathrm{mmol} .{ }^{1} \mathrm{H}$ NMR ( 400 $\left.\mathrm{MHz}, \mathrm{DMSO}-d_{6}\right) \delta \mathrm{ppm} 11.05-11.04(\mathrm{~m}, 1 \mathrm{H}), 9.02(\mathrm{dd}, J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.59$ (dd, $J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.60(\mathrm{~s}, 1 \mathrm{H}), 7.24(\mathrm{~s}, 1 \mathrm{H}), 7.19(\mathrm{t}, J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 4.01$
(s, 3H), $3.95(\mathrm{~s}, 3 \mathrm{H}), 3.58$ (obs.), 2.76 (t, $J=6.0 \mathrm{~Hz}, 2 \mathrm{H}$ ), 2.63 (b.s., 4 H ), 1.75 (b.s., 4H). LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{4}$, 396.2; found $397.1[\mathrm{M}+\mathrm{H}]^{+}$.

3-hydroxy-12-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxamide hydrochloride (28). To a $10-20 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar was dissolved methyl 2-amino-4-methoxypyridine-3-carboxylate (65) (400 mg, 2.20 mmol ) in anhydrous toluene (14 mL ). Argon was bubbled through the solution. 2,3-dibromonapthalene (66) (731 $\mathrm{mg}, 2.43 \mathrm{mmol})$, cesium carbonate ( $1 \mathrm{~g}, 3.07 \mathrm{mmol}$ ), Xantphos (191 mg, 0.329 mmol ), and palladium(II) acetate ( $50 \mathrm{mg}, 0.220 \mathrm{mmol}$ ) were added and the vial was sealed. The mixture was heated for 2 hours at $130^{\circ} \mathrm{C}$ using microwaves. The reaction mixture was cooled to room temperature and diluted with water. The aqueous layer was extracted $2 x$ with EtOAc. Combined organic layers were washed 1 x with water, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography: silica gel, Merck 70 $\mathrm{g}, 15-40 \mu \mathrm{~m}$, dry loading, eluent: heptane/DCM to give methyl 2-[(3-bromo-2-napthyl)amino]-4-methoxy-pyridine-3-carboxylate (67) (391 mg, $1.01 \mathrm{mmol}, 46 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 9.58(\mathrm{~s}, 1 \mathrm{H}), 8.77$ (s, $1 \mathrm{H}), 8.32(\mathrm{~s}, 1 \mathrm{H}), 8.29(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.84(\mathrm{t}, J=8.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.51(\mathrm{dd}, J=$ $8.0,8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.43(\mathrm{dd}, J=10.0,8.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.80(\mathrm{~d}, J=4.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.91(\mathrm{~s}$, $3 H$ ), 3.89 (s, 3H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{18} \mathrm{H}_{15} \mathrm{BrN}_{2} \mathrm{O}_{3}, 386.0$; found 387.2 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a suspension of methyl 2-[(3-bromo-2-napthyl)amino]-4-methoxypyridine-3-carboxylate (67) (391 mg, 1.01 mmol ) in $\mathrm{MeOH}(7 \mathrm{~mL})$ was added $1 \mathrm{M} \mathrm{NaOH}(2.1 \mathrm{~mL}, 2.12 \mathrm{mmol})$. The mixture was stirred overnight at $59^{\circ} \mathrm{C}$ then for 3 hours at $65{ }^{\circ} \mathrm{C}$. The reaction mixture was cooled to room temperature and the solvent concentrated in vacuo. The residue was acidified to $\mathrm{pH} \sim 4$ with 1 N citric acid. The precipitate that formed was filtered, washed with a minimum amount of water, and dried on vacuo at $35{ }^{\circ} \mathrm{C}$ to give 2-[(3-bromo-2-napthyl)amino]-4-methoxy-pyridine-3-carboxylic acid ( $386 \mathrm{mg}, 1.03 \mathrm{mmol}$ ) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $d_{6}$ ) $\delta$ ppm $10.21(\mathrm{~s}, 1 \mathrm{H}), 8.92(\mathrm{~s}, 1 \mathrm{H})$, $8.29(\mathrm{~s}, 1 \mathrm{H}), 8.25(\mathrm{~d}, J=4.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.81(\mathrm{t}, J=8.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.49(\mathrm{dd}, J=8.0,8.0$ $\mathrm{Hz}, 1 \mathrm{H}), 7.39(\mathrm{dd}, J=8.0,8.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.75(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.88(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{17} \mathrm{H}_{13} \mathrm{BrN}_{2} \mathrm{O}_{3}, 372.0$; found $373.1[\mathrm{M}+\mathrm{H}]^{+}$.

To a 2-5 mL microwave vial charged with a magnetic stir bar was dissolved 2-[(3-bromo-2-napthyl)amino]-4-methoxypyridine-3-carboxylic acid (224 mg, $0.600 \mathrm{mmol})$, TBTU (275 mg, 0.840 mmol ), $N$-(2-aminoethyl)pyrrolidine (109 $\mu \mathrm{L}$, 0.840 mmol ), and DIPEA ( $314 \mu \mathrm{~L}, 1.80 \mathrm{mmol}$ ) successively in anhydrous DCM (5 $\mathrm{mL})$. The vial was sealed and heated overnight at $45^{\circ} \mathrm{C}$. The reaction mixture was cooled to room temperature and quenched with a saturated solution of $\mathrm{NaHCO}_{3}$. The aqueous layer was extracted with DCM ( $3 \times 50 \mathrm{~mL}$ ). Combined organic layers were washed with water, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography on a 30 g SI 60 15-40 $\mu \mathrm{m}$ column with $\mathrm{DCM} / \mathrm{MeOH} 0-10 \%$ to give 2-[(3-bromo-2-napthyl)amino]-4-methoxy- $N$-(2-pyrrolidin-1-ylethyl)pyridine-3-carboxamide (68) (226 mg, 0.481
mmol, $80 \%$ yield). ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm} 10.86$ (s, 1H), 8.87 (s, $1 \mathrm{H}), 8.60(\mathrm{t}, J=4.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.29(\mathrm{~s}, 1 \mathrm{H}), 8.26(\mathrm{~d}, J=4.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.49(\mathrm{dd}, J=$ 8.0, 8.0 Hz, 1H), 7.41 (dd, $J=8.0,4.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.79(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.95(\mathrm{~s}$, 3H), 3.49 ( $q, J=8.0 \mathrm{~Hz}, 2 H$ ), 2.75-2.66 (m, 6H), 1.72 (s, 4H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{25} \mathrm{BrN}_{4} \mathrm{O}_{2}$, 468.1; found $469.1[\mathrm{M}+\mathrm{H}]^{+}$.

To a 2-5 mL microwave vial charged with a magnetic stir bar was added anhydrous toluene ( 3 mL ). Carbon monoxide was bubbled to saturate the solvent. Then 2-[(3-bromo-2-napthyl)amino]-4-methoxy-N-(2-pyrrolidin-1-ylethyl)pyridine-3-carboxamide (68) (50 mg, 0.107 mmol ), palladium(II) acetate ( $2.4 \mathrm{mg}, 0.011$ $\mathrm{mmol})$, Xantphos ( $9.2 \mathrm{mg}, 0.016 \mathrm{mmol}$ ), Xantphos Pd G3 ( $5.3 \mathrm{mg}, 0.006 \mathrm{mmol}$ ), and $\mathrm{K}_{3} \mathrm{PO}_{4}$ ( $69 \mathrm{mg}, 0.320 \mathrm{mmol}$ ) were added successively. The mixture was bubbled again with carbon monoxide for 5 minutes then sealed. The mixture was heated for 2 hours at $130{ }^{\circ} \mathrm{C}$ using microwaves. By LCMS, the hydroxyl byproduct looked to form instead of the methoxy product. The reaction mixture was cooled to room temperature and filtered on Talc. Rinsed with EtOAc and the filtrate was concentrated in vacuo. Purified via automated normal phase liquid chromatography on a 15 g SI60 15-40 $\mu \mathrm{m}$ column with DCM/MeOH 0-10\% to give 3-hydroxy-12-oxo-N-(2-pyrrolidin-1-ylethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide ( $6 \mathrm{mg}, 0.015 \mathrm{mmol}, 14 \%$ yield). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{3}, 402.2$; found $403.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 50 mL round-bottom flask charged with a magnetic stir bar was dissolved 3 -hydroxy-12-oxo- N -(2-pyrrolidin-1-ylethyl)-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxamide ( $6 \mathrm{mg}, 0.015 \mathrm{mmol}$ ) in a $1: 1$ mixture of $\mathrm{DCM} / \mathrm{MeOH}$ ( 1 mL ). A solution of 4 M HCl in 1,4-dioxane ( $11 \mu \mathrm{~L}, 0.045 \mathrm{mmol}$ ) was added dropwise and the mixture was stirred for 1 hour at room temperature. Diethyl ether was then added ( $10-20 \mathrm{~mL}$ ) until precipitation of a yellow solid. The solid was collected by filtration and dried on vacuo overnight to give 3-hydroxy-12-oxo- N -(2-pyrrolidin-1-ylethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride ( $5.7 \mathrm{mg}, 0.013 \mathrm{mmol}, 87 \%$ yield). ${ }^{1} \mathrm{H}$ NMR ( 600 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta$ ppm $11.18(\mathrm{t}, J=6 \mathrm{~Hz}, 1 \mathrm{H}), 9.86$ (b.s., 1 H$), 9.00(\mathrm{~s}, 1 \mathrm{H}), 8.62(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H})$, $8.25(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.09(\mathrm{~s}, 1 \mathrm{H}), 8.04(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.73(\mathrm{dd}, J=9.6$, $6.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.57(\mathrm{dd}, J=8.4,6.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.49(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 3.76(\mathrm{q}, J=$ $6.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.64-3.62(\mathrm{~m}, 2 \mathrm{H}), 3.40(\mathrm{q}, J=6.6 \mathrm{~Hz}, 2 \mathrm{H}), 3.10-3.07(\mathrm{~m}, 2 \mathrm{H}), 2.04-$ $2.01(\mathrm{~m}, 2 \mathrm{H}), 1.91-1.87(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{3}, 402.2$; found $403.3[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(pyrrolidin-1-yl)ethyl)naphtho[2',3':4,5]imidazo[1,2-a]pyridine-4-carboxamide hydrochloride (29). To a 2-5 mL microwave vial charged with a magnetic stir bar was dissolved methyl 2-chloropyridine-3-carboxylate (69) (100 mg, 0.58 mmol$)$ in anhydrous toluene ( 3 mL ). The mixture was purged three times with argon, then 2-bromonapthalen-1-amine (70) (163 mg, 0.70 mmol ), palladium(II) acetate (13 $\mathrm{mg}, 0.06 \mathrm{mmol}$ ), Xantphos ( $51 \mathrm{mg}, 0.09 \mathrm{mmol}$ ), and cesium carbonate ( 266 mg , 0.82 mmol ) were added. The mixture was purged again three times with argon, sealed, and heated for 2 hours at $130^{\circ} \mathrm{C}$ using microwaves. The reaction mixture was cooled to room temperature and diluted with water. The aqueous layer was extracted with EtOAc ( $3 \times 50 \mathrm{~mL}$ ). Combined organic layers were washed with
brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated under reduced pressure. Purified via automated normal phase liquid chromatography on a 30 g SI 60 15-40 $\mu \mathrm{m}$ column with EtOAc/Heptane (0-50\%) to give methyl naphtho[ $\left.1^{\prime}, 2^{\prime}: 4,5\right]$ imidazo[1,2-a]pyridine-11-carboxylate (74) (30 mg, 0.11 mmol , $18 \%$ yield) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 9.47-9.41$ $(\mathrm{m}, 1 \mathrm{H}), 8.71(\mathrm{~d}, J=8.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.44(\mathrm{~d}, J=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.22-8.17(\mathrm{~m}, 1 \mathrm{H}), 8.14$ (d, $J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.90(\mathrm{~d}, J=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.63(\mathrm{~m}, 1 \mathrm{H})$, $7.24(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 4.01(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{2}, 276.1$; found $277.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 50 mL round-bottom flask charged with a magnetic stir bar was dissolved methyl naphtho[1',2':4,5]imidazo[1,2-a]pyridine-11-carboxylate (74) (25 $\mathrm{mg}, 0.09 \mathrm{mmol})$ in $\mathrm{MeOH}(2 \mathrm{~mL})$. A solution of $1 \mathrm{M} \mathrm{NaOH}(190 \mu \mathrm{~L}, 0.19 \mathrm{mmol})$ was added dropwise and the mixture was stirred overnight at room temperature. The mixture was acidified to $\mathrm{pH} \sim 3-4$ with 1 N HCl . A solid precipitated but some of the product was still soluble, so MeOH was evaporated under reduced pressure and water was added to the mixture. The precipitate was filtered, washed with water, and dried overnight in vacuo to give naphtho[ $\left.1^{\prime}, 2^{\prime}: 4,5\right]$ imidazo[1,2-a]pyridine-11carboxylic acid ( $21 \mathrm{mg}, 0.08 \mathrm{mmol}, 88 \%$ yield) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR (400 $\left.\mathrm{MHz}, \mathrm{DMSO}_{6}-\mathrm{d}_{6}\right) \delta \mathrm{ppm} 9.62(\mathrm{~s}, 1 \mathrm{H}), 8.90(\mathrm{~s}, 1 \mathrm{H}), 8.54(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.45(\mathrm{~s}$, $1 \mathrm{H}), 8.20(\mathrm{~d}, J=6.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.05(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.81(\mathrm{~s}, 1 \mathrm{H}), 7.74(\mathrm{~s}, 1 \mathrm{H})$, $7.50(\mathrm{~s}, 1 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{16} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{2}, 262.1$; found $263.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 5 mL vial charged with a magnetic stir bar was added naphtho[1',2':4,5]imidazo[1,2-a]pyridine-11-carboxylic acid (18 mg, 0.07 mmol ), TBTU ( $31 \mathrm{mg}, 0.10 \mathrm{mmol}$ ), and DIPEA ( $48 \mu \mathrm{~L}, 0.28 \mathrm{mmol}$ ) in DMF ( 1 mL ). The resulting mixture was stirred for 5 minutes at room temperature before adding N -(2-aminoethyl)pyrrolidine ( $22 \mu \mathrm{~L}, 0.14 \mathrm{mmol}$ ). The vial was sealed and the reaction was stirred overnight at room temperature. The reaction was quenched with a saturated solution of $\mathrm{NaHCO}_{3}$ then the organic layer was extracted with EtOAc (3 $x 30 \mathrm{~mL}$ ). Combined organic layers were washed with brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography on a 15 g SI60 15-40 $\mu \mathrm{m}$ column with MeOH/DCM (0-10\%) to give $\quad N$-(2-(pyrrolidin-1-yl)ethyl)naphtho[1',2':4,5]imidazo[1,2-a]pyridine-11carboxamide ( $17 \mathrm{mg}, 0.05 \mathrm{mmol}, 68 \%$ yield) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR (400 $\left.\mathrm{MHz}, \mathrm{DMSO}-d_{6}\right) \delta \mathrm{ppm} 10.86(\mathrm{~d}, J=4.9 \mathrm{~Hz}, 1 \mathrm{H}), 9.41(\mathrm{dd}, J=6.8,1.3 \mathrm{~Hz}, 1 \mathrm{H})$, 8.77 (d, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.47(\mathrm{~d}, J=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.35(\mathrm{dd}, J=7.1,1.3 \mathrm{~Hz}, 1 \mathrm{H})$, $8.16(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.93(\mathrm{~d}, J=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.65(\mathrm{~m}, 2 \mathrm{H}), 7.32(\mathrm{t}, J=$ $6.9 \mathrm{~Hz}, 1 \mathrm{H}), 3.69(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 2.80(\mathrm{~s}, 2 \mathrm{H}), 2.69(\mathrm{~s}, 4 \mathrm{H}), 1.87(\mathrm{~s}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{22} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}, 358.2$; found $359.4[\mathrm{M}+\mathrm{H}]^{+}$.

To a 50 mL round-bottom flask was dissolved N -(2-(pyrrolidin-1-yl)ethyl)naphtho[1',2':4,5]imidazo[1,2-a]pyridine-11-carboxamide (17 mg, 0.05 mmol ) in a $\mathrm{DCM} / \mathrm{MeOH}$ 1:1 mixture ( 3 mL ). A solution of 4 M HCl in 1,4-dioxane ( $36 \mu \mathrm{~L}, 0.14 \mathrm{mmol}$ ) was added dropwise, and the reaction was stirred for 1 hour at room temperature. Diethyl ether was then added (10-20 mL) until precipitation of a yellow solid. A solid precipitated but was too thin to be filtered so solvents were
removed under vacuo to give $N$-(2-(pyrrolidin-1-yl)ethyl)naphtho[1',2':4,5]imidazo[1,2-a]pyridine-11-carboxamide hydrochloride ( $17.7 \mathrm{mg}, 0.04 \mathrm{mmol}, 95 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d ) $\delta$ ppm 10.65 (s, 1H), 10.35 (s, 1H), 9.49 (d, $J=10.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.95(\mathrm{~d}, J=10.0 \mathrm{~Hz}$, $1 \mathrm{H}), 8.50(\mathrm{~d}, J=10.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.41(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.17(\mathrm{~d}, J=10.0 \mathrm{~Hz}, 1 \mathrm{H})$, 7.97 (d, J = $10.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{dt}, J=35.0,7.5 \mathrm{~Hz}, 2 \mathrm{H}), 7.39(\mathrm{t}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H})$, 3.99-3.96 (obs.), 3.75-3.70 (obs.), 3.53 (q, J=7.5, 2H), 3.18-3.11 (m, 2H), 2.05$2.00(\mathrm{~m}, 2 \mathrm{H}), 1.92-1.88(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{22} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}, 358.2$; found $359.1[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(pyrrolidin-1-yl)ethyl)pyrido[2', 1':2,3]imidazo[4,5-b]quinoline-10-carboxamide hydrochloride (30). To a $10-20 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar was added 3 -bromoquinoline ( 75 ) ( $301 \mathrm{mg}, 1.42 \mathrm{mmol}$ ) in toluene ( 12 mL ). The mixture was bubbled with argon, then methyl 2-aminopyridine-3-carboxylate (60) ( $200 \mathrm{mg}, 1.29 \mathrm{mmol}$ ), cesium carbonate ( $591 \mathrm{mg}, 1.80 \mathrm{mmol}$ ), Xantphos ( 112 mg , 0.19 mmol ), and palladium(II) acetate ( $29 \mathrm{mg}, 0.29 \mathrm{mmol}$ ) were added. The vial was sealed and heated for 2 hours at $130^{\circ} \mathrm{C}$ using microwaves. The reaction was cooled to room temperature and quenched with water. The aqueous layer was extracted 2 x with EtOAc. Combined organic layers were washed 1 x with water, 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered over a pad of talc, and evaporated to dryness to give a brown solid. The crude was taken up with EtOAc to give a suspension that was filtered. The residue was collected and dried on vacuum at $35^{\circ} \mathrm{C}$ to give 210 mg of a beige solid. The filtrate was evaporated to dryness and the process repeated to give an additional 36 mg . The residues were combined to
give methyl 2-(quinolin-3-ylamino)nicotinate (76) (246 mg, $0.88 \mathrm{mmol}, 68 \%$ yield) as a beige solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.36(\mathrm{~s}, 1 \mathrm{H}), 9.02(\mathrm{~d}, \mathrm{~J}=$ $2.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{~d}, J=2.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.53(\mathrm{dd}, J=4.8,1.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.34(\mathrm{dd}, J=$ 7.8, $2.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.00-7.88 (m, 2H), 7.67-7.53 (m, 2H), $7.02(\mathrm{dd}, J=7.8,4.8 \mathrm{~Hz}$, 1H), 3.96 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{16} \mathrm{H}_{13} \mathrm{~N}_{3} \mathrm{O}_{2}$, 279.1; found 280.3 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a suspension of methyl 2-(quinolin-3-ylamino)nicotinate (76) (138 mg, 0.49 mmol ) in DCM ( 4.6 mL ) was added N -bromosuccinimide ( $97 \mathrm{mg}, 0.54 \mathrm{mmol}$ ) and the reaction was stirred overnight at room temperature. The reaction mixture was quenched with water and the aqueous layer was extracted 2 x with DCM. Combined organic layers were washed 1 x with water, 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and evaporated to dryness to give a yellow solid. Purified by flash chromatography: RediSep Gold $24 \mathrm{~g}, 30 \mu \mathrm{~m}$ spheric, eluent: $\mathrm{MeOH} / \mathrm{DCM}$, liquid loading to give methyl pyrido[2',1':2,3]imidazo[4,5-b]quinoline-10-carboxylate (77) $\left(65 \mathrm{mg}, 0.23 \mathrm{mmol}, 47 \%\right.$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 600 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta$ ppm $9.30(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.92(\mathrm{~s}, 1 \mathrm{H}), 8.39(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.26(\mathrm{dd}, J=$ $21.3,8.1 \mathrm{~Hz}, 2 \mathrm{H}), 7.82(\mathrm{dd}, J=8.4,5.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.67(\mathrm{dd}, J=8.4,5.4 \mathrm{~Hz}, 1 \mathrm{H})$, $7.15(\mathrm{t}, \mathrm{J}=3.6 \mathrm{~Hz}, 1 \mathrm{H}), 3.98(\mathrm{~s}, 3 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): m / z$ calcd for $\mathrm{C}_{16} \mathrm{H}_{11} \mathrm{~N}_{3} \mathrm{O}_{2}$, 277.1; found $278.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a suspension of methyl pyrido[2',1':2,3]imidazo[4,5-b]quinoline-10carboxylate (77) (78 mg, 0.28 mmol$)$ in $\mathrm{MeOH}(5 \mathrm{~mL})$ was added $1 \mathrm{M} \mathrm{NaOH}(0.70$ $\mathrm{mL}, 0.70 \mathrm{mmol}$ ) and the reaction mixture was stirred overnight at room
temperature. The solvent was evaporated and the residue was taken up with water and extracted with DCM. The aqueous layer was collected and acidified with 1 N HCl to give a suspension that was filtered. The residue was collected and dried on vacuum at $35^{\circ} \mathrm{C}$ to give pyrido[2', $1^{\prime}: 2,3$ ]imidazo[4,5-b]quinoline-10-carboxylic acid ( $63 \mathrm{mg}, 0.24 \mathrm{mmol}, 84 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta$ ppm $14(\mathrm{~m}, 1 \mathrm{H}), 9.73(\mathrm{~d}, J=6.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.97(\mathrm{~s}, 1 \mathrm{H}), 8.88(\mathrm{~d}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H})$, $8.44(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.02-7.93(\mathrm{~m}, 1 \mathrm{H}), 7.83(\mathrm{t}, J=$ $7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.70(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}) . \mathrm{LCMS}(\mathrm{ESI}): m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{9} \mathrm{~N}_{3} \mathrm{O}_{2}, 263.1$; found $264.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a suspension of pyrido[2',1':2,3]imidazo[4,5-b]quinoline-10-carboxylic acid $(63 \mathrm{mg}, 0.24 \mathrm{mmol})$ in DMF $(2 \mathrm{~mL})$ was added TBTU $(110 \mathrm{mg}, 0.34 \mathrm{mmol})$ and DIPEA $(0.17 \mathrm{~mL}, 0.96 \mathrm{mmol})$ to give a red solution. Then, N -(2aminoethyl)pyrrolidine ( $0.043 \mathrm{~mL}, 0.34 \mathrm{mmol}$ ) was added and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was quenched with water and extracted $2 x$ with EtOAc. Combined organic layers were washed $1 x$ with water, $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered and evaporated to dryness to give a red solid. Purified by flash chromatography: silica gel, RediSep Gold $12 \mathrm{~g}, 30 \mu \mathrm{~m}$ spheric, eluent: $\mathrm{MeOH} / \mathrm{DCM}$, liquid loading to give N -(2-(pyrrolidin-1-yl)ethyl)pyrido[2',1':2,3]imidazo[4,5-b]quinoline-10-carboxamide (47 $\mathrm{mg}, 0.13 \mathrm{mmol}, 55 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $\left.400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}\right) \delta \mathrm{ppm}$ $10.31(\mathrm{~s}, 1 \mathrm{H}), 9.29(\mathrm{dd}, J=6.7,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{~s}, 1 \mathrm{H}), 8.54(\mathrm{dd}, J=7.1,1.4 \mathrm{~Hz}$, $1 \mathrm{H}), 8.32(\mathrm{~d}, \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.26(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.89-7.80(\mathrm{~m}, 1 \mathrm{H}), 7.69(\mathrm{t}$, $J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.26(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 3.64(\mathrm{q}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.75(\mathrm{~d}, J=6.3$
$\mathrm{Hz}, 2 \mathrm{H}$ ), $2.55(\mathrm{~s}, 4 \mathrm{H}), 1.79(\mathrm{~s}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{21} \mathrm{~N}_{5} \mathrm{O}, 359.2$; found $360.3[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of N -(2-(pyrrolidin-1-yl)ethyl)pyrido[2',1':2,3]imidazo[4,5-b]quinoline-10-carboxamide ( $47 \mathrm{mg}, 0.13 \mathrm{mmol}$ ) in $1: 1 \mathrm{MeOH} / \mathrm{DCM}(4 \mathrm{~mL})$ was added 4 M HCl in 1,4 -dioxane ( $0.098 \mathrm{~mL}, 0.39 \mathrm{mmol}$ ). The reaction mixture was stirred for 1 hour at room temperature. Diethyl ether was added and a suspension formed. The precipitate was filtered, washed with diethyl ether, and dried on vacuo at $35^{\circ} \mathrm{C}$ to give $N$-(2-(pyrrolidin-1-yl)ethyl)pyrido[2', 1':2,3]imidazo[4,5-b]quinoline-10-carboxamide hydrochloride ( $57.1 \mathrm{mg}, 0.14 \mathrm{mmol}$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO-d 6 ) $\delta \mathrm{ppm} 10.40$ (b.s., 1 H ), 10.21-10.19 (m, 1H), 9.40 (d, J=7.8 Hz, 1H), 8.92 (s, 1H), $8.67-8.66(\mathrm{~m}, 1 \mathrm{H}), 8.34(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.27(\mathrm{~d}, J=8.5$ $\mathrm{Hz}, 1 \mathrm{H}), 7.87(\mathrm{t}, \mathrm{J}=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.72(\mathrm{t}, \mathrm{J}=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.39-7.37(\mathrm{~m}, 1 \mathrm{H}), 3.91$ ( $\mathrm{q}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}$ ), $3.68-3.64(\mathrm{~m}, 2 \mathrm{H}), 3.47(\mathrm{q}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.15-3.08(\mathrm{~m}, 2 \mathrm{H})$, 2.06-1.99 (m, 2H), 1.93-1.89 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{2} \mathrm{~N}_{5} \mathrm{O}, 359.2$; found $360.4[\mathrm{M}+\mathrm{H}]^{+}$.

7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxylic acid (73). To a $10-20 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar, methyl 2-chloropyridine-3carboxylate ( 69 ) ( $1 \mathrm{~g}, 5.83 \mathrm{mmol}$ ) was dissolved in anhydrous toluene ( 15 mL ). The mixture was purged three times with argon. Then, 2-bromonapthalen-1-amine (70) ( $1499 \mathrm{mg}, 6.41 \mathrm{mmol}$ ), palladium(II) acetate ( $53 \mathrm{mg}, 0.233 \mathrm{mmol}$ ), rac-BINAP $(218 \mathrm{mg}, 0.350 \mathrm{mmol})$, and cesium carbonate ( $2659 \mathrm{mg}, 8.16 \mathrm{mmol}$ ) were successively added. The mixture was purged again three times with argon before
the vial was sealed. The reaction was heated for 2 hours at $130{ }^{\circ} \mathrm{C}$ using microwaves. The reaction mixture was cooled to room temperature, water was added, and the mixture was extracted with EtOAc. Combined organic layers were washed with brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. The crude material was washed with diethyl ether until purity $>90 \%$ to give methyl 2-((2-bromonaphthalen-1-yl)amino)nicotinate (71) (810 mg, $2.27 \mathrm{mmol}, 34 \%$ yield) as a grey solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta$ ppm 9.64 (s, 1H), 8.28 (dd, $J=$ $7.8,1.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.09(\mathrm{dd}, J=4.7,1.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.87(\mathrm{~d}, J$ $=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.85-7.76(\mathrm{~m}, 1 \mathrm{H}), 7.55(\mathrm{~m}, 1 \mathrm{H}), 6.81(\mathrm{dd}, \mathrm{J}=7.8,4.7 \mathrm{~Hz}, 1 \mathrm{H}), 3.96$ (s, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{17} \mathrm{H}_{13} \mathrm{BrN}_{2} \mathrm{O}_{2}, 356.0$; found $356.9[\mathrm{M}+\mathrm{H}]^{+}$.

To a 40 mL vial charged with a magnetic stir bar was dissolved methyl 2-((2-bromonaphthalen-1-yl)amino)nicotinate (71) (1 g, 2.80 mmol$)$ in anhydrous toluene ( 15 mL ). The mixture was purged three times with argon then palladium(II) acetate ( $63 \mathrm{mg}, 0.28 \mathrm{mmol}$ ), Xantphos ( $243 \mathrm{mg}, 0.42 \mathrm{mmol}$ ), Xantphos-Pd-G3 $(140 \mathrm{mg}, 0.14 \mathrm{mmol})$, and $\mathrm{K}_{3} \mathrm{PO}_{4}(1808 \mathrm{mg}, 8.40 \mathrm{mmol})$ were added. The mixture was purged again three times before adding hexakis(oxomethylidene)molybdenum ( $739 \mathrm{mg}, 2.80 \mathrm{mmol}$ ). The vial was sealed and stirred overnight at $115{ }^{\circ} \mathrm{C}$. The mixture was cooled to room temperature, filtered over a pad of talc, and rinsed with EtOAc. The filtrate was concentrated in vacuo. Purified via automated normal phase liquid chromatography using a 90 g SI60 15-40 $\mu \mathrm{m}$ column ( $0-75 \%$ EtOAc/heptane) to give methyl 7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxylate (72) (310 mg, $1.02 \mathrm{mmol}, 30 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6$ ) $\delta \mathrm{ppm} 9.08$ (dd, $J=7.3,1.6$
$\mathrm{Hz}, 2 \mathrm{H}), 9.00(\mathrm{~s}, 1 \mathrm{H}), 8.24-8.15(\mathrm{~m}, 3 \mathrm{H}), 8.09(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 2 \mathrm{H}), 7.94(\mathrm{~d}, J=8.8$ $\mathrm{Hz}, 2 \mathrm{H}), 7.90-7.76(\mathrm{~m}, 4 \mathrm{H}), 7.69-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.41-7.24(\mathrm{~m}, 4 \mathrm{H}), 4.05(\mathrm{~s}, 5 \mathrm{H})$, 1.65 (d, $J=21.6 \mathrm{~Hz}, 1 \mathrm{H}$ ). LCMS (ESI): m/z calcd for $\mathrm{C}_{18} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{3}, 304.1$; found $305.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 50 mL round-bottom flask charged with a magnetic stir bar was dissolved methyl 7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxylate (310 $\mathrm{mg}, 1.02 \mathrm{mmol})(\mathbf{7 2})$ in $\mathrm{MeOH}(10 \mathrm{~mL})$. A solution of $1 \mathrm{M} \mathrm{NaOH}(2.14 \mathrm{~mL}, 2.14$ mmol ) was added dropwise and the mixture was stirred overnight at room temperature. The mixture was acidified using 1 N HCl until $\mathrm{pH} \sim 2-3$, then the product precipitated as a yellow solid. The solid was filtered and dried in vacuo to give 7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxylic acid ( $250 \mathrm{mg}, 0.86$ mmol, $84 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm} 16.81$ (m, 1H), $9.22(\mathrm{~d}, \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.75-8.65(\mathrm{~m}, 2 \mathrm{H}), 8.28-8.15(\mathrm{~m}, 2 \mathrm{H}), 8.03(\mathrm{~d}, \mathrm{~J}$ $=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.92(\mathrm{dd}, J=6.1,3.2 \mathrm{~Hz}, 2 \mathrm{H}), 7.45(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{17} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{3}, 290.1$; found $291.1[\mathrm{M}+\mathrm{H}]^{+}$.

Method E: Synthesis of amide analogs with commercially available amine. N -(2-(dimethylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-
carboxamide (37 free base). To a 10 mL vial charged with a magnetic stir bar was added 73 (14 mg, 0.048 mmol$)$, TBTU ( $22 \mathrm{mg}, 0.068 \mathrm{mmol}$ ), and DIPEA ( $25.3 \mu \mathrm{~L}$, 0.145 mmol ) in anhydrous DMF ( 0.4 mL ). The resulting mixture was stirred overnight at room temperature then quenched with a saturated solution of $\mathrm{NaHCO}_{3}$. The aqueous layer was extracted with EtOAc and DCM. Combined
organic layers were washed with brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography using a $12 \mathrm{~g} \mathrm{SI} 6015-40 \mu \mathrm{~m}$ column (10\% MeOH/DCM) to give $N$-(2-(dimethylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide ( $10 \mathrm{mg}, 0.028 \mathrm{mmol}, 55 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $d_{6}$ ) $\delta$ ppm $10.67(\mathrm{~d}, J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 9.18(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 9.10(\mathrm{~d}, J=$ $7.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.74(\mathrm{~d}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.22(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.12(\mathrm{~d}, J=7.4 \mathrm{~Hz}$, $1 \mathrm{H}), 7.96(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.86(\mathrm{p}, J=6.4 \mathrm{~Hz}, 2 \mathrm{H}), 7.37(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.70$ (q, $J=5.7 \mathrm{~Hz}, 2 \mathrm{H}), 2.62(\mathrm{t}, J=5.9 \mathrm{~Hz}, 2 \mathrm{H}), 2.32(\mathrm{~s}, 6 \mathrm{H}) . \mathrm{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}_{2}, 360.2$; found $361.4[\mathrm{M}+1]^{+}$.

Method F: Synthesis of hydrochloride analogs. N-(2-(dimethylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2, 1-b]quinazoline-12-carboxamide hydrochloride (37). To a 100 mL round bottom flask charged with a magnetic stir bar, N -(2-(dimethylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide ( $8.5 \mathrm{mg}, 0.024 \mathrm{mmol}$ ) was dissolved in a $1: 1$ mixture of $\mathrm{DCM} / \mathrm{MeOH}$ ( 2 mL ). A solution of 4 M HCl in 1,4-dioxane ( $18 \mu \mathrm{~L}, 0.071 \mathrm{mmol}$ ) was added dropwise and the reaction mixture was stirred for 1 hour at room temperature. Diethyl ether was then added $(50-100 \mathrm{~mL})$ until precipitation of a yellow solid. The solid was filtered and dried in vacuo overnight to give N -(2-(dimethylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxamide hydrochloride ( 8 mg , $0.020 \mathrm{mmol}, 85 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ $10.66(\mathrm{~s}, 1 \mathrm{H}), 10.10(\mathrm{~s}, 1 \mathrm{H}), 9.19(\mathrm{~d}, J=6.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.84(\mathrm{~s}, 1 \mathrm{H}), 8.61(\mathrm{~d}, J=6.7$ $\mathrm{Hz}, 1 \mathrm{H}), 8.23(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.14(\mathrm{~s}, 1 \mathrm{H}), 7.98(\mathrm{~d}, J=9.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.90(\mathrm{~s}$,
$2 H), 7.39(\mathrm{~s}, 1 \mathrm{H}), 4.04-3.98(\mathrm{~m}, 2 \mathrm{H}), 2.90(\mathrm{~s}, 6 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}_{2}, 360.2$; found $361.5[\mathrm{M}+1]^{+}$.

13-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-13H-benzo[f]pyrido[2,1-b]quinazoline-8carboxamide hydrochloride (32). To a 100 mL round-bottom flask charged with a magnetic stir bar was added 2-bromonicotinic acid (78) (2 g, 9.9 mmol ), diethyl ether ( 11 mL ), MeOH ( $0.63 \mathrm{~mL}, 15.64 \mathrm{mmol}$ ), and DEAD ( $1.61 \mathrm{~mL}, 10.2 \mathrm{mmol}$ ). A pressure-equalized addition funnel was fitted and a solution of triphenylphosphine $(2.67 \mathrm{~g}, 10.2 \mathrm{mmol})$ in diethyl ether $(11 \mathrm{~mL})$ was added dropwise over a period of 15 minutes at room temperature. Stirring continued for 17 hours at room temperature. The reaction mixture was vacuum filtered and the precipitate was washed with diethyl ether. The filtrate was concentrated to give a yellow solid. Purified via automated normal phase liquid chromatography using an 80 g silica cartridge (10-40\% EtOAc/heptane) to give methyl 2-bromonicotinate (79) (1.74 g, $8.05 \mathrm{mmol}, 81 \%$ yield) as a clear colorless oil. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta \mathrm{ppm}$ 8.52-8.46 (m, 1H), 8.12-8.05 (m, 1H), 7.39-7.32 (m, 1H), $3.96(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{7} \mathrm{H}_{6} \mathrm{BrNO}_{2}, 215.0$; found $215.9[\mathrm{M}+\mathrm{H}]^{+}$.

To a 250 mL round-bottom flask was added napthalen-2-amine (80) (2 g, $13.97 \mathrm{mmol})$ and DMF ( 28 mL ). The resulting mixture was cooled to $0^{\circ} \mathrm{C}$. Freshly recrystallized $N$-bromosuccinimide ( $2.61 \mathrm{~g}, 14.67 \mathrm{mmol}$ ) was added at $0^{\circ} \mathrm{C}$ and the reaction mixture was allowed to warm to room temperature. Stirred for 5 minutes at room temperature. Diluted with EtOAc and washed 1x with sat. $\mathrm{NaHCO}_{3}$. The aqueous layer was extracted $4 x$ with additional EtOAc. Combined
organic layers were washed $1 x$ with $10 \% \mathrm{LiCl}, 1 x$ with water, $1 x$ with sat. brine, dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography using a 120 g silica cartridge (6-50\% EtOAc/heptane) to give 1-bromonaphthalen-2-amine (81) $(2.34 \mathrm{~g}, 10.54 \mathrm{mmol}$, $75 \%$ yield) as a peach-colored solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 7.83$ (d, $J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.72(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.66(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.51-7.44$ ( $\mathrm{m}, 1 \mathrm{H}$ ), $7.25-7.19(\mathrm{~m}, 1 \mathrm{H}), 7.12(\mathrm{~d}, \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{10} \mathrm{H}_{8} \mathrm{BrN}, 221.0$; found $222.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a 10-20 mL microwave vial charged with a magnetic stir bar was added methyl 2-bromonicotinate (79) (500 mg, 2.31 mmol ), 1-bromonaphthalen-2-amine (81) ( $616.8 \mathrm{mg}, 2.78 \mathrm{mmol}$ ), rac-BINAP-Pd-G3 (229.69 mg, 0.23 mmol ), cesium carbonate ( $1.51 \mathrm{~g}, 4.63 \mathrm{mmol}$ ), and 1,4-dioxane ( 15 mL ). The resulting mixture was capped, sealed, and bubbled with nitrogen for 10 min , then heated for 45 minutes at $100^{\circ} \mathrm{C}$. The reaction was allowed to cool to room temperature, diluted with DCM, and poured into sat. brine. The aqueous layer was extracted $3 x$ with additional DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography using a 120 g silica cartridge (6-50\% EtOAc/heptane). Clean fractions were collected and set aside. Mixed fractions were subjected to another automated normal phase column using an 80 g silica cartridge (6-50\% EtOAc/heptane). Clean fractions were combined to give methyl 2-((1-bromonaphthalen-2-yl)amino)nicotinate (82) (431.9 mg, $1.21 \mathrm{mmol}, 52 \%$ yield) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 10.60$ (s, 1H),
$8.55(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.45(\mathrm{dd}, J=4.7,2.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.35(\mathrm{dd}, J=7.8,1.9 \mathrm{~Hz}$, $1 \mathrm{H}), 8.14(\mathrm{dd}, J=8.5,1.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.95(\mathrm{dd}, J=8.9,2.5 \mathrm{~Hz}, 2 \mathrm{H}), 7.68-7.63(\mathrm{~m}$, 1H), $7.55-7.49(\mathrm{~m}, 1 \mathrm{H}), 7.02(\mathrm{dd}, J=7.8,4.8 \mathrm{~Hz}, 1 \mathrm{H}), 3.95(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{17} \mathrm{H}_{13} \mathrm{BrN}_{2} \mathrm{O}_{2}, 356.0$; found $358.0[\mathrm{M}+2]^{+}$.

To a 10-20 mL microwave vial charged with a magnetic stir bar was added methyl 2-((1-bromonaphthalen-2-yl)amino)nicotinate (82) (300 mg, 0.84 mmol ), palladium(II) acetate ( $94.28 \mathrm{mg}, 0.42 \mathrm{mmol}$ ), Xantphos ( $485.96 \mathrm{mg}, 0.84 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}$ (534.83 mg, 2.52 mmol ) and 1,4-dioxane ( 6 mL ). The resulting mixture was capped, sealed, and bubbled with carbon monoxide for 10 min , then heated for 3 hours at $100^{\circ} \mathrm{C}$. The reaction was allowed to cool to room temperature, then was diluted with EtOAc and filtered through a pad of Celite. The filtrate was washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with additional EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography using a 40 g silica cartridge (6-50\% EtOAc/heptane) to give methyl 13-oxo-13H-benzo[f]pyrido[2,1-b]quinazoline-8-carboxylate (83) (220.2 mg, 0.72 mmol, $86 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 9.87$ (d, $J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 9.23(\mathrm{dd}, J=7.3,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.36(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.17(\mathrm{dd}$, $J=6.8,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.12(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.88-7.81(\mathrm{~m}, 1 \mathrm{H}), 7.74-7.68(\mathrm{~m}$, $2 \mathrm{H}), 7.31(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.97(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{18} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{3}$, 304.1; found $305.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added methyl 13-oxo-13H-benzo[f]pyrido[2,1-b]quinazoline-8-carboxylate (100 mg, 0.33 mmol ), MeOH ( 7 mL ), and 2 N NaOH ( $0.33 \mathrm{~mL}, 0.66 \mathrm{mmol}$ ). The resulting mixture was stirred for 2 days at room temperature. Concentrated to dryness to give 13-0xo-13Hbenzo[ $f$ ]pyrido[2,1-b]quinazoline-8-carboxylic acid ( $125.2 \mathrm{mg}, 0.43 \mathrm{mmol}$ ) as an off-white solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta$ ppm $9.90(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 9.01(\mathrm{dd}, J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.28(\mathrm{~d}, J=9.0 \mathrm{~Hz}$, $1 \mathrm{H}), 8.09-8.03(\mathrm{~m}, 1 \mathrm{H}), 7.84-7.76(\mathrm{~m}, 1 \mathrm{H}), 7.70(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.68-7.63(\mathrm{~m}$, 1H), 7.48 (dd, $J=6.6,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.23-7.17(\mathrm{~m}, 1 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{17} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{3}, 290.1$; found $291.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 13-oxo-13Hbenzo[ $f]$ pyrido[2,1-b]quinazoline-8-carboxylic acid (115 mg, 0.40 mmol ), TBTU ( $190.81 \mathrm{mg}, 0.59 \mathrm{mmol}$ ), DMF ( 3 mL ), DIPEA ( $0.21 \mathrm{~mL}, 1.19 \mathrm{mmol}$ ), and 2-(pyrrolidin-1-yl)ethan-1-amine ( $0.08 \mathrm{~mL}, 0.59 \mathrm{mmol}$ ). The resulting mixture was stirred for 16 hours at room temperature. An additional 1.5 equivalents of TBTU and 1.5 equivalents of 2-(pyrrolidin-1-yl)ethan-1-amine were added to push the reaction forward. Stirring continued for 3 days at room temperature. At this time, another 1.5 equivalents of TBTU and 1.5 equivalents of 2-(pyrrolidin-1-yl)ethan-1amine were added and stirring continued for 3 hours at room temperature. The reaction mixture was diluted with water and the formed precipitate was vacuum filtered and washed with additional water. Purified via automated reverse phase liquid chromatography using a $30 \times 75$ LUNA column (10-60\% ACN/water) to give 13-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-13H-benzo[f]pyrido[2,1-b]quinazoline-8-
carboxamide trifluoroacetate ( $34.9 \mathrm{mg}, 0.0697 \mathrm{mmol}, 18 \%$ yield) as an orangebrown oil. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.16$ (t, $J=6.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), 9.83 (d, $J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 9.64(\mathrm{~s}, 1 \mathrm{H}), 9.36(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.77(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H})$, 8.45 (d, $J=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.15(\mathrm{~d}, \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, \mathrm{~J}=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.87(\mathrm{t}$, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.73(\mathrm{t}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.44(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.89(\mathrm{q}, J=6.2$ $\mathrm{Hz}, 2 \mathrm{H}), 3.75-3.67(\mathrm{~m}, 2 \mathrm{H}), 3.50(\mathrm{q}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.18-3.10(\mathrm{~m}, 2 \mathrm{H}), 2.09-1.99$ (m, 2H), 1.91-1.83 (m, 2H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}$, 386.2; found $387.0[\mathrm{M}+\mathrm{H}]^{+}$.

13-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-13H-benzo[f]pyrido[2,1-b]quinazoline-8carboxamide trifluoroacetate ( $34 \mathrm{mg}, 0.0679 \mathrm{mmol}$ ) was taken up in DCM ( 6 mL ) and washed $1 x$ with sat. $\mathrm{NaHCO}_{3}$. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were concentrated in vacuo and taken back up in 1,4dioxane ( 2 mL ). 4 M HCl in 1,4-dioxane ( $0.03 \mathrm{~mL}, 0.11 \mathrm{mmol}$ ) was added and the resulting mixture was stirred for 3 hours at room temperature. The resulting suspension was concentrated to dryness to give 13-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-13H-benzo[f]pyrido[2,1-b]quinazoline-8-carboxamide hydrochloride (23.1, $0.055 \mathrm{mmol}, 97 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d ) $\delta$ ppm 11.15 (t, $J=5.8 \mathrm{~Hz}, 1 \mathrm{H}), 9.97(\mathrm{~s}, 1 \mathrm{H}), 9.84(\mathrm{~d}, \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 9.38-9.33(\mathrm{~m}$, $1 \mathrm{H}), 8.80-8.75(\mathrm{~m}, 1 \mathrm{H}), 8.45(\mathrm{~d}, \mathrm{~J}=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.18-8.11(\mathrm{~m}, 2 \mathrm{H}), 7.87(\mathrm{t}, \mathrm{J}=$ $7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.73(\mathrm{t}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.44(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.90(\mathrm{q}, J=6.1 \mathrm{~Hz}$, 2 H ), 3.73-3.66 (m, 2H), 3.53-3.46 (m, 2H), 3.18-3.07 (m, 2H), 2.07-1.99 (m, 2H), 1.92-1.81 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}$, 386.2; found 387.0 $[\mathrm{M}+\mathrm{H}]^{+}$.

13-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-13H-pyridazino[4,5-f]pyrido[2,1-b]quinazoline-8-carboxamide hydrochloride (33). To a $2-5 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar and fitted with a Teflon cap was dissolved 6-bromopthalazine (84) ( $100 \mathrm{mg}, 0.48 \mathrm{mmol}$ ) in anhydrous toluene ( 3 mL ). Under argon atmosphere, methyl 2-aminopyridine-3-carboxylate ( 60 ) ( $74 \mathrm{mg}, 0.48 \mathrm{mmol}$ ), cesium carbonate $(218 \mathrm{mg}, 0.67 \mathrm{mmol})$, Xantphos ( $42 \mathrm{mg}, 0.072 \mathrm{mmol}$ ), and palladium(II) acetate ( $11 \mathrm{mg}, 0.048 \mathrm{mmol}$ ) were added and the vial was sealed with a Teflon cap before heating for 2 hours at $130^{\circ} \mathrm{C}$ using microwaves. The reaction mixture was cooled to room temperature, diluted with water, and the aqueous layer was extracted with EtOAc. Combined organic layers were washed with brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, and concentrated in vacuo. Purified via automated normal phase liquid chromatography using a 12 g silica cartridge ( $0-50 \% \mathrm{EtOAc} /$ heptane ). The column was then rinsed with $10 \% \mathrm{MeOH} / \mathrm{DCM}$ to give methyl 2-(phthalazin-6ylamino) nicotinate ( 85 ) ( $74 \mathrm{mg}, 0.18 \mathrm{mmol}$ ) as a pale brown solid, used directly in the next step. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{12} \mathrm{~N}_{4} \mathrm{O}_{2}, 280.1$; found $281.1[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of methyl 2-(phthalazin-6-ylamino)nicotinate (85) (74 mg, 0.26 mmol ) in a $2: 1$ mixture of $\mathrm{DCM} / \mathrm{MeOH}(6 \mathrm{~mL})$, benzyltrimethylammonium tribromide ( $37 \mathrm{mg}, 0.093 \mathrm{mmol}$ ) was added and the reaction mixture was stirred for 1.5 hours at room temperature. An additional 0.25 equivalents benzyltrimethylammonium tribromide was added and the reaction was stirred for 2.5 hours at room temperature. Water was added and the mixture was acidified to $\mathrm{pH} \sim 1$ using 1 N HCl . The mixture was then extracted with EtOAc. Combined organic layers were washed with water and brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, and
concentrated under reduced pressure. Purified via automated normal phase liquid chromatography using a 4 g silica cartridge (10\% MeOH/DCM) to give methyl 2-((5-bromophthalazin-6-yl)amino)nicotinate (86) (30.9 mg, 0.09 mmol$)$ as a pale orange solid, used directly in the next step, contains residual benzyltrimethylammonia. ${ }^{1} \mathrm{H}$ NMR $\left(400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}\right) \delta \mathrm{ppm} 8.19(\mathrm{~d}, \mathrm{~J}=8.0 \mathrm{~Hz}$, 2H), 7.98-7.94 (m, 3H), $7.80(\mathrm{~d}, \mathrm{~J}=12.0 \mathrm{~Hz}, 2 \mathrm{H}), 1.06(\mathrm{t}, \mathrm{J}=8.0 \mathrm{~Hz}, 3 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{11} \mathrm{BrN}_{4} \mathrm{O}_{2}, 358.0$; found $359.9[\mathrm{M}+2]^{+}$.

To a $10-20 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar was dissolved methyl 2-((5-bromophthalazin-6-yl)amino)nicotinate (86) (31 mg, 0.086 mmol ) in anhydrous toluene ( 1 mL ). The mixture was purged 3 times with argon, then palladium(II) acetate ( $1.9 \mathrm{mg}, 0.0086 \mathrm{mmol})$, Xantphos $(7.5 \mathrm{mg}, 0.013 \mathrm{mmol})$, Xantphos Pd G3 (4.3 mg, 0.0043 mmol$)$, and $\mathrm{K}_{3} \mathrm{PO}_{4}(56 \mathrm{mg}, 0.258 \mathrm{mmol})$ were added. The mixture was again purged 3 times with argon before adding hexakis(oxomethylene)molybdenum ( $23 \mathrm{mg}, 0.086 \mathrm{mmol}$ ). The vial was sealed and heated overnight at $115{ }^{\circ} \mathrm{C}$. The mixture was cooled to room temperature, filtered over a pad of talc, and rinsed with EtOAc. The filtrate was evaporated to give crude product. Purified via automated normal phase liquid chromatography using a 4 g silica cartridge (0-60\% EtOAc/heptane) to give methyl 13-0xo-13H-pyridazino[4,5-f]pyrido[2,1-b]quinazoline-8-carboxylate (87) (1.6 mg, 0.01 mmol ) as an orange solid. LCMS (ESI): m/z calcd for $\mathrm{C}_{16} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{O}_{3}$, 306.1; found 307.1 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a 10 mL vial charged with a magnetic stir bar was added methyl 13-oxo-13H-pyridazino[4,5-f]pyrido[2,1-b]quinazoline-8-carboxylate ( $5 \mathrm{mg}, 0.0163 \mathrm{mmol}$ ) in $\mathrm{MeOH}(1 \mathrm{~mL})$, and $1 \mathrm{M} \mathrm{NaOH}(0.034 \mathrm{~mL}, 0.0343 \mathrm{mmol})$. The resulting mixture was stirred for 2 days at room temperature. The mixture was acidified to $\mathrm{pH} \sim 3-4$ with conc. HCl . The yellow solid that formed was triturated with MeOH , filtered, washed with MeOH , and dried under vacuum to give 13-oxo-13H-pyridazino[4,5$f$ ]pyrido[2,1-b]quinazoline-8-carboxylic acid ( $11.5 \mathrm{mg}, 0.04 \mathrm{mmol}$ ) as a yellow solid, used without further purification. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{8} \mathrm{~N}_{4} \mathrm{O}_{3}, 292.1$; found $293.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 10 mL vial charged with a magnetic stir bar and fitted with a Teflon cap was added 13-oxo-13H-pyridazino[4,5-f]pyrido[2,1-b]quinazoline-8-carboxylic acid $(11.5 \mathrm{mg}, 0.04 \mathrm{mmol})$, TBTU ( $9 \mathrm{mg}, 0.0275 \mathrm{mmol})$, DIPEA ( $0.010 \mathrm{~mL}, 0.059 \mathrm{mmol}$ ), and anhydrous DMF ( 0.21 mL ). The resulting mixture was stirred for 15 minutes at room temperature. before addition of 2-(pyrrolidin-1-yl)ethan-1-amine ( 0.036 mL , 0.0275 mmol ). The resulting mixture was stirred overnight at $45^{\circ} \mathrm{C}$. The reaction mixture was hydrolyzed with sat. $\mathrm{NaHCO}_{3}$ and extracted $3 x$ with EtOAc and DCM. Combined organic layers were washed with brine, dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. Purified via automated normal phase liquid chromatography using a 4 g silica cartridge ( $10 \% \mathrm{MeOH} / \mathrm{DCM}$ ). The column was rinsed with $15 \% \mathrm{MeOH} / \mathrm{DCM}+1 \% \mathrm{Et}_{3} \mathrm{~N}$ and the solvent was evaporated under vacuum to give 13-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-13H-pyridazino[4,5-f]pyrido[2,1-b]quinazoline-8-carboxamide ( $4.6 \mathrm{mg}, 0.01 \mathrm{mmol}, 60 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.17 (s, 1H), 9.84 (s, 1H), 9.37 (d, J = 8.0 Hz,
$1 \mathrm{H}), 8.89(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.61(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.26(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H})$, $7.54(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.62-3.61(\mathrm{~m}, 2 \mathrm{H}), 2.77-2.76(\mathrm{~m}, 2 \mathrm{H}), 2.64(\mathrm{~m}, 3 \mathrm{H}), 1.85$ ( $\mathrm{m}, 3 \mathrm{H}$ ).

13-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-13H-pyridazino[4,5-f]pyrido[2,1-b]quinazoline-8-carboxamide ( $4.6 \mathrm{mg}, 0.0118 \mathrm{mmol}$ ) was dissolved in a $1: 1$ mixture of $\mathrm{DCM} / \mathrm{MeOH}(2 \mathrm{~mL})$ and 4 M HCl in 1,4-dioxane ( $0.089 \mathrm{~mL}, 0.0355 \mathrm{mmol}$ ) was added. The resulting mixture was stirred for 1.5 hours at room temperature. Diethyl ether was added and the solid was filtered and dried under vacuum overnight to give 13-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-13H-pyridazino[4,5$f$ ]pyrido[2,1-b]quinazoline-8-carboxamide hydrochloride ( $2 \mathrm{mg}, 0.0047 \mathrm{mmol}, 36 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.20$ (s, 1H), 10.90$10.88(\mathrm{~m}, 1 \mathrm{H}), 9.85(\mathrm{~s}, 1 \mathrm{H}), 9.68(\mathrm{~b} . \mathrm{s} ., 1 \mathrm{H}), 9.41(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.88(\mathrm{~d}, J=$ $6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.58(\mathrm{dd}, J=24.0,12.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.56(\mathrm{t}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.89(\mathrm{q}, J=$ $6.0 \mathrm{~Hz}, 2 \mathrm{H}$ ), 3.69-3.67 (m, 3H), $3.50(\mathrm{q}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}$ ), 3.14-3.13 (obs.), 2.04$2.03(\mathrm{~m}, 2 \mathrm{H}), 1.89-1.87(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{20} \mathrm{~N}_{6} \mathrm{O}_{2}, 388.2$; found $389.2[\mathrm{M}+\mathrm{H}]^{+}$.

7-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide hydrochloride (31). This compound was synthesized from 73 (172 $\mathrm{mg}, 0.593 \mathrm{mmol}$ ) and $N$-(2-aminoethyl)pyrrolidine ( $98 \mu \mathrm{~L}, 0.773 \mathrm{mmol}$ ) according to method E followed by method F to give $82 \mathrm{mg}, 0.19 \mathrm{mmol}, 84 \%$ yield. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta \mathrm{ppm} 10.67(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 9.17(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}$, $1 \mathrm{H}), 8.83(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.60(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.21(\mathrm{~d}, J=8.8 \mathrm{~Hz}$,
$1 \mathrm{H}), 8.15-8.10(\mathrm{~m}, 1 \mathrm{H}), 7.99-7.85(\mathrm{~m}, 3 \mathrm{H}), 7.38(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.99(\mathrm{q}, \mathrm{J}=$ $6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.64$ (s, 2H), 3.50 (s, 2H), 3.11 (s, 2H), 2.05-1.79 (m, 4H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 386.2$; found $387.3[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-hydroxyethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxamide hydrochloride (34). This compound was synthesized from 73 ( $28 \mathrm{mg}, 0.096 \mathrm{mmol}$ ) and 2-aminoethan-1-ol ( $7.28 \mu \mathrm{~L}, 0.122 \mathrm{mmol}$ ) according to method E followed by method F to give $7 \mathrm{mg}, 0.019 \mathrm{mmol}, 20 \%$ yield. ${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta$ ppm 11.00-10.98(m, 1H), 9.19-9.16 (m, 2H), $8.74(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.21(\mathrm{~d}, J=$ $8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.96(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.87(\mathrm{dd}, J=7.8$, $6.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.80(\mathrm{dd}, J=8.1,5.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.38(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 3.78-3.76$ (obs.), 3.69-3.66 (obs.). LCMS (ESI): m/z calcd for $\mathrm{C}_{19} \mathrm{H}_{15} \mathrm{~N}_{3} \mathrm{O}_{3}, 333.1$; found 334.2 $[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-aminoethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxamide hydrochloride (35). This compound was synthesized from 73 ( $40 \mathrm{mg}, 0.138 \mathrm{mmol}$ ) and tert-butyl N -(2-aminoethyl)carbamate ( $31.5 \mathrm{mg}, 0.193 \mathrm{mmol}$ ) according to method E followed by method F to give $20 \mathrm{mg}, 0.050 \mathrm{mmol}, 36 \%$ yield. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d ${ }^{2}$ ) $\delta$ ppm $10.64(t, J=5.8 \mathrm{~Hz}, 1 \mathrm{H}), 9.25-9.16(\mathrm{~m}, 1 \mathrm{H}), 8.85-$ $8.79(\mathrm{~m}, 1 \mathrm{H}), 8.62(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.27-8.21(\mathrm{~m}, 1 \mathrm{H}), 8.15(\mathrm{dd}, J=9.8$, $8.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{dd}, J=21.4,8.8 \mathrm{~Hz}, 3 \mathrm{H}), 7.94-7.84(\mathrm{~m}, 2 \mathrm{H}), 7.39(\mathrm{t}, J=7.1 \mathrm{~Hz}$, $1 \mathrm{H}), 5.75(\mathrm{~s}, 1 \mathrm{H}), 3.86(\mathrm{~d}, \mathrm{~J}=12.5 \mathrm{~Hz}, 7 \mathrm{H}), 3.17(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{19} \mathrm{H}_{16} \mathrm{~N}_{4} \mathrm{O}_{2}, 332.1$; found $333.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(methylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide hydrochloride (36). This compound was synthesized from 73 ( 40 mg , 0.138 mmol ) and tert-butyl (2-aminoethyl)methylcarbamate ( $33.6 \mathrm{mg}, 0.193 \mathrm{mmol}$ ) according to method $E$ followed by method $F$ to give $9.3 \mathrm{mg}, 0.024 \mathrm{mmol}, 17 \%$ yield. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.70(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 9.18(\mathrm{dd}, J=$ $7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.86(\mathrm{~s}, 2 \mathrm{H}), 8.82-8.78(\mathrm{~m}, 1 \mathrm{H}), 8.63(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H})$, $8.22(\mathrm{~d}, \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.15-8.09(\mathrm{~m}, 1 \mathrm{H}), 7.97(\mathrm{~d}, \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.93-7.85$ $(\mathrm{m}, 2 \mathrm{H}), 7.39(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.92(\mathrm{q}, J=6.2 \mathrm{~Hz}, 2 \mathrm{H}), 3.30-3.24(\mathrm{~m}, 2 \mathrm{H}), 2.64$ $(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 3 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): m / z$ calcd for $\mathrm{C}_{20} \mathrm{H}_{18} \mathrm{~N}_{4} \mathrm{O}_{2}, 346.1$; found 347.0 $[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(diethylamino)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide hydrochloride (38). This compound was synthesized from 73 ( 40 mg , 0.138 mmol ) and $N, N$-diethylethylenediamine ( $22.4 \mathrm{mg}, 0.193 \mathrm{mmol}$ ) according to method $E$ followed by method $F$ to give $26 \mathrm{mg}, 0.059 \mathrm{mmol}, 43 \%$ yield. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta$ ppm $10.60(t, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.12(\mathrm{~s}, 1 \mathrm{H}), 9.18(\mathrm{dd}, J=$ $7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.88-8.83(\mathrm{~m}, 1 \mathrm{H}), 8.58(\mathrm{dd}, J=7.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.23(\mathrm{~d}, J=8.8$ $\mathrm{Hz}, 1 \mathrm{H}), 8.16-8.11(\mathrm{~m}, 1 \mathrm{H}), 7.98(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.93-7.86(\mathrm{~m}, 2 \mathrm{H}), 7.38(\mathrm{t}, J$ $=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.00(\mathrm{q}, J=6.5 \mathrm{~Hz}, 2 \mathrm{H}), 3.41(\mathrm{dq}, J=14.1,6.9 \mathrm{~Hz}, 2 \mathrm{H}), 3.33-3.21$ ( $\mathrm{m}, 4 \mathrm{H}$ ), $1.27(\mathrm{t}, \mathrm{J}=7.3 \mathrm{~Hz}, 6 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 388.2$; found $389.0[\mathrm{M}+\mathrm{H}]^{+}$.

7-oxo-N-(2-(piperidin-1-yl)ethyl)-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide hydrochloride (39). This compound was synthesized from 73 ( 40 mg ,
0.138 mmol ) and 2-(1-piperidyl)ethanamine ( $24.7 \mathrm{mg}, 0.193 \mathrm{mmol}$ ) according to method E followed by method F to give $18 \mathrm{mg}, 0.038 \mathrm{mmol}, 28 \%$ yield). ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.65(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 9.19(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}$, 1 H ), $8.87-8.82(\mathrm{~m}, 1 \mathrm{H}), 8.60(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.23(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H})$, $8.17-8.11(\mathrm{~m}, 1 \mathrm{H}), 8.05-7.96(\mathrm{~m}, 1 \mathrm{H}), 7.94-7.86(\mathrm{~m}, 2 \mathrm{H}), 7.39(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H})$, $4.02(\mathrm{q}, J=6.4 \mathrm{~Hz}, 2 \mathrm{H}), 3.61(\mathrm{~d}, \mathrm{~J}=11.7 \mathrm{~Hz}, 2 \mathrm{H}), 3.42(\mathrm{q}, J=6.5 \mathrm{~Hz}, 2 \mathrm{H}), 3.03$ (q, $J=8.9 \mathrm{~Hz}, 2 \mathrm{H}), 1.83$ (d, $J=14.3 \mathrm{~Hz}, 2 \mathrm{H}$ ), 1.70 (d, $J=12.6 \mathrm{~Hz}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{24} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 400.2$; found $401.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-methylpiperazin-1-yl)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxamide hydrochloride (40). This compound was synthesized from 73 (40 $\mathrm{mg}, 0.138 \mathrm{mmol}$ ) and 2-(4-methylpiperazin-1-yl)ethanamine ( $27.6 \mathrm{mg}, 0.193$ mmol ) according to method E followed by method F to give $19 \mathrm{mg}, 0.042 \mathrm{mmol}$, $30 \%$ yield. ${ }^{1} \mathrm{H}$ NMR ( 600 MHz , DMSO-d $\mathrm{d}_{6}$ ) ppm 11.60 (b.s., 1H), 10.65 (s, 1H), 9.17 (d, $J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.85(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.61(\mathrm{~d}, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.22$ (d, $J=6.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.13(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.97(\mathrm{~d}, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.94-7.87$ (m, 2H), 7.38 (t, J = 6.0 Hz, 1H), 4.09-4.02 (obs.), 3.56-3.37 (obs.), 2.82 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{25} \mathrm{~N}_{5} \mathrm{O}_{2}, 415.2$; found $416.2[\mathrm{M}+\mathrm{H}]^{+}$.
(S)-N-(2-(3-fluoropyrrolidin-1-yl)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-
b]quinazoline-12-carboxamide hydrochloride (41). This compound was synthesized from 73 ( $40 \mathrm{mg}, 0.138 \mathrm{mmol}$ ) and ( $S$ )-2-(3-fluoropyrrolidin-1-yl)ethan1 -amine hydrochloride ( $74.9 \mathrm{mg}, 0.444 \mathrm{mmol}$, prepared according to method B) according to method E followed by method F to give $5 \mathrm{mg}, 0.011 \mathrm{mmol}, 8 \%$ yield).
${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.70(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 10.56(\mathrm{~s}, 1 \mathrm{H}), 9.19$ (d, J=7.1 Hz, 1H), 8.86-8.81 (m, 1H), 8.65-8.59 (m, 1H), $8.23(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H})$, $8.16-8.12(\mathrm{~m}, 1 \mathrm{H}), 7.98(\mathrm{~d}, \mathrm{~J}=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.89(\mathrm{dd}, J=6.1,2.9 \mathrm{~Hz}, 2 \mathrm{H}), 7.39(\mathrm{t}$, $J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 5.48(\mathrm{dd}, J=53.5,23.7 \mathrm{~Hz}, 1 \mathrm{H}), 4.00(\mathrm{q}, J=6.3 \mathrm{~Hz}, 3 \mathrm{H}), 3.83(\mathrm{~d}$, $J=7.9 \mathrm{~Hz}, 1 \mathrm{H}$ ), 3.63-3.55 (obs.), 2.24-2.06 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{21} \mathrm{FN}_{4} \mathrm{O}_{2}$, 404.2; found $405.1[\mathrm{M}+\mathrm{H}]^{+}$.
(R)-N-(2-(3-fluoropyrrolidin-1-yl)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-
b]quinazoline-12-carboxamide (42). This compound was synthesized from 73 (50 $\mathrm{mg}, 0.172 \mathrm{mmol}$ ) and ( $R$ )-2-(3-fluoropyrrolidin-1-yl)ethan-1-amine ( $74.9 \mathrm{mg}, 0.444$ mmol, prepared according to method B ) according to method E followed by method F to give $1 \mathrm{mg}, 0.002 \mathrm{mmol} .{ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.69(\mathrm{~d}, J=$ $5.7 \mathrm{~Hz}, 1 \mathrm{H}), 10.46(\mathrm{~s}, 1 \mathrm{H}), 9.19(\mathrm{dd}, \mathrm{J}=7.1,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.86-8.82(\mathrm{~m}, 1 \mathrm{H}), 8.65-$ $8.59(\mathrm{~m}, 1 \mathrm{H}), 8.23(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.14(\mathrm{~d}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.99(\mathrm{~d}, J=8.8 \mathrm{~Hz}$, $1 \mathrm{H}), 7.93-7.86(\mathrm{~m}, 2 \mathrm{H}), 7.40(\mathrm{t}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 5.56-5.33(\mathrm{~m}, 1 \mathrm{H}), 4.00(\mathrm{p}, J=$ $6.4 \mathrm{~Hz}, 2 \mathrm{H}$ ), 3.88-3.73 (m, 1H), 3.63-3.57 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{2} 1 \mathrm{FN}_{4} \mathrm{O}_{2}$, 404.2; found $405.0[\mathrm{M}+\mathrm{H}]^{+}$.

## N-(3-(dimethylamino)propyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-

 carboxamide hydrochloride (43). This compound was synthesized from 73 ( 40 mg , 0.138 mmol ) and $N, N$-dimethylpropane-1,3-diamine ( $20.8 \mathrm{mg}, 0.204 \mathrm{mmol}$ ) according to method $E$ followed by method $F$ to give $34 \mathrm{mg}, 0.083 \mathrm{mmol}, 60 \%$ yield. ${ }^{1} \mathrm{H}$ NMR ( 600 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.58(\mathrm{t}, \mathrm{J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}), 9.87(\mathrm{~s}, 1 \mathrm{H})$, 9.17 (dd, $J=7.1,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.81(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.60(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}$,$1 \mathrm{H}), 8.23(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.14(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.98(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H})$, $7.94-7.86(\mathrm{~m}, 2 \mathrm{H}), 7.38(\mathrm{t}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.67(\mathrm{q}, J=6.9 \mathrm{~Hz}, 2 \mathrm{H}), 3.22(\mathrm{dt}, J=$ 10.6, $5.5 \mathrm{~Hz}, 2 \mathrm{H}), 2.79(\mathrm{~d}, J=4.9 \mathrm{~Hz}, 6 \mathrm{H}), 2.13(\mathrm{p}, J=7.3 \mathrm{~Hz}, 2 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}):$ $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{22} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}$, 374.2; found $375.0[\mathrm{M}+\mathrm{H}]^{+}$.

7-oxo-N-(3-(pyrrolidin-1-yl)propyl)-7H-benzo[h]pyrido[2, 1-b]quinazoline-12carboxamide hydrochloride (44). This compound was synthesized from 73 (40 mg, 0.138 mmol ) and 3-(pyrrolidin-1-yl)propan-1-amine (18.2 mg, 0.142 mmol ) according to method $E$ followed by method $F$ to give $35.5 \mathrm{mg}, 0.081 \mathrm{mmol}, 59 \%$ yield. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta \mathrm{ppm} 10.58(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), 10.50 (b.s., $1 \mathrm{H}), 9.17(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.81(\mathrm{~d}, J=10.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.60(\mathrm{~d}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H})$, $8.22(\mathrm{~d}, J=10.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.14(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.98-7.94(\mathrm{~m}, 2 \mathrm{H}), 7.90-7.87$ $(\mathrm{m}, 1 \mathrm{H}), 7.38(\mathrm{t}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 3.68(\mathrm{q}, J=5.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.57-3.54(\mathrm{~m}, 2 \mathrm{H}), 3.31-$ $3.27(\mathrm{~m}, 2 \mathrm{H}), 3.02-2.98 \mathrm{~m}, 2 \mathrm{H}), 2.18-2.15(\mathrm{~m}, 2 \mathrm{H}), 2.00-1.97(\mathrm{~m}, 2 \mathrm{H}), 1.90-1.87$ (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 400.2$; found $401.1[\mathrm{M}+\mathrm{H}]^{+}$. N-(azetidin-3-yl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxamide hydrochloride (45). This compound was synthesized from 73 ( $40 \mathrm{mg}, 0.138 \mathrm{mmol}$ ) and tert-butyl 3-aminoazetidine-1-carboxylate ( $56 \mu \mathrm{~L}, 0.355 \mathrm{mmol}$ ) according to method $E$ followed by method $F$ to give $35 \mathrm{mg}, 0.082 \mathrm{mmol}, 59 \%$ yield. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta \mathrm{ppm} 10.76(\mathrm{~d}, J=6.5 \mathrm{~Hz}, 2 \mathrm{H}), 9.17(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}$, $2 \mathrm{H}), 8.86-8.81(\mathrm{~m}, 2 \mathrm{H}), 8.54(\mathrm{dd}, J=7.0,1.6 \mathrm{~Hz}, 2 \mathrm{H}), 8.22(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 2 \mathrm{H})$, 8.17-8.11 (m, 2H), $7.98(\mathrm{~d}, J=8.9 \mathrm{~Hz}, 2 \mathrm{H}), 7.94-7.86(\mathrm{~m}, 4 \mathrm{H}), 7.38(\mathrm{t}, J=7.1 \mathrm{~Hz}$,
$2 \mathrm{H}), 5.75(\mathrm{~s}, 1 \mathrm{H}), 5.04(\mathrm{~h}, J=7.8 \mathrm{~Hz}, 2 \mathrm{H}), 4.38(\mathrm{~s}, 4 \mathrm{H}), 4.27(\mathrm{~d}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{20} \mathrm{H}_{16} \mathrm{~N}_{4} \mathrm{O}_{2}$, 344.1; found $345.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(1-methylpiperidin-4-yl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxamide hydrochloride (46). This compound was synthesized from 73 ( 40 mg , 0.138 mmol ) and 1-methylpiperidin-4-amine ( $22 \mathrm{mg}, 0.193 \mathrm{mmol}$ ) according to method $E$ followed by method $F$ to give $42.2 \mathrm{mg}, 0.1 \mathrm{mmol}, 72 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6$ ) $\delta \mathrm{ppm} 10.66(\mathrm{~m}, 2 \mathrm{H}), 9.17(\mathrm{~d}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H})$, $8.78(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.63(\mathrm{~d}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.22(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.15-$ $8.12(\mathrm{~m}, 2 \mathrm{H}), 7.97(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.89(\mathrm{t}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.37(\mathrm{t}, J=7.3 \mathrm{~Hz}$, 1H), 4.30-4.25 (obs.), 3.23-3.20 (m, 2H), $2.80(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 3 \mathrm{H}), 2.32-2.30(\mathrm{~m}$, 2H), 2.12 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}$, 386.2; found 387.1 $[\mathrm{M}+\mathrm{H}]^{+}$.

4-methyl-11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6carboxamide hydrochloride (47). To a mixture of 2-chloropyridine-3-carboxylic acid (57) (517 mg, 3.281 mmol ) and 2-amino-3-methylbenzoic acid (88a) (566 mg, $3.744 \mathrm{mmol})$ in a $1: 1$ mixture of DMF/water $(4 \mathrm{~mL})$ was added sulfuric acid ( 2 mL , $36.770 \mathrm{mmol}, 98 \% \mathrm{w} / \mathrm{w}$ ). The resulting mixture was heated at $130^{\circ} \mathrm{C}$ for 9 hours. Additional sulfuric acid ( $2 \mathrm{~mL}, 36.770 \mathrm{mmol}, 98 \%$ ), DMF ( 1 mL ), and water ( 1 mL ) were added, and the resulting mixture was heated at $110{ }^{\circ} \mathrm{C}$ for 24 hours. The reaction mixture was allowed to cool to room temperature, diluted with water, and extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was dry
loaded onto a 25 g silica cartridge and purification was attempted (0-10\% $\mathrm{MeOH} / \mathrm{DCM}$ ), but separation was unsuccessful. Material was carried forward without further purification (125 mg). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{14} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{3}, 254.1$; found $255.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 4-methyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (89a) (125 mg, 0.492 mmol$)$, TBTU ( $760 \mathrm{mg}, 2.367 \mathrm{mmol}$ ), and DIPEA ( 0.8 $\mathrm{mL}, 4.581 \mathrm{mmol})$ in DMF ( 4 mL ) was added $N$-(2-aminoethyl)pyrrolidine ( 0.2 mL , $1.576 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with water and extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was dry loaded onto a 25 g silica cartridge and was purified via automated NPLC $0-10 \% \mathrm{MeOH} / \mathrm{DCM}$ to give $35 \mathrm{mg}, 0.1 \mathrm{mmol}, 13 \%$ yield as a yellow solid. LCMS (ESI): m/z calcd for $\mathrm{C}_{20} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 350.2$; found $351.1[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 4-methyl-11-oxo-N-(2-pyrrolidin-1-ylethyl)pyrido[2,1-b]quinazoline-6-carboxamide ( $35 \mathrm{mg}, 0.1 \mathrm{mmol}$ ) in a mixture of DCM ( 2 mL ) and $\mathrm{MeOH}(0.5 \mathrm{~mL})$ was added $2 \mathrm{M} \mathrm{HCl}(0.5 \mathrm{~mL}, 1 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 1 hour then concentrated to dryness. The yellow residue was taken up in $\mathrm{DCM}(2 \mathrm{~mL})$ and $\mathrm{MeOH}(0.2 \mathrm{~mL})$ and $\mathrm{iPr}_{2} \mathrm{O}(8 \mathrm{~mL})$ was slowly added dropwise with continuous stirring. A precipitate formed, which was filtered, washed with $\mathrm{iPr}_{2} \mathrm{O}(1 \times 2 \mathrm{~mL})$, and dried overnight at $40^{\circ} \mathrm{C}$ to give 19 mg , $0.049 \mathrm{mmol}, 50 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d6) $\delta \mathrm{ppm}$
$11.26(\mathrm{t}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}), 9.98(\mathrm{~s}, 1 \mathrm{H}), 9.05(\mathrm{dd}, J=10.0,5.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.66(\mathrm{~d}, J=$ $5.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.21(\mathrm{~d}, J=10.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.90(\mathrm{~d}, J=5.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.51(\mathrm{t}, J=10.0$ $\mathrm{Hz}, 1 \mathrm{H}), 7.25(\mathrm{t}, \mathrm{J}=10.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.85(\mathrm{q}, J=10.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.45(\mathrm{q}, J=10.0 \mathrm{~Hz}$, 3H), 3.12-3.10 (m, 2H), 2.69 (s, 3H), 2.03-1.86 (m, 4H). LCMS (ESI): m/z calcd for $\mathrm{C}_{20} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 350.2$; found $351.0[\mathrm{M}+\mathrm{H}]^{+}$.

11-oxo-4-phenyl-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6carboxamide hydrochloride (48). To each of $410-20 \mathrm{~mL}$ microwave vials was added 2-amino-3-bromobenzoic acid (90) ( $800 \mathrm{mg}, 3.703 \mathrm{mmol}$ ), DMF ( 12.6 mL ), and water ( 2.1 mL ). Argon was bubbled through the mixture, then phenylboronic acid ( $542 \mathrm{mg}, 4.445 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(1.95 \mathrm{~g}, 9.058 \mathrm{mmol})$ and $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(126 \mathrm{mg}$, 0.109 mmol ) were added. The vial was sealed and heated at $150^{\circ} \mathrm{C}$ for 15 minutes using microwaves. The reaction mixture was allowed to cool to room temperature and the vials were combined. The reaction mixture was diluted with water and extracted $2 x$ with ethyl acetate. The aqueous layer was collected and acidified with 1 N HCl until $\mathrm{pH}=3$. The mixture was extracted $3 x$ with ethyl acetate. Combined organic layers were dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. The crude material was taken up in $\mathrm{Et}_{2} \mathrm{O}$ and filtered (repeated 2 x ). The filtrate was concentrated in vacuo and purified via automated RPLC 10-100\% ACN/water ( $0.05 \%$ TFA) to give $1.4 \mathrm{~g}, 6.566 \mathrm{mmol}, 45 \%$ yield. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) ठ ppm 7.79 (dd, J = 8.0, 1.7 Hz, 1H), 7.53-7.45 (m, 2H), 7.43-7.36 (m, 3H), 7.17 (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 6.65$ (dd, $J=8.0,7.3 \mathrm{~Hz}, 1 \mathrm{H}$ ). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{13} \mathrm{H}_{11} \mathrm{NO}_{2}$, 213.1; found $214.3[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 2-chloropyridine-3-carboxylic acid (57) (2.07 g, 13.139 $\mathrm{mmol})$ in $1 \mathrm{M} \mathrm{HCl}(66 \mathrm{~mL}, 66 \mathrm{mmol})$ was added 2-amino-3-phenyl-benzoic acid (91) $(1.4 \mathrm{~g}, 6.566 \mathrm{mmol})$. The resulting mixture was heated at $110^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature, filtered, and the precipitate washed with a minimal amount of water. The resulting solid was collected, taken up in EtOH, and filtered. The resulting solid was collected, taken up in DCM, and filtered. The resulting filtrate was collected and concentrated to dryness to give $394 \mathrm{mg}, 1.246 \mathrm{mmol}, 19 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 16.10 (s, 1H), 9.09 (dd, $J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.63 (dd, $J=$ $7.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.42(\mathrm{dd}, J=8.1,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.00(\mathrm{dd}, J=7.3,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.75-$ $7.67(\mathrm{~m}, 1 \mathrm{H}), 7.63-7.46(\mathrm{~m}, 5 \mathrm{H}), 7.29(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{19} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{3}, 316.1$; found $317.1[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 11-oxo-4-phenyl-pyrido[2,1-b]quinazoline-6-carboxylic acid (92) ( $50 \mathrm{mg}, 0.158 \mathrm{mmol}$ ), TBTU ( $73 \mathrm{mg}, 0.227 \mathrm{mmol}$ ), and DIPEA ( 0.138 mL , $0.790 \mathrm{mmol})$ in DMF ( 2 mL ) was added $N$-(2-aminoethyl)pyrrolidine ( $29 \mu \mathrm{~L}, 0.221$ $\mathrm{mmol})$. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with water and extracted $2 x$ with DCM. Combined organic layers were washed $1 x$ with water, $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-5\% MeOH/DCM, 10 g silica cartridge) to give $52 \mathrm{mg}, 0.126 \mathrm{mmol}, 80 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 10.50(\mathrm{t}, \mathrm{J}=5.7 \mathrm{~Hz}$, $1 \mathrm{H}), 9.03(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.63(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.39(\mathrm{dd}, J=8.1$, $1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.93(\mathrm{dd}, J=7.3,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.71-7.55(\mathrm{~m}, 5 \mathrm{H}), 7.55-7.47(\mathrm{~m}, 1 \mathrm{H})$,
$7.21(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.13(\mathrm{q}, J=6.7 \mathrm{~Hz}, 2 \mathrm{H}), 2.47-2.13(\mathrm{~m}, 4 \mathrm{H}), 2.11-1.88(\mathrm{~m}$, 2 H ), 1.72-1.50 (m, 4H). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}$, 412.2; found 413.2 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of 11-oxo-4-phenyl-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6-carboxamide ( $50 \mathrm{mg}, 0.121 \mathrm{mmol}$ ) in a $1: 1$ mixture of DCM/MeOH ( 8 mL ) was added dropwise 4 M HCl in 1,4-dioxane ( $91 \mu \mathrm{~L}, 0.364 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 1 hour. $\mathrm{Et}_{2} \mathrm{O}$ was added (1020 mL ) until formation of a yellow precipitate. The solid was filtered and dried on vacuo overnight to give $30 \mathrm{mg}, 0.067 \mathrm{mmol}, 55 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d $\mathrm{d}_{6}$ ) $\delta$ ppm $10.65(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), 10.16-9.98 (m, 1H), 9.07 (dd, $J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.64$ (dd, $J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.41$ (dd, $J=8.1,1.5 \mathrm{~Hz}$, $1 \mathrm{H}), 7.96$ (dd, $J=7.3,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.74-7.51(\mathrm{~m}, 6 \mathrm{H}), 7.25(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H})$, 3.45-3.27 (m, 4H), 2.95-2.79 (m, 4H), 2.05-1.89 (m, 2H), 1.89-1.74 (m, 2H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 412.2$; found $413.2[\mathrm{M}+\mathrm{H}]^{+}$.

## 3-methyl-11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6-

 carboxamide hydrochloride (49). To a mixture of 2-chloropyridine-3-carboxylic acid (57) ( $110 \mathrm{mg}, 0.698 \mathrm{mmol}$ ) and 2-amino-4-methylbenzoic acid (88b) ( 106 mg , 0.701 mmol ) in EtOH ( 3 mL ) was added conc. $\mathrm{HCl}(0.15 \mathrm{~mL}, 1.827 \mathrm{mmol})$. The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 2 days. Additional conc. $\mathrm{HCl}(0.4 \mathrm{~mL}$, $4.871 \mathrm{mmol})$ and $\mathrm{EtOH}(0.5 \mathrm{~mL})$ were added, and the resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 1 day. The reaction mixture was allowed to cool to room temperature and the resulting precipitate was filtered to give $85 \mathrm{mg}, 0.334 \mathrm{mmol}, 49 \%$ yield asa yellow solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta$ ppm 9.07 (dd, $J=8.0,4.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.66(\mathrm{dd}, J=8.0,4.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.25(\mathrm{~d}, J=8.0$ $\mathrm{Hz}, 1 \mathrm{H}), 7.73(\mathrm{~s}, 1 \mathrm{H}), 7.47(\mathrm{dd}, J=8.0,4.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 2.55$ (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{14} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{3}, 254.1$; found $255.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 3-methyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (89b) ( $58 \mathrm{mg}, 0.228 \mathrm{mmol}$ ), TBTU (152 mg, 0.473 mmol ), and DIPEA ( 0.1 mL , $0.573 \mathrm{mmol})$ in DMF ( 2 mL ), was added $N$-(2-aminoethyl)pyrrolidine ( $31 \mu \mathrm{~L}, 0.244$ $\mathrm{mmol})$. The resulting mixture was stirred at room temperature overnight. The resulting precipitate was filtered, washed with $\mathrm{Et}_{2} \mathrm{O}(2 \times 2 \mathrm{~mL})$, and dried overnight at $40^{\circ} \mathrm{C}$ under vacuum to give $18 \mathrm{mg}, 0.051 \mathrm{mmol}, 23 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.14 (s, 1H), 10.20 (s, 1H), 9.02 (dd, $J=7.2$, $1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.62(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.24(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.89(\mathrm{~s}, 1 \mathrm{H})$, $7.44(\mathrm{dd}, J=8.3,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.20(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.86(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H})$, $3.66(\mathrm{~s}, 2 \mathrm{H}), 3.52-3.38(\mathrm{~m}, 2 \mathrm{H}), 3.10(\mathrm{~s}, 2 \mathrm{H}), 2.56(\mathrm{~s}, 3 \mathrm{H}), 1.95(\mathrm{dd}, \mathrm{J}=40.5,7.6$ $\mathrm{Hz}, 4 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{20} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 350.2$; found $351.3[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of 3-methyl-11-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6-carboxamide ( $18 \mathrm{mg}, 0.051 \mathrm{mmol}$ ) in a $1: 1$ mixture of $\mathrm{DCM} / \mathrm{MeOH}$ ( 1.8 mL ) was added 2 M HCl in $\mathrm{Et}_{2} \mathrm{O}(91 \mu \mathrm{~L}, 0.182 \mathrm{mmol})$. The reaction mixture was stirred at room temperature for 1.5 hours. $\mathrm{Et}_{2} \mathrm{O}(4 \mathrm{~mL})$ was added, and the reaction mixture was cooled to $0^{\circ} \mathrm{C}$ without stirring. The resulting precipitate was filtered, washed with $\mathrm{Et}_{2} \mathrm{O}(2 \times 2 \mathrm{~mL})$, and dried overnight at $40^{\circ} \mathrm{C}$ to give 18.6 mg , $0.048 \mathrm{mmol}, 94 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (DMSO-d6, 500 MHz ) $\delta \mathrm{ppm}$
10.91-11.24 (m, 1H), 10.08-10.63 (m, 1H), 9.02 (dd, $J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.62(\mathrm{dd}$, $J=7.1,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.23(\mathrm{~d}, J=8.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.91(\mathrm{~s}, 1 \mathrm{H}), 7.44(\mathrm{dd}, J=8.3,1.5$ $\mathrm{Hz}, 1 \mathrm{H}), 7.20(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.86(\mathrm{q}, \mathrm{J}=6.2 \mathrm{~Hz}, 2 \mathrm{H}), 3.58-3.73(\mathrm{~m}, 2 \mathrm{H}), 3.45$ ( $\mathrm{q}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}$ ), 3.02-3.16 (m, 2H), $2.56(\mathrm{~s}, 3 \mathrm{H}), 1.96-2.07(\mathrm{~m}, 2 \mathrm{H}), 1.82-1.93$ (m, 2H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{20} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 350.2$; found $351.0[\mathrm{M}+\mathrm{H}]^{+}$. 11-oxo-3-phenyl-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6carboxamide hydrochloride (50). To a mixture of 2-chloropyridine-3-carboxylic acid (57) ( $156 \mathrm{mg}, 0.990 \mathrm{mmol}$ ) and 3-aminobiphenyl-4-carboxylic acid (88c) (235 mg, 1.102 mmol ) in EtOH ( 4 mL ) was added conc. $\mathrm{HCl}(0.8 \mathrm{~mL}, 9.742 \mathrm{mmol})$. The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 2 days. The reaction mixture was allowed to cool to room temperature and the resulting precipitate was filtered, washed with EtOH ( $2 \times 2 \mathrm{~mL}$ ), and dried at $40^{\circ} \mathrm{C}$ for 2 hours under vacuum to give $70 \mathrm{mg}, 0.221$ mmol, $22 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (DMSO-d6, 400 MHz ) $\delta \mathrm{ppm} 9.10$ (dd, $J=8.0,4,0 \mathrm{~Hz}, 1 \mathrm{H}), 8.69(\mathrm{dd}, J=8.0,4.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.42(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.26$ ( $\mathrm{s}, 1 \mathrm{H}$ ), 7.98-7.94 (m, 3H), 7.60-7.49 (m, 3H), 7.31 (t, J = $8.0 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{19} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{3}, 316.1$; found $317.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 11-oxo-3-phenyl-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid ( 89 c ) ( $70 \mathrm{mg}, 0.221 \mathrm{mmol}$ ), TBTU ( $138 \mathrm{mg}, 0.430 \mathrm{mmol}$ ), and DIPEA ( 0.35 $\mathrm{mL}, 2.004 \mathrm{mmol}$ ) in DMF ( 3 mL ) was added $N$-(2-aminoethyl)pyrrolidine ( $40 \mu \mathrm{~L}$, $0.315 \mathrm{mmol})$. The resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with sat. $\mathrm{NaHCO}_{3}$ and the aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $2 x$ with sat.
$\mathrm{NaHCO}_{3}$ and sat. $\mathrm{NH}_{4} \mathrm{Cl}$, dried over a phase separator and concentrated in vacuo to give $81 \mathrm{mg}, 0.196 \mathrm{mmol}, 89 \%$ yield as a red solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO$\left.d_{6}\right) \delta \operatorname{ppm} 11.29(t, J=4.7 \mathrm{~Hz}, 1 \mathrm{H}), 9.03(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.67(\mathrm{dd}, J=$ $7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.40(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.02(\mathrm{~s}, 1 \mathrm{H}), 7.91-7.80(\mathrm{~m}, 3 \mathrm{H}), 7.66-$ $7.47(\mathrm{~m}, 3 \mathrm{H}), 7.21(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.59(\mathrm{~d}, J=5.5 \mathrm{~Hz}, 2 \mathrm{H}), 2.76(\mathrm{~s}, 2 \mathrm{H}), 2.63$ (s, 4H), 1.67 (s, 4H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}$, 412.2; found 413.4 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of 11-oxo-3-phenyl- N -(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6-carboxamide ( $81 \mathrm{mg}, 0.196 \mathrm{mmol}$ ) in a $2: 1$ mixture of $\mathrm{DCM} / \mathrm{MeOH}$ $(3 \mathrm{~mL})$ was added 2 M HCl in $\mathrm{Et}_{2} \mathrm{O}(0.4 \mathrm{~mL}, 0.800 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was slowly added dropwise to $\mathrm{Et}_{2} \mathrm{O}(6 \mathrm{~mL})$ with continuous stirring. The resulting precipitate was filtered, washed with $\mathrm{Et}_{2} \mathrm{O}(3 \times 3 \mathrm{~mL})$, and dried at $40^{\circ} \mathrm{C}$ overnight to give 64.2 mg , $0.143 \mathrm{mmol}, 73 \%$ yield as a pale pink solid. ${ }^{1} \mathrm{H}$ NMR (DMSO-d6, 500 MHz ) $\delta \mathrm{ppm}$ $11.16(\mathrm{t}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 10.78(\mathrm{br} \mathrm{s}, 1 \mathrm{H}), 9.04(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.65(\mathrm{dd}$, $J=6.8,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.49(\mathrm{~d}, J=1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.39(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.94-7.97$ $(\mathrm{m}, 2 \mathrm{H}), 7.92(\mathrm{dd}, J=8.4,1.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.48-7.61(\mathrm{~m}, 3 \mathrm{H}), 7.23(\mathrm{t}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H})$, 3.91 ( $\mathrm{q}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.60-3.74(\mathrm{~m}, 2 \mathrm{H}), 3.46(\mathrm{q}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.95-3.18(\mathrm{~m}$, $2 H$ ), 1.72-2.11 (m, 4H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 412.2$; found 413.0 $[\mathrm{M}+\mathrm{H}]^{+}$.

3,4-dimethyl-11-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2, 1-b]quinazoline-6carboxamide hydrochloride (51). A mixture of 2-chloropyridine-3-carboxylic acid
(57) (900 mg, 5.712 mmol$)$ and 2-amino-3,4-dimethylbenzoic acid (88d) (1049 mg, $6.350 \mathrm{mmol})$ in $1 \mathrm{M} \mathrm{HCl}(40 \mathrm{~mL}, 40 \mathrm{mmol})$ was heated at $105^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature and was extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-10\% MeOH/DCM, 90 g silica cartridge) to give $112 \mathrm{mg}, 0.417$ mmol, $7 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 17.35$ (s, $1 \mathrm{H}), 9.05(\mathrm{dd}, J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.66(\mathrm{dd}, J=7.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.13(\mathrm{~d}, J=8.3$ $\mathrm{Hz}, 1 \mathrm{H}), 7.47(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}) . \mathrm{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{15} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{3}, 268.1$; found $269.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 3,4-dimethyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6carboxylic acid (89d) ( $40 \mathrm{mg}, 0.149 \mathrm{mmol}$ ), TBTU ( $96 \mathrm{mg}, 0.299 \mathrm{mmol}$ ), and DIPEA ( $0.13 \mathrm{~mL}, 0.744 \mathrm{mmol}$ ) in DMF ( 4 mL ) was added N -(2-aminoethyl)pyrrolidine (38 $\mu \mathrm{L}, 0.298 \mathrm{mmol})$. The resulting mixture was stirred at room temperature overnight. Additional TBTU ( $96 \mathrm{mg}, 0.299 \mathrm{mmol}$ ), DIPEA ( $0.13 \mathrm{~mL}, 0.744 \mathrm{mmol}$ ), and N -(2aminoethyl)pyrrolidine ( $38 \mu \mathrm{~L}, 0.298 \mathrm{mmol}$ ) were added, and the resulting mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with sat. $\mathrm{NaHCO}_{3}$ and extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC ( $0-10 \% \mathrm{MeOH} / \mathrm{DCM}, 90 \mathrm{~g}$ silica cartridge) to give $23 \mathrm{mg}, 0.063 \mathrm{mmol}, 42 \%$ yield as a yellow solid. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 364.2$; found $365.4[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of 3,4-dimethyl-11-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-11H-pyrido[2,1-b]quinazoline-6-carboxamide ( $23 \mathrm{mg}, 0.063 \mathrm{mmol}$ ) in a $1: 1$ mixture of DCM/MeOH ( 4 mL ) was added 4 M HCl in 1,4-dioxane ( $47 \mu \mathrm{~L}, 0.189 \mathrm{mmol}$ ). The
 added until the precipitation of a yellow solid. The resulting precipitate was filtered and dried overnight under vacuum to give $21.2 \mathrm{mg}, 0.053 \mathrm{mmol}, 84 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.14(\mathrm{t}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 10.14$ $(\mathrm{s}, 1 \mathrm{H}), 9.01(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.60(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H})$, $7.44(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.21(\mathrm{t}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.86(\mathrm{q}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.45(\mathrm{q}$, $J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.09(\mathrm{~m}, 4 \mathrm{H}), 2.02(\mathrm{~m}, 4 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}$, 364.2; found $365.0[\mathrm{M}+\mathrm{H}]^{+}$.

2,2-difluoro-6-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-6H-[1,3]dioxolo[4,5-h]pyrido[2,1-b]quinazoline-11-carboxamide hydrochloride (52). To a solution of 2,2-difluoro-1,3-benzodioxol-4-amine (93) (2.5 g, 14.441 mmol$)$ in DCM $(30 \mathrm{~mL})$ at $0^{\circ} \mathrm{C}$ was added $N$-bromosuccinimide ( $2.59 \mathrm{~g}, 14.552 \mathrm{mmol}$ ). The resulting mixture was stirred at 0 ${ }^{\circ} \mathrm{C}$ for 30 minutes then allowed to warm to room temperature and stirred for 1 hour. The reaction mixture was quenched with $1 \mathrm{M} \mathrm{Na}{ }_{2} \mathrm{~S}_{2} \mathrm{O}_{3}$, diluted with water, and extracted $2 x$ with DCM. Combined organic layers were dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0$25 \%$ EtOAc/heptane, 90 g silica cartridge) to give $2.53 \mathrm{~g}, 10.039 \mathrm{mmol}, 70 \%$ yield as an off-white solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 7.23(\mathrm{~d}, J=6.5 \mathrm{~Hz}$, 1H), 6.56 (d, J = 7.0 Hz, 1H), 5.80 (s, 2H).

To a 2-5 mL microwave vial was added methyl 2-chloropyridine-3carboxylate (69) (150 mg, 0.874 mmol ) and toluene ( 2.5 mL ). The mixture was purged three times with argon (reduced pressure was applied in vial until formation of bubbles, let 15-30 seconds then flushed with argon). 5-bromo-2,2-difluorobenzo[d][1,3]dioxol-4-amine (94) (288 mg, 1.143 mmol$), \mathrm{Pd}(\mathrm{OAc})_{2}(10 \mathrm{mg}$, $0.044 \mathrm{mmol})$, DPEphos ( $35 \mathrm{mg}, 0.065 \mathrm{mmol}$ ) and KOtBu ( $135 \mathrm{mg}, 1.203 \mathrm{mmol}$ ) were added, the mixture was purged again three times with argon, and the vial was sealed. The resulting mixture was heated at $100{ }^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature, quenched with water, and diluted with DCM. The layers were separated, and the aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-25\% EtOAc/heptane, 30 g silica cartridge) to give 80 mg , $0.207 \mathrm{mmol}, 18 \%$ yield as an off-white solid. ${ }^{1} \mathrm{H}$ NMR ( $\left.400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}\right) \delta \mathrm{ppm}$ $10.12(\mathrm{~s}, 2 \mathrm{H}), 8.36(\mathrm{~s}, 3 \mathrm{H}), 8.27(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 2 \mathrm{H}), 8.13(\mathrm{dd}, J=7.5,2.0 \mathrm{~Hz}, 1 \mathrm{H})$, $7.62(\mathrm{~d}, J=8.7 \mathrm{~Hz}, 2 \mathrm{H}), 7.38(\mathrm{~d}, J=8.7 \mathrm{~Hz}, 2 \mathrm{H}), 7.21(\mathrm{dd}, J=7.5,4.9 \mathrm{~Hz}, 2 \mathrm{H})$, 7.11 (dd, $J=7.5,4.9 \mathrm{~Hz}, 1 \mathrm{H}), 4.06(\mathrm{~s}, 6 \mathrm{H}), 3.93(\mathrm{~s}, 3 \mathrm{H}), 3.82(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{14} \mathrm{H}_{9} \mathrm{BrF}_{2} \mathrm{~N}_{2} \mathrm{O}_{4}, 386.0$; found $388.9[\mathrm{M}+2]^{+}$.

To a 2-5 mL microwave vial was added methyl 2-((5-bromo-2,2-difluorobenzo[d][1,3]dioxol-4-yl)amino)nicotinate (95) (130 mg, 0.336 mmol ) and toluene ( 3 mL ). The mixture was purged three times with argon (reduced pressure was applied in vial until formation of bubbles, let 15-30 seconds then flushed with argon). $\mathrm{Pd}(\mathrm{OAc})_{2}(7.5 \mathrm{mg}, 0.033 \mathrm{mmol})$, XantPhos (29 mg, 0.050 mmol$)$, XantPhos

Pd G3 (17 mg, 0.018 mmol$)$, and $\mathrm{K}_{3} \mathrm{PO}_{4}(217 \mathrm{mg}, 1.008 \mathrm{mmol})$ were added, the mixture was purged again three times before adding $\mathrm{Mo}(\mathrm{CO})_{6}(133 \mathrm{mg}, 0.504$ $\mathrm{mmol})$. The vial was sealed and heated at $115{ }^{\circ} \mathrm{C}$ for 8 hours. Additional $\mathrm{Pd}(\mathrm{OAc})_{2}$ (7.5 mg, 0.033 mmol$)$, XantPhos (29 mg, 0.050 mmol$)$, and XantPhos Pd G3 (17 $\mathrm{mg}, 0.018 \mathrm{mmol})$ were added and the reaction mixture was heated at $115{ }^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature, quenched with water, and diluted with DCM. The layers were separated, and the aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-25\% EtOAc/heptane, 30 g silica cartridge) to give $36 \mathrm{mg}, 0.108 \mathrm{mmol}, 32 \%$ yield as a yellow gum. ${ }^{1} \mathrm{H}$ NMR (400 $\left.\mathrm{MHz}, \mathrm{DMSO}-d_{6}\right) \delta \mathrm{ppm} 8.93(\mathrm{dd}, J=7.3,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.24(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H})$, 8.10 (dd, $J=6.8,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.64(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.20-7.12(\mathrm{~m}, 1 \mathrm{H}), 3.93(\mathrm{~s}$, 3H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{8} \mathrm{~F}_{2} \mathrm{~N}_{2} \mathrm{O}_{5}, 334.0$; found $335.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of methyl 2,2-difluoro-6-oxo-6H-[1,3]dioxolo[4,5-h]pyrido[2,1-b]quinazoline-11-carboxylate (96) (36 mg, 0.108 mmol ) in MeOH ( 1 mL ) was added $1 \mathrm{M} \mathrm{HCl}(1 \mathrm{~mL}, 1 \mathrm{mmol})$. The resulting mixture was heated at $100^{\circ} \mathrm{C}$ for 5 hours. The reaction mixture was allowed to cool to room temperature, diluted with water, and extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give 17 mg , $0.053 \mathrm{mmol}, 49 \%$ yield as a yellow solid. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{14} \mathrm{H}_{6} \mathrm{~F}_{2} \mathrm{~N}_{2} \mathrm{O}_{5}$, 320.0; found $321.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a mixture of 2,2-difluoro-6-oxo-6H-[1,3]dioxolo[4,5-h]pyrido[2,1-b]quinazoline-11-carboxylic acid ( $17 \mathrm{mg}, 0.053 \mathrm{mmol}$ ), TBTU ( $35 \mathrm{mg}, 0.109 \mathrm{mmol}$ ), and DIPEA ( $41 \mu \mathrm{~L}, 0.235 \mathrm{mmol}$ ) in DMF ( 1 mL ) was added N -(2aminoethyl)pyrrolidine ( $20 \mu \mathrm{~L}, 0.158 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 3 hours. Additional TBTU ( $17 \mathrm{mg}, 0.053 \mathrm{mmol}$ ), DIPEA ( 20 $\mu \mathrm{L}, 0.115 \mathrm{mmol}$ ), and N -(2-aminoethyl)pyrrolidine ( $10 \mu \mathrm{~L}, 0.079 \mathrm{mmol}$ ) were added, and the resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was quenched with sat. $\mathrm{NaHCO}_{3}$ and extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-10\% MeOH/DCM, 15 g silica cartridge) to give $7 \mathrm{mg}, 0.017 \mathrm{mmol}, 32 \%$ yield as a yellow solid. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{20} \mathrm{H}_{18} \mathrm{~F}_{2} \mathrm{~N}_{4} \mathrm{O}_{4}, 416.1$; found $417.1[\mathrm{M}+\mathrm{H}]^{+}$.

To a solution of 2,2-difluoro-6-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-6H-[1,3]dioxolo[4,5-h]pyrido[2,1-b]quinazoline-11-carboxamide ( $7 \mathrm{mg}, 0.017 \mathrm{mmol}$ ) in a 1:1 mixture of $\mathrm{DCM} / \mathrm{MeOH}(1 \mathrm{~mL})$ was added 4 M HCl in 1,4 -dioxane ( $12 \mu \mathrm{~L}$, 0.048 mmol ). The resulting mixture was stirred at room temperature for 1 hour. $\mathrm{Et}_{2} \mathrm{O}(10-20 \mathrm{~mL})$ was added until precipitation of a yellow solid occurred. The resulting solid was filtered and dried overnight under vacuum to give $7 \mathrm{mg}, 0.015$ mmol, $88 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 600 MHz , DMSO-d ) $\delta \mathrm{ppm} 10.43$ (s, $1 \mathrm{H}), 9.75(\mathrm{~s}, 1 \mathrm{H}), 9.06$ (d, $J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.60(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.28(\mathrm{~d}, J=$ $12.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.68(\mathrm{~d}, \mathrm{~J}=12.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.82-3.65(\mathrm{~m}, 2 \mathrm{H})$,
$3.65(\mathrm{~m}, 2 \mathrm{H}), 3.43(\mathrm{~m}, 2 \mathrm{H}), 3.12(\mathrm{~m}, 2 \mathrm{H}), 1.94(\mathrm{~m}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{20} \mathrm{H}_{18} \mathrm{~F}_{2} \mathrm{~N}_{4} \mathrm{O}_{4}, 416.1$; found $416.9[\mathrm{M}+\mathrm{H}]^{+}$

Cell culture. Performed by Daming Chen. A375 melanoma cells were maintained at $37{ }^{\circ} \mathrm{C}$ in a humidified atmosphere containing $5 \% \mathrm{CO}_{2}$. A375 cells were cultured in DMEM supplemented with $10 \%$ fetal bovine serum (FBS) and 4 mM glutamine.

RPA194 Degradation Assay. Performed by Daming Chen. A375 cells were seeded on 96-well plates (PerkinElmer ViewPlate-96 Black, catalog \# 6005182) and treated with the compounds at $0.01,0.03,0.1,0.3,1,3,10$, and $30 \mu \mathrm{M}$ or treated with vehicle (DMSO) for 4 h . After treatment, cells were washed with phosphate-buffered saline (PBS), fixed in 3.5-4\% paraformaldehyde, permeabilized with 0.1-0.5\% NP-40, and blocked with 1-3\% bovine serum albumin (BSA). Cells were incubated with primary antibody, anti-RPA194 (C1) [sc-48385, Santa Cruz Biotechnology], for 2 h at $37^{\circ} \mathrm{C}$ and washed three times with PBS. Cells were incubated with secondary antibody, Alexa 594-conjugated anti-mouse (A11005, Invitrogen) or Alexa 488-conjugated anti-mouse (A11001, Thermo Fisher), for 1 h at $37^{\circ} \mathrm{C}$, washed three times with PBS, and DNA was stained with Hoechst 33342 (H-21492, Invitrogen). Images were acquired using a Molecular Devices ImageXpress Micro XLS High Content Imager (20X objective, 9 fields/well) and processed using MetaXpress High Content Software-6. The fold change to control was determined. IC50 was determined using GraphPad Prism for Windows (version 6.01) using a three or four-parameter fit.
hERG Inhibition Assay. hERG inhibition analysis was performed at Evotec. Studies were performed using HEK293 cells stably transfected with hERG cDNA. QPatch was primed with appropriate extracellular (bath) and intracellular (pipette) solutions prior to conducting a study. The composition of the extracellular solution was: $\mathrm{NaCl}(137 \mathrm{mM}), \mathrm{KCl}(4 \mathrm{mM}), \mathrm{CaCl}_{2}(1.8 \mathrm{mM}), \mathrm{MgCl}_{2}(1 \mathrm{mM})$, glucose (10 $\mathrm{mM})$, HEPES ( 10 mM ), pH 7.4 with NaOH . The composition of the intracellular solution was: $\mathrm{KCl}(130 \mathrm{mM}), \mathrm{MgCl}_{2}(1 \mathrm{mM})$, EGTA ( 5 mM ), Mg-ATP ( 5 mM ), HEPES ( 10 mM ), pH 7.2 with KOH . Extracellular and intracellular solutions were filtered through a $0.2 \mu \mathrm{~m}$ polycarbonate membrane filter upon formulation. A 48well plate (QPlate, Sophion Biosciences A/S), used for the QPatch was loaded into the system and primed before preparing cells suspension in the bath solution. Test substances were formulated in DMSO. Stock solutions were diluted in extracellular solutions to final perfusion concentrations ( $0.01,0.1,1,10$, and $30 \mu \mathrm{M}$ ).

A schematic diagram of the voltage protocol used is indicated. The standard voltage profile will be as follows: step from -80 mV to -50 mV for $200 \mathrm{~ms},+20 \mathrm{mV}$ for 4.8 s , step to -50 mV for 5 s then step to the holding potential of -80 mV . The step from -80 mV to the test command ( +20 mV ) results in an outward current (i.e. current flows out of the cell) and the step from the test command ( +20 mV ) to -50 mV results in the tail current (the tail current represents deactivation of the current over time).


The voltage protocol was run and recorded continuously during the experiment. The stability of recording was assessed through initial wash with bath solution alone. The vehicle (extracellular solution + 0.3\% DMSO and 0.05\% Pluronic F-68) was then applied for 3 minutes followed by the test substance. The test substance was applied in triplicate to ensure adequate mixing. The standard combined exposure time was 5 minutes. If the quality of the recording deteriorates, then the experiment may be finished at that point and cell data was not analyzed and reported. Each test substance was tested in at least 2 cells. Finally, to verify and confirm assay quality, the hERG pharmacological standard E-4031 was tested at 5 concentrations ( $0.003,0.011,0.033,0.1$, and $0.3 \mu \mathrm{M}$ ) at least in duplicate, as a positive control.

The average of tail current amplitude values recorded from 4 sequential voltage pulses will be used to calculate for each cell the effect of the test substance by calculating the residual current (\% control) compared with vehicle pre-treatment. The data was plotted and an $\mathrm{IC}_{50}$ value was estimated from the concentrationresponse relationship, if appropriate. If a test compound blocks less than $50 \%$ at
the maximum concentration, only percentage of block for each tested concentration is reported, rather than an extrapolated $\mathrm{IC}_{50}$ value. The computer systems used on this study to acquire and quantify data include QPatch assay software v5.6 (Sophion Biosciences), GraphPad (Prism), and Excel 2010 (Microsoft Office).

CYP1A2 Inhibition Assay. CYP1A2 inhibition analysis was performed at Evotec. Recombinant human hepatic CYP450s (baculovirus-insect-cell expression system) expressing the isoform 1A2 were obtained from BD GenTest Corp. Test compounds were received in DMSO at a concentration of 10 mM . Probe substrate was formulated in DMF. 3-cyano-7-ethoxycoumarin (CEC) was used as a probe substrate. Furafylline was used as a control inhibitor. The following buffers were prepared: 0.5 M filtered potassium phosphate and 1 M magnesium chloride. Test compounds were pre-dissolved in 10\% DMSO and aliquots transferred to individual wells containing phosphate buffer ( 0.1 M final concentration) and probe substrate. Final concentration of DMSO in the incubations was $<1 \%$. A source of reducing equivalents was added containing glucose-6-phosphate dehydrogenase (1 unit/mL), glucose-6-phoshate ( 7.9 mM ), and NADP (3mM). Magnesium chloride was added to each well to a final concentration of 7.5 mM . CEC was added to a final concentration of 0.01 mM . Protein concentration for CYP1A2 was $26 \mu \mathrm{~g} / \mathrm{mL}$. For the determination of $\mathrm{IC}_{50}$, the final concentrations of test compounds ranged from 0.023 to $50 \mu \mathrm{M}$ (3-fold dilution, 8-concentration ranges). Control incubations contained furafylline in place of test compounds. Positive and negative controls representing minimum and maximum probe substrate degradation contained
known inhibitor at high concentration or $10 \%$ DMSO, respectively. After a 5 minute pre-incubation, the reactions were initiated by addition of protein. After the appropriate incubation time, fluorescence was measured using a plate reader at the following wavelengths: excitation - 390 nm , emission - 455 nm . Fluorescence values obtained at each concentration of test compound were converted to percent inhibition based on positive and negative controls. Calculation of $\mathrm{IC}_{50}$, where required, was from fitting a 4-parameter logistic equation. Where $\mathrm{IC}_{50}$ is greater than that of top concentration $(50 \mu \mathrm{M})$, data is reported as $>50 \mu \mathrm{M}$. $\mathrm{IC}_{50}$ determined for control inhibitors were compared with historic and literature values to ensure assay functionality. Control compounds were compared with historic and literature values to ensure assay functionality.

In Vivo Pharmacokinetic Study. Pharmacokinetic analysis was performed at Pharmaron. Male CD1 mice were treated with a single dose administered intravenously ( $1 \mathrm{mg} / \mathrm{kg}, \mathrm{n}=3$ ), orally ( $30 \mathrm{mg} / \mathrm{kg}, \mathrm{n}=3$ ), or intraperitoneally (30 $\mathrm{mg} / \mathrm{kg}, \mathrm{n}=3$ ) and blood samples were taken at timepoints $0,0.083,0.25,0.5,1$, $2,4,8$, and 24 h for $\mathrm{IV} ; 0,0.25,0.5,1,2,4,8$, and 24 h for PO and IP. IV compound was formulated in 30\% dimethylacetamide (DMA) + 10\% polyethylene glycol 200 (PEG200) $+5 \%$ Kolliphor ELP in Milli-Q water. PO and IP compound was formulated in 0.2 M phosphate buffer, pH 6.8 .

Microsomal Stability Assay. Performed at Evotec. A mixture of mouse liver microsomes ( $0.5 \mathrm{mg} / \mathrm{mL}$ protein) and test compound $(1 \mu \mathrm{M})$ was preincubated at $37{ }^{\circ} \mathrm{C}$ for 5 min , then NADPH was added to the mixture. The time course ( 0 to 45
min) concentration of the test compound was determined by LC-MS/MS. Time (minutes) is plotted against the natural logarithm of the percent compound remaining to determine the slope. Apparent intrinsic clearance was determined from the slope $\left(\mathrm{min}^{-1}\right)$, the volume of incubation $(\mathrm{mL})$, and the amount of protein (mg). Apparent intrinsic clearance values are expressed as $\mu \mathrm{L} / \mathrm{min} / \mathrm{mg}$ protein.

$$
\begin{gather*}
t_{\frac{1}{2}}=\frac{\operatorname{Ln}(2)}{\text { Slope }}  \tag{1}\\
C l_{\text {int }, \text { app }}=\frac{L n(2)}{t_{\frac{1}{2}}} \times \frac{\text { Vol.incubation }}{\text { Amount of protein }} \tag{2}
\end{gather*}
$$

Hepatocyte Stability Assay. Performed at Evotec. A mixture of cryopreserved rat hepatocytes ( 1 million cells $/ \mathrm{mL}$ ) and test compound ( $1 \mu \mathrm{M}$ ) was incubated at 37 ${ }^{\circ} \mathrm{C}$ for 2 h . The time course ( 0 to 2 h ) concentration of the test compound was determined by LC-MS/MS. Time (minutes) is plotted against the natural logarithm of the percent compound remaining to determine the slope. Apparent intrinsic clearance was determined from the slope $\left(\mathrm{min}^{-1}\right)$, the volume of incubation ( mL ), and the number of cells (million cells). Apparent intrinsic clearance values are expressed as $\mu \mathrm{L} / \mathrm{min} /$ million cells. A scaling factor of 6.5 was applied to apparent intrinsic clearance values to provide scaled intrinsic clearance. Scaled intrinsic clearance values are expressed as $\mathrm{mL} / \mathrm{min} / \mathrm{kg}$.

$$
\begin{gather*}
t_{\frac{1}{2}}=\frac{L n(2)}{\text { Slope }}  \tag{1}\\
C l_{\text {int,app }}=\frac{L n(2)}{t_{\frac{1}{2}}} \times \frac{\text { Vol.incubation }}{\text { Number of cells }} \tag{3}
\end{gather*}
$$

Cell Viability Assay. Cell viability analysis was performed at Evotec. A375 cells were plated in 384-well plates at a density of 800 cells/well and incubated for 3 days with the compounds at $37{ }^{\circ} \mathrm{C}$ in $5 \% \mathrm{CO}_{2}$. Viability was determined using CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Experiment was reperformed to provide at least 3 replicates.

RNA Isolation and qPCR. Performed by Daming Chen and Hester Liu. A375 cells were treated with the compounds $(1 \mu \mathrm{M})$ for 6 hours, collected by scraping, and pelleted by centrifugation. RNA was isolated using Qiagen RNeasy kit. Total RNA $(4 \mu \mathrm{~g})$ was reverse transcribed and used to perform qPCR in triplicate with SYBR GREEN master mix (Fisher Scientific) on ABI QuantStudio 12K Flex System using primer pairs:

$$
\text { F= forward; } R=
$$

GENE $\quad$ Reverse $\quad$ Primer sequence 5’ - 3'

| GAPDH | F | ACCCAGAAGACTGTGGATGG |
| :--- | :--- | :--- |
|  | R | TTCAGCTCAGGGATGACCTT |
| 5 5'ETS851 | F | GAACGGTGGTGTGTCGTT |
|  | R | GCGTCTCGTCTCGTCTCACT |
| DHFR | F | GCTGCTGTCATGGTTGGTTC |
|  | R | AGAGGTTGTGGTCATTCTCTGG |
|  |  |  |
| tRNA Valine | F | TCCGTAGTGTAGTGGTTATCACG |

$R \quad$ GTTTCGAACCGGGGACCT

Transcript quantification was measured by $\Delta \Delta \mathrm{Ct}$ method. All results were normalized against GAPDH, and coefficient of variation was calculated. The experiment was conducted using three biological repeats in triplicate.

Statistical Analysis. Values were calculated and statistical significance was assessed using ordinary one-way ANOVA in GraphPad Prism 9.3.1.

## References

(1) Hein, N.; Hannan, K. M.; George, A. J.; Sanij, E.; Hannan, R. D. The Nucleolus: An Emerging Target for Cancer Therapy. Trends Mol. Med. 2013, 19 (11), 643-654. https://doi.org/10.1016/j.molmed.2013.07.005.
(2) Ferreira, R.; Schneekloth, J. S.; Panov, K. I.; Hannan, K. M.; Hannan, R. D. Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age. Cells 2020, 9 (2), 266. https://doi.org/10.3390/cells9020266.
(3) Derenzini, M.; Montanaro, L.; Treré, D. What the Nucleolus Says to a Tumour Pathologist. Histopathology 2009, 54 (6), 753-762. https://doi.org/10.1111/j.1365-2559.2008.03168.x.
(4) Bywater, M. J.; Pearson, R. B.; McArthur, G. A.; Hannan, R. D. Dysregulation of the Basal RNA Polymerase Transcription Apparatus in Cancer. Nat. Rev. Cancer 2013, 13 (5), 299-314. https://doi.org/10.1038/nrc3496.
(5) Drygin, D.; Rice, W. G.; Grummt, I. The RNA Polymerase I Transcription Machinery: An Emerging Target for the Treatment of Cancer. Annu. Rev. Pharmacol. Toxicol. 2010, 50 (1), 131-156. https://doi.org/10.1146/annurev.pharmtox.010909.105844.
(6) Deisenroth, C.; Zhang, Y. Ribosome Biogenesis Surveillance: Probing the Ribosomal Protein-Mdm2-P53 Pathway. Oncogene 2010, 29 (30), 42534260. https://doi.org/10.1038/onc.2010.189.
(7) Bursac, S.; Brdovcak, M. C.; Donati, G.; Volarevic, S. Activation of the Tumor Suppressor P53 upon Impairment of Ribosome Biogenesis. Biochim. Biophys. Acta - Mol. Basis Dis. 2014, 1842 (6), 817-830. https://doi.org/10.1016/j.bbadis.2013.08.014.
(8) Peltonen, K.; Colis, L.; Liu, H.; Trivedi, R.; Moubarek, M. S.; Moore, H. M.; Bai, B.; Rudek, M. A.; Bieberich, C. J.; Laiho, M. A Targeting Modality for Destruction of RNA Polymerase I That Possesses Anticancer Activity. Cancer Cell 2014, 25 (1), 77-90. https://doi.org/10.1016/j.ccr.2013.12.009.
(9) Bywater, M. J.; Poortinga, G.; Sanij, E.; Hein, N.; Peck, A.; Cullinane, C.; Wall, M.; Cluse, L.; Drygin, D.; Anderes, K.; Huser, N.; Proffitt, C.; Bliesath, J.; Haddach, M.; Schwaebe, M. K.; Ryckman, D. M.; Rice, W. G.; Schmitt, C.; Lowe, S. W.; Johnstone, R. W.; Pearson, R. B.; McArthur, G. A.; Hannan, R. D. Inhibition of RNA Polymerase I as a Therapeutic Strategy to Promote Cancer-Specific Activation of P53. Cancer Cell 2012, 22 (1), 5165. https://doi.org/10.1016/J.CCR.2012.05.019.
(10) Burger, K.; Mühl, B.; Harasim, T.; Rohrmoser, M.; Malamoussi, A.; Orban, M.; Kellner, M.; Gruber-Eber, A.; Kremmer, E.; Hölzel, M.; Eick, D. Chemotherapeutic Drugs Inhibit Ribosome Biogenesis at Various Levels. J. Biol. Chem. 2010, 285 (16), 12416-12425. https://doi.org/10.1074/jbc.M109.074211.
(11) Bruno, P. M.; Lu, M.; Dennis, K. A.; Inam, H.; Moore, C. J.; Sheehe, J.; Elledge, S. J.; Hemann, M. T.; Pritchard, J. R. The Primary Mechanism of Cytotoxicity of the Chemotherapeutic Agent CX-5461 Is Topoisomerase II Poisoning. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (8), 4053-4060. https://doi.org/10.1073/PNAS.1921649117/-/DCSUPPLEMENTAL.
(12) Colis, L.; Ernst, G.; Sanders, S.; Liu, H.; Sirajuddin, P.; Peltonen, K.; Depasquale, M.; Barrow, J. C.; Laiho, M. Design, Synthesis, and StructureActivity Relationships of Pyridoquinazolinecarboxamides as RNA Polymerase i Inhibitors. J. Med. Chem. 2014, 57 (11), 4950-4961. https://doi.org/10.1021/jm5004842.
(13) Jamieson, C.; M. Moir, E.; Rankovic, Z.; Wishart, G. Medicinal Chemistry of HERG Optimizations: Highlights and Hang-Ups. J. Med. Chem. 2006, 49 (17), 5029-5046. https://doi.org/10.1021/jm060379l.
(14) Gunes, A.; Dahl, M. L. Variation in CYP1A2 Activity and Its Clinical Implications: Influence of Environmental Factors and Genetic Polymorphisms. Pharmacogenomics 2008, 9 (5), 625-637. https://doi.org/10.2217/14622416.9.5.625/FORMAT/EPUB.
(15) Ivashkevich, A.; Redon, C. E.; Nakamura, A. J.; Martin, R. F.; Martin, O. A. Use of the $\gamma$-H2AX Assay to Monitor DNA Damage and Repair in Translational Cancer Research. Cancer Lett. 2012, 327 (1-2), 123-133. https://doi.org/10.1016/j.canlet.2011.12.025.
(16) Peltonen, K.; Colis, L.; Liu, H.; Jäämaa, S.; Moore, H. M.; Enbäck, J.; Laakkonen, P.; Vaahtokari, A.; Jones, R. J.; af Hällström, T. M.; Laiho, M. Identification of Novel P53 Pathway Activating Small-Molecule Compounds Reveals Unexpected Similarities with Known Therapeutic Agents. PLoS ONE 2010, 5 (9), e12996. https://doi.org/10.1371/journal.pone.0012996.
(17) Xu, T.; Alper, H. Synthesis of Pyrido[2,1- b ]Quinazolin-11-Ones and Dipyrido[1,2-a:2',3'- d ]Pyrimidin-5-Ones by Pd/DIBPP-Catalyzed Dearomatizing Carbonylation. Org. Lett. 2015, 17 (6), 1569-1572. https://doi.org/10.1021/acs.orglett.5b00452.

## Chapter. 3 High-Throughput Screening Efforts Towards the Discovery of New RNA Polymerase I Inhibitors

## Introduction

RNA polymerase $\mathrm{I}(\mathrm{Pol} \mathrm{I})$ is a DNA-dependent RNA polymerase that is responsible for transcription of the 47S pre-ribosomal RNA (rRNA) and serves as the rate-limiting step in ribosome biogenesis. Many types of cancers exhibit dysregulated rates of Pol I transcription, reflecting a need for increased ribosome synthesis to generate proteins to sustain heightened growth rates. Cancer cells may be selectively vulnerable to agents that inhibit Pol I transcription, providing an attractive therapeutic strategy for cancer treatment. ${ }^{1}$ BMH-21 is the first specific and selective ${ }^{2}$ Pol I inhibitor and does so by intercalating into GC-rich rDNA and creating a transcription block, leading to the ubiquitination and proteasomal degradation of the large catalytic subunit, RPA194. ${ }^{3}$ Notably, it accomplishes this independently of p 53 and without eliciting a DNA damage response. ${ }^{4} \mathrm{BMH}-21$ is the first of only a small number of compounds, such as amodiaquine, ${ }^{5}$ hernandonine, ${ }^{6}$ and sempervirine, ${ }^{7}$ to exhibit the RPA194 degradation phenotype, and a quantitative cell-based assay has been developed ${ }^{3}$ to measure the extent of RPA194 degradation caused by compound treatment. Recently, in collaboration with Evotec, this assay was translated from 96-well plate format to 384 -well plate format to facilitate high-throughput screening (HTS) efforts. Herein, we describe efforts to discover new Pol I inhibitors via high-throughput screening and to generate new structure-activity relationships (SARs).

## Results and Discussion

The RPA194 degradation assay relies on fluorescence microscopy to detect the fluorescence intensity of a fluorescent antibody specific for RPA194 after compound treatment (Figure 3-1). BMH-21 and related compounds ${ }^{8-10}$ cause the proteasomal degradation of RPA194, resulting in a decrease in fluorescence intensity relative to control, whereas compounds such as actinomycin $D$ (ActD) cause localization of RPA194 into nucleolar cap structures at the periphery of the nucleolus, without affecting the levels of RPA194 or its stability. The formation of nucleolar caps reduces the homogeneity of the RPA194 fluorescence signal. By utilizing fluorescence microscopy, these two discrete phenotypes can be distinguished and used to classify screening molecules. In collaboration with Evotec, the RPA194 degradation assay was translated from 96-well plate format to 384 -well plate format and a primary HTS of 251 K compounds from the Evotec library was screened at a single $10 \mu \mathrm{M}$ concentration in singlicate. Hits were chosen and assigned to two groups, BMH-21-like or ActD-like, based on either a $>20 \%$ degradation of RPA194 signal or a $>20 \%$ decrease in RPA194 homogeneity, respectively. The screening cascade workflow is represented in Figure 3-2.

Based on these criteria, the BMH-21-like phenotype had a $0.7 \%$ hit rate and a $0.3 \%$ false positive rate, while the ActD-like phenotype had a $0.3 \%$ hit rate and a $0.07 \%$ false positive rate. Hits were retested at $10 \mu \mathrm{M}$ in triplicate to confirm the activity exhibited in the primary screen. Of the BMH-21-like hits, $44 \%$ were confirmed active while only $16 \%$ of ActD hits met the confirmation criteria.


Figure 3-1. Fluorescent microscopy images of RPA194 degradation assay. A375 cells were treated with BMH-21 ( $1 \mu \mathrm{M}$ ) or ActD $(50 \mathrm{ng} / \mathrm{mL})$ for 3 hr and stained for RPA194 and NCL. Merged image with DNA staining is shown below. Scale bar, $10 \mu \mathrm{~m}$. Adapted from Peltonen et al., $2014^{3}$ with permission from Elsevier.

Importantly, autofluorescent compounds that interfered with the assay readout by increasing the measured RPA194 signal were discarded. Confirmed hits were further profiled with an eleven-point titration at 1:2 dilution starting from $30 \mu \mathrm{M}$ to determine their RPA194 degradation IC50 as well as secondary assays measuring $y \mathrm{H} 2 \mathrm{AX}$ activation, cell viability, and Pol I transcription inhibition via quantitative polymerase chain reaction (qPCR). At this point, all ActD hits were discontinued for several reasons: almost all were $\gamma \mathrm{H} 2 \mathrm{AX}$-positive, some triggered alerts for panassay interference compounds (PAINS), ${ }^{11}$ and some were positive for mutagenicity via Ames testing. ${ }^{12}$ Further development of the ActD-like hits would require more de-risking by in-silico safety methods and a deeper literature review. Instead, the BMH-21-like hits were prioritized, advanced to medicinal chemistry review, and were grouped into clusters based on chemical structure. The most attractive cluster was the bicyclic aromatics, providing two hits, $\mathbf{1}$ and $\mathbf{2}$ shown in Figure 3-3, both containing a quinazoline core structure.

Hit expansion began with substructure-searching for quinazolines


Figure 3-2. High-throughput screening cascade of the Evotec library. substituted only at the two- and four-position of the heterocycle, resulting in over 2,000 documented compounds. These were broken down into another round of clustering, and 127 commercially available compounds were purchased to begin SAR studies by surveying diverse chemical space. Unfortunately, none of these compounds exhibited any activity for RPA194 degradation. Thus, further SAR efforts required a more conservative design of quinazoline analogs.

We set out to improve the RPA194 degradation potency to improve the $\mathrm{IC}_{50}$ below $10 \mu \mathrm{M}$ with the end goal of achieving an $\mathrm{IC}_{50}$ of $0.5 \mu \mathrm{M}$ or below, while remaining negative for $\gamma \mathrm{H} 2 \mathrm{AX}$ activation. Potent RPA194 degraders would justify additional follow-up with secondary assays, such as cell viability and qPCR assays. Desirable IC50's for these secondary assays would be equivalent or lower to that of the RPA194 degradation $\mathrm{IC}_{50}$. Analogs that meet these criteria would be




| Example | $\mathbf{1}$ | $\mathbf{2}$ |
| :---: | :---: | :---: |
| RPA194 IC $_{50}(\mu \mathrm{M})^{\mathbf{a}}$ | 3.9 | 12 |
| $\boldsymbol{\gamma H 2 A X} \mathrm{IC}_{50}(\mu \mathrm{M})^{\mathbf{b}}$ | $>30$ | $>30$ |
| Cell Viability IC $50(\mu \mathrm{M})^{\mathrm{c}}$ | 4.2 | 6.9 |
| qPCR IC $_{50}(\mu \mathrm{M})^{\mathrm{d}}$ | 2.8 | $>10$ |

Figure 3-3. Profile of hits 1 and 2. aRPA194 degradation measured in A375 cells. IC ${ }_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate. ${ }^{b} y \mathrm{H} 2 \mathrm{AX}$ activation measured in A375 cells. IC50 represents the mean of duplicate independent biological experiments performed in triplicate. ${ }^{\circ}$ Cell viability analysis measured in A375 cells CellTiter-Glo ${ }^{\circledR}$ Luminescent Cell Viability Assay. IC50 represents the mean of duplicate independent biological experiments performed in triplicate. ${ }^{d}$ qPCR analysis in A375 cells performed with EXPRESS SYBR® GreenER ${ }^{\text {TM }}$ qPCR SuperMix Universal. $\mathrm{IC}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
further profiled for absorption, distribution, metabolism, and excretion (ADME) properties as well as physical properties such as solubility and logD.

We began by modifying the quinazoline core of 1 by making simple substitutions at either the two- or four-position while holding the other position constant. Summarized in Table 3-1, this resulted in two groups of analogs. The first group was produced by replacing the 2-hydroxy-3-methoxyphenyl ring at the twoposition while holding the $N$-methylethanolamine substituent at the four-position constant. By removing the substituents on the phenyl ring, $\mathbf{3}$ showed a complete loss of activity $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$, implying that the substituents on the phenyl ring are

|  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Ex. | R2 | $\begin{aligned} & \text { RPA194 } \\ & \text { IC }_{50}(\mu \mathrm{M})^{\mathrm{a}} \end{aligned}$ | Ex. | R4 | $\begin{aligned} & \text { RPA194 } \\ & \text { IC } \mathrm{C}_{50}(\mu \mathrm{M})^{\mathrm{a}} \end{aligned}$ |
| 3 |  | >10 | 7 |  | >10 |
| 4 |  | >30 | 8 | $\square_{N}$ | >10 |
| 5 | $\begin{gathered} \mathrm{H}_{3} \mathrm{C}_{-} \mathrm{N} \text { 氯 } \\ \mathrm{H}_{3} \mathrm{C} \end{gathered}$ | >30 | 9 |  | 1.5 |
| 6 |  | >10 | 10 |  | 6.6 |
|  |  |  | 11 |  | >10 |
|  |  |  | 12 |  | >10 |

Table 3-1. Analogs of 1 substituted at the 2- or 4-position. ${ }^{\text {aRPA1 }} 194$ degradation measured in A375 cells. IC ${ }_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
important for activity. It is possible that these substituents engage in hydrogenbonding with the rDNA, a part of the Pol I complex, or intermolecularly with itself. Likewise, replacing the phenyl ring with aniline 4 also resulted in a loss of activity $\left(\mathrm{IC}_{50}>30 \mu \mathrm{M}\right)$. Previously, we have observed that an extended pi system is important for potency. ${ }^{9,10}$ Given the structural similarity that the quinazoline hits share with the internal quinazolinone moiety in the BMH-21 tetracycle, it is possible that some aspects of the BMH-21 SARs may carry over to the new series of molecules. Replacing phenyl with aniline places the aromatic ring further away
from the quinazoline core and introduces another degree of freedom in the molecule, possibly affecting its intercalative ability. ${ }^{13}$ Replacing the aromatic ring with both acyclic 5 and cyclic 6 amines also resulted in a loss of activity $\left(\mathrm{IC}_{50}>30\right.$ and $>10 \mu \mathrm{M}$, respectively), highlighting the requirement for an extended pi system and an aromatic ring at the two-position of the quinazoline core.

The second group of analogs was generated by holding the 2-hydroxy-3methoxyphenyl ring at the two-position constant while varying substituents at the four-position of the quinazoline core (Table 3-1). Shortening the N methylethanolamine substituent into dimethylamine 7 or wrapping into a ring 8 lead to a loss of activity $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$. It is possible that substituents at this position reach towards the phosphate backbone of the rDNA or a part of the Pol I complex and engage in hydrogen-bonding interactions. With this in mind, we replaced the hydroxyl group at the end of the $N$-methylethanolamine in 1 with dimethylamine and varied the carbon linker length between the two nitrogen atoms (Compounds 9 and 10). To our excitement, the two-carbon linker 9 resulted in more than a twofold increase in potency ( $\mathrm{IC}_{50} 1.5 \mu \mathrm{M}$ ). Again reflecting previously observed SARs, ${ }^{9,10}$ the two-carbon linker was superior to the three-carbon linker (Compounds 9 and 10, $\mathrm{IC}_{50} 1.5$ and $6.6 \mu \mathrm{M}$, respectively), reflecting an ideal substituent length to place the terminal amine in a position to engage in putative hydrogen-bonding interactions. Also worth noting is that the $\mathrm{N}, \mathrm{N}$ dimethylaminoethylamine moiety is shared with the side chain of BMH-21. We believe that this terminal amine is protonated at physiological pH , further supporting the possibility of interactions with the negatively charged phosphate
backbone. Replacing the aliphatic substituent with anilines 11 and 12 also resulted in a loss of activity ( $\mathrm{IC}_{50}>10$ and $>10 \mu \mathrm{M}$, respectively), highlighting the need for an aliphatic hydrogen bonding partner at the four-position. With little improvement over hit compound 1 to be found in terms of RPA194 degradation potency, we instead turned to analogs of 2 to see if potency could be improved from a weak starting point (IC50 $12 \mu \mathrm{M}$ ).

Employing a similar strategy, we set out to generate analogs that would alter substituents at either the two- or four-position while holding the other position constant. Summarized in Table 3-2, the first group of analogs was generated by substituting the two-position pyrrolidine of 2 and holding the four-position 2,4dimethoxyaniline substituent constant. Notably, replacing pyrrolidine with the smaller dimethylamine 13 resulted in a loss of activity ( $\mathrm{IC}_{50}>30 \mu \mathrm{M}$ ), further highlighting the sensitivity of the SAR. As seen previously with 1, substituting the two-position with aniline 14 resulted in a loss of activity ( $\mathrm{IC}_{50}>10 \mu \mathrm{M}$ ). Likewise, replacing pyrrolidine with a plain phenyl ring 15 also resulted in loss of activity (IC50 $>10 \mu \mathrm{M}$ ). Curiously, introducing the same 2-hydroxy-3-methoxyphenyl ring 16 found in 1 did not rescue potency $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$, possibly pointing towards the fourposition aromatic substituent as being the cause for the lack in potency between the two hits. Substitution with electron-deficient aromatic rings 17 and 18 also resulted in a loss of activity $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$. Similar to hit quinazoline 1, substitution at the two-position was completely intolerable, showcasing the heightened sensitivity to change. With this observation, we again turned to four-position substitutions to explore potency improvements of 2.

Another group of analogs, summarized in Table 3-2, was generated by holding the two-position pyrrolidine constant while modifying substituents at the four-position. Intriguingly, an almost two-fold increase in potency was observed by removing the methoxy substituents on the aniline ring $19\left(\mathrm{IC}_{50} 6.0 \mu \mathrm{M}\right)$. This opposes the trend observed so far of aromatic groups not being tolerated at the four-position. One possible explanation is that 1 and 2 exhibit different binding orientations when intercalating into the rDNA. Considering that the substitution patterns in 1 and 2 are essentially mirrored (1: two-position - aromatic, fourposition - aliphatic; 2: two-position - aliphatic, four-position - aromatic), it is possible that key binding interactions are mediated by changes in binding orientation. Furthermore, 1 possibly binds in a more optimal orientation than 2 when comparing key profile data (Figure 3-3). An electron-deficient aromatic aniline 20 was not tolerated, agreeing with the overall trend ( $\left.\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$. Small improvements in potency could be gained by replacing the aniline substituent with alkyl groups like dimethylamine 21 and pyrrolidine 22 ( $\mathrm{IC}_{50} 11$ and $8.6 \mu \mathrm{M}$, respectively). However, without any additional aromatic substituents present, the potency increase was limited, likely due to decreased intercalation ability.

The improvement in potency observed by incorporating the dimethylaminoethylamine side chain to the four-position of the quinazoline 9 was promising. However, when compared to $\mathrm{BMH}-21$, the side chain is on the opposite
( 14

Table 3-2. Analogs of 2 substituted at the 2- or 4-position. aRPA194 degradation measured in A375 cells. $\mathrm{IC}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
hemisphere of the heterocyclic core. Likewise, when evaluating similar tetracycle cores with the amide side chain on the northern hemisphere (23 and 24, $\mathrm{IC}_{50} \mathbf{> 1 0}$ and $>10 \mu \mathrm{M}$, respectively), we observed a loss of activity (Table 3-3). There are
Ex.

Table 3-3. Amide side-chain at the incorrect position of the tetracycle core and addition of the amide side chain to the 8-position of the quinazoline core. aRPA194 degradation measured in A375 cells. IC $\mathrm{C}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
several possible contributing factors to the loss of activity observed. One contribution may originate from the amide side chain being on the wrong side of the tetracycle, affecting interactions with the phosphate backbone. Indeed, we have routinely observed very sensitive SARs regarding modifications to the linker
length and terminal amine that resulted in drastic potency losses as described in Chapter 2. The direction of the bend in the tetracycle may also be unfavorable for intercalation in the binding pocket. In the previous chapter, severe differences in potency were observed between the up- and down-direction of the bend in the tetracycle. The direction of the bend in the tetracycle was able to directly affect potency regardless of incorporation of the optimal amide side chain. Another factor affecting $\mathbf{2 4}$ is the lack of the carbonyl in the central quinazolinone ring. In the previous chapter, deletion of the carbonyl to form a 6-5-6-6 ring system resulted in a decrease in potency but not a complete loss of activity. To test this idea, 25 and 26 were designed to reflect the general structure of the two hits while incorporating the ideal amide side chain in the southern hemisphere at the eight-position of the quinazoline core (Table 3-3). While 25 was modestly active (IC50 $4.7 \mu \mathrm{M}$ ), reflecting a possible favored hit-like structure, 26 was inactive ( $\mathrm{IC}_{50}>10 \mu \mathrm{M}$ ). An attempt was made to incorporate the 2-hydroxy-3-methoxyphenyl ring at the two-position in an effort to further improve the potency compared to 25. Unfortunately, purification issues caused by lack of solubility prevented the generation of this analog. Future efforts may address purification of this analog and its evaluation. Instead, priority was shifted to a new series of aryl quinazolinone compounds, that arguably would be able to test the same hypothesis as the analog in question.

Summarized in Table 3-4, the aryl quinazolinone series (27-29) was designed to act as a hybrid structure between the pyridoquinazolinone tetracycle of BMH-21 and the bicyclic quinazoline core of the two hits. The new series would incorporate the favorable aromatic phenyl ring at the two-position while possessing
(

Table 3-4. Aryl quinazolinone series. Designed to attempt to create a barrier to rotation in the biaryl C-C bond (blue) to establish pseudo-planarity by way of intermolecular hydrogen-bonding (red) between the quinazolinone core (green) and the two-position (and/or six-position) phenyl substituent. aRPA194 degradation measured in A375 cells. IC50 represents the mean of duplicate independent biological experiments performed in triplicate.
differing amounts of potential hydrogen bonding participants. Instead of aliphatic amine substitution at the four-position, switching to a quinazolinone scaffold would
provide the carbonyl hydrogen bond acceptor observed to be important for potency. Providing sites for intermolecular hydrogen bonding could raise the barrier to rotation in the quinazolinone - phenyl $C-C$ bond, ideally allowing the molecule to adopt a more planar orientation, helping with its intercalative ability, without the requisite extended fused aromatic ring system of BMH-21. With this hypothesis, 28 and 29 which contain hydrogen bonding partners, ideally should be more potent than the plain phenyl substituent 27. Unfortunately, this was not the case, and the opposite result was observed. Compound 27 was modestly potent, while both 28 and 29 were inactive ( $\mathrm{IC}_{50} 2.4,>10$, and $>10 \mu \mathrm{M}$, respectively).

While 9 and 27 marginally improved RPA194 degradation potency as compared to 1, unfortunately, the SAR proved to be very sensitive to not only substitution of the quinazoline core but also substitution of the two-position phenyl ring. Similarly, potency of analogs of 2 could be improved by approximately twofold (19), but this scaffold exhibited sensitive SAR, especially involving substitution of the two-position pyrrolidine. Incorporation of the ideal amide side chain from previous BMH-21 SARs showed that potency could be somewhat improved, however this was still not enough to bring analogs into the desired $0.5 \mu \mathrm{M}$-andbelow range. Since no compounds were able to meet this threshold, secondary assays were not performed to further profile these compounds. However, RPA194 degradation potency correlates well with the ability to kill cancer cells, ${ }^{3}$ so it is likely that these compounds would exhibit limited potency in secondary assays as well. Although simple substitutions to the quinazoline core were largely ineffective, it is still possible that with additional time and resources that potent Pol I inhibitors may
still be generated from this scaffold. Potential avenues for follow-up include attaching the amide side chain to the other free positions on the distal ring of the quinazoline core or even to the two-position phenyl ring itself. Different heterocycle substitutions at the two- and four-position as well as other free positions of the quinazoline are likely yet to be explored, especially since the initial hit expansion substructure-searching only included quinazolines substituted at the two- and fourposition. With the two- and four-positions being sensitive to alteration, the six- and 7-positions of the quinazoline core may offer an exit vector to develop further SAR. A deeper investigation into the binding orientations of the two hits may also reveal a possible favorable configuration. Structural studies and target identification methods have long been a desirable direction of this research project and will be discussed in the next chapter.

## Chemistry

In general, most compounds were able to be quickly synthesized through a two-step process. Summarized in Scheme 3-1, common intermediate 31 could be generated through an $S_{N} A R$ reaction between 2,4-dichloroquinazoline 30 and 2(methylamino)ethanol at room temperature. This intermediate allowed for modular functionalization of the two-position of the quinazoline either through SuzukiMiyaura coupling with various commercially available boronic acids to give compounds 1 and 3 or through a second $S_{N} A R$ reaction with various amines at elevated temperatures to provide compounds 4-6. When generating analogs variable at the four-position, a similar $S_{N} A R$ followed by Suzuki-Miyaura coupling
approach was utilized to furnish compounds $\mathbf{7 - 1 2}$. $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}$ was chosen as the catalyst-ligand system versus the standard $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}$ catalyst seen in SuzukiMiyaura couplings after a report from Pulipati et al. ${ }^{14}$ observed that the dcpf ligand performed the best in Suzuki-Miyaura couplings to generate 4-amino-2arylquinazolines.

A similar approach was used to generate the compounds depicted in


Scheme 3-1. General scheme for the synthesis to provide compounds 1, 3-12. Reagents and conditions: (a) 2-(methylamino)ethanol, DIPEA, DCM, rt. (b) Boronic acid, $\mathrm{Na}_{2} \mathrm{CO}_{3}, \mathrm{Pd}_{( }\left(\mathrm{PPh}_{3}\right)_{4}(10$ $\mathrm{mol} \%$ ), EtOH, toluene, $120^{\circ} \mathrm{C}$. (c) amine, MeCN, $76-110^{\circ} \mathrm{C}$ or amine, TEA, $90-110^{\circ} \mathrm{C}$. (d) amine, $\mathrm{NaOAc}, 3: 1$ 1,4-dioxane/water, $65^{\circ} \mathrm{C}$ or amine, NaOAc, 3:1 THF/water, $65^{\circ} \mathrm{C}$. (e) 2-hydroxy-3methoxyphenylboronic acid, $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(10 \mathrm{~mol} \%), 1,4$-dioxane, $100{ }^{\circ} \mathrm{C}$.

Scheme 3-2. Common intermediate 33 was synthesized from 30 after $S_{n A R}$ with 2,4-dimethoxyaniline and subsequent $S_{N A R}$ with various amines to give compounds 2, 13-14 or Suzuki-Miyaura coupling with commercially available boronic acids to give compounds 15-18 allowed for facile functionalization. Likewise, compounds 19-22 could be synthesized through two rounds of $S_{N} A R$ reactions. In general, substitution of 2,4-dichloroquinazoline 30 with amines occurs


Scheme 3-2. General scheme for the synthesis to provide compounds 2, 13-18. Reagents and conditions: (a) 2,4-dimethoxyaniline, NaOAc, 1:1 THF/water, $70^{\circ} \mathrm{C}$ or 2,4-dimethoxyaniline, $\mathrm{NaOAc}, 3: 1$ 1,4-dioxane/water, $65^{\circ} \mathrm{C}$. (b) 2M dimethylamine in THF, THF, $75{ }^{\circ} \mathrm{C}$ or aniline, DMF, $80^{\circ} \mathrm{C}$. (c) boronic acid, $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(11-14 \mathrm{~mol} \%), 6: 1 \mathrm{DMF} /$ water, $100{ }^{\circ} \mathrm{C}$ or 2-hydroxy-3methoxyphenylboronic acid, $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}\left(10 \mathrm{~mol} \%\right.$ ), 1,4-dioxane, $100{ }^{\circ} \mathrm{C}$. (d) amine, $\mathrm{NaOAc}, 3: 1$ 1,4-dioxane/water, $65{ }^{\circ} \mathrm{C}$ or amine, $\mathrm{NaOAc}, 3: 1 \mathrm{THF} /$ water, $65^{\circ} \mathrm{C}$. (e) pyrrolidine, MeCN, $100-110{ }^{\circ} \mathrm{C}$.
first at the four-position then at the two-position with elevated temperatures. ${ }^{15}$ Using this strategy, a library of compounds can be quickly generated due to the regioselectivity observed.

Compounds 23 and 24, summarized in Scheme 3-3, were synthesized bases on a report from Maity et al. ${ }^{16}$ that utilized a one-pot approach to generate linear and angular fused quinazolinones. Following Fischer esterification of 34 and $N$-bromosuccinimide-mediated benzylic bromination of 35, reaction with isoquinoline-1-amine generated the tetracyclic angular fused quinazoline scaffold. According to Maity et al., the eight-position methylene should auto-oxidize to the quinazolinone, ${ }^{16}$ however, we observed a mixture of products, the partially oxidized hydroxyl 36 and the unoxidized methylene 37 with very little fully oxidized quinazolinone product observed. However, both scaffolds could be carried forward and separated after ester saponification and amide coupling. The methylene 24


Scheme 3-3. Synthesis of compounds 23 and 24. Reagents and conditions: (a) Conc. $\mathrm{H}_{2} \mathrm{SO}_{4}$, $\mathrm{MeOH}, 76^{\circ} \mathrm{C}$. (b) N -bromosuccinimide, $\mathrm{MeCN}, 94^{\circ} \mathrm{C}$. (c) isoquinoline-1-amine, DMF, $85^{\circ} \mathrm{C}$ then $\mathrm{CsCO}_{3}$, Cul ( $10 \mathrm{~mol} \%$ ), L-proline ( $20 \mathrm{~mol} \%$ ), DMF, $85^{\circ} \mathrm{C}$, air. (d) $2 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (e) $\mathrm{N}, \mathrm{N}$ dimethylethylenediamine, TBTU, DIPEA, DMF, rt. (f) Dess-Martin periodinane, DCM, rt.
remained unoxidized throughout this sequence, while the partially oxidized hydroxyl was converted to the quinazolinone 23 after oxidation with Dess-Martin periodinane.

Incorporation of the amide side chain to the eight-position of the quinazoline core required a rebuild of the 2,4-dichloroquinazoline intermediate 40, shown in Scheme 3-4. Following amidation of 38 and cyclization with 1,1'carbonyldiimidazole to generate the corresponding quinazoline-2,4-dione 39, reaction with phosphorus oxychloride was able to produce the 2,4dichloroquinazoline intermediate 40 . It should be noted that 40 is relatively unstable at ambient conditions and will hydrolyze and revert to the quinazoline-2,4-dione 39 within 24-48 hours. Care should also be taken during the workup


Scheme 3-4. Synthesis of compounds 25 and 26. Reagents and conditions: (a) $\mathrm{NH}_{4} \mathrm{OH}(28 \%$ $\mathrm{NH}_{3}$ in water), TBTU, DIPEA, DMF, rt. (b) CDI, $\mathrm{K}_{2} \mathrm{CO}_{3}$, DMF, $90^{\circ} \mathrm{C}$. (c) $\mathrm{POCl}_{3}$, DIPEA, $100{ }^{\circ} \mathrm{C}$. (d) amine, $\mathrm{NaOAC}, 2: 1 \mathrm{THF} /$ water, $65{ }^{\circ} \mathrm{C}$. (e) phenylboronic acid, $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(10 \mathrm{~mol} \%), 1,4-$ dioxane, $100^{\circ} \mathrm{C}$. (f) $2 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (g) 2-pyrrolidin-1-ylethanamine, TBTU, DIPEA, DMF, rt.
procedure to prevent the generation of exotherms as hydrolysis and reversion can occur during this process as well. Therefore, long-term storage of this intermediate is difficult and newly synthesized batches of 40 should be used right away. Fortunately, batches of 40 that have reverted to 39 can be regenerated with phosphorus oxychloride, allowing for forward synthesis to continue. Following $S_{N A R}$ of 40 with various amines and Suzuki-Miyaura coupling with phenylboronic acid, intermediate 42a-b undergoes ester saponification and amide coupling to produce compounds 25-26.

Finally, Scheme 3-5 summarizes the synthesis of compounds 27-29. This series makes use of a common intermediate 40 that can be synthesized as shown in Scheme 3-4. Partial hydrolysis of the 2,4-dichloroquinazoline intermediate 40 with potassium hydroxide as reported by Holmes et al. ${ }^{17}$ provided quinazolinone 43 that underwent amide coupling and Suzuki-Miyaura coupling to generate compounds 27-29. Notably, TBTU-mediated amide coupling was unsuccessful with this series of compounds. After a small screening of various amide coupling strategies and reagents, Ghosez reagent ${ }^{18}$ was able to successfully produce the desired amide intermediate.

In summary, the ability to translate the RPA194 degradation assay from 96well plate format to 384-well plate format allowed for high-throughput screening efforts, in collaboration with Evotec, to be carried out. While searching for compounds that exhibited either BMH-21-like phenotypes or ActD like phenotypes, the former was prioritized after hit profiling revealed several undesirable properties


Scheme 3-5. Synthesis of compounds 27-29. Reagents and conditions: (a) $2 \mathrm{M} \mathrm{KOH}, 105^{\circ} \mathrm{C}$. (b) 2-pyrrolidin-1-ylethanamine, Ghosez reagent, DIPEA, DCM, rt. (c) boronic acid, $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}$ ( $10 \mathrm{~mol} \%$ ), 1,4-dioxane, $100^{\circ} \mathrm{C}$.
of the ActD-like hits. High-throughput screening of 251K compounds from the Evotec library resulted in two hits, 1 and 2, that both share the same 2,4substitution pattern on a quinazoline scaffold. Although SAR by catalog and inhouse SAR efforts were largely unsuccessful, small improvements in RPA194 degradation potency could be achieved (compounds 9 and 19). The optimal pyrrolidine amide side chain could be incorporated into the eight-position of the quinazoline core, but unfortunately did not result in a large improvement to potency despite the previous SARs carried out to study influence of the amide side chain on potency. Finally, the aryl quinazoline series was designed and synthesized in an effort to create a hybrid structure of BMH-21 and the HTS hits, utilizing intermolecular hydrogen bonding to increase the rigidity and planarity of the molecule. Unfortunately, the opposite effect was observed, hinting that other steric factors may be at play when it comes to the compound's orientation in the binding site. Overall, the SAR data generated suggests that RPA194 degradation potency is highly sensitive to structural changes in the molecule that affect key interactions between the molecule and its target or even the orientation of the molecule in its binding site. Therefore, obtaining structural information about how the molecule
binds to its target is particularly important for the informed generation of future analogs.

## Experimental

Synthesis. General Methods. All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on an ISCO CombiFlash Rf or Biotage Isolera using Biotage Flash cartridges with peak detection at 254 nm . Reverse phase purification was accomplished using a Gilson 215 liquid handler equipped with a Phenomenex C18 column (150 mm x 20 mm i.d., $5 \mu \mathrm{~m}$ ). Peak collection was triggered by UV detection at 214 or $254 \mathrm{~nm} .{ }^{1} \mathrm{H}$ NMR spectra were recorded on a Bruker 400 instrument operating at 400 MHz with tetramethylsilane or residual protonated solvent used as a reference. Analytical LC/MS was performed using an Agilent 1260 equipped with autosampler (Agilent Poroshell 120 C18 column (50 $\mathrm{mm} \times 4.6 \mathrm{~mm}$ i.d., $3.5 \mu \mathrm{~m}$ ); 0.05\% TFA in water/acetonitrile gradient; UV detection at 215 and 254 nm ) and electrospray ionization. All final compounds showed purity greater than $95 \%$ at 215 and 254 nm using this method. Compounds 1-6, 13-15, and 17-18 were synthesized at Evotec.

2-(4-((2-hydroxyethyl)(methyl)amino)quinazolin-2-yl)-6-methoxyphenol (1). To a 25 mL microwave vial charged with a magnetic stir bar was added 2-((2-chloroquinazolin-4-yl)(methyl)amino)ethan-1-ol (31) (100 mg, 0.421 mmol ), toluene ( 3 mL ), and EtOH ( 1.8 mL ). The resulting mixture was purged $3 x$ with argon (reduced pressure was applied in vial until formation of bubbles, let 15-30 seconds
then flushed with argon). Then (2-hydroxy-3-methoxyphenyl)boronic acid (106 mg, $0.631 \mathrm{mmol}), \mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(49 \mathrm{mg}, 0.042 \mathrm{mmol})$, and $2 \mathrm{M} \mathrm{Na} 2 \mathrm{CO}_{3}(0.84 \mathrm{~mL}, 1.68$ mmol ) were added successively. The resulting mixture was purged again $3 x$ with argon before the vial was sealed. The reaction mixture was heated at $120^{\circ} \mathrm{C}$ for 1 hour using microwaves. The reaction mixture was allowed to cool to room temperature, quenched with water, and diluted with DCM. The layers were separated, and the aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (EtOAc/heptane 0-50\%, 15 g silica cartridge) to give $50 \mathrm{mg}, 0.154 \mathrm{mmol}, 37 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (600 MHz, DMSO-d6) $\delta \mathrm{ppm} 14.86(\mathrm{~s}, 1 \mathrm{H}), 8.33(\mathrm{dd}, \mathrm{J}$ $=8.51,0.73 \mathrm{~Hz}, 1 \mathrm{H}), 8.00(\mathrm{dd}, J=8.07,1.61 \mathrm{~Hz}, 1 \mathrm{H}), 7.73-7.86(\mathrm{~m}, 2 \mathrm{H}), 7.44-$ $7.51(\mathrm{~m}, 1 \mathrm{H})$, 6.99-7.10 (m, 1H), 6.80-6.88 (m, 1H), 4.89-5.01 (m, 1H), 3.92-3.99 $(\mathrm{m}, 2 \mathrm{H}), 3.83-3.89(\mathrm{~m}, 2 \mathrm{H}), 3.78-3.81(\mathrm{~m}, 3 \mathrm{H}), 3.50(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{18} \mathrm{H}_{19} \mathrm{~N}_{3} \mathrm{O}_{3}, 325.1$; found $326.2[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2,4-dimethoxyphenyl)-2-(pyrrolidin-1-yl)quinazolin-4-amine (2). To a vial under inert atmosphere and charged with a magnetic stir bar was added 2-chloro- N -(2,4-dimethoxyphenyl)quinazolin-4-amine (33) (100 mg, 0.317 mmol$)$, THF ( 3 mL ), and pyrrolidine ( $0.50 \mathrm{~mL}, 5.99 \mathrm{mmol}$ ). The resulting mixture was heated at $75^{\circ} \mathrm{C}$ for 8 hours. The reaction mixture was allowed to cool to room temperature and was concentrated in vacuo. The resulting residue was taken up in water, sat. $\mathrm{NH}_{4} \mathrm{Cl}$, and EtOAc, forming an emulsion. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with water, $1 x$ with sat. brine, dried over
a phase separator, and concentrated in vacuo. Crude material was recrystallized in EtOH and oven-dried at $50^{\circ} \mathrm{C}$ for 12 hours to give $31 \mathrm{mg}, 0.088 \mathrm{mmol}, 28 \%$ yield as a pale gray solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.67(\mathrm{~s}, 1 \mathrm{H}), 8.07$ $(\mathrm{d}, J=3.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.64-7.53(\mathrm{~m}, 1 \mathrm{H}), 7.36(\mathrm{~d}, J=7.9$ $\mathrm{Hz}, 1 \mathrm{H}), 7.14(\mathrm{t}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.03(\mathrm{~d}, J=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 6.67(\mathrm{dd}, J=8.9,3.1 \mathrm{~Hz}$, 1H), $3.86(\mathrm{~s}, 3 \mathrm{H}), 3.75(\mathrm{~s}, 3 \mathrm{H}), 3.59-3.49(\mathrm{~m}, 4 \mathrm{H}), 2.00-1.83(\mathrm{~m}, 4 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{20} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}$, 350.2; found $351.3[\mathrm{M}+\mathrm{H}]^{+}$.

2-(methyl(2-phenylquinazolin-4-yl)amino)ethan-1-ol (3). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-((2-chloroquinazolin-4$\mathrm{yl})$ (methyl)amino)ethan-1-ol (31) (100 mg, 0.421 mmol ), toluene ( 3 mL ), and EtOH $(1.8 \mathrm{~mL})$. The resulting mixture was purged $3 x$ with argon (reduced pressure was applied in vial until formation of bubbles, let 15-30 seconds then flushed with argon). Then phenylboronic acid ( $77 \mathrm{mg}, 0.632 \mathrm{mmol}$ ), $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(49 \mathrm{mg}, 0.042$ mmol ), and $2 \mathrm{M} \mathrm{Na}_{2} \mathrm{CO}_{3}(0.84 \mathrm{~mL}, 1.68 \mathrm{mmol})$ were added successively. The resulting mixture was purged again $3 x$ with argon before the vial was sealed. The reaction mixture was heated at $120^{\circ} \mathrm{C}$ for 1 hour using microwaves. The reaction mixture was allowed to cool to room temperature, quenched with water, and diluted with DCM. The layers were separated, and the aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (EtOAc/heptane $0-50 \%$, 15 g silica cartridge) to give 42 mg , $0.150 \mathrm{mmol}, 36 \%$ yield as a white solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d6) $\delta \mathrm{ppm} 8.43-$ $8.51(\mathrm{~m}, 2 \mathrm{H}), 8.29(\mathrm{~d}, J=8.07 \mathrm{~Hz}, 1 \mathrm{H}), 7.80-7.84(\mathrm{~m}, 1 \mathrm{H}), 7.73-7.79(\mathrm{~m}, 1 \mathrm{H}), 7.48-$
$7.54(\mathrm{~m}, 3 \mathrm{H}), 7.42-7.47(\mathrm{~m}, 1 \mathrm{H}), 4.94(\mathrm{t}, J=5.26 \mathrm{~Hz}, 1 \mathrm{H}), 3.91(\mathrm{~d}, J=5.14 \mathrm{~Hz}$, 2H), 3.84-3.89 (m, 2H), 3.47 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{17} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}, 279.1$; found $279.9[\mathrm{M}+\mathrm{H}]^{+}$.

2-(methyl(2-(phenylamino)quinazolin-4-yl)amino)ethan-1-ol (4). To a $2-5 \mathrm{~mL}$ microwave vial charged with a magnetic stir bar was added 2-((2-chloroquinazolin-$4-\mathrm{yl})($ methyl $)$ amino)ethan-1-ol (31) (150 mg, 0.631 mmol ), $\mathrm{MeCN}(3 \mathrm{~mL})$, and aniline ( $0.17 \mathrm{~mL}, 1.86 \mathrm{mmol})$. The resulting mixture was sealed and heated at 110 ${ }^{\circ} \mathrm{C}$ for 30 minutes using microwaves. Additional aniline ( $0.17 \mathrm{~mL}, 1.86 \mathrm{mmol}$ ) and $E t_{3} \mathrm{~N}(0.17 \mathrm{~mL}, 1.22 \mathrm{mmol})$ were added. The vial was sealed again and heated at $90^{\circ} \mathrm{C}$ overnight (classic heat). The reaction mixture was heated at $90^{\circ} \mathrm{C}$ for 4 additional days with an addition of aniline ( $0.17 \mathrm{~mL}, 1.86 \mathrm{mmol}$ ) and $\mathrm{Et}_{3} \mathrm{~N}(0.17$ $\mathrm{mL}, 1.22 \mathrm{mmol}$ ) each day ( 4 x additions). The reaction mixture was allowed to cool to room temperature and diluted with water. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-75\% EtOAc/heptane, 15 g silica cartridge) to give 19 mg , $0.065 \mathrm{mmol}, 10 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ $9.05(\mathrm{~s}, 1 \mathrm{H}), 8.09(\mathrm{~d}, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.88(\mathrm{~d}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.58-7.57(\mathrm{~m}, 1 \mathrm{H})$, $7.46(\mathrm{~d}, \mathrm{~J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.26-7.24(\mathrm{~m}, 2 \mathrm{H}), 7.14(\mathrm{~m}, 1 \mathrm{H}), 6.90-6.88(\mathrm{~m}, 1 \mathrm{H}), 4.87-$ $4.85(\mathrm{~m}, 1 \mathrm{H}), 3.82-3.77(\mathrm{~m}, 4 \mathrm{H}), 3.33(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{17} \mathrm{H}_{18} \mathrm{~N}_{4} \mathrm{O}, 294.1$; found $295.3[\mathrm{M}+\mathrm{H}]^{+}$.

2-((2-(dimethylamino)quinazolin-4-yl)(methyl)amino)ethan-1-ol (5). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-((2-chloroquinazolin-4-yl)(methyl)amino)ethan-1-ol (31) ( $150 \mathrm{mg}, 0.631 \mathrm{mmol}$ ), $\mathrm{MeCN}(3 \mathrm{~mL})$, and 2M N -methylmethanamine in THF ( $2.5 \mathrm{~mL}, 5 \mathrm{mmol}$ ). The resulting mixture was sealed and heated at $76^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature and diluted with water. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give $150 \mathrm{mg}, 0.609 \mathrm{mmol}, 97 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 7.99$ (d, $J=5.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.48$7.46(\mathrm{~m}, 1 \mathrm{H}), 7.33-7.31(\mathrm{~m}, 1 \mathrm{H}), 7.02-7.00(\mathrm{~m}, 1 \mathrm{H}), 4.85(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.80-$ 3.77 (m, 2H), $3.70(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.27$ (s, 3H), 3.13 (s, 6H). LCMS (ESI): m/z calcd for $\mathrm{C}_{13} \mathrm{H}_{18} \mathrm{~N}_{4} \mathrm{O}, 246.1$; found $247.2[\mathrm{M}+\mathrm{H}]^{+}$.

2-(methyl(2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)ethan-1-ol (6). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-((2-chloroquinazolin-$4-\mathrm{yl})($ methyl)amino)ethan-1-ol (31) (200 mg, 0.841 mmol ), $\mathrm{MeCN}(4 \mathrm{~mL})$, and pyrrolidine ( $0.21 \mathrm{~mL}, 2.52 \mathrm{mmol})$. The resulting mixture was sealed and heated at $110^{\circ} \mathrm{C}$ for 30 minutes using microwaves. The reaction mixture was allowed to cool to room temperature and a precipitate formed. The resulting solid was filtered, washed with MeCN , and dried under vacuum to give $140 \mathrm{mg}, 0.514 \mathrm{mmol}, 61 \%$ yield as a white solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}^{2} \mathrm{~d}_{6}$ ) $\delta \mathrm{ppm} 7.98$ (dd, $J=8.31$, $0.73 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.47 (ddd, $J=8.25,6.91,1.22 \mathrm{~Hz}, 1 \mathrm{H}), 7.31(\mathrm{~d}, J=7.82 \mathrm{~Hz}, 1 \mathrm{H})$, 6.93-7.04 (m, 1H), $4.85(\mathrm{t}, \mathrm{J}=5.38 \mathrm{~Hz}, 1 \mathrm{H}), 3.75-3.85(\mathrm{~m}, 2 \mathrm{H}), 3.65-3.73(\mathrm{~m}, 2 \mathrm{H})$,
3.43-3.60 (m, 4H), 3.27 (s, 3H), 1.90 ( $\mathrm{m}, \mathrm{J}=6.40,3.50,3.50 \mathrm{~Hz}, 4 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{15} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}$, 272.2; found $273.0[\mathrm{M}+\mathrm{H}]^{+}$.

2-(4-(dimethylamino)quinazolin-2-yl)-6-methoxyphenol hydrochloride (7). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro- $\mathrm{N}, \mathrm{N}$ -dimethyl-quinazolin-4-amine (32a) ( $100 \mathrm{mg}, 0.482 \mathrm{mmol}$ ), (2-hydroxy-3-methoxyphenyl)boronic acid ( $122 \mathrm{mg}, 0.726 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(307 \mathrm{mg}, 1.45 \mathrm{mmol}$ ), and 1,4dioxane ( 3.5 mL ). The resulting mixture was bubbled with nitrogen for 1 minute, then $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(37 \mathrm{mg}, 0.049 \mathrm{mmol})$ was added, and the reaction mixture was capped, sealed, and heated at $100^{\circ} \mathrm{C}$ for 22 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed 1 x with sat. brine. The aqueous layer was extracted 3 x with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (6-50\% EtOAc/heptane, 20 g silica cartridge) to give 2-[4-(dimethylamino)quinazolin-2-yl]-6-methoxy-phenol ( $115 \mathrm{mg}, 0.389 \mathrm{mmol}, 81 \%$ yield) as a pale orange solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm} 14.84$ (s, 1H), $8.26-8.20(\mathrm{~m}, 1 \mathrm{H}), 8.03(\mathrm{dd}, \mathrm{J}=8.1,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.85-7.75(\mathrm{~m}, 2 \mathrm{H}), 7.52-7.45$ (m, 1H), 7.06 (dd, $J=8.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 6.84(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.81(\mathrm{~s}, 3 \mathrm{H}), 3.46$ (s, 6H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{17} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{2}$, 295.1; found $296.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-[4-(dimethylamino)quinazolin-2-yll-6-methoxy-phenol ( $50 \mathrm{mg}, 0.169 \mathrm{mmol}$ ), DCM $(3.5 \mathrm{~mL})$, and 4 M HCl in 1,4-dioxane ( $0.04 \mathrm{~mL}, 0.16 \mathrm{mmol}$ ). The resulting mixture
was stirred at room temperature for 18 hours then concentrated to dryness to give 2-[4-(dimethylamino)quinazolin-2-yl]-6-methoxy-phenol hydrochloride $(66 \mathrm{mg}$, $0.199 \mathrm{mmol})$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.36-8.30(\mathrm{~m}$, $1 \mathrm{H}), 7.97-7.87(\mathrm{~m}, 2 \mathrm{H}), 7.80(\mathrm{~d}, \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.65-7.57(\mathrm{~m}, 1 \mathrm{H}), 7.22-7.16(\mathrm{~m}$, $1 \mathrm{H}), 6.95(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.86(\mathrm{~s}, 3 \mathrm{H}), 3.56(\mathrm{~s}, 6 \mathrm{H}) . \mathrm{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{17} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{2}$, 295.1; found $296.1[\mathrm{M}+\mathrm{H}]^{+}$.

2-methoxy-6-(4-(pyrrolidin-1-yl)quinazolin-2-yl)phenol hydrochloride (8). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro-4-pyrrolidin-1-yl-quinazoline (32b) (100 mg, 0.428 mmol ), (2-hydroxy-3-methoxyphenyl)boronic acid ( $108 \mathrm{mg}, 0.643 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(273 \mathrm{mg}, 1.29 \mathrm{mmol})$, and 1,4dioxane ( 3 mL ). The resulting mixture was bubbled with nitrogen for 1 minute before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(33 \mathrm{mg}, 0.044 \mathrm{mmol})$. The reaction mixture was then capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 22 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and vacuum filtered through a pad of Celite. The filtrate was washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (6-50\% EtOAc/heptane, 20 g silica cartridge) to give 2-methoxy-6-(4-pyrrolidin-1-ylquinazolin-2-yl)phenol ( $88 \mathrm{mg}, 0.274 \mathrm{mmol}$, $64 \%$ yield) as a pale orange solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 14.91$ (s, $1 \mathrm{H}), 8.33(\mathrm{dd}, J=8.5,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.03(\mathrm{dd}, J=8.1,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.84-7.72(\mathrm{~m}$, $2 \mathrm{H}), 7.52-7.44(\mathrm{~m}, 1 \mathrm{H}), 7.05(\mathrm{dd}, J=8.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 6.82(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H})$,
$3.98(\mathrm{~s}, 4 \mathrm{H}), 3.80(\mathrm{~s}, 3 \mathrm{H}), 2.07-1.98(\mathrm{~m}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{19} \mathrm{H}_{19} \mathrm{~N}_{3} \mathrm{O}_{2}$, 321.1; found $322.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-methoxy-6-(4-pyrrolidin-1-ylquinazolin-2-yl)phenol ( $50 \mathrm{mg}, 0.156 \mathrm{mmol}$ ), DCM ( 3 mL ), and 4M HCl in 1,4-dioxane $(0.04 \mathrm{~mL}, 0.16 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 18 hours then concentrated to dryness to give 2-methoxy-6-(4-pyrrolidin-1-ylquinazolin-2-yl)phenol hydrochloride ( $65 \mathrm{mg}, 0.182 \mathrm{mmol}$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.42(\mathrm{~d}, \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.95$ (d, $J=4.2 \mathrm{~Hz}, 2 \mathrm{H}), 7.82(\mathrm{dd}, J=8.1,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.67-7.60(\mathrm{~m}, 1 \mathrm{H}), 7.21$ (dd, $J$ $=8.1,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 6.96(\mathrm{t}, \mathrm{J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 4.07(\mathrm{~s}, 5 \mathrm{H}), 3.86(\mathrm{~s}, 3 \mathrm{H}), 2.05(\mathrm{~s}, 4 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{19} \mathrm{H}_{19} \mathrm{~N}_{3} \mathrm{O}_{2}$, 321.1; found $322.2[\mathrm{M}+\mathrm{H}]^{+}$.

## 2-(4-((2-(dimethylamino)ethyl)(methyl)amino)quinazolin-2-yl)-6-methoxyphenol

 hydrochloride (9). To a 2-5 mL microwave vial charged with a magnetic stir bar was added (2-hydroxy-3-methoxy-phenyl)boronic acid (172 mg, 1.02 mmol ), $\mathrm{K}_{3} \mathrm{PO}_{4}$ (433 mg, 2.04 mmol$), N^{\prime}$-(2-chloroquinazolin-4-yl)- $N, N, N$ '-trimethyl-ethane-1,2diamine (32c) ( $180 \mathrm{mg}, 0.680 \mathrm{mmol}$ ), and 1,4-dioxane ( 3.5 mL ). The resulting mixture was bubbled with nitrogen for 2 minutes. before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}$ ( 52 $\mathrm{mg}, 0.069 \mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $100^{\circ} \mathrm{C}$ for 10 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified viaautomated RPLC (10-50\% MeCN/water, $50 \times 150$ LUNA, load in MeOH). Collected fractions were diluted with DCM and basified to $\mathrm{pH} \sim 9$ with sat. $\mathrm{NaHCO}_{3}$. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give 2-[4-[2-(dimethylamino)ethyl-methyl-amino]quinazolin-2-yl]-6-methoxy-phenol (14 $\mathrm{mg}, 0.040 \mathrm{mmol}, 6 \%$ yield) as a yellow solid. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{20} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}$, 352.2; found $353.2[\mathrm{M}+\mathrm{H}]^{+}$.

In a 20 mL vial charged with a magnetic stir bar was dissolved 2-[4-[2-(dimethylamino)ethyl-methyl-amino]quinazolin-2-yl]-6-methoxy-phenol (14 mg, 0.040 mmol ) in DCM ( 1 mL ). 4 M HCl in 1,4-dioxane ( $0.02 \mathrm{~mL}, 0.08 \mathrm{mmol}$ ) was added, and the resulting mixture was stirred overnight at room temperature then concentrated to dryness to give 2-[4-[2-(dimethylamino)ethyl-methyl-amino]quinazolin-2-yl]-6-methoxy-phenol hydrochloride (17 mg, 0.044 mmol ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.32(\mathrm{~d}, \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.96$ $(\mathrm{d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.90-7.80(\mathrm{~m}, 2 \mathrm{H}), 7.60-7.52(\mathrm{~m}, 1 \mathrm{H}), 7.11(\mathrm{dd}, J=8.0,1.5$ $\mathrm{Hz}, 1 \mathrm{H}), 6.88(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 4.30(\mathrm{t}, J=6.7 \mathrm{~Hz}, 2 \mathrm{H}), 3.82(\mathrm{~s}, 3 \mathrm{H}), 3.56-3.50$ (obs.), 2.88 (d, $J=4.9 \mathrm{~Hz}, 6 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{20} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 352.2$; found $353.2[\mathrm{M}+\mathrm{H}]^{+}$.

## 2-(4-((3-(dimethylamino)propyl)(methyl)amino)quinazolin-2-yl)-6-methoxyphenol

 hydrochloride (10). To a 2-5 mL microwave vial charged with a magnetic stir bar was added (2-hydroxy-3-methoxy-phenyl)boronic acid (199 mg, 1.18 mmol ), $\mathrm{K}_{3} \mathrm{PO}_{4}$ (503 mg, 2.37 mmol$), \quad N^{\prime}$-(2-chloroquinazolin-4-yl)- $N, N, N^{\prime}$-trimethyl-propane-1,3-diamine (32d) (220 mg, 0.789 mmol ), and 1,4-dioxane ( 4 mL ). The resulting mixture was bubbled with nitrogen for 2 minutes before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(60 \mathrm{mg}, 0.079 \mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 10 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed 1x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated RPLC (10-50\% MeCN/water, $50 \times 150$ LUNA, load in methanol). Collected fractions were diluted with DCM and basified to $\mathrm{pH} \sim 9$ with sat. $\mathrm{NaHCO}_{3}$. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give 2-[4-[3-(dimethylamino)propyl-methyl-amino]quinazolin-2-yl]-6-methoxy-phenol ( $64 \mathrm{mg}, 0.175 \mathrm{mmol}, 22 \%$ yield) as a brown oil. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{21} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}, 366.2$; found $367.2[\mathrm{M}+\mathrm{H}]^{+}$.

In a 20 mL vial charged with a magnetic stir bar was dissolved 2-[4-[3-(dimethylamino)propyl-methyl-amino]quinazolin-2-yl]-6-methoxy-phenol (60 mg, $0.164 \mathrm{mmol})$ in $\mathrm{DCM}(2 \mathrm{~mL}) .4 \mathrm{M} \mathrm{HCl}$ in 1,4-dioxane ( $0.08 \mathrm{~mL}, 0.32 \mathrm{mmol}$ ) was added, and the mixture was stirred at room temperature overnight then concentrated to dryness to give 2-[4-[3-(dimethylamino)propyl-methyl-amino]quinazolin-2-yl]-6-methoxy-phenol hydrochloride ( $67 \mathrm{mg}, 0.166 \mathrm{mmol}$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.69(\mathrm{~s}, 1 \mathrm{H}), 8.34(\mathrm{~d}, \mathrm{~J}=8.5$ $\mathrm{Hz}, 1 \mathrm{H}), 7.99-7.86(\mathrm{~m}, 2 \mathrm{H}), 7.80(\mathrm{~d}, \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.66-7.57(\mathrm{~m}, 1 \mathrm{H}), 7.18(\mathrm{dd}$, $J=8.1,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 6.94(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 4.02(\mathrm{t}, J=7.2 \mathrm{~Hz}, 2 \mathrm{H}), 3.85(\mathrm{~s}, 3 \mathrm{H})$,
3.61 (s, 3H), 3.22-3.13 (m, 2H), 2.74 (d, J = $4.9 \mathrm{~Hz}, 6 \mathrm{H}), 2.29-2.18(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{21} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}, 366.2$; found $367.2[\mathrm{M}+\mathrm{H}]^{+}$.

2-methoxy-6-(4-(phenylamino)quinazolin-2-yl)phenol hydrochloride (11). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro- N -phenyl-quinazolin-4-amine (32e) (100 mg, 0.391 mmol ), (2-hydroxy-3-methoxyphenyl)boronic acid ( $99 \mathrm{mg}, 0.589 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(250 \mathrm{mg}, 1.18 \mathrm{mmol}$ ), and 1,4dioxane ( 3 mL ). The resulting mixture was bubbled with nitrogen for 1 minute, then $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(30 \mathrm{mg}, 0.040 \mathrm{mmol})$ was added, and the mixture was capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 22 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (6-50\% EtOAc/heptane, 20 g silica cartridge) to give 2-(4-anilinoquinazolin-2-yl)-6-methoxy-phenol ( $82 \mathrm{mg}, 0.239 \mathrm{mmol}, 61 \%$ yield) as an orange solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 14.63(\mathrm{~s}, 1 \mathrm{H}), 10.21(\mathrm{~s}, 1 \mathrm{H})$, 8.66-8.60 (m, 1H), 7.99-7.83(m, 5H), 7.73-7.66 (m, 1H), $7.53(\mathrm{dd}, \mathrm{J}=8.4,7.4$ $\mathrm{Hz}, 2 \mathrm{H}), 7.31-7.25(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{dd}, J=8.1,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 6.86(\mathrm{t}, J=8.0 \mathrm{~Hz}, 1 \mathrm{H})$, 3.83 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{2}, 343.1$; found $344.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-(4-anilinoquinazolin-2-yl)-6-methoxy-phenol ( $50 \mathrm{mg}, 0.146 \mathrm{mmol}$ ), DCM ( 3 mL ), and 4 M HCl in 1,4-dioxane ( $0.04 \mathrm{~mL}, 0.16 \mathrm{mmol}$ ). The resulting mixture was stirred at
room temperature for 18 hours then concentrated to dryness to give 2-(4-anilinoquinazolin-2-yl)-6-methoxy-phenol hydrochloride ( $56 \mathrm{mg}, 0.147 \mathrm{mmol}$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.64(\mathrm{~s}, 1 \mathrm{H}), 8.68(\mathrm{~d}, \mathrm{~J}=8.3$ $\mathrm{Hz}, 1 \mathrm{H}), 8.01-7.90(\mathrm{~m}, 2 \mathrm{H}), 7.85-7.77(\mathrm{~m}, 3 \mathrm{H}), 7.72$ (ddd, $J=8.3,6.7,1.5 \mathrm{~Hz}$, $1 \mathrm{H}), 7.52(\mathrm{t}, J=7.9 \mathrm{~Hz}, 2 \mathrm{H}), 7.33-7.26(\mathrm{~m}, 1 \mathrm{H}), 7.11(\mathrm{dd}, J=8.0,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 6.88$ (t, $J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.81(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{2}, 343.1$; found $344.2[\mathrm{M}+\mathrm{H}]^{+}$.

## 2-methoxy-6-(4-((4-(trifluoromethyl)phenyl)amino)quinazolin-2-yl)phenol

hydrochloride (12). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro- $N$-[4-(trifluoromethyl)phenyl]quinazolin-4-amine (32f) (100 $\mathrm{mg}, 0.309 \mathrm{mmol}$ ), (2-hydroxy-3-methoxy-phenyl)boronic acid ( $78 \mathrm{mg}, 0.464$ $\mathrm{mmol}), \mathrm{K}_{3} \mathrm{PO}_{4}(197 \mathrm{mg}, 0.928 \mathrm{mmol})$, and 1,4-dioxane ( 2.5 mL ). The resulting mixture was bubbled with nitrogen for 1 minute before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(24 \mathrm{mg}$, $0.032 \mathrm{mmol})$. The mixture was then capped, sealed, and heated at $100^{\circ} \mathrm{C}$ for 22 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and vacuum filtered through a pad of Celite. The filtrate was washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated NPLC (6-50\% EtOAc/heptane, 20 g silica cartridge) to give 2-methoxy-6-[4-[4-(trifluoromethyl)anilino]quinazolin-2yl]phenol ( $85 \mathrm{mg}, 0.207 \mathrm{mmol}, 67 \%$ yield) as a pale orange solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \operatorname{ppm} 14.61(\mathrm{~s}, 1 \mathrm{H}), 10.38(\mathrm{~s}, 1 \mathrm{H}), 8.64(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.17$ $(\mathrm{d}, J=8.4 \mathrm{~Hz}, 2 \mathrm{H}), 8.00-7.83(\mathrm{~m}, 5 \mathrm{H}), 7.75-7.67(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{dd}, J=8.1,1.6$
$\mathrm{Hz}, 1 \mathrm{H}), 6.89(\mathrm{t}, \mathrm{J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}$ ), $3.81(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{22} \mathrm{H}_{16} \mathrm{~F}_{3} \mathrm{~N}_{3} \mathrm{O}_{2}$, 411.1; found $412.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-methoxy-6-[4-[4-(trifluoromethyl)anilino]quinazolin-2-yl]phenol (50 mg, 0.122 mmol ), DCM $(2.5 \mathrm{~mL})$, and 4 M HCl in 1,4-dioxane ( $0.03 \mathrm{~mL}, 0.12 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 18 hours then concentrated to dryness to give 2-methoxy-6-[4-[4-(trifluoromethyl)anilino]quinazolin-2-yl]phenol hydrochloride (56 $\mathrm{mg}, 0.125 \mathrm{mmol}$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.71$ (s, $1 \mathrm{H}), 8.73(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.17(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 2 \mathrm{H}), 8.02-7.91(\mathrm{~m}, 2 \mathrm{H}), 7.90-$ $7.82(\mathrm{~m}, 3 \mathrm{H}), 7.77-7.70(\mathrm{~m}, 1 \mathrm{H}), 7.15-7.10(\mathrm{~m}, 1 \mathrm{H}), 6.91(\mathrm{t}, \mathrm{J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 3.82$ (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{22} \mathrm{H}_{16} \mathrm{~F}_{3} \mathrm{~N}_{3} \mathrm{O}_{2}, 411.1$; found $412.2[\mathrm{M}+\mathrm{H}]^{+}$.
$N^{4}$-(2,4-dimethoxyphenyl)- $N^{2}, N^{2}$-dimethylquinazoline-2,4-diamine (13). To a vial under inert atmosphere and charged with a magnetic stir bar was added 2-chloro-$N$-(2,4-dimethoxyphenyl)quinazolin-4-amine (33) (100 mg, 0.317 mmol ), THF (3 mL ), and 2 M N -methylmethanamine in THF ( $1 \mathrm{~mL}, 2 \mathrm{mmol}$ ). The resulting mixture was heated at $75^{\circ} \mathrm{C}$ for 8 hours. The reaction mixture was allowed to cool to room temperature and was concentrated in vacuo. The resulting residue was taken up in water, sat. $\mathrm{NH}_{4} \mathrm{Cl}$, and EtOAc, forming an emulsion. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with water, $1 x$ with sat. brine, dried over a phase separator, and concentrated in vacuo. Crude material was recrystallized in EtOH and oven-dried at $50{ }^{\circ} \mathrm{C}$ for 12 hours to give $29 \mathrm{mg}, 0.089 \mathrm{mmol}, 28 \%$ yield as a gray solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta$
ppm $8.75(\mathrm{~s}, 1 \mathrm{H}), 8.06(\mathrm{dd}, J=8.3,1.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.82(\mathrm{~d}, J=3.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.57$ (ddd, $J=8.3,7.0,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.35(\mathrm{dd}, J=8.4,0.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.14(\mathrm{ddd}, J=8.1,7.0,1.2$ $\mathrm{Hz}, 1 \mathrm{H}), 7.03(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.70(\mathrm{dd}, J=8.9,3.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.83(\mathrm{~s}, 3 \mathrm{H}), 3.74$ (s, 3H), 3.13 (s, 6H). LCMS (ESI): m/z calcd for $\mathrm{C}_{18} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}_{2}$, 324.2; found 325.0 $[\mathrm{M}+\mathrm{H}]^{+}$.
$N^{4}$-(2,4-dimethoxyphenyl)- $N^{2}$-phenylquinazoline-2,4-diamine (14). To a vial under inert atmosphere and charged with a magnetic stir bar was added 2-chloro- N -(2,4-dimethoxyphenyl)quinazolin-4-amine (33) ( $215 \mathrm{mg}, 0.681 \mathrm{mmol}$ ), DMF ( 6 mL ), and aniline ( $0.3 \mathrm{~mL}, 3.29 \mathrm{mmol}$ ) dropwise. The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 12 hours. The reaction mixture was allowed to cool to room temperature, diluted with water, and extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over a phase separator, and concentrated in vacuo. Crude material was purified via automated NPLC (0-100\% EtOAc/heptane, 10 g silica cartridge, dry loaded). Combined fractions were concentrated in vacuo. The resulting residue was triturated in $\mathrm{iPr}_{2} \mathrm{O}$, filtered, washed with pentane, and ovendried at $50^{\circ} \mathrm{C}$ for 2 hours to give $71 \mathrm{mg}, 0.191 \mathrm{mmol}, 28 \%$ yield as an off-white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm $9.18(\mathrm{~s}, 1 \mathrm{H}), 9.07(\mathrm{~s}, 1 \mathrm{H}), 8.24(\mathrm{~d}, \mathrm{~J}=$ $7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.74(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.70-7.57(\mathrm{~m}, 1 \mathrm{H}), 7.46(\mathrm{~d}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H})$, $7.36(\mathrm{~d}, J=3.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.30-7.20(\mathrm{~m}, 1 \mathrm{H}), 7.13(\mathrm{t}, J=7.9 \mathrm{~Hz}, 2 \mathrm{H}), 7.08(\mathrm{~d}, J=$ $9.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.91-6.75(\mathrm{~m}, 2 \mathrm{H}), 3.76(\mathrm{~s}, 3 \mathrm{H}), 3.74(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{22} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}_{2}, 372.2$; found $373.4[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2,4-dimethoxyphenyl)-2-phenylquinazolin-4-amine (15). To a vial under inert atmosphere and charged with a magnetic stir bar was added 2 -chloro- N -(2,4-dimethoxyphenyl)quinazolin-4-amine (33) (210 mg, 0.665 mmol ), phenylboronic acid (125 mg, 1.03 mmol$), \mathrm{K}_{3} \mathrm{PO}_{4}(534 \mathrm{mg}, 2.48 \mathrm{mmol})$, DMF ( 6 mL ), and water (1 $\mathrm{mL})$. The resulting mixture was bubbled with nitrogen for 5 minutes, then $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}$ $(79 \mathrm{mg}, 0.068 \mathrm{mmol})$ was added, the vial was sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 16 hours. The reaction mixture was allowed to cool to room temperature and filtered over a pad of Celite. The resulting filtrate was diluted with water and extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over a phase separator, and concentrated in vacuo. Crude material was purified via automated NPLC (0-100\% EtOAc/heptane, 10 g silica cartridge, dry loaded). Combined fractions were concentrated in vacuo. The resulting residue was triturated in $\mathrm{iPr}_{2} \mathrm{O}$, filtered, washed with pentane, and oven-dried at $50{ }^{\circ} \mathrm{C}$ for 2 hours to give $61 \mathrm{mg}, 0.171 \mathrm{mmol}, 26 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta$ ppm $9.31(\mathrm{~s}, 1 \mathrm{H}), 8.44(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.42-8.33(\mathrm{~m}, 2 \mathrm{H}), 7.87$ (d, J = 3.8 Hz, 2H), 7.73 (d, J = $3.1 \mathrm{~Hz}, 1 \mathrm{H}$ ), $7.60(\mathrm{dt}, J=8.2,4.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.51-$ 7.42 (m, 3H), 7.11 (d, $J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.83(\mathrm{dd}, J=8.9,3.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.81(\mathrm{~s}, 3 \mathrm{H})$, 3.80 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{22} \mathrm{H}_{19} \mathrm{~N}_{3} \mathrm{O}_{2}, 357.1$; found $358.3[\mathrm{M}+\mathrm{H}]^{+}$.

## 2-(4-((2,4-dimethoxyphenyl)amino)quinazolin-2-yl)-6-methoxyphenol

 hydrochloride (16). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro- N -(2,4-dimethoxyphenyl)quinazolin-4-amine (33) (100 mg, $0.317 \mathrm{mmol})$, (2-hydroxy-3-methoxy-phenyl)boronic acid ( $80 \mathrm{mg}, 0.475 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(202 \mathrm{mg}, 0.952 \mathrm{mmol})$, and 1,4-dioxane ( 2.5 mL ). The resulting mixture wasbubbled with nitrogen for 1 minute before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(24 \mathrm{mg}, 0.032 \mathrm{mmol})$. The mixture was capped, sealed, and heated at $100^{\circ} \mathrm{C}$ for 22 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and vacuum filtered through a pad of Celite. The filtrate was washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (6-50\% EtOAc/heptane, 20 g silica cartridge) to give 2-[4-(2,4-dimethoxyanilino)quinazolin-2-yl]-6-methoxyphenol ( $60 \mathrm{mg}, 0.149 \mathrm{mmol}, 47 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, $\left.\mathrm{DMSO}_{6}\right) \delta \mathrm{ppm} 14.15(\mathrm{~s}, 1 \mathrm{H}), 9.83(\mathrm{~s}, 1 \mathrm{H}), 8.50(\mathrm{~d}, J=8.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.92-7.84$ (m, 1H), 7.83-7.76 (m, 2H), 7.64-7.56 (m, 1H), $7.40(d, J=8.6 H z, 1 H), 6.98(d d$, $J=8.1,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 6.79-6.71(\mathrm{~m}, 2 \mathrm{H}), 6.66(\mathrm{dd}, J=8.6,2.7 \mathrm{~Hz}, 1 \mathrm{H}), 3.85(\mathrm{~s}, 3 \mathrm{H})$, 3.75 (s, 6H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{21} \mathrm{~N}_{3} \mathrm{O}_{4}$, 403.2; found $404.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-[4-(2,4-dimethoxyanilino)quinazolin-2-yll-6-methoxy-phenol ( $30 \mathrm{mg}, 0.074 \mathrm{mmol}$ ), DCM (2 mL ), and 4 M HCl in 1,4-dioxane ( $0.02 \mathrm{~mL}, 0.08 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 18 hours then concentrated to dryness to give 2-[4-(2,4-dimethoxyanilino)quinazolin-2-yl]-6-methoxy-phenol hydrochloride ( 37 mg , $0.084 \mathrm{mmol})$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.63(\mathrm{~s}, 1 \mathrm{H})$, $8.61(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.00-7.93(\mathrm{~m}, 2 \mathrm{H}), 7.79-7.67(\mathrm{~m}, 2 \mathrm{H}), 7.40(\mathrm{~d}, J=8.6$ $\mathrm{Hz}, 1 \mathrm{H}), 7.10(\mathrm{~d}, \mathrm{~J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.85(\mathrm{t}, \mathrm{J}=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.80(\mathrm{~d}, \mathrm{~J}=2.7 \mathrm{~Hz}, 1 \mathrm{H})$, 6.68 (dd, $J=8.6,2.6 \mathrm{~Hz}, 1 \mathrm{H}$ ), $3.85(\mathrm{~s}, 3 \mathrm{H}), 3.77(\mathrm{~d}, J=2.5 \mathrm{~Hz}, 5 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{21} \mathrm{~N}_{3} \mathrm{O}_{4}, 403.2$; found $404.2[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2,4-dimethoxyphenyl)-2-(4-(trifluoromethyl)phenyl)quinazolin-4-amine (17). To a vial under inert atmosphere and charged with a magnetic stir bar was added 2-chloro- N -(2,4-dimethoxyphenyl)quinazolin-4-amine (33) ( $160 \mathrm{mg}, 0.507 \mathrm{mmol}$ ), [4(trifluoromethyl)phenyl]boronic acid ( $147 \mathrm{mg}, 0.774 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(392 \mathrm{mg}, 1.82$ $\mathrm{mmol})$, DMF ( 6 mL ), and water ( 1 mL ). The resulting mixture was bubbled with nitrogen for 5 minutes, then $\operatorname{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(64 \mathrm{mg}, 0.055 \mathrm{mmol})$ was added, and the vial was sealed and heated at $100^{\circ} \mathrm{C}$ for 2 hours. The reaction mixture was allowed to cool to room temperature and filtered over a pad of Celite. The resulting filtrate was diluted with water and EtOAc. The aqueous layer was extracted $2 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over a phase separator, and concentrated in vacuo. Crude material was purified via automated NPLC ( $0-100 \%$ EtOAc/heptane, 10 g silica cartridge, dry loaded). Combined fractions were concentrated in vacuo. The resulting solid was triturated in $\mathrm{Pr}_{2} \mathrm{O}$, filtered, washed with pentane, and oven-dried at $50^{\circ} \mathrm{C}$ for 2 hours to give $70 \mathrm{mg}, 0.165 \mathrm{mmol}, 33 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR $\left(400 \mathrm{MHz}\right.$, DMSO- $\left.\mathrm{d}_{6}\right) \delta$ ppm 9.47 (s, 1H), 8.53 (d, J = $8.1 \mathrm{~Hz}, 2 \mathrm{H}$ ), 8.49 (d, J= $8.3 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.91 (d, J = 3.3 Hz, 2H), 7.86 (d, $J=8.4 \mathrm{~Hz}, 2 \mathrm{H}), 7.65$ (ddd, $J=8.2,4.9,3.3 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.60 (d, $J=$ $3.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.12(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.86(\mathrm{dd}, J=9.0,3.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.80(\mathrm{~s}, 3 \mathrm{H})$, 3.79 ( $\mathrm{s}, 3 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{18} \mathrm{~F}_{3} \mathrm{~N}_{3} \mathrm{O}_{2}, 425.1$; found $426.4[\mathrm{M}+\mathrm{H}]^{+}$. N-(2,4-dimethoxyphenyl)-2-(pyridin-4-yl)quinazolin-4-amine (18). To a vial under inert atmosphere and charged with a magnetic stir bar was added 2-chloro- N -(2,4-dimethoxyphenyl)quinazolin-4-amine (33) ( $150 \mathrm{mg}, 0.475 \mathrm{mmol}$ ), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine ( $159 \mathrm{mg}, 0.775 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}$ ( 556
$\mathrm{mg}, 2.58 \mathrm{mmol})$, DMF ( 6 mL ), and water ( 1 mL ). The resulting mixture was bubbled with nitrogen for 5 minutes then $\mathrm{Pd}\left(\mathrm{PPh}_{3}\right)_{4}(72 \mathrm{mg}, 0.062 \mathrm{mmol})$ was added, and the vial was sealed and heated at $100^{\circ} \mathrm{C}$ for 2 hours. The reaction mixture was allowed to cool to room temperature and filtered over a pad of Celite. The resulting filtrate was diluted with water and EtOAc. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over a phase separator, and concentrated in vacuo. Crude material was purified via automated NPLC (0-4\% MeOH/DCM, 25 g silica cartridge, dry loaded). Combined fractions were concentrated in vacuo. The resulting solid was triturated in $\mathrm{iPr}_{2} \mathrm{O}$, filtered, washed with $\mathrm{iPr}_{2} \mathrm{O}$, and oven-dried at $50^{\circ} \mathrm{C}$ for 2 hours to give 16 mg , $0.045 \mathrm{mmol}, 9 \%$ yield as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 9.53$ $(\mathrm{s}, 1 \mathrm{H}), 8.72(\mathrm{~d}, J=4.7 \mathrm{~Hz}, 2 \mathrm{H}), 8.50(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.18(\mathrm{~d}, J=5.9 \mathrm{~Hz}, 2 \mathrm{H})$, $7.92(\mathrm{~d}, J=3.5 \mathrm{~Hz}, 2 \mathrm{H}), 7.67(\mathrm{ddd}, J=8.3,4.9,3.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.57(\mathrm{~d}, J=3.1 \mathrm{~Hz}$, $1 \mathrm{H}), 7.12(\mathrm{~d}, \mathrm{~J}=9.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.87(\mathrm{dd}, J=9.0,3.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.80(\mathrm{~s}, 3 \mathrm{H}), 3.79(\mathrm{~s}$, $3 H)$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{21} \mathrm{H}_{18} \mathrm{~N}_{4} \mathrm{O}_{2}, 358.1$; found $359.3[\mathrm{M}+\mathrm{H}]^{+}$.

N-phenyl-2-(pyrrolidin-1-yl)quinazolin-4-amine hydrochloride (19). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2 -chloro- $N$-phenyl-quinazolin-4-amine (32e) (100 mg, 0.391 mmol$)$, $\mathrm{MeCN}(2 \mathrm{~mL})$, and pyrrolidine $(0.15 \mathrm{~mL}, 1.17 \mathrm{mmol})$. The reaction mixture was sealed and heated at $110{ }^{\circ} \mathrm{C}$ for 30 minutes. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. Crude material was purified via automated NPLC (1-10\% $\mathrm{MeOH} / \mathrm{DCM}, 12 \mathrm{~g}$ silica cartridge) to give $N$-phenyl-2-pyrrolidin-1-yl-quinazolin-4amine ( $77 \mathrm{mg}, 0.2665 \mathrm{mmol}, 68 \%$ yield) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz ,

DMSO-d6) $\delta$ ppm $9.41(\mathrm{~s}, 1 \mathrm{H}), 8.34-8.27(\mathrm{~m}, 1 \mathrm{H}), 8.02-7.95(\mathrm{~m}, 2 \mathrm{H}), 7.60-7.51$ $(\mathrm{m}, 1 \mathrm{H}), 7.40-7.32(\mathrm{~m}, 3 \mathrm{H}), 7.16-7.10(\mathrm{~m}, 1 \mathrm{H}), 7.09-7.03(\mathrm{~m}, 1 \mathrm{H}), 3.55(\mathrm{~s}, 3 \mathrm{H})$, 1.97-1.88 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{18} \mathrm{H}_{18} \mathrm{~N}_{4}, 290.2$; found 291.2 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added $N$-phenyl-2-pyrrolidin-1-yl-quinazolin-4-amine ( $50 \mathrm{mg}, 0.172 \mathrm{mmol}$ ), DCM ( 3.5 mL ), and 4 M HCl in 1,4-dioxane ( $0.04 \mathrm{~mL}, 0.16 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 2 days then concentrated to dryness to give $N$-phenyl-2-pyrrolidin-1-yl-quinazolin-4-amine hydrochloride ( $56 \mathrm{mg}, 0.171 \mathrm{mmol}, 99 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 12.14 (s, 1H), 10.78 (s, 1H), 8.64 (d, J $=8.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.93-7.80(\mathrm{~m}, 4 \mathrm{H}), 7.54-7.44(\mathrm{~m}, 3 \mathrm{H}), 7.30-7.24(\mathrm{~m}, 1 \mathrm{H}), 3.70-$ $3.57(\mathrm{~m}, 4 \mathrm{H}), 2.10-1.87(\mathrm{~m}, 5 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{18} \mathrm{H}_{18} \mathrm{~N}_{4}, 290.2$; found $291.2[\mathrm{M}+\mathrm{H}]^{+}$.

2-(pyrrolidin-1-yl)-N-(4-(trifluoromethyl)phenyl)quinazolin-4-amine hydrochloride (20). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro- $N$-[4-(trifluoromethyl)phenyl]quinazolin-4-amine (32f) (200 mg, 0.618 mmol ), MeCN ( 3.5 mL ), and pyrrolidine ( $0.24 \mathrm{~mL}, 1.85 \mathrm{mmol}$ ). The resulting mixture was capped, sealed, and heated at $100^{\circ} \mathrm{C}$ for 1 hour. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (1-10\%
$\mathrm{MeOH} / \mathrm{DCM}, \quad 12 \mathrm{~g}$ silica cartridge) to give 2-pyrrolidin-1-yl-N-[4-(trifluoromethyl)phenyl]quinazolin-4-amine (188 $\mathrm{mg}, 0.525 \mathrm{mmol}, 85 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d 6 ) $\delta$ ppm $9.72(\mathrm{~s}, 1 \mathrm{H}), 8.36-8.29(\mathrm{~m}, 1 \mathrm{H})$, 8.25 (d, $J=8.5 \mathrm{~Hz}, 2 \mathrm{H}), 7.73(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 2 \mathrm{H}), 7.63-7.55(\mathrm{~m}, 1 \mathrm{H}), 7.38(\mathrm{~d}, J=$ $8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.20-7.11(\mathrm{~m}, 1 \mathrm{H}), 3.57(\mathrm{~s}, 5 \mathrm{H}), 1.94(\mathrm{~s}, 1 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{19} \mathrm{H}_{17} \mathrm{~F}_{3} \mathrm{~N}_{4}, 358.1$; found $359.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-pyrrolidin-1-yl- $N$-[4-(trifluoromethyl)phenyl]quinazolin-4-amine (50 mg, 0.140 mmol ), DCM (3 mL ), and 4 M HCl in 1,4-dioxane ( $0.04 \mathrm{~mL}, 0.16 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 18 hours then concentrated to dryness to give 2-pyrrolidin-1-yl-N-[4-(trifluoromethyl)phenyl]quinazolin-4-amine hydrochloride (54 $\mathrm{mg}, 0.137 \mathrm{mmol}, 98 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( $\left.400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}\right) \delta \mathrm{ppm}$ $12.37(\mathrm{~s}, 1 \mathrm{H}), 10.81(\mathrm{~s}, 1 \mathrm{H}), 8.66(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.17(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 2 \mathrm{H})$, 7.90-7.75 (m, 3H), 7.50-7.40 (m, 1H), $3.66(t, J=6.6 \mathrm{~Hz}, 4 \mathrm{H}), 1.99(\mathrm{~d}, J=24.2$ $\mathrm{Hz}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{19} \mathrm{H}_{17} \mathrm{~F}_{3} \mathrm{~N}_{4}, 358.1$; found $359.2[\mathrm{M}+\mathrm{H}]^{+}$.

N,N-dimethyl-2-(pyrrolidin-1-yl)quinazolin-4-amine hydrochloride (21). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro- $\mathrm{N}, \mathrm{N}$-dimethyl-quinazolin-4-amine (32a) (100 mg, 0.482 mmol$)$, $\mathrm{MeCN}(2.5 \mathrm{~mL})$, and pyrrolidine $(0.18 \mathrm{~mL}, 1.44 \mathrm{mmol})$. The reaction mixture was heated at $110^{\circ} \mathrm{C}$ for 30 min . The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. Crude material was purified via automated NPLC (1-10\% MeOH/DCM, 12 g silica cartridge) to give $N, N$-dimethyl-2-pyrrolidin-1-yl-quinazolin-4-amine (98
$\mathrm{mg}, 0.404 \mathrm{mmol}, 84 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ 7.97 (dd, $J=8.5,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.61-7.54(\mathrm{~m}, 1 \mathrm{H}), 7.49(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.15-$ $7.08(\mathrm{~m}, 1 \mathrm{H}), 3.56(\mathrm{q}, \mathrm{J}=3.6 \mathrm{~Hz}, 4 \mathrm{H}), 3.38-3.32(\mathrm{~m}, 6 \mathrm{H}), 1.93(\mathrm{~d}, \mathrm{~J}=4.1 \mathrm{~Hz}, 4 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{14} \mathrm{H}_{18} \mathrm{~N}_{4}, 242.2$; found $243.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added $\mathrm{N}, \mathrm{N}$-dimethyl-2-pyrrolidin-1-yl-quinazolin-4-amine ( $50 \mathrm{mg}, 0.206 \mathrm{mmol}$ ), $\mathrm{DCM}(4.5 \mathrm{~mL}$ ), and 4 M HCl in 1,4-dioxane ( $0.05 \mathrm{~mL}, 0.2 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 2 days then concentrated to dryness to give $\mathrm{N}, \mathrm{N}$-dimethyl-2-pyrrolidin-1-yl-quinazolin-4-amine hydrochloride ( $56 \mathrm{mg}, 0.201 \mathrm{mmol}, 97 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d 6 ) $\delta \mathrm{ppm} 11.93$ (s, 1H), 8.18 (dd, J = $8.5,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.88(\mathrm{dd}, J=8.4,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.82-7.76(\mathrm{~m}, 1 \mathrm{H}), 7.40-7.34(\mathrm{~m}$, $1 \mathrm{H}), 3.65(\mathrm{t}, J=6.7 \mathrm{~Hz}, 5 \mathrm{H}), 3.46(\mathrm{~s}, 7 \mathrm{H}), 1.99(\mathrm{~d}, J=35.8 \mathrm{~Hz}, 5 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{14} \mathrm{H}_{18} \mathrm{~N}_{4}, 242.2$; found $243.2[\mathrm{M}+\mathrm{H}]^{+}$.

2,4-di(pyrrolidin-1-yl)quinazoline hydrochloride (22). To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro-4-pyrrolidin-1-yl-quinazoline (32b) ( $100 \mathrm{mg}, 0.428 \mathrm{mmol}$ ), $\mathrm{MeCN}(2.5 \mathrm{~mL})$, and pyrrolidine ( $0.16 \mathrm{~mL}, 1.28$ $\mathrm{mmol})$. The resulting mixture was capped, sealed, and heated at $110{ }^{\circ} \mathrm{C}$ for 1.5 hours. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. Crude material was purified via automated NPLC (1-10\% $\mathrm{MeOH} / \mathrm{DCM}, 4 \mathrm{~g}$ silica cartridge) to give 2,4-dipyrrolidin-1-ylquinazoline ( 87 mg , $0.324 \mathrm{mmol}, 76 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ $8.07(\mathrm{dd}, J=8.5,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.55(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.43(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H})$,
$7.09(\mathrm{t}, \mathrm{J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 3.85(\mathrm{~s}, 3 \mathrm{H}), 3.55(\mathrm{~s}, 1 \mathrm{H}), 2.02-1.85(\mathrm{~m}, 7 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}):$ $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{16} \mathrm{H}_{20} \mathrm{~N} 4,268.2$; found $269.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2,4-dipyrrolidin1 -ylquinazoline ( $50 \mathrm{mg}, 0.186 \mathrm{mmol}$ ), DCM ( 4 mL ), and 4M HCI in 1,4-dioxane $(0.05 \mathrm{~mL}, 0.2 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 2 days then concentrated to dryness to give 2,4-dipyrrolidin-1-ylquinazoline hydrochloride ( $58 \mathrm{mg}, 0.190 \mathrm{mmol}$ ) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO$\left.d_{6}\right) \delta \mathrm{ppm} 11.87(\mathrm{~s}, 1 \mathrm{H}), 8.26-8.21(\mathrm{~m}, 1 \mathrm{H}), 7.88(\mathrm{dd}, J=8.4,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.82-$ $7.76(\mathrm{~m}, 1 \mathrm{H}), 7.42-7.35(\mathrm{~m}, 1 \mathrm{H}), 4.17-4.05(\mathrm{~m}, 2 \mathrm{H}), 3.86-3.75(\mathrm{~m}, 1 \mathrm{H}), 3.65(\mathrm{t}, \mathrm{J}$ $=6.7 \mathrm{~Hz}, 4 \mathrm{H}$ ), 2.10-1.87 (m, 9H). LCMS (ESI): m/z calcd for $\mathrm{C}_{16} \mathrm{H}_{2} 0 \mathrm{~N} 4,268.2$; found $269.2[\mathrm{M}+\mathrm{H}]^{+}$.

## N -(2-(dimethylamino)ethyl)-8-oxo-8H-isoquinolino[1,2-b]quinazoline-9-

carboxamide trifluoroacetate (23). To a round bottom flask charged with a magnetic stir bar was added methyl 8 -hydroxy-8H-isoquinolino[1,2-b]quinazoline-9carboxylate (36) ( $1.23 \mathrm{~g}, 4.015 \mathrm{mmol}$ ), $\mathrm{MeOH}(25 \mathrm{~mL})$, and $2 \mathrm{M} \mathrm{NaOH}(4 \mathrm{~mL}, 8$ $\mathrm{mmol})$. The resulting mixture was stirred at room temperature for 3 days. The reaction mixture was acidified with $\sim 8 \mathrm{~mL} 1 \mathrm{M} \mathrm{HCl}$ to $\mathrm{pH} \sim 5$ and concentrated in vacuo to give 1.85 g of a beige solid, may contain NaCl . Used without further purification. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.68(\mathrm{~s}, 1 \mathrm{H}), 7.78-7.33(\mathrm{~m}, 7 \mathrm{H})$, 6.75 (s, 1H), 4.38 (s, 1H), 1.03 (d, $J=6.1 \mathrm{~Hz}, 3 \mathrm{H}$ ). LCMS (ESI): m/z calcd for $\mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{3}$, 292.1; found $293.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a 40 mL vial charged with a magnetic stir bar was added 8-hydroxy-8H-isoquinolino[1,2-b]quinazoline-9-carboxylic acid (1 g, 3.42 mmol ), TBTU (1.2 g, $3.74 \mathrm{mmol})$, DMF ( 15 mL ), and DIPEA ( $1.83 \mathrm{~mL}, 10.26 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 15 minutes prior to addition of $N^{\prime}, N^{\prime}-$ dimethylethane-1,2-diamine ( $0.56 \mathrm{~mL}, 5.13 \mathrm{mmol}$ ). Following addition of the amine, the reaction was stirred at room temperature for 21 hours. Over the course of reaction, 2 additional equivalents of TBTU and 2 equivalents of amine were added to push reaction forward, but a small portion of starting material remained unreacted. Proceeded to workup. Poured reaction mixture into 250 mL of cold water and stirred for 10 minutes. Transferred to separatory funnel and extracted $3 x$ with EtOAc. Combined organics were washed $1 x$ with $10 \% \mathrm{LiCl}, 1 x$ with sat. brine, dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo to give 580 mg of an orange oily solid. Used without further purification. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}$, 362.2; found $363.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a round-bottom flask charged with a magnetic stir bar was added N -[2-(dimethylamino)ethyl]-8-hydroxy-8H-isoquinolino[1,2-b]quinazoline-9carboxamide ( $490 \mathrm{mg}, 1.35 \mathrm{mmol}$ ), DCM (14 mL), and Dess-Martin periodinane (2833 mg, 6.68 mmol ). The resulting mixture was stirred at room temperature overnight. The reaction was diluted with DCM and washed 1 x with $12 \mathrm{~mL} 1: 11 \mathrm{M}$ $\mathrm{Na}_{2} \mathrm{~S}_{2} \mathrm{O}_{3}$ : sat. $\mathrm{NaHCO}_{3}, 1 \mathrm{x}$ with water, and 1 x with sat. brine. The aqueous layer was extracted $3 x$ with DCM and combined organics were washed $1 x$ with sat. brine, dried over MgSO4, filtered, and concentrated in vacuo. Crude material was purified via automated RPLC (10-50\% MeCN/water 50x250 LUNA) to give N -[2-
(dimethylamino)ethyl]-8-oxo-isoquinolino[1,2-b]quinazoline-9-carboxamide trifluoroacetate ( $30 \mathrm{mg}, 0.063 \mathrm{mmol}, 5 \%$ yield) as an orange solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $d_{6}$ ) $\delta$ ppm $9.03-8.97(\mathrm{~m}, 1 \mathrm{H}), 8.58(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.48(\mathrm{t}, J=5.8$ $\mathrm{Hz}, 1 \mathrm{H}), 8.02-7.95(\mathrm{~m}, 2 \mathrm{H}), 7.95-7.87(\mathrm{~m}, 2 \mathrm{H}), 7.82-7.76(\mathrm{~m}, 1 \mathrm{H}), 7.49(\mathrm{dd}, \mathrm{J}=$ $6.2,2.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.39(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 3.70-3.61(\mathrm{~m}, 2 \mathrm{H}), 3.42-3.35(\mathrm{~m}, 2 \mathrm{H})$, 2.96 ( $\mathrm{d}, J=4.9 \mathrm{~Hz}, 6 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{20} \mathrm{~N}_{4} \mathrm{O}_{2}, 360.4$; found 361.2 $[\mathrm{M}+\mathrm{H}]^{+}$.

## N-(2-(dimethylamino)ethyl)-8H-isoquinolino[1,2-b]quinazoline-9-carboxamide

(24). To a 20 mL vial charged with a magnetic stir bar was added methyl 8 H -isoquinolino[1,2-b]quinazoline-9-carboxylate (37) (60 mg, 0.207 mmol$)$, MeOH (3 mL ), and 2 M NaOH ( $0.21 \mathrm{~mL}, 0.42 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 4 days. The reaction mixture was concentrated in vacuo and the residue was taken up in isopropanol, acidified $(\mathrm{pH} \sim 5)$ with 1 N HCl , and vacuum filtered to give $8 H$-isoquinolino[1,2-b]quinazoline-9-carboxylic acid (33 $\mathrm{mg}, 0.121 \mathrm{mmol}, 57 \%$ yield) as a beige solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ $13.56(\mathrm{~s}, 1 \mathrm{H}), 9.07(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.12-8.03(\mathrm{~m}, 2 \mathrm{H}), 8.00-7.87(\mathrm{~m}, 3 \mathrm{H}), 7.81$ $(\mathrm{d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.62-7.57(\mathrm{~m}, 1 \mathrm{H}), 7.54(\mathrm{t}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 6.00(\mathrm{~s}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{17} \mathrm{H}_{12} \mathrm{~N}_{2} \mathrm{O}_{2}$, 276.1; found $277.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 8 H -isoquinolino[1,2-b]quinazoline-9-carboxylic acid ( $33 \mathrm{mg}, 0.121 \mathrm{mmol}$ ), TBTU (58 $\mathrm{mg}, 0.181 \mathrm{mmol})$, DMF ( 2 mL ), and DIPEA ( $0.04 \mathrm{~mL}, 0.239 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 15 minutes before adding $\mathrm{N}, \mathrm{N}$ -
dimethylethylenediamine ( $0.02 \mathrm{~mL}, 0.79 \mathrm{mmol}$ ). The reaction mixture was stirred at room temperature for 2.5 hours. The reaction mixture was diluted with water and stirred at room temperature for 1 hour. The mixture was transferred to a separatory funnel using EtOAc to rinse the vial $3 x$. The contents of the separatory funnel were washed 1 x with $10 \% \mathrm{LiCl}$ and the aqueous layer was extracted 3 x with EtOAc. Combined organics were washed 1 x with sat. brine, dried over MgSO4, filtered, and concentrated in vacuo. Crude materials were purified via automated RPLC 10$50 \% \mathrm{MeCN} /$ water (Gilson) to give N -[2-(dimethylamino)ethyl]-8H-isoquinolino[1,2-b]quinazoline-9-carboxamide trifluoroacetate ( $7 \mathrm{mg}, 0.015 \mathrm{mmol}, 12 \%$ yield) as a yellow oily solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d $)^{\text {( }} \delta \mathrm{ppm} 11.89(\mathrm{~s}, 1 \mathrm{H}), 9.74(\mathrm{~s}, 1 \mathrm{H})$, $8.97-8.83(\mathrm{~m}, 2 \mathrm{H}), 8.10(\mathrm{~d}, \mathrm{~J}=4.2 \mathrm{~Hz}, 2 \mathrm{H}), 7.96(\mathrm{dd}, J=8.1,4.6 \mathrm{~Hz}, 2 \mathrm{H}), 7.65(\mathrm{t}$, $J=6.2 \mathrm{~Hz}, 2 \mathrm{H}), 7.55(\mathrm{~d}, J=4.5 \mathrm{~Hz}, 2 \mathrm{H}), 5.82(\mathrm{~s}, 2 \mathrm{H}), 3.67-3.60(\mathrm{~m}, 2 \mathrm{H}), 3.36-$ $3.28(\mathrm{~m}, 2 \mathrm{H}), 2.89(\mathrm{~d}, J=4.5 \mathrm{~Hz}, 6 \mathrm{H}), 2.69(\mathrm{~d}, J=0.9 \mathrm{~Hz}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}, 346.2$; found $347.2[\mathrm{M}+\mathrm{H}]^{+}$.

## 2-phenyl-4-(pyrrolidin-1-yl)-N-(2-(pyrrolidin-1-yl)ethyl)quinazoline-8-carboxamide

 hydrochloride (25). To a 20 mL vial charged with a magnetic stir bar was added methyl 2-phenyl-4-pyrrolidin-1-yl-quinazoline-8-carboxylate (42a) (235 mg, 0.705 $\mathrm{mmol})$, $\mathrm{MeOH}(7 \mathrm{~mL})$, and $2 \mathrm{M} \mathrm{NaOH}(0.7 \mathrm{~mL}, 1.4 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 2 days. Added an additional 2 equivalents of 2 M NaOH and stirred at room temperature for 24 hours. Added an additional 1 equivalent of 2 M NaOH and stirred at room temperature for 3 days. Concentrated in vacuo, residue taken up in water and neutralized with 2 N HCl . Vacuum filtered and washed with water to give 2-phenyl-4-pyrrolidin-1-yl-quinazoline-8-carboxylicacid (164 $\mathrm{mg}, 0.514 \mathrm{mmol}$ ) as a brown solid. Used without further purification. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{19} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{2}$, 319.1; found $320.7[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-phenyl-4-pyrrolidin-1-yl-quinazoline-8-carboxylic acid (160 mg, 0.501 mmol ), TBTU (242 $\mathrm{mg}, 0.754 \mathrm{mmol}$ ), DMF ( 5 mL ), DIPEA ( $0.27 \mathrm{~mL}, 1.50 \mathrm{mmol}$ ) and 2-pyrrolidin-1ylethanamine ( $0.1 \mathrm{~mL}, 0.752 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 17 hours. Poured into $\sim 50 \mathrm{~mL}$ water with stirring and vacuum filtered to give 2-phenyl-4-pyrrolidin-1-yl- N -(2-pyrrolidin-1-ylethyl)quinazoline-8carboxamide ( $169 \mathrm{mg}, 0.407 \mathrm{mmol}, 81 \%$ yield) as a brown solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm $11.38(\mathrm{~s}, 1 \mathrm{H}), 8.59(\mathrm{~d}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.50-8.38(\mathrm{~m}, 3 \mathrm{H})$, $7.59-7.51(\mathrm{~m}, 4 \mathrm{H}), 4.00(\mathrm{~s}, 5 \mathrm{H}), 3.63(\mathrm{~d}, J=5.6 \mathrm{~Hz}, 2 \mathrm{H}), 2.76(\mathrm{~s}, 2 \mathrm{H}), 2.02(\mathrm{~s}, 5 \mathrm{H})$, $1.64(\mathrm{~s}, 5 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{25} \mathrm{H}_{29} \mathrm{~N}_{5} \mathrm{O}$, 415.2 ; found $416.8[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-phenyl-4-pyrrolidin-1-yl- N -(2-pyrrolidin-1-ylethyl)quinazoline-8-carboxamide (165 mg, 0.397 $\mathrm{mmol})$, $\mathrm{DCM}(4 \mathrm{~mL})$, and 4 M HCl in 1,4-dioxane ( $0.2 \mathrm{~mL}, 0.8 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 1 hour then concentrated to dryness to give 2-phenyl-4-pyrrolidin-1-yl- N -(2-pyrrolidin-1-ylethyl)quinazoline-8carboxamide hydrochloride ( $222 \mathrm{mg}, 0.491 \mathrm{mmol}$ ) as a brown solid. ${ }^{1} \mathrm{H}$ NMR (400 $\left.\mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta$ ppm $16.20(\mathrm{~s}, 1 \mathrm{H}), 11.94(\mathrm{~s}, 1 \mathrm{H}), 10.81(\mathrm{~s}, 1 \mathrm{H}), 9.50(\mathrm{~s}, 1 \mathrm{H}), 8.39-$ $8.17(\mathrm{~m}, 2 \mathrm{H}), 7.83-7.62(\mathrm{~m}, 3 \mathrm{H}), 4.19(\mathrm{~d}, \mathrm{~J}=64.5 \mathrm{~Hz}, 5 \mathrm{H}), 3.90(\mathrm{~s}, 1 \mathrm{H}), 3.25(\mathrm{~s}$, 2H), 2.46-1.96 (m, 12H). LCMS (ESI): m/z calcd for $\mathrm{C}_{25} \mathrm{H}_{29} \mathrm{~N}_{5} \mathrm{O}, 415.2$; found $416.8[\mathrm{M}+\mathrm{H}]^{+}$. (26). To a 20 mL vial charged with a magnetic stir bar was added methyl 4-anilino-2-phenyl-quinazoline-8-carboxylate (42b) (180 mg, 0.507 mmol$)$, MeOH ( 5 mL ), and $2 \mathrm{M} \mathrm{NaOH}(0.51 \mathrm{~mL}, 1.02 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 1 day. Concentrated in vacuo, residue taken up in water and neutralized with 2N HCl. Vacuum filtered to give 4-anilino-2-phenyl-quinazoline-8carboxylic acid ( $152 \mathrm{mg}, 0.445 \mathrm{mmol}, 88 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 8.63 (d, $J=8.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.37$ (d, $J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.17$ (dd, $J=6.8,3.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.69(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 2 \mathrm{H}), 7.59(\mathrm{dd}, J=5.0,1.9 \mathrm{~Hz}, 3 \mathrm{H}), 7.44$ $(\mathrm{t}, J=7.9 \mathrm{~Hz}, 2 \mathrm{H}), 7.16(\mathrm{t}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{15} \mathrm{~N}_{3} \mathrm{O}_{2}$, 341.1; found $342.6[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 4-anilino-2-phenyl-quinazoline-8-carboxylic acid (145 mg, 0.425 mmol ), TBTU ( $205 \mathrm{mg}, 0.638$ mmol), DMF ( 4.5 mL ), DIPEA ( $0.23 \mathrm{~mL}, 1.27 \mathrm{mmol}$ ), and 2-pyrrolidin-1ylethanamine ( $0.08 \mathrm{~mL}, 0.637 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 17 hours. Poured into $\sim 50 \mathrm{~mL}$ water with stirring and vacuum filtered to give 4-anilino-2-phenyl- $N$-(2-pyrrolidin-1-ylethyl)quinazoline-8carboxamide ( $174 \mathrm{mg}, 0.398 \mathrm{mmol}, 94 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , $\left.\mathrm{CDCl}_{3}\right) \delta$ ppm $11.49(\mathrm{~s}, 1 \mathrm{H}), 8.91(\mathrm{~d}, J=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.44(\mathrm{~d}, J=4.3 \mathrm{~Hz}, 3 \mathrm{H}), 8.05$ (d, J = 8.0 Hz, 1H), 7.86 (d, J = $8.1 \mathrm{~Hz}, 2 \mathrm{H}), 7.69-7.60(\mathrm{~m}, 1 \mathrm{H}), 7.57-7.45(\mathrm{~m}, 6 \mathrm{H})$, 3.84 (s, 3H), 2.90 (d, $J=10.4 \mathrm{~Hz}, 3 \mathrm{H}$ ), 2.66 (s, 5H), 1.78 (s, 6H). LCMS (ESI): m/z calcd for $\mathrm{C}_{27} \mathrm{H}_{27} \mathrm{~N}_{5} \mathrm{O}$, 437.2 ; found $438.8[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 4-anilino-2-phenyl- $N$-(2-pyrrolidin-1-ylethyl)quinazoline-8-carboxamide (170 mg, 0.389 mmol), DCM ( 4 mL ), and 4 M HCl in 1,4-dioxane ( $0.19 \mathrm{~mL}, 0.76 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 1 hour then concentrated to dryness to give 4-anilino-2-phenyl- $N$-(2-pyrrolidin-1-ylethyl)quinazoline-8carboxamide hydrochloride ( $229 \mathrm{mg}, 0.483 \mathrm{mmol}$ ) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm $11.00(\mathrm{~s}, 1 \mathrm{H}), 10.60(\mathrm{~s}, 1 \mathrm{H}), 8.94(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.68$ (d, $J=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.36-8.25(\mathrm{~m}, 2 \mathrm{H}), 7.92(\mathrm{~d}, J=8.0 \mathrm{~Hz}, 2 \mathrm{H}), 7.80(\mathrm{t}, J=7.9 \mathrm{~Hz}$, $1 \mathrm{H}), 7.63(\mathrm{~s}, 2 \mathrm{H}), 7.52(\mathrm{t}, J=7.6 \mathrm{~Hz}, 2 \mathrm{H}), 7.27(\mathrm{t}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 3.91(\mathrm{~d}, J=6.2$ $\mathrm{Hz}, 2 \mathrm{H}), 3.64(\mathrm{~s}, 2 \mathrm{H}), 3.48(\mathrm{~d}, \mathrm{~J}=6.2 \mathrm{~Hz}, 2 \mathrm{H}), 3.09(\mathrm{~s}, 2 \mathrm{H}), 1.99(\mathrm{~s}, 2 \mathrm{H}), 1.86(\mathrm{~s}$, 2H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{27} \mathrm{H}_{27} \mathrm{~N}_{5} \mathrm{O}, 437.2$; found $438.8[\mathrm{M}+\mathrm{H}]^{+}$.

4-oxo-2-phenyl-N-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide hydrochloride (27). To a 40 mL vial charged with a magnetic stir bar was added 2-chloro-4-oxo-3,4-dihydroquinazoline-8-carboxylic acid (43) (500 mg, 2.23 mmol ), DCM (6 mL), and 1-Chloro-N,N,2-trimethyl-1-propenylamine ( $0.32 \mathrm{~mL}, 2.45$ $\mathrm{mmol})$. The resulting mixture was stirred at room temperature for 3 hours. In a separate vial, 2-pyrrolidin-1-ylethanamine ( $0.31 \mathrm{~mL}, 2.45 \mathrm{mmol}$ ), DIPEA ( 1.19 mL , 6.68 mmol ), and DCM ( 5 mL ) were mixed and added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 18 hours. Diluted with DCM and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give 2-chloro-4-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide (610 mg, 1.91 mmol )
as a brown oil. Used without further purification. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{17} \mathrm{ClN}_{4} \mathrm{O}_{2}$, 320.1 ; found $319.0[\mathrm{M}-\mathrm{H}]$.

To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro-4-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide $(200 \mathrm{mg}, 0.624 \mathrm{mmol})$, phenylboronic acid ( $115 \mathrm{mg}, 0.943 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(530 \mathrm{mg}$, 2.50 mmol ), and 1,4-dioxane ( 3.5 mL ). The resulting mixture was bubbled with nitrogen for 2 minutes before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}$ ( $48 \mathrm{mg}, 0.064 \mathrm{mmol}$ ). The reaction mixture was capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 10 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed 1 x with sat. brine. The aqueous layer was extracted 3 x with EtOAc. Combined organic layers were dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated RPLC 10-50\% MeCN/water (50x150 LUNA), loaded in MeOH. Collected fractions were concentrated in vacuo, diluted with EtOAc, and basified with sat. $\mathrm{NaHCO}_{3}$ to $\mathrm{pH} \sim 9$. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. The resulting mixture was taken up in DCM ( 1 mL ) and 4 M HCl in 1,4-dioxane ( $0.03 \mathrm{~mL}, 0.12 \mathrm{mmol}$ ) was added. The resulting mixture was stirred at room temperature for 18 hours then concentrated to dryness. Repurified via automated RPLC 10-50\% MeCN/water (50x150 Gemini) to give 4-oxo-2-phenyl-N-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide ( $9 \mathrm{mg}, 0.025 \mathrm{mmol}$ ) as a white solid. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 362.2$; found $363.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 4-oxo-2-phenyl-$N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide ( $9 \mathrm{mg}, 0.025$ mmol), $\mathrm{DCM}(1 \mathrm{~mL})$, and 4 M HCl in 1,4-dioxane ( $0.01 \mathrm{~mL}, 0.04 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature overnight then concentrated to dryness to give 4-oxo-2-phenyl- $N$-(2-pyrrolidin-1-ylethyl)-3H-quinazoline-8carboxamide hydrochloride (11mg, 0.028 mmol ) as a white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm $10.36(\mathrm{t}, \mathrm{J}=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.09(\mathrm{~s}, 1 \mathrm{H}), 8.48(\mathrm{dd}, J=7.6$, $1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.35(\mathrm{dd}, \mathrm{J}=7.8,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.18-8.11(\mathrm{~m}, 2 \mathrm{H}), 7.71-7.58(\mathrm{~m}, 4 \mathrm{H})$, 3.88-3.77 (obs.), 3.41 (q, J = 6.2 Hz, 2H), 3.16-2.97 (m, 3H), 2.03-1.89 (m, 3H), 1.88-1.74 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{2}, 362.2$; found 363.0 $[\mathrm{M}+\mathrm{H}]^{+}$.

2-(2-hydroxyphenyl)-4-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8carboxamide (28). To a 40 mL vial charged with a magnetic stir bar was added 2-chloro-4-oxo-3,4-dihydroquinazoline-8-carboxylic acid (43) (500 mg, 2.23 mmol ), DCM (6 mL), and 1-Chloro-N,N,2-trimethyl-1-propenylamine ( $0.32 \mathrm{~mL}, 2.45$ $\mathrm{mmol})$. The resulting mixture was stirred at room temperature for 3 hours. In a separate vial, 2-pyrrolidin-1-ylethanamine ( $0.31 \mathrm{~mL}, 2.45 \mathrm{mmol}$ ), DIPEA ( 1.19 mL , 6.68 mmol ), and DCM ( 5 mL ) were mixed and added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with DCM and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give 2-chloro-4-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide (610 mg,
1.91 mmol ) as a brown oil. Used without further purification. LCMS (ESI): m/z calcd for $\mathrm{C}_{15} \mathrm{H}_{17} \mathrm{ClN}_{4} \mathrm{O}_{2}$, 320.1; found $319.0[\mathrm{M}-\mathrm{H}]^{-}$.

To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro-4-oxo- N -(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide (200 mg, 0.624 mmol$)$, (2-hydroxyphenyl)boronic acid (129 mg, 0.935 mmol ), $\mathrm{K}_{3} \mathrm{PO}_{4}(530 \mathrm{mg}, 2.49 \mathrm{mmol})$, and 1,4-dioxane ( 3.5 mL ). The resulting mixture was bubbled with nitrogen for 2 minutes before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(48 \mathrm{mg}, 0.064$ $\mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 10 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated RPLC 10-50\% MeCN/water (50x150 LUNA), loaded in MeOH to give 2-(2-hydroxyphenyl)-4-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8carboxamide ( $2 \mathrm{mg}, 0.005 \mathrm{mmol}$ ) as a yellow oil. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{3}, 378.2$; found $379.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was dissolved 2-(2-hydroxyphenyl)-4-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8carboxamide (2 mg, 0.005 mmol ) in DCM (1 mL). 4M HCl in 1,4-dioxane (26 $\mu \mathrm{L}$, 0.011 mmol ) was added, and the reaction mixture was stirred at room temperature overnight then concentrated to dryness to give 2-(2-hydroxyphenyl)-4-oxo- N -(2-pyrrolidin-1-ylethyl)-3H-quinazoline-8-carboxamide hydrochloride (3 mg, 0.007
mmol) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 12.99(\mathrm{~s}, 1 \mathrm{H}), 12.66$ $(\mathrm{s}, 1 \mathrm{H}), 9.95(\mathrm{~s}, 1 \mathrm{H}), 9.47-9.37(\mathrm{~m}, 1 \mathrm{H}), 8.34-8.24(\mathrm{~m}, 1 \mathrm{H}), 8.15(\mathrm{dd}, J=17.3,7.7$ $\mathrm{Hz}, 2 \mathrm{H}), 7.62(\mathrm{t}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.47(\mathrm{t}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.04(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H})$, 6.98 (t, J = 7.6 Hz, 1H), 3.76-3.58 (obs.), 3.16-3.00 (obs.), 2.07-1.94 (m, 3H), 1.93-1.79 (m, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{21} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{3}, 378.2$; found 379.2 $[\mathrm{M}+\mathrm{H}]^{+}$.

2-(2,6-dimethoxyphenyl)-4-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide (29). To a 40 mL vial charged with a magnetic stir bar was added 2-chloro-4-oxo-3,4-dihydroquinazoline-8-carboxylic acid (43) $(2430 \mathrm{mg}, 10.82 \mathrm{mmol})$, DCM $(20 \mathrm{~mL}), 1$-Chloro- $\mathrm{N}, \mathrm{N}, 2$-trimethyl-1-propenylamine ( $1.57 \mathrm{~mL}, 11.9 \mathrm{mmol}$ ), and DIPEA ( $5.79 \mathrm{~mL}, 32.46 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature. In a separate vial, 2-pyrrolidin-1-ylethanamine ( $1.51 \mathrm{~mL}, 11.9 \mathrm{mmol}$ ) was diluted in DCM ( 5 mL ) and added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 5 days. The reaction mixture was diluted with DCM and washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give 2-chloro-4-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide $(2.61 \mathrm{~g}, 8.11 \mathrm{mmol})$ as a brown oil. Used without further purification. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{15} \mathrm{H}_{17} \mathrm{CIN}_{4} \mathrm{O}_{2}$, 320.1; found $318.9[\mathrm{M}-\mathrm{H}]^{-}$.

To a 2-5 mL microwave vial charged with a magnetic stir bar was added 2-chloro-4-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide
( $200 \mathrm{mg}, 0.624 \mathrm{mmol}$ ), (2,6-dimethoxyphenyl)boronic acid ( $171 \mathrm{mg}, 0.940 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(530 \mathrm{mg}, 2.49 \mathrm{mmol})$, and 1,4-dioxane ( 3.5 mL ). The resulting mixture was bubbled with nitrogen for 2 minutes before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(48 \mathrm{mg}, 0.064$ $\mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $100^{\circ} \mathrm{C}$ for 10 hours. The reaction mixture was allowed to cool to room temperature and washed 1 x with sat. brine. The aqueous layer was extracted 3 x with EtOAc. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated RPLC 10-50\% MeCN/water (50x150 LUNA), loaded in MeOH to give 2-(2,6-dimethoxyphenyl)-4-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide $\quad(5 \mathrm{mg}$, 0.012 mmol ) as a light brown oil. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{23} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{4}, 422.2$; found $423.2[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was dissolved 2-(2,6-dimethoxyphenyl)-4-oxo- $N$-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8carboxamide ( $5 \mathrm{mg}, 0.012 \mathrm{mmol}$ ) in $\mathrm{DCM}(1 \mathrm{~mL}) .4 \mathrm{M} \mathrm{HCl}$ in 1,4-dioxane ( $59 \mu \mathrm{~L}$, 0.024 mmol ) was added, and the mixture was stirred at room temperature overnight then concentrated to dryness to give 2-(2,6-dimethoxyphenyl)-4-oxo- $N$ -(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinazoline-8-carboxamide hydrochloride (6 $\mathrm{mg}, 0.0126 \mathrm{mmol})$ as a yellow residue. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta \mathrm{ppm} 12.73$ (s, 1H), 10.47 (t, J=5.9 Hz, 1H), 10.08 (s, 2H), 8.51 (dd, $J=7.7,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.34$ (dd, $J=7.9,1.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.66(\mathrm{t}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.51(\mathrm{t}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 6.85(\mathrm{~d}$, $J=8.5 \mathrm{~Hz}, 2 \mathrm{H}$ ), 3.80 (s, 5H), 3.73-3.63 (obs.), 3.32-3.23 (obs.), 3.02-2.90 (m,
$3 H), 1.94-1.83(m, 3 H), 1.80-1.69(m, 3 H)$. LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{4}$, 422.2; found $423.2[\mathrm{M}+\mathrm{H}]^{+}$.

2-((2-chloroquinazolin-4-yl)(methyl)amino)ethan-1-ol (31). To a solution of 2,4dichloroquinazoline (30) (2 g, 10.05 mmol ) in DCM (30 mL) was added 2(methylamino)ethanol ( $1.2 \mathrm{~mL}, 15 \mathrm{mmol}$ ) and DIPEA ( $3.4 \mathrm{~mL}, 19.47 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 1 hour. A precipitate formed that was filtered and washed with DCM to give 2-((2-chloroquinazolin-4$\mathrm{yl})$ (methyl)amino)ethan-1-ol ( $2.05 \mathrm{~g}, 8.625 \mathrm{mmol}, 86 \%$ yield) as an off-white solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO- $\mathrm{d}_{6}$ ): $\delta \mathrm{ppm} 8.31$ (d, $J=7.8 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.78 (ddd , $J=8.3$, $7.0,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.65(\mathrm{dd}, J=8.3,1.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.47(\mathrm{ddd}, J=8.4,7.0,1.5 \mathrm{~Hz}, 1 \mathrm{H})$, $4.94(\mathrm{t}, \mathrm{J}=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 3.76-3.86(\mathrm{~m}, 4 \mathrm{H}), 3.42(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{11} \mathrm{H}_{12} \mathrm{ClN}_{3} \mathrm{O}, 237.1$; found $238.1[\mathrm{M}+\mathrm{H}]^{+}$.

Method A: Synthesis of intermediates 32a-f. 2-chloro-N,N-dimethylquinazolin-4amine (32a). To a 40 mL vial charged with a magnetic stir bar was added 2,4dichloroquinazoline (30) (500 mg, 2.51 mmol$), \mathrm{NaOAc}(351 \mathrm{mg}, 4.28 \mathrm{mmol}), 1,4-$ dioxane ( 18 mL ), water ( 6 mL ), and 2M dimethylamine in THF ( $1.38 \mathrm{~mL}, 2.76$ mmol ) (dropwise). The reaction mixture was heated at $65{ }^{\circ} \mathrm{C}$ for 2 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with ethyl acetate. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (6-50\% EtOAc/heptane, 20 g silica cartridge) to give 2-chloro- $\mathrm{N}, \mathrm{N}$-dimethyl-
quinazolin-4-amine ( $490 \mathrm{mg}, 2.360 \mathrm{mmol}, 94 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 8.25-8.20 (m, 1H), 7.82-7.75 (m, 1H), 7.67-7.61 (m, 1H), 7.51-7.44 (m, 1H), $3.37(\mathrm{~s}, 6 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{10} \mathrm{H}_{10} \mathrm{CIN}_{3}$, 207.1; found $208.2[\mathrm{M}+\mathrm{H}]^{+}$.

2-chloro-4-(pyrrolidin-1-yl)quinazoline (32b). This compound was synthesized from 2,4-dichloroquinazoline (30) (500 mg, 2.51 mmol ) and pyrrolidine ( 0.23 mL , 2.76 mmol ) according to Method A to give 2-chloro-4-(pyrrolidin-1-yl)quinazoline ( $550 \mathrm{mg}, 2.353 \mathrm{mmol}, 94 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \operatorname{ppm} 8.31(\mathrm{dd}, J=8.6,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.81-7.74(\mathrm{~m}, 1 \mathrm{H}), 7.62(\mathrm{dd}, J=8.3,1.3 \mathrm{~Hz}$, 1H), 7.51-7.43(m, 1H), $3.87(\mathrm{~s}, 4 \mathrm{H}), 1.97(\mathrm{~s}, 4 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{12} \mathrm{H}_{12} \mathrm{CIN}_{3}$, 233.1; found $234.0[\mathrm{M}+\mathrm{H}]^{+}$.

Method B: Alternate synthesis of intermediates 32a-f. $N^{1}$-(2-chloroquinazolin-4-yl)$N^{1}, N^{2}, N^{2}$-trimethylethane-1,2-diamine (32c). To a 40 mL vial charged with a magnetic stir bar was added 2,4-dichloroquinazoline (30) (1000 mg, 5.02 mmol ), NaOAc (701 mg, 8.54 mmol ), THF (18 mL), water ( 6 mL ), and $N, N, N^{\prime}-$ trimethylethylenediamine $(0.72 \mathrm{~mL}, 5.53 \mathrm{mmol})$. The resulting mixture was heated at $65^{\circ} \mathrm{C}$ for 2 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed 1x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated RPLC 5-50\% MeCN/water ( $50 \times 250 \mathrm{~mm}$ LUNA) to give $N^{\prime}$-(2-chloroquinazolin-4-yl)- $N, N, N$ '-trimethyl-ethane-1,2-diamine ( $180 \mathrm{mg}, 0.680 \mathrm{mmol}$ )
as a clear colorless oil. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{13} \mathrm{H}_{17} \mathrm{CIN} 4,264.1$; found 265.2 $[\mathrm{M}+\mathrm{H}]^{+}$.
$N^{1}$-(2-chloroquinazolin-4-yl)- $N^{1}, N^{3}, N^{3}$-trimethylpropane-1,3-diamine (32d). This compound was synthesized from 2,4-dichloroquinazoline (30) (1000 mg, 5.02 mmol ) and $N, N, N^{\prime}$-trimethyl-1,3-propanediamine ( $0.81 \mathrm{~mL}, 5.53 \mathrm{mmol}$ ) according to Method B to give $N^{1}$-(2-chloroquinazolin-4-yl)- $N^{1}, N^{3}, N^{3}$-trimethylpropane-1,3diamine ( $220 \mathrm{mg}, 0.789 \mathrm{mmol}, 16 \%$ yield) as a clear colorless oil. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 9.68 (s, 1H), 8.25 (dd, $J=8.6,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.89-7.77$ (m, $1 \mathrm{H}), 7.67(\mathrm{dd}, J=8.3,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.57-7.47(\mathrm{~m}, 1 \mathrm{H}), 3.79(\mathrm{t}, J=7.2 \mathrm{~Hz}, 2 \mathrm{H})$, 3.48 (s, 3H), 3.21-3.11 (m, 2H), 2.81 (d, J=4.8 Hz, 6H), 2.15-2.04 (m, 1H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{14} \mathrm{H}_{19} \mathrm{ClN}_{4}$, 278.1; found $279.1[\mathrm{M}+\mathrm{H}]^{+}$.

2-chloro-N-phenylquinazolin-4-amine (32e). This compound was synthesized from 2,4-dichloroquinazoline (30) (500 mg, 2.51 mmol ) and aniline ( $0.25 \mathrm{~mL}, 2.76 \mathrm{mmol}$ ) according to Method $B$ to give 2-chloro- $N$-phenylquinazolin-4-amine ( 470 mg , $1.838 \mathrm{mmol}, 73 \%$ yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm}$ $10.21(\mathrm{~s}, 1 \mathrm{H}), 8.62-8.54(\mathrm{~m}, 1 \mathrm{H}), 7.93-7.86(\mathrm{~m}, 1 \mathrm{H}), 7.81-7.75(\mathrm{~m}, 2 \mathrm{H}), 7.74-7.70$ (m, 1H), 7.69-7.63 (m, 1H), 7.48-7.40 (m, 2H), 7.24-7.18 (m, 1H). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{14} \mathrm{H}_{10} \mathrm{ClN}, 255.1$; found $256.1[\mathrm{M}+\mathrm{H}]^{+}$.

2-chloro-N-(4-(trifluoromethyl)phenyl)quinazolin-4-amine (32f). This compound was synthesized from 2,4-dichloroquinazoline (30) (2000 mg, 10.05 mmol ) and 4aminobenzotrifluoride ( $1.39 \mathrm{~mL}, 11.05 \mathrm{mmol}$ ) according to Method A to give 2-chloro- N -(4-(trifluoromethyl)phenyl)quinazolin-4-amine (1.27 g, $3.923 \mathrm{mmol}, 39 \%$
yield) as a white solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 10.42$ (s, 1H), 8.61 (dd, $J=8.4,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.12-8.04(\mathrm{~m}, 2 \mathrm{H}), 7.97-7.89(\mathrm{~m}, 1 \mathrm{H}), 7.84-7.74(\mathrm{~m}$, 3H), 7.73-7.66 (m, 1H). LCMS (ESI): m/z calcd for $\mathrm{C}_{15} \mathrm{H}_{9} \mathrm{CIF}_{3} \mathrm{~N}_{3}, 323.0$; found $324.0[\mathrm{M}+\mathrm{H}]^{+}$.

2-chloro-N-(2,4-dimethoxyphenyl)quinazolin-4-amine (33). To a round-bottom flask charged with a magnetic stir bar was added 2,4-dichloroquinazoline (30)(1.5 $\mathrm{g}, 7.54 \mathrm{mmol})$, 2,4-dimethoxyaniline ( $1.25 \mathrm{~g}, 8.16 \mathrm{mmol}$ ), $\mathrm{NaOAc}(1.02 \mathrm{~g}, 12.28$ mmol ), THF ( 20 mL ), and water ( 20 mL ). The resulting mixture was heated at 70 ${ }^{\circ} \mathrm{C}$ for 9 hours. The reaction mixture was allowed to cool to room temperature and diluted with sat. $\mathrm{NaHCO}_{3}$ and EtOAc. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed with water, sat. $\mathrm{NaHCO}_{3}$, and sat. brine then dried over a phase separator and concentrated under vacuum at $40^{\circ} \mathrm{C}$ to give 2-chloro- N -(2,4-dimethoxyphenyl) quinazolin-4-amine ( $2.35 \mathrm{~g}, 7.443 \mathrm{mmol}$, $99 \%$ yield) as a black solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 9.87$ (s, 1H), $8.47(\mathrm{~d}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.88(\mathrm{ddd}, J=8.3,7.0,1.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.71(\mathrm{~d}, J=7.6 \mathrm{~Hz}$, $1 \mathrm{H}), 7.63(\mathrm{ddd}, J=8.2,7.0,1.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.20(\mathrm{~d}, J=3.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.10(\mathrm{~d}, J=9.0$ $\mathrm{Hz}, 1 \mathrm{H}), 6.89(\mathrm{dd}, J=9.0,3.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.76(\mathrm{~d}, J=3.7 \mathrm{~Hz}, 6 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{16} \mathrm{H}_{14} \mathrm{ClN}_{3} \mathrm{O}_{2}$, 315.1; found $316.0[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 3-bromo-2-(bromomethyl)benzoate (35). To a solution of 3-bromo-2methylbenzoic acid ( $2 \mathrm{~g}, 9.30 \mathrm{mmol}$ ) in $\mathrm{MeOH}(24 \mathrm{~mL})$ was added $\mathrm{H}_{2} \mathrm{SO}_{4}$ (conc.) $(2.32 \mathrm{~mL}, 43.52 \mathrm{mmol})$. The resulting mixture was heated at $76{ }^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature and was concentrated
in vacuo but not to dryness. It was then diluted with water and neutralized with sat. $\mathrm{NaHCO}_{3}$ to $\mathrm{pH} \sim 9$. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ each with $10 \% \mathrm{NaHCO}_{3}$, water, and sat. brine before being dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo to give methyl 3-bromo-2-methylbenzoate ( $1.98 \mathrm{~g}, 8.644 \mathrm{mmol}, 93 \%$ yield) as an orange liquid, used without further purification. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm} 7.82$ (dd, J $=8.0,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.71(\mathrm{dd}, J=7.7,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.27-7.22(\mathrm{~m}, 1 \mathrm{H}), 3.84(\mathrm{~s}, 3 \mathrm{H})$, 2.51 ( $\mathrm{s}, 3 \mathrm{H}$ ). LCMS (ESI): m/z calcd for $\mathrm{C}_{9} \mathrm{H}_{9} \mathrm{BrO}_{2}$, 228.0; found $229.3[\mathrm{M}+\mathrm{H}]^{+}$.

To a round-bottom flask charged with a magnetic stir bar was added methyl 3-bromo-2-methyl-benzoate (1.94 g, 8.47 mmol$)$, $\mathrm{MeCN}(28 \mathrm{~mL})$, and N bromosuccinimide ( $1.58 \mathrm{~g}, 8.89 \mathrm{mmol}$ ). The resulting mixture was heated at $94{ }^{\circ} \mathrm{C}$ for 3 days. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and washed $1 x$ with $10 \% \mathrm{NaHCO}_{3}$. The aqueous layer was extracted $3 x$ with EtOAc. Combined organics were washed $1 x$ with sat. brine, dried over $\mathrm{MgSO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (6-50\% EtOAc/heptane, 120 g silica cartridge) to give methyl 3-bromo-2-(bromomethyl)benzoate ( $1.87 \mathrm{~g}, 6.072 \mathrm{mmol}, 72 \%$ yield) as a clear colorless oil. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $d_{6}$ ) $\delta \mathrm{ppm} 7.93(\mathrm{dd}, J=8.1,1.2 \mathrm{~Hz}, 1 \mathrm{H})$, $7.86(\mathrm{dd}, \mathrm{J}=7.8,1.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.42(\mathrm{t}, J=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 5.03(\mathrm{~s}, 2 \mathrm{H}), 3.89(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{9} \mathrm{H}_{8} \mathrm{Br}_{2} \mathrm{O}_{2}$, 305.9; found $307.7[\mathrm{M}+2 \mathrm{H}]^{+}$.

Methyl 8-hydroxy-8H-isoquinolino[1,2-b]quinazoline-9-carboxylate (36). To a round bottom flask charged with a magnetic stir bar was added methyl 3-bromo-2-
(bromomethyl)benzoate (35) (1709 mg, 5.55 mmol$)$, isoquinolin-1-amine $(800 \mathrm{mg}$, $5.55 \mathrm{mmol})$, and DMF ( 30 mL ). The resulting mixture was heated at $85{ }^{\circ} \mathrm{C}$ overnight. Then $\mathrm{Cul}(106 \mathrm{mg}, 0.557 \mathrm{mmol}), \mathrm{Cs}_{2} \mathrm{CO}_{3}(2713 \mathrm{mg}, 8.33 \mathrm{mmol})$, and $\mathrm{L}-$ proline ( $128 \mathrm{mg}, 1.11 \mathrm{mmol}$ ) were added. The resulting mixture was stirred at 85 ${ }^{\circ} \mathrm{C}$ for 2 days. The reaction mixture was heated at $85^{\circ} \mathrm{C}$ for an additional 24 hours open to the air to encourage autooxidation but no change in product distribution was observed. The reaction was allowed to cool to room temperature and diluted with $\sim 80 \mathrm{~mL}$ cold water with stirring. The resulting solid was vacuum filtered and washed with additional cold water to give 1.28 g of a solid containing a mixture of methyl 8-hydroxy-8H-isoquinolino[1,2-b]quinazoline-9-carboxylate (36) and methyl $8 H$-isoquinolino[1,2-b]quinazoline-9-carboxylate (37). Used without further purification. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 8.63(\mathrm{~d}, J=8.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.70-$ 7.57 (m, 3H), 7.57-7.42 (m, 5H), $7.27(\mathrm{dd}, J=7.3,5.0 \mathrm{~Hz}, 1 \mathrm{H}), 6.98(\mathrm{~d}, J=7.4$ $\mathrm{Hz}, 1 \mathrm{H}), 6.71(\mathrm{~d}, \mathrm{~J}=7.4 \mathrm{~Hz}, 1 \mathrm{H}), 3.89(\mathrm{~s}, 3 \mathrm{H}), 3.85(\mathrm{~s}, 1 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{18} \mathrm{H}_{14} \mathrm{~N}_{2} \mathrm{O}_{3}, 306.1$; found $307.0[\mathrm{M}+\mathrm{H}]^{+} ; m / z$ calcd for $\mathrm{C}_{18} \mathrm{H}_{14} \mathrm{~N}_{2} \mathrm{O}_{2}, 290.1$; found $291.0[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 8H-isoquinolino[1,2-b]quinazoline-9-carboxylate (37). To a 20 mL vial charged with a magnetic stir bar was added methyl 3-bromo-2(bromomethyl)benzoate (35) (182 mg, 0.591 mmol ), isoquinolin-1-amine ( 86 mg , 0.597 mmol ), and DMF ( 4 mL ). The resulting mixture was heated at $85^{\circ} \mathrm{C}$ for 6 hours. Then $\mathrm{Cs}_{2} \mathrm{CO}_{3}(318 \mathrm{mg}, 0.976 \mathrm{mmol})$, $\mathrm{Cul}(13 \mathrm{mg}, 0.068 \mathrm{mmol})$, and $\mathrm{L}-$ proline ( $15 \mathrm{mg}, 0.130 \mathrm{mmol}$ ) were added. The resulting mixture was stirred at 85 ${ }^{\circ} \mathrm{C}$ overnight. The reaction mixture was allowed to cool to room temperature, the
cap was opened to air, and stirring continued overnight. The reaction mixture was diluted with water and the precipitate was vacuum filtered. The residue was air dried overnight to give methyl 8 H -isoquinolino[1,2-b]quinazoline-9-carboxylate (37) (112 $\mathrm{mg}, 0.386 \mathrm{mmol}$ ) as a yellow solid, used without further purification. LCMS (ESI): m/z calcd for $\mathrm{C}_{18} \mathrm{H}_{14} \mathrm{~N}_{2} \mathrm{O}_{2}$, 290.1; found $291.1[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxylate (39). To a 250 mL round-bottom flask charged with a magnetic stir bar was added 2-amino-3-methoxycarbonyl-benzoic acid (38) (5000 mg, 25.62 mmol ), TBTU ( 9049 mg , $28.18 \mathrm{mmol})$, DMF ( 26 mL ), and DIPEA ( $9.14 \mathrm{~mL}, 51.24 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 5 minutes before adding $\mathrm{NH}_{4} \mathrm{OH}$ (4.28 $\left.\mathrm{mL}, 30.74 \mathrm{mmol}, 28 \% \mathrm{NH}_{3}\right)$. The reaction mixture was stirred at room temperature for 23 hours. The reaction mixture was diluted with $\sim 200 \mathrm{~mL}$ water, stirred at room temperature, and vacuum filtered to give methyl 2-amino-3-carbamoyl-benzoate (4527 mg, $23.312 \mathrm{mmol}, 91 \%$ yield) as a yellow solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.04(\mathrm{~s}, 4 \mathrm{H}), 7.99-7.87(\mathrm{~m}, 1 \mathrm{H})$, 7.80 (dd, $J=7.7,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.42-7.30(\mathrm{~m}, 2 \mathrm{H}), 6.61-6.52(\mathrm{~m}, 1 \mathrm{H}), 3.80(\mathrm{~s}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{9} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{3}$, 194.1; found $195.6[\mathrm{M}+\mathrm{H}]^{+}$.

To a 500 mL round-bottom flask charged with a magnetic stir bar was added methyl 2-amino-3-carbamoyl-benzoate ( $4500 \mathrm{mg}, 23.17 \mathrm{mmol}$ ), $\mathrm{K}_{2} \mathrm{CO}_{3}(9928 \mathrm{mg}$, 71.84 mmol ), 1,1 '-carbonyldiimidazole ( $11273 \mathrm{mg}, 69.52 \mathrm{mmol}$ ), and DMF ( 47 mL ). The resulting mixture was heated at $90^{\circ} \mathrm{C}$ for 4 hours. The reaction mixture was allowed to cool to room temperature and diluted with water. The aqueous layer
was extracted 2 x with EtOAc. The aqueous layer was collected, neutralized to pH $\sim 7$ with 2 N HCl , and vacuum filtered to give methyl 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxylate ( $4079 \mathrm{mg}, 18.526 \mathrm{mmol}, 80 \%$ yield) as a white solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm} 8.25$ (dd, $J=7.8,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.19(\mathrm{dd}, J=7.8,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.30(\mathrm{t}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H})$, 3.92 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{10} \mathrm{H}_{8} \mathrm{~N}_{2} \mathrm{O}_{4}, 220.0$; found $221.6[\mathrm{M}+\mathrm{H}]^{+}$. Methyl 2,4-dichloroquinazoline-8-carboxylate (40). To a 40 mL vial charged with a magnetic stir bar was added methyl 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8carboxylate (39) (1000 mg, 4.54 mmol$), \mathrm{POCl}_{3}(4 \mathrm{~mL}, 42.78 \mathrm{mmol})$, and DIPEA $(1.22 \mathrm{~mL}, 6.81 \mathrm{mmol})$ (dropwise). The resulting mixture was heated at $100^{\circ} \mathrm{C}$ for 6 hours. The reaction mixture was allowed to cool to room temperature and was added to $\sim 125 \mathrm{~mL}$ ice water dropwise with stirring. The resulting precipitate was vacuum filtered and washed with water to give methyl 2,4-dichloroquinazoline-8carboxylate ( $1040 \mathrm{mg}, 4.046 \mathrm{mmol}, 89 \%$ yield) as a light brown solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 8.53-8.47(\mathrm{~m}, 1 \mathrm{H}), 8.44-$ $8.38(\mathrm{~m}, 1 \mathrm{H}), 7.96(\mathrm{dd}, J=8.5,7.3 \mathrm{~Hz}, 1 \mathrm{H}), 3.95(\mathrm{~s}, 1 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{10} \mathrm{H}_{6} \mathrm{Cl}_{2} \mathrm{~N}_{2} \mathrm{O}_{2}$, 256.0; found $256.5[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 2-chloro-4-(pyrrolidin-1-yl)quinazoline-8-carboxylate (41a). To a 40 mL vial charged with a magnetic stir bar was added methyl 2,4-dichloroquinazoline-8carboxylate (40) (500 mg, 1.94 mmol$), \mathrm{NaOAc}(272 \mathrm{mg}, 3.31 \mathrm{mmol})$, THF ( 12 mL ), water ( 6 mL ), and pyrrolidine ( $0.18 \mathrm{~mL}, 2.14 \mathrm{mmol}$ ) (dropwise). The resulting mixture was heated at $65{ }^{\circ} \mathrm{C}$ for 45 minutes. The reaction mixture was allowed to
cool to room temperature, diluted with DCM, and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give methyl 2-chloro-4-pyrrolidin-1-yl-quinazoline-8-carboxylate ( $500 \mathrm{mg}, 1.714 \mathrm{mmol}$ ) as a yellow solid, used without further purification. LCMS (ESI): m/z calcd for $\mathrm{C}_{14} \mathrm{H}_{14} \mathrm{ClN}_{3} \mathrm{O}_{2}$, 291.1; found $292.6[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 2-chloro-4-(phenylamino)quinazoline-8-carboxylate (41b). To a 40 mL vial charged with a magnetic stir bar was added methyl 2,4-dichloroquinazoline-8carboxylate (40) (1000 mg, 3.89 mmol ), $\mathrm{NaOAc}(543 \mathrm{mg}, 6.61 \mathrm{mmol})$, THF (16 mL ), water ( 8 mL ), and aniline ( $0.39 \mathrm{~mL}, 4.28 \mathrm{mmol}$ ) (dropwise). The resulting mixture was heated at $65^{\circ} \mathrm{C}$ for 45 min . The reaction mixture was allowed to cool to room temperature, diluted with DCM, and washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo to give methyl 4-anilino-2-chloro-quinazoline-8-carboxylate ( $755 \mathrm{mg}, 2.406 \mathrm{mmol}, 62 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta \mathrm{ppm} 10.37$ (s, 1H), 8.74 (dd, $J=8.5,1.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.08(\mathrm{dd}, J=7.3,1.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.73(\mathrm{~m}, 2 \mathrm{H}), 7.70$ (dd, $J=8.4,7.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.45(\mathrm{dd}, \mathrm{J}=8.5,7.4 \mathrm{~Hz}, 2 \mathrm{H}), 7.27-7.19(\mathrm{~m}, 1 \mathrm{H}), 3.90$ (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{16} \mathrm{H}_{12} \mathrm{CIN}_{3} \mathrm{O}_{2}, 313.1$; found $313.6[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 2-phenyl-4-(pyrrolidin-1-yl)quinazoline-8-carboxylate (42a). To a 30 mL microwave vial charged with a magnetic stir bar was added methyl 2-chloro-4-pyrrolidin-1-yl-quinazoline-8-carboxylate (41a) (225 mg, 0.77 mmol ),
phenylboronic acid ( $142 \mathrm{mg}, 1.16 \mathrm{mmol}$ ), $\mathrm{K}_{3} \mathrm{PO}_{4}(492 \mathrm{mg}, 2.31 \mathrm{mmol}$ ), and 1,4dioxane ( 5.5 mL ). The resulting mixture was bubbled with nitrogen for 5 minutes before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}(59 \mathrm{mg}, 0.078 \mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 15 hours. The reaction mixture was allowed to cool to room temperature then vacuum filtered through a pad of Celite and washed with DCM. The filtrate was washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC (0-20\% MeOH/DCM, 40 g silica cartridge) to give methyl 2-phenyl-4-pyrrolidin-1-yl-quinazoline-8-carboxylate ( $246 \mathrm{mg}, 0.738 \mathrm{mmol}, 96 \%$ yield) as a light brown solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta \mathrm{ppm} 8.58(\mathrm{~s}, 4 \mathrm{H}), 8.27$ (d, $J=8.6 \mathrm{~Hz}, 2 \mathrm{H}), 7.98(\mathrm{~d}, J=7.4 \mathrm{~Hz}, 2 \mathrm{H}), 7.46(\mathrm{dd}, J=5.5,1.9 \mathrm{~Hz}, 3 \mathrm{H}), 4.08(\mathrm{~s}$, $3 H), 4.02(\mathrm{~s}, 2 \mathrm{H}), 2.08(\mathrm{~s}, 2 \mathrm{H}), 1.26(\mathrm{~s}, 1 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{20} \mathrm{H}_{19} \mathrm{~N}_{3} \mathrm{O}_{2}$, 333.1; found $333.8[\mathrm{M}+\mathrm{H}]^{+}$.

Methyl 2-phenyl-4-(phenylamino)quinazoline-8-carboxylate (42b). To a 30 mL microwave vial charged with a magnetic stir bar was added methyl 4-anilino-2-chloro-quinazoline-8-carboxylate (41b) (360 mg, 1.15 mmol ), phenylboronic acid $(210 \mathrm{mg}, 1.72 \mathrm{mmol}), \mathrm{K}_{3} \mathrm{PO}_{4}(731 \mathrm{mg}, 3.44 \mathrm{mmol})$, and 1,4-dioxane ( 8 mL ). The resulting mixture was bubbled with nitrogen for 5 minutes before adding $\mathrm{Pd}(\mathrm{dcpf}) \mathrm{Cl}_{2}$ ( $87 \mathrm{mg}, 0.115 \mathrm{mmol}$ ). The reaction mixture was capped, sealed, and heated at $100{ }^{\circ} \mathrm{C}$ for 14 hours. The reaction mixture was allowed to cool to room temperature, vacuum filtered through a pad of Celite, and washed with DCM. The filtrate was washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with

DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated NPLC (1060\% EtOAc/heptane, 80 g silica cartridge) to give methyl 4-anilino-2-phenyl-quinazoline-8-carboxylate (188 mg, 0.529 mmol ) as a yellow solid. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{22} \mathrm{H}_{17} \mathrm{~N}_{3} \mathrm{O}_{2}, 355.1$; found $355.7[\mathrm{M}+\mathrm{H}]^{+}$.

2-chloro-4-oxo-3,4-dihydroquinazoline-8-carboxylic acid (43). To a 250 mL roundbottom flask charged with a magnetic stir bar was added methyl 2,4-dichloroquinazoline-8-carboxylate (40) (4.65 g, 18.09 mmol$)$ and 2M KOH(37 mL, $74 \mathrm{mmol})$. The resulting mixture was heated at $105^{\circ} \mathrm{C}$ for 5 minutes then allowed to cool to room temperature. The reaction mixture was cooled in an ice-water bath and acidified to $\mathrm{pH} \sim 5$ with 2 N HCl . The resulting precipitate was vacuum filtered and washed with water to give 2-chloro-4-oxo-3,4-dihydroquinazoline-8-carboxylic acid ( $4.27 \mathrm{~g}, 19.012 \mathrm{mmol}$ ), used without further purification. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d ${ }_{6}$ ) $\delta$ ppm $14.38(\mathrm{~s}, 1 \mathrm{H}), 8.24-8.15(\mathrm{~m}, 2 \mathrm{H}), 7.54(\mathrm{t}, J=7.7 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{10} \mathrm{H}_{7} \mathrm{ClN}_{2} \mathrm{O}_{3}, 224.0$; found $225.0[\mathrm{M}+\mathrm{H}]^{+}$.

Cell culture. Performed by Daming Chen. A375 melanoma cells were maintained at $37{ }^{\circ} \mathrm{C}$ in a humidified atmosphere containing $5 \% \mathrm{CO}_{2}$. A 375 cells were cultured in DMEM supplemented with $10 \%$ fetal bovine serum (FBS) and 4 mM glutamine.

RPA194 Degradation and $y \mathrm{H} 2 \mathrm{AX}$ Activation Assay. Performed at Evotec. A375 cells were seeded on 384 -well plates and treated with the compounds at 0.01 , $0.03,0.1,0.3,1,3,10$, and $30 \mu \mathrm{M}$ or treated with vehicle (DMSO) for 4 h . After treatment, cells were washed with phosphate-buffered saline (PBS), fixed in 3.5-

4\% paraformaldehyde, permeabilized with $0.1-0.5 \%$ NP-40, and blocked with 1$3 \%$ bovine serum albumin (BSA). Cells were incubated with primary antibodies, anti-RPA194 (C1) [sc-48385, Santa Cruz Biotechnology] and anti- $\gamma \mathrm{H} 2 \mathrm{AX}$ [81299, Abcam], for 2 h at $37^{\circ} \mathrm{C}$ and washed three times with PBS. Cells were incubated with secondary antibodies, Alexa 594-conjugated anti-mouse (A11005, Invitrogen) or Alexa 488-conjugated anti-mouse (A11001, Thermo Fisher) and Alexa 647conjugated anti-rabbit (A21443, Thermo Fisher), for 1 hour at $37^{\circ} \mathrm{C}$, washed three times with PBS, and DNA was stained with Hoechst 3334 (H-21492, Invitrogen). Images were acquired using an Operetta CLS device (40X objective, 9 fields/well) and processed using ColumbusD with the Acapella script: SCR_RPA194_Pol1_complete_v1.script and Pol1.par. The fold change to control was determined. IC50 was determined using GraphPad Prism for Windows (version 6.01) using a three or four-parameter fit.

Cell Viability Assay. Cell viability analysis was performed at Evotec. A375 cells were plated in 384-well plates at a density of 800 cells/well and incubated for 3 days with the compounds at $37{ }^{\circ} \mathrm{C}$ in $5 \% \mathrm{CO}_{2}$. Viability was determined using CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Experiment was reperformed to provide at least 3 replicates.

RNA Isolation and qPCR. Performed at Evotec. A375 cells were treated with the compounds for 6 hours, collected by scraping, and pelleted by centrifugation. RTqPCR performed in triplicate with EXPRESS SYBR® GreenER ${ }^{\text {TM }}$ qPCR SuperMix

Universal (Fisher Scientific). Transcript quantification was measured by $\Delta \Delta C q$ method. The experiment was conducted using three biological repeats in triplicate.

## References

(1) Hein, N.; Hannan, K. M.; George, A. J.; Sanij, E.; Hannan, R. D. The Nucleolus: An Emerging Target for Cancer Therapy. Trends Mol. Med. 2013, 19 (11), 643-654. https://doi.org/10.1016/j.molmed.2013.07.005.
(2) Jacobs, R. Q.; Fuller, K. B.; Cooper, S. L.; Carter, Z. I.; Laiho, M.; Lucius, A. L.; Schneider, D. A. RNA Polymerase I Is Uniquely Vulnerable to the SmallMolecule Inhibitor BMH-21. Cancers 2022, 14 (22), 5544. https://doi.org/10.3390/cancers14225544.
(3) Peltonen, K.; Colis, L.; Liu, H.; Trivedi, R.; Moubarek, M. S.; Moore, H. M.; Bai, B.; Rudek, M. A.; Bieberich, C. J.; Laiho, M. A Targeting Modality for Destruction of RNA Polymerase I That Possesses Anticancer Activity. Cancer Cell 2014, 25 (1), 77-90. https://doi.org/10.1016/j.ccr.2013.12.009.
(4) Colis, L.; Peltonen, K.; Sirajuddin, P.; Liu, H.; Sanders, S.; Ernst, G.; Barrow, J. C.; Laiho, M. DNA Intercalator BMH-21 Inhibits RNA Polymerase I Independent of DNA Damage Response. Oncotarget 2014, 5 (12), 43614369. https://doi.org/10.18632/oncotarget. 2020.
(5) Espinoza, J. A.; Zisi, A.; Kanellis, D. C.; Carreras-Puigvert, J.; Henriksson, M.; Hühn, D.; Watanabe, K.; Helleday, T.; Lindström, M. S.; Bartek, J. The Antimalarial Drug Amodiaquine Stabilizes P53 through Ribosome Biogenesis Stress, Independently of Its Autophagy-Inhibitory Activity. Cell Death Differ. 2020, 27 (2), 773-789. https://doi.org/10.1038/s41418-019-0387-5.
(6) Chen, Y. T.; Chen, J. J.; Wang, H. T. Targeting RNA Polymerase i with Hernandonine Inhibits Ribosomal RNA Synthesis and Tumor Cell Growth. Mol. Cancer Res. 2019, 17 (11), 2294-2305. https://doi.org/10.1158/1541-7786.MCR-19-0402/82067/AM/TARGETING-RNA-POLYMERASE-I-WITHHERNANDONINE.
(7) Caggiano, C.; Guida, E.; Todaro, F.; Bielli, P.; Mori, M.; Ghirga, F.; Quaglio, D.; Botta, B.; Moretti, F.; Grimaldi, P.; Rossi, P.; Jannini, E. A.; Barchi, M.; Dolci, S. Sempervirine Inhibits RNA Polymerase I Transcription Independently from P53 in Tumor Cells. Cell Death Discov. 2020, 6 (1), 115. https://doi.org/10.1038/s41420-020-00345-4.
(8) Peltonen, K.; Colis, L.; Liu, H.; Jäämaa, S.; Zhang, Z.; Hällström, T. A.; Moore, H. M.; Sirajuddin, P.; Laiho, M. Small Molecule BMH-Compounds That Inhibit RNA Polymerase I and Cause Nucleolar Stress. Mol. Cancer Ther. 2014, 13 (11), 2537-2546. https://doi.org/10.1158/1535-7163.MCT-14-0256.
(9) Colis, L.; Ernst, G.; Sanders, S.; Liu, H.; Sirajuddin, P.; Peltonen, K.; DePasquale, M.; Barrow, J. C.; Laiho, M. Design, Synthesis, and Structure-Activity Relationships of Pyridoquinazolinecarboxamides as RNA Polymerase I Inhibitors. J. Med. Chem. 2014, 57 (11), 4950-4961. https://doi.org/10.1021/jm5004842.
(10) Dorado, T. E.; de León, P.; Begum, A.; Liu, H.; Chen, D.; Rajeshkumar, N. V.; Rey-Rodriguez, R.; Hoareau-Aveilla, C.; Alcouffe, C.; Laiho, M.; Barrow, J. C. Discovery and Evaluation of Novel Angular Fused Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors. ACS Med. Chem. Lett. 2022, 13 (4), 608-614.
https://doi.org/10.1021/acsmedchemlett.1c00660.
(11) Baell, J. B.; Holloway, G. A. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. J. Med. Chem. 2010, 53 (7), 2719-2740. https://doi.org/10.1021/jm901137j.
(12) Ames, B. N. The Detection of Chemical Mutagens with Enteric Bacteria. In Chemical Mutagens: Principles and Methods for Their Detection Volume 1; Hollaender, A., Ed.; Springer US: Boston, MA, 1971; pp 267-282. https://doi.org/10.1007/978-1-4615-8966-2_9.
(13) Bissantz, C.; Kuhn, B.; Stahl, M. A Medicinal Chemist's Guide to Molecular Interactions. J. Med. Chem. 2010, 53 (14), 5061-5084.
https://doi.org/10.1021/jm100112j.
(14) Pulipati, Y.; Gurram, V.; Laxmi, S. V.; Satyanarayana, Y.; Singh, K.; Kumar, V.; Sharma, S.; Pottabathini, N.; Iska, V. B. R. Suzuki-Miyaura Coupling of Quinazolines Containing an Unprotected NH2 Group: Synthesis and Biological Testing of Quinazoline Derivatives. Synth. Commun. 2017, 47 (12), 1142-1150. https://doi.org/10.1080/00397911.2017.1315672.
(15) Van Horn, K. S.; Burda, W. N.; Fleeman, R.; Shaw, L. N.; Manetsch, R. Antibacterial Activity of a Series of N2, N4- Disubstituted Quinazoline-2,4Diamines. J. Med. Chem. 2014, 57 (7), 3075-3093. https://doi.org/10.1021/jm500039e.
(16) Maity, A.; Mondal, S.; Paira, R.; Hazra, A.; Naskar, S.; Sahu, K. B.; Saha, P.; Banerjee, S.; Mondal, N. B. A Novel Approach for the One-Pot Synthesis
of Linear and Angular Fused Quinazolinones. Tetrahedron Lett. 2011, 52 (23), 3033-3037. https://doi.org/10.1016/j.tetlet.2011.04.019.
(17) Holmes, J. L.; Almeida, L.; Barlaam, B.; Croft, R. A.; Dishington, A. P.; Gingipalli, L.; Hassall, L. A.; Hawkins, J. L.; Ioannidis, S.; Johannes, J. W.; McGuire, T. M.; Moore, J. E.; Patel, A.; Pike, K. G.; Pontz, T.; Wu, X.; Wang, T.; Zhang, H. J.; Zheng, X. Synthesis of Novel HydroxymethylSubstituted Fused Heterocycles. Synth. Ger. 2016, 48 (8), 1226-1234. https://doi.org/10.1055/s-0035-1561355.
(18) Haveaux, B.; Dekoker, A.; Rens, M.; Sidani, A. R.; Toye, J.; Ghosez, L. AChloro Enamines, Reactive Intermediates for Synthesis: 1-Chloro- $N, N$,2Trimethylpropenylamine: Propenylamine, 1-chloro- $N, N, 2$-trimethyl-. Org. Synth. 1979, 59, 26. https://doi.org/10.1002/0471264180.os059.05.

## Chapter. 4 Progress Towards Generation of a Photoaffinity Labeling Chemical Probe to Determine the Molecular Target of BMH-21 Introduction

BMH-21, shown in Figure 4-1, is the first selective specific inhibitor of RNA polymerase I (Pol I). It was originally discovered in a high-throughput screen (HTS) searching for p53 activators. ${ }^{1}$ Later, it was determined that while BMH-21 acts as an non-genotoxic p53 activator, it also potently repressed growth in p53-null and mutant cell lines. ${ }^{2}$ The compound inhibits Pol I transcription by intercalating into ribosomal DNA (rDNA) with a preference for GC-rich DNA, creating a transcription block, and causing the proteasomal-mediated degradation of the large catalytic subunit, RPA194. Although several rounds of structure-activity relationship (SAR) studies have been carried out, few analogs have retained substantial potency, indicating low tolerance to structural change. Notably, all SARs have been determined by phenotypic measurements of RPA194 degradation. Without structural data to inform compound design, efforts to identify vectors for additional SARs will likely remain difficult. Therefore, gaining insight into how BMH-21 interacts with the rDNA and possibly the Pol I complex could provide rationale for the improved design of future compounds. Herein, we describe efforts to transform BMH-21 into a chemical probe to gain structural insight about its binding interactions and to identify its molecular target.

## Results and Discussion

Several years ago, an attempt was made to perform ${ }^{1} \mathrm{H}$ and NOESY NMR experiments with BMH-21 and a short 10-mer double-stranded DNA
oligonucleotide. Unfortunately, the data collected were not informative and no further experiments were carried out. Instead, we turned to X-ray crystallography as a method to potentially discover specific interactions between BMH-21 and the DNA.

We started this endeavor after noting the structural similarity that BMH-21 shared with 9-amino-DACA, a topoisomerase II (TOP2) poison, along with its reported crystal structure bound to the palindromic DNA hexanucleotide $\mathrm{d}(\mathrm{CGTACG})_{2}$ (PDB: 465D). ${ }^{3}$ BMH-21 does not exhibit any TOP2 inhibitory activity. ${ }^{1}$ However, 9-amino-DACA bears the same dimethylamino carboxamide side chain as $\mathrm{BMH}-21$ attached at the four-position of the acridine and pyridoquinazolinone core for 9-amino-DACA and BMH-21, respectively, highlighted in Figure 4-1. With this striking resemblance, we questioned whether the reported crystallization conditions could be adapted to facilitate BMH-21 - DNA co-crystallization and if BMH-21 would possess a similar orientation to that of 9-amino-DACA when intercalated into dsDNA.


BMH-21


9-amino-DACA

Figure 4-1. Chemical structures of BMH-21 and 9-amino DACA. The dimethylamino carboxamide side chain (highlighted in blue) is observed in both compounds and is attached at the same four-position of the respective fused heteroaromatic core.

Using the same sequence of DNA and starting from the ideal crystallization conditions for 9 -amino-DACA, ${ }^{3}$ several rounds of screening crystallization conditions were conducted to determine optimal conditions for BMH-21 crystallization utilizing vapor diffusion in sitting drops in 96-well format (CrystalQuick 96 Well, Greiner 609101, Hampton Research). Screening conditions included changes to the pH of the inner drop, the percent-concentration ( $\% \mathrm{v} / \mathrm{v}$ ) of precipitant in the inner drop as well as in the outer reservoir, and addition of various divalent cations. The 9-amino-DACA crystallization conditions provided a valuable starting point for further optimization for BMH-21, preventing the need to conduct sparse matrix screening. ${ }^{4}$ This is important given the context with the lowthroughput nature of preparing screening plates and the time required to observe crystal formation (~3-4 weeks). Eventually, optimal conditions for BMH-21 - DNA co-crystallization were determined to be a $2 \mu \mathrm{~L}$ drop containing 0.5 mM DNA, 40 mM sodium cacodylate buffer ( pH 6.0 ), $10 \mathrm{mM} \mathrm{MgCl} 2,2 \mathrm{mM} \mathrm{CoCl} 2,5 \mathrm{mM}$ spermine $\cdot 4 \mathrm{HCl}, 1 \mathrm{mM} \mathrm{BMH}-21 \cdot 2 \mathrm{HCl}$, and $5 \%$ MPD equilibrated against $200 \mu \mathrm{~L}$ of a solution of $31 \%$ MPD in water. With these conditions in hand, acceptably sized and defined crystals containing BMH-21 and DNA could be reliably generated after $\sim 4$ weeks of incubation at $16{ }^{\circ} \mathrm{C}$. The yellow rhombus-shaped crystals were harvested, cryopreserved, and submitted to the Brookhaven National Laboratory Synchrotron Light Source. To our delight, most crystals diffracted at a resolution of $1.5-1.7 \AA$, and a molecular replacement model could be determined (PDB: 1FN1). ${ }^{5}$ Unfortunately, the compound exhibited multiple binding orientations throughout the crystal resulting in a lack of specific base-pair - compound
interactions. The most important information we sought to learn about was specifically how the side chain interacts with the DNA, especially with the sensitivity to change in the side chain we observed with previous SARs. However, due to the amount of variability in the binding orientation throughout the crystal, we were unable to gain any detailed information about the position of the side chain. Crystallization efforts were then discontinued in favor of synthesizing a chemical probe analog of BMH-21 to attempt photoaffinity labeling target identification studies.

Photoaffinity labeling is a popular tool for drug target identification. ${ }^{6,7}$ In general, a small molecule is modified to incorporate a photoreactive group, such as benzophenone, aryl azide, or diazirine. Cells are then treated with the photoprobe and irradiated with UV light, generating a highly reactive intermediate that is able to form a covalent bond between the photoprobe and the biological target. Bioorthogonal conjugation reactions such as the copper (I)-catalyzed azidealkyne cycloaddition ${ }^{8}$ (CuAAC, also referred to as "click chemistry") allows for the incorporation of various reporter groups, such as a fluorophore for detection or biotin for enrichment. ${ }^{9}$ Combined with chemical proteomics, proteome-wide small molecule - protein interactions can be identified using a mass spectrometry-based affinity chromatography approach. ${ }^{10}$ Recent advances have led to the commercial availability of 'all-in-one' chemical reagents that contain both the photoreactive group and the click chemistry handle. ${ }^{6}$

Thus, the goal of this work was to chemically modify BMH-21 to incorporate a photoreactive diazirine moiety and an alkyne "click" handle that would facilitate the addition of a biotin-azide affinity tag. After affinity purification with streptavidin and elution from the bead, mass spectrometry chemical proteomics would be used to identify the molecular target. Recently, several cryo-EM structures of the human Pol I complex have been reported. ${ }^{11-13}$ If the photoprobe were able to pulldown any of the Pol I subunits, the binding site information could potentially be used to model the compound within the reported Pol I structures, allowing for the informed design of more optimal Pol I inhibitors. Diazirine was chosen as the photoreactive moiety because of its small size, ideally limiting the physical perturbation of its incorporation, especially regarding how size-limited the system is as the previous SARs have demonstrated. Furthermore, diazirine has other advantages such as the activation absorption wavelength (350-380 nm) avoiding significant damage to the biological system and its chemical stability in a variety of conditions including strongly basic, strongly acidic, oxidizing, and reducing agents. ${ }^{6}$ In addition to the wide variety of commercially available diazirine building blocks, aliphatic diazirines can typically be synthesized by a well-established three-step route. ${ }^{14}$ For the click chemistry aspect, several biotin-alkyne and biotin-azide reagents with varying linker lengths are commercially available. However, it is generally preferable to incorporate the alkyne tag to the probe to minimize non-specific proteomic labeling. ${ }^{15,16}$ Generation of a BMH-21 chemical probe would require a new SAR study to identify potential sites to incorporate the diazirine and alkyne moieties. Ideally, the chemical probe would exhibit similar RPA194 degradation potency to
that of $\mathrm{BMH}-21$ with an $\mathrm{IC}_{50}<1 \mu \mathrm{M}$. Comparable potencies between the probe and the lead molecule would give confidence that the additional functional groups are noninfluential in binding and activity, supporting that the probe is acting by a similar mechanism of action as the lead molecule. Highlighted in Figure 4-2, we observed that pyrrolidine 2 and piperidine analogs 3-4 of BMH-21 1 retained substantial potency and potentially offered a vector to introduce the diazirine moiety. Importantly, placing the diazirine on the amide side chain is preferable because from previous studies ${ }^{17,18}$ we have observed drastic changes in potency influenced by the side chain, implying that the side chain takes part in key interactions with


1
0.33


3
0.18


2
0.37


Figure 4-2. BMH-21 (1) and previously reported potent analogs (2-4). aRPA194 degradation measured in A375 cells. IC $\mathrm{C}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
the target. To reduce quenching of the reactive carbene intermediate by water that lowers crosslinking yields, placing the photoreactive diazirine close in proximity to the target binding site is critical. ${ }^{19}$ Incorporating the diazirine on the amide side chain would also be preferable from a synthetic chemistry perspective, owing to the large variety of commercially available pyrrolidine and piperidine building blocks. The alkyne click handle initially made more sense to incorporate into the tetracycle core because of the planar nature of the tetracycle and the ease of synthesis from halogenated aromatic building blocks. The specific placement of the alkyne is less crucial, compared to the photoreactive group, as long as the alkyne has sufficient space to participate in the CuAAC reaction and does not lead to a reduction in potency.

We began by surveying the effects that alkyne modifications to the core had on RPA194 degradation potency, shown in Table 4-1. While incorporation of the alkyne was slightly more favorable at the three-position 5 of the tricycle compared to the two-position 6 ( $\mathrm{IC}_{50} 14$ and $>10 \mu \mathrm{M}$, respectively), shortening the tetracycle to a tricycle was overall not tolerated, a previously observed trend (see Chapter 2). Efforts were made to synthesize the 2,3-dialkyne tricycle. However, the acidpromoted cyclocondensation reaction was not amenable with the dihalogenated substrate. Other halogenated 2-aminonicotinic acids and anthranilic acids were also unable to undergo $S_{N} A R$ successfully to produce halogenated tricycles, summarized in Scheme 4-1. Bromination of the tetracycle at the six-position of the tetracycle could be achieved, however, solubility issues after generation of the TIPS-protected alkyne intermediate stunted forward progress (Scheme 4-1). It is
Example

Table 4-1. Modified cores with alkyne handle. aRPA194 degradation measured in A375 cells. $\mathrm{IC}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.



Successful, but poor solubility limits forward synthetic progress.

Scheme 4-1. General synthetic route for various brominated tricycles and attempts to brominate the BMH-21 tetracycle. Except for 39 (Scheme 4-3) and 44 (Scheme 4-4), the acidpromoted cyclocondensation of all other combinations of variously substituted 2-chloronicotinic acids and anthranilic acids was unsuccessful. The BMH-21 tetracycle could be successfully brominated at the six-position. However, poor solubility limited forward synthetic progress.
possible that if the solubility issues could be overcome, integration of the alkyne at the six-position of the tetracycle may provide a potent core as hinted by previous analogs (Chapter 2).

Incorporation of the diazirine moiety on pyrrolidine and piperidine side chains was then surveyed, summarized in Table 4-2. To produce the smallest structural perturbation, spirocyclic diazirines were synthesized from the corresponding pyrrolidinone or piperidinone building blocks. Unfortunately, in all cases 7-12, the spirocyclic diazirine led to a loss of activity $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$. This is troubling considering that the related 4,4-piperidinediol 4 exhibited comparable potency to that of $\mathrm{BMH}-21$ (1) ( $\mathrm{IC}_{50} 0.61$ and $0.33 \mu \mathrm{M}$, respectively). Testing the three- and four-position diazirine piperidine analogs with another known active tetracycle core provided 13 and 14 , but unfortunately, these were inactive as well $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$, confirming that the spirocyclic diazirines were responsible for the loss in activity. One possible explanation could be that the diazirine reduced the basicity of the pyrrolidine/piperidine nitrogen, an important characteristic previously observed to be critical for potency. ${ }^{18}$ To attempt to combat this, methyl groups were added to the linker (10-11) or the two-position of the piperidine (12) to try to restore some electron density to the piperidine nitrogen. Unexpectedly, these compounds were all inactive as well, implying that the inductive reduction in basicity by the diazirine could not be offset by a single methyl group. A second possibility is that the spirocyclic diazirine analogs forced the heterocycle into an unfavorable confirmation for activity. A series of freely rotatable diazirine analogs was then proposed and evaluated, shown in Table 4-3.


Table 4-2. Spirocyclic diazirine analogs. aRPA194 degradation measured in A375 cells. IC50 represents the mean of duplicate independent biological experiments performed in triplicate.
Example

Table 4-3. Freely rotatable diazirine analogs. aRPA194 degradation measured in A375 cells. IC50 represents the mean of duplicate independent biological experiments performed in triplicate.

A minimal addition of the diazirine to the dimethylamino side chain 15 resulted in a loss of activity $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$, possibly reflecting a reduction in amine basicity by the diazirine. Giving the diazirine freedom of rotation and allowing the heterocyclic amine to have freedom to ring-flip led to an improvement in potency. While pyrrolidine 16 remained weakly active ( $\mathrm{IC}_{50} 20 \mu \mathrm{M}$ ), restoring the freedom of rotation to the bond between the diazirine and the piperidine ring had a larger effect (17 and 18, $\mathrm{IC}_{50} 5.4$ and $2.7 \mu \mathrm{M}$, respectively). Interestingly, substitution of the piperidine ring at the four-position 18 resulted in a 2-fold improvement in potency
compared to substitution at the three-position 17, possibly explained by a reduction in the proposed inductive effect by placing the diazirine slightly further away from the piperidine nitrogen. Although small gains in potency were achieved with the freely rotatable diazirine series, these compounds were still outside the desired <1 $\mu \mathrm{M} \mathrm{IC}_{50}$ and lacked an alkyne click handle. Additional work would need to be done to determine where the alkyne handle could be attached without greatly reducing potency.

In order to confirm that the four-position of piperidine could serve as a vector for further SAR, a series of non-diazirine analogs were synthesized and evaluated, represented in Table 4-4. Replacement of the 4,4-diol (4) with 4,4-dimethyl (19) led to a 5 -fold reduction in potency ( $\mathrm{IC}_{50} 0.61$ vs $3.3 \mu \mathrm{M}$, respectively), hinting that the four-position is placed in an environment that is capable of hydrogen bonding. This possibly explains why the spirocyclic diazirines (9, 11-12, and 14) and freely rotatable diazirine 18 were either inactive or exhibited reduced potency. Although the diazirine nitrogens can act as hydrogen bond acceptors, a hydrogen bond donor may be more favorable at the four-position. Creating a spirocycle with cyclopropane 20 also led to a reduction in potency ( $\mathrm{IC}_{50} 2.7 \mu \mathrm{M}$ ), again indicating that while the prevention of ring flipping may contribute to unfavorable binding orientations, the inductive reduction in amine basicity by the diazirine may exhibit a more pronounced effect on potency. Adding a methyl group to the linker between the amide and piperidine (21 and 22 ) or pyrrolidine (23) provided robustly potent analogs ( $\mathrm{IC}_{50} 0.50,0.50$, and $0.06 \mu \mathrm{M}$, respectively). While these analogs were evaluated as racemic mixtures of the two enantiomers, it is possible that isolation


Table 4-4. Non-diazirine amide analogs. aRPA194 degradation measured in A375 cells. IC $5_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
of each stereoisomer could reveal a more favorable stereochemical orientation at the alpha- and beta-positions relative to the amine. Substitution at the four-position of piperidine with large groups such as tert-butyl 24 and phenyl 25 unsurprisingly resulted in a loss of activity ( $\mathrm{IC}_{50}>10 \mu \mathrm{M}$ ), reflecting the space confinements that restrict further building out from the amine. Intriguingly, providing a hydrogen donor in the form of an amine at the four-position of piperidine gave a modestly potent compound (26, $\mathrm{IC}_{50} 1.0 \mu \mathrm{M}$ ) with potential for further functionalization with a trifunctional chemical probe building block. Building from the aliphatic dimethylamine or heterocyclic piperidine with bifunctional building blocks provided 27 and 28, setting a baseline for activity to compare with diazirine incorporation with trifunctional building blocks. Although the potency was still less than desired, the substituted piperidine seemed to have an advantage against the long chain alkyl design (27 and $\mathbf{2 8}$, $\mathrm{IC}_{50} 6.9$ vs $2.5 \mu \mathrm{M}$, respectively).

Finally, a series of bifunctional amide analogs was prepared by utilizing commercially available trifunctional building blocks, summarized in Table 4-5. Notably, although 27 and 28 were weakly potent, addition of the diazirine to the same scaffold caused a complete loss of activity ( 29 and 30 , $\mathrm{IC}_{50}>10 \mu \mathrm{M}$ ). Consistently, the addition of a diazirine moiety to the molecule resulted in severe losses of potency, even with a spatially conservative change from a methylene group to the diazirine. Removing the hydrogen bond accepting carbonyl from the terminal amide of 30 and replacing it with a hydrogen bond donating amine linker restored some activity, albeit weakly, in pyrrolidine (31, IC50 $15 \mu \mathrm{M}$ ). However, using the amine linker at the four-position of the piperidine provided a modestly
Example

Table 4-5. Bifunctional amide analogs. aRPA194 degradation measured in A375 cells. IC ${ }_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
potent bifunctional probe candidate (32, IC $502.1 \mu \mathrm{M}$ ). The presence of a hydrogen bond donor at the four-position of the piperidine seemed important for potency, as
piperazine 33 and diazepane 34 hydrogen bond acceptors were much less potent ( $\mathrm{IC}_{50}>10$ and $6.1 \mu \mathrm{M}$, respectively). Although no photoprobe compound met the desired potency threshold, 32 was chosen to advance to pulldown experiments given its ability to consistently produce full-dose responsive inhibition. The modest potency remains a concern, but we felt that 32 would help establish a baseline for future probes. Critically, addition of the probe components i.e., the diazirine, the alkyne handle, and the alkyl linker only resulted in a roughly 2 -fold decrease in potency, reflecting a moderately tolerable pocket in which the probe arm may extend (Figure 4-3, 26 and 32, $\mathrm{IC}_{50} 1.0$ vs $2.1 \mu \mathrm{M}$ ).

Occasionally, related BMH-21-like compounds and some HTS analogs



Example
RPA194 IC 50 ( $\mu \mathrm{M})^{\mathrm{a}}$

26
1.0

32
2.1

Figure 4-3. Comparison of parent compound 26 and photoprobe 32. aRPA194 degradation measured in A375 cells. IC50 represents the mean of duplicate independent biological experiments performed in triplicate.
exhibited autofluorescence, preventing accurate assessment of RPA194 degradation. As a check to ensure that the diazirine group was not interfering with the fluorescence microscopy readouts of the RPA194 degradation assay, an orthogonal cell viability assay using the CellTiter-Glo reagent was performed, a valid analytic method due to the strong correlation between the decrease in RPA194 and reduction in cancer cell viability. ${ }^{2}$ Importantly, the CellTiter-Glo cell viability relies on luminescence rather than fluorescence as the detection method, preventing the opportunity for autofluorescent compounds to interfere with the readouts. A cell viability survey of twelve compounds including non-diazirine and diazirine equivalents was conducted and compared against the measured RPA194 potencies, summarized in Table 4-6. In general, cell viability IC50's and RPA194 degradation IC50's correlated relatively well. Compounds that potently caused

| Example | Cell <br> Viability <br> IC $_{50}(\mu \mathrm{M})^{\mathbf{a}}$ | RPA194 $^{\text {IC }}{ }_{50}$ <br> $(\mu \mathrm{M})^{\mathbf{b}}$ | Example | Cell <br> Viability <br> IC $_{50}(\boldsymbol{\mu M})^{\mathbf{a}}$ | RPA194 <br> IC $_{50}$ <br> $(\mu \mathrm{M})^{\mathbf{b}}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{1}$ | 0.53 | 0.33 | $\mathbf{1 5}$ | $>10$ | $>10$ |
| $\mathbf{2}$ | 0.20 | 0.37 | $\mathbf{1 6}$ | 18 | 20 |
| $\mathbf{3}$ | 0.82 | 0.18 | $\mathbf{1 8}$ | 4.5 | 2.7 |
| $\mathbf{7}$ | $>10$ | $>10$ | $\mathbf{2 1}$ | 1.6 | 0.5 |
| $\mathbf{9}$ | 4.1 | $>10$ | $\mathbf{2 8}$ | 3.9 | 2.5 |
| $\mathbf{1 1}$ | $>10$ | $>10$ | $\mathbf{3 0}$ | $>10$ | $>10$ |

Table 4-6. Cell viability of selected amide analogs. ${ }^{\text {a Cell viability analysis was determined using }}$ CellTiter-Glo ${ }^{\circledR}$ Luminescent Cell Viability Assay in A375 cells. Data shown represents a single independent biological experiment performed in triplicate. ${ }^{\text {b RPA194 }}$ degradation measured in A375 cells. $\mathrm{IC}_{50}$ represents the mean of duplicate independent biological experiments performed in triplicate.
degradation of RPA194 also potently reduced cell viability, while the opposite was also true. Of possible interest is the discrepancy in potencies between the two assays for compound 9 . While being inactive in the RPA194 degradation assay $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$, it exhibited modest potency in the cell viability assay $\left(\mathrm{IC}_{50} 4.1 \mu \mathrm{M}\right)$. When reviewing the images captured and the raw RPA194 assay data for 9 , there was no apparent autofluorescence observed, indicating that there is potentially a secondary mechanism other than RPA194 degradation that results in reduced cell viability.

A live-cell photoaffinity labelling experiment with 32 was performed in A375 cells by adapting the protocol from Head and Liu. ${ }^{20}$ The general workflow is represented by Figure 4-4 and will be shortly summarized here. A375 cells were treated with one of three conditions: DMSO negative control, $5 \mu \mathrm{M}$ photoprobe (32), or $5 \mu \mathrm{M}$ photoprobe (32) $+1 \mu \mathrm{M}$ competitor (1). Cells were incubated at 37 ${ }^{\circ} \mathrm{C}$ for 15 minutes before washing to remove excess compound and then irradiated with UV light to induce crosslinking. Cells were collected, sonicated, and boiled at $95{ }^{\circ} \mathrm{C}$ to complete lysis and denature proteins. Click chemistry was used to install a biotin handle, and samples were affinity purified using high-capacity streptavidin agarose beads. Whole-sample proteomics sample prep included dithiothreitol reduction, iodoacetamide capping, cleaning with an S-Trap micro spin column, trypsin digestion. and desalting,

Proteins that are pulled down in the DMSO sample are considered nonspecific binders and are treated as the background. Including a competition sample


Figure 4-4. General application of photoaffinity probes. This figure was adapted from Bush et al., 2013. ${ }^{21}$
allows for the determination of specific-binding proteins. Since the competitor (1) is about six-fold more potent than the photoprobe (32) in the RPA194 degradation assay (IC50 0.33 vs $2.1 \mu \mathrm{M}$, respectively), it is expected that 1 should be able to 'outcompete' 32 for specific-binding to target proteins, resulting in a decreased proteomics signal intensity. In general, the concentration of the competitor compound should be at least 20x the concentration of the photoprobe. ${ }^{20}$ However, due to the reduced RPA194 potency exhibited by 32, it was unfeasible to treat cells with an exceptionally high concentration of 1 . Furthermore, $1 \mu \mathrm{M}$ treatment of 1 induces robust Pol I transcription inhibition within 15 minutes, translocation of nucleolin (NCL) out of the nucleolus within 30 minutes, and RPA194 degradation at 2-3 hours post-treatment. ${ }^{2}$ Therefore, the concentrations of both 32 and 1 as
well as the initial incubation period are important to consider for future experiments. The concentration of the photoprobe will influence how many proteins are pulled down: too high of a concentration will lead to increased non-specific binding and background noise while too low of a concentration will lead to reduced signal and may miss low abundance proteins all-together. Fortunately, Pol I related proteins, especially in A375 cells, are exceptionally abundant and should have no problems with signal detection. Similarly, initial incubation times can be experimented with to provide a balance between adequate time for the compound to reach the target but shortly enough to prevent the degradation or translocation of target proteins prior to UV-crosslinking.

In total, 65 proteins were pulled down across the three sample conditions, with 36 proteins identified only in the photoprobe sample and 11 proteins identified in both the photoprobe and competition samples but with enhanced intensity observed in the photoprobe sample relative to the competition sample. The top 10 results for both categories are summarized in Table 4-7. Unfortunately, there were no Pol I transcription related proteins identified in the total list. This could imply that the photoprobe has altered characteristics and does not reach the presumed target in the nucleolus, although the cell-based activity assay readouts do require target engagement in the nucleolus. Confirmation that the photoprobe does inhibit Pol I transcription can be achieved by qPCR and should be performed as a next step. In addition, validation of the top identified proteins such as pyruvate kinase (PKM) and $\alpha$-enolase (ENO1) can be

| Identified in Photoprobe Sample <br> Only | Identified with Enhanced Intensity <br> in Photoprobe Sample Relative to <br> Competition Sample |
| :---: | :---: |
| PKM | ENO1 |
| APOD | TUBB4B |
| PIP | TFRC |
| PFN1 | EPHA2 |
| PRDX2 | TUBA1B |
| EEF2 | EEF1A1 |
| AZGP1 | CFL1 |
| SQSTM1 | HSP90AB1 |
| ALDOA | HSPA8 |
| ACTN1 | UBB |

Table 4-7. Top proteins identified by mass spectrometry proteomics after photoaffinity pulldown with photoprobe 32 in A375 cells.
accomplished by western blot using antibodies for the respective proteins following the biotin pull-down. Since this initial experiment utilized an already relatively high concentration of photoprobe $(5 \mu \mathrm{M})$, it may be necessary to revisit the probe design to identify a more potent compound, requiring further SAR. It is also possible that the probe does not explicitly target the Pol I complex or any transcription related proteins. Many chemotherapeutics inhibit Pol I to some degree, but it often involves DNA damage caused by crosslinking-adducts or topoisomerase poisoning (Chapter 1). BMH-21 does not induce DNA damage nor exhibit any topoisomerase inhibition. ${ }^{2,22}$ BMH-21 and the photoprobe may only intercalate into DNA and
possibly even RNA. Rather than proteomics analysis, it may be more appropriate to attempt to identify nucleic acid binding sites by sequencing. ${ }^{23}$

## Chemistry

The synthesis of compounds 1-4 has been previously reported ${ }^{17,18}$ and is summarized in Scheme 4-2. The acid-promoted cyclocondensation reaction between 2-chloronicotinic acid and 3-amino-2-napthoic acid furnishes the tetracyclic core intermediate 37. Subsequent TBTU-mediated amide coupling is able to provide 1-4.

Scheme 4-3 details the synthesis of 5. In a similar fashion, the acidpromoted cyclocondensation reaction successfully yielded the brominated tricyclic core intermediate 39. The conversion of the carboxylic acid to the ethyl ester was necessary to avoid proto-decarboxylation during the Sonogashira reaction to provide 40. Notably, the length of the alkyl chain portion of the ester was important to hinder the decarboxylation side reaction as carboxylic acid as well as methyl ester were both susceptible to decarboxylation. The ethyl ester was not immune to decarboxylation; however, it occurred on a much slower timescale, allowing for completion of the desired Sonogashira product with minimal degradation and decarboxylation observed. Even so, prolonged heating time would still eventually result in decarboxylation. The palladium ligand choice as well as temperature were also optimized. Typically, Sonogashira reactions utilize a copper cocatalyst that coordinates with the alkyne during the catalytic cycle, ${ }^{24,25}$ however, copper-free methods have been developed ${ }^{26}$ and could be applied to this substrate. A small


Scheme 4-2. General scheme for the synthesis to provide compounds 1-4. Reagents and conditions: (a) HCI, EtOH, reflux. (b) Amine, TBTU, DIPEA, DMF, rt.


Scheme 4-3. Synthesis of compound 5. Reagents and conditions: (a) HCl, EtOH, reflux. (b) TBTU, DIPEA, EtOH, DMF, rt. (c) (Triisopropylsilyl)acetylene, Pd(amphos) ${ }_{2} \mathrm{Cl}_{2}(5 \mathrm{~mol} \%)$, DIPEA, DMF, $120^{\circ} \mathrm{C}$. (d) $6 \mathrm{M} \mathrm{HCl}, 80^{\circ} \mathrm{C}$. (e) 1 M TBAF in THF, DCM, rt. (f) 1 -(2-aminoethyl)piperidine-4,4diol hydrochloride (42), TBTU, DIPEA, DMF, rt.
palladium ligand screen was conducted to overcome the initial issues observed with the lack of oxidative addition. While $\mathrm{PdCl}_{2}\left(\mathrm{PPh}_{3}\right)_{2}$ was unable to show signs of oxidative addition, highly electron dense phosphine ligands such as $\mathrm{P}(\mathrm{tBu})_{3}$ were overreactive and led to increased amounts of the decarboxylated degradation product. A balance of steric and electronic properties was found with $\mathrm{Pd}($ amphos $) \mathrm{Cl}_{2} .{ }^{27,28}$ Using this ligand, the desired product could be obtained with
relatively short reaction times (<2 hours) and with virtually no degradation observed as long as heating times were not substantially lengthened. Temperature was also important for aiding the oxidative addition step. Even with $\mathrm{P}(\mathrm{tBu})_{3}$, temperatures in excess of $100^{\circ} \mathrm{C}$ were required to observe any oxidative addition progress by LCMS. After installation of the alkyne, hydrolysis of the ethyl ester and deprotection of the silyl protecting group on the alkyne provided intermediate 41, which could undergo amide coupling to give 5 .

A similar process was used to produce 6, summarized in Scheme 4-4. Acidpromoted cyclocondensation successfully provided intermediate 44, however, this substrate was unable to produce the ethyl ester by the same TBTU-mediated reaction used in Scheme 4-3. Instead, formation of the acid chloride with oxalyl chloride, followed by the slow addition of ethanol was able to provide the desired ethyl ester. Using the same optimized Sonogashira coupling conditions described above, the alkyne was able to be installed. After hydrolysis of the ethyl ester and deprotection of the alkyne, amide coupling provided 6.

Scheme 4-5 summarizes the general synthetic route to access the spirocyclic diazirine analogs 7-12 utilizing a protocol adapted from Martyloga et al. ${ }^{29}$ The spirocyclic diazirines could be prepared from the respective ketone starting material (47a-f) utilizing a three-step process. Treatment of the ketone with




Scheme 4-4. Synthesis of compound 6. Reagents and conditions: (a) HCI, EtOH, reflux. (b) (COCI)2, DMF (10 mol \%), DCM, rt. (c) EtOH, DCM, rt. (d) (Triisopropylsilyl)acetylene, $\mathrm{Pd}(\mathrm{amphos})_{2} \mathrm{Cl}_{2}(5 \mathrm{~mol} \%)$, DIPEA, DMF, $120^{\circ} \mathrm{C}$. (e) $6 \mathrm{M} \mathrm{HCl}, 80^{\circ} \mathrm{C}$. (f) $1 \mathrm{M} \mathrm{TBAF} \mathrm{in} \mathrm{THF}, \mathrm{DCM}, \mathrm{rt}$. (g) 1-(2-aminoethyl)piperidine-4,4-diol hydrochloride (42), TBTU, DIPEA, DMF, rt.


Scheme 4-5. General scheme for the synthesis to provide compounds 7-12. Reagents and conditions: (a) $7 \mathrm{~N} \mathrm{NH}_{3}$ in MeOH , rt. (b) Hydroxylamine-O-sulfonic acid, $-40^{\circ} \mathrm{C}-\mathrm{rt}$. (c) $\mathrm{I}_{2}, \mathrm{TEA}$, MeOH , rt. (d) 4 M HCl in 1,4-dioxane, MeOH , rt. (e) 2-(Boc-amino)ethyl bromide, DIPEA, MeCN, rt or tert-butyl $N$-(2-bromo-1-methyl-ethyl)carbamate, DIPEA, MeCN, rt. (f) HATU, DIPEA, DMF, rt.
$7 \mathrm{~N} \mathrm{NH}_{3}$ in MeOH followed by hydroxylamine-O-sulfonic acid resulted in a diaziridine intermediate that was subsequently oxidized in the presence of iodine and triethylamine to give the desired diazirine 48a-f. Piperidinone starting materials provided moderate yields of the resultant diazirine product while pyrrolidinone gave substantially lower yields, a pattern also observed by Martyloga et al. ${ }^{29}$ Acidic
deprotection of the Boc-group followed by alkylation with the respective 2-(Bocamino)ethyl bromide and subsequent deprotection yielded the various diamines 49a-f which could be coupled with 37 to provide the diazirine-containing amide analogs 7-12. Scheme 4-6 summarizes the previously reported ${ }^{18}$ synthesis of the angular fused pyridoquinazolinone core 53. Buchwald-Hartwig coupling followed by palladium catalyzed cyclocarbonylation afforded the angular tetracyclic core 52. After saponification of the methyl ester, amide coupling with the respective threeor four-position diazirine substituted piperidine diamine produces 13 and 14.

The same diazirine preparation workflow could also be applied to the ketone substrates with freedom of rotation 54a-d, shown in Scheme 4-7. After a Boc-


Scheme 4-6. Synthesis of compounds 13-14. Reagents and conditions: (a) $\mathrm{Pd}(\mathrm{OAc})_{2}(4 \mathrm{~mol} \%)$, rac-BINAP ( $6 \mathrm{~mol} \%$ ), $\mathrm{Cs}_{2} \mathrm{CO}_{3}$, toluene, $130^{\circ} \mathrm{C}$. (b) $\mathrm{Pd}(\mathrm{OAc})_{2}(10 \mathrm{~mol} \%)$, Xantphos ( $15 \mathrm{~mol} \%$ ), Xantphos Pd G3 (5 mol \%), $\mathrm{K}_{3} \mathrm{PO}_{4}, \mathrm{Mo}(\mathrm{CO})_{6}$, toluene, $100^{\circ} \mathrm{C}$. (c) $2 \mathrm{M} \mathrm{NaOH}, \mathrm{MeOH}$, rt. (d) Amine, HATU, DIPEA, DMF, rt.


Scheme 4-7. General scheme for the synthesis to provide compounds 15-18. Reagents and conditions: (a) $7 \mathrm{~N} \mathrm{NH}_{3}$ in MeOH , rt. (b) Hydroxylamine-O-sulfonic acid, $-40^{\circ} \mathrm{C}-\mathrm{rt}$. (c) $\mathrm{I}_{2}$, TEA, MeOH , rt. (d) 4M HCl in 1,4-dioxane, MeOH, rt. (e) 2-(Boc-amino)ethyl bromide, DIPEA, MeCN, rt. (f) HATU, DIPEA, DMF, rt.
deprotection - alkylation - Boc-deprotection sequence, the resulting diamines 56a-d could undergo amide coupling to provide 15-18.

Synthesis of the non-diazirine containing amide analogs 19-25 is summarized in Scheme 4-8. For any diamines that were not commercially available, they could be synthesized in two steps following the previously described ${ }^{18}$ alkylation - Boc-deprotection sequence followed by amide coupling to provide 19-20 and 24-25. For diamines that were commercially available, the respective amide analogs (21-23) were quickly afforded by simple amide coupling.

Scheme 4-9 details the synthesis of 26 and 27. A two-step amide coupling


Scheme 4-8. General scheme for the synthesis to provide compounds 19-25. Reagents and conditions: (a) 2-(Boc-amino)ethyl bromide, DIPEA, MeCN, rt. (b) 4M HCl in 1,4-dioxane, MeOH , rt. (c) HATU, DIPEA, DMF, rt.
followed by Boc-deprotection was sufficient to produce 26, which could later be used to prepare the photoprobe 32. Similarly, amide coupling followed by Bocdeprotection was able to provide 64. Freeing the amine from the HCl salt was necessary to generate moderate yields of 27 after alkylation with 7-iodoheptyne (62), generated via Finkelstein reaction from 7-chloroheptyne.

The synthesis of 28 is detailed in Scheme 4-10. Alkylation of the foursubstituted piperidine 65 with benzyl (2-bromoethyl)carbamate followed by deprotection of the CBz-group with 10\% palladium on carbon under a hydrogen atmosphere yielded diamine 66 that underwent amide coupling with 37 to generate

60
26

$61 \quad 62$

63
37
64
27

Scheme 4-9. Synthesis of compounds 26-27. Reagents and conditions (a) HATU, DIPEA, DMF, rt. (b) 4 M HCl in 1,4-dioxane, DCM, rt. (c) NaI, Acetone, $80^{\circ} \mathrm{C}$. (d) TBTU, DIPEA, DMF, rt. (e) 4M HCl in 1,4-dioxane, DCM , rt then sat. $\mathrm{NaHCO}_{3}, \mathrm{DCM}$, rt. (f) 62, $\mathrm{K}_{2} \mathrm{CO}_{3}$, DMF, rt.




Scheme 4-10. Synthesis of compound 28. Reagents and conditions: (a) Benzyl (2bromoethyl)carbamate, DIPEA, MeCN, rt. (b) $\mathrm{H}_{2}$ (balloon), Pd/C (10 wt. \%), MeOH, rt. (c) TBTU, DIPEA, DMF, rt. (d) TFA, DCM, rt. (e) hept-6-yn-1-amine, TBTU, DIPEA, DMF, rt.
67. After hydrolysis of the tert-butyl ester with trifluoroacetic acid, a second amide coupling was able to provide 28.

Scheme 4-11 summarizes the synthesis of 29 and 30 . Similar to the synthesis of 27 , the free-base of intermediate 64 could be used to alkylate the trifunctional diazirine-containing building block. In this case, 64 was sufficiently nucleophilic, giving a moderate yield of 29. Less nucleophilic amines provided lower yields, and when amine bases like triethylamine were used, the elimination product was more prevalent than the desired alkylation product. To combat this, several inorganic bases and solvents were screened, and potassium carbonate in DMF was determined to be optimal. In the case of $\mathbf{3 0}$, alkylation of 65 with 2-(Boc-

64
29


67
30

Scheme 4-11. Synthesis of compounds 29-30. Reagents and conditions: (a) 3-but-3-ynyl-3-(2iodoethyl)diazirine, $\mathrm{K}_{2} \mathrm{CO}_{3}$, DMF, rt. (b) 2-(Boc-amino)ethyl bromide, DIPEA, MeCN, rt. (c) 4 M HCl in 1,4-dioxane, $0^{\circ} \mathrm{C}$ - rt. (d) HATU, DIPEA, DMF, $50^{\circ} \mathrm{C}$. (e) TFA, DCM, rt. (f) 2-(3-but-3-ynyldiazirin-$3-\mathrm{yl}$ )ethanamine, HATU, DIPEA, rt.
amino)ethyl bromide and selective deprotection of the Boc-group in the presence of a tert-butyl ester according to Han et al. ${ }^{30}$ afforded diamine 68 that underwent amide coupling with 37 to produce intermediate 67. In a similar fashion to 28,
hydrolysis of the tert-butyl ester followed by amide coupling with a trifunctional diazirine-containing building block provided 30.

The synthesis of $\mathbf{3 1}$ and $\mathbf{3 2}$ is shown in Scheme 4-12. Alkylation of $\mathbf{6 9}$ with benzyl (2-bromoethyl)carbamate provided orthogonally protected primary amines on either side of the molecule and allowed for step-wise deprotection and regioselective functionalization. Deprotection of the CBz-group provided 70, which after amide coupling with 37 gave 71. Then, deprotection of the Boc-group, freeing of the HCl salt, and alkylation with the trifunctional building block afforded 31. Notably, the final alkylation to provide 31 required elevated temperatures even with the optimal alkylation conditions, reflecting the lack of nucleophilicity of the primary amine. In a similar fashion, 32 could be generated from 26 after freeing of the HCl salt and alkylation, also requiring elevated temperatures.

Finally, Scheme 4-13 summarizes the synthesis of 33 and 34. The commercially available diamine 72 could be coupled with 37 to provide intermediate 73. After Boc-deprotection, freeing of the HCl salt, and alkylation, albeit with elevated temperatures to drive the reaction to completion, 33 was produced. In the case of 34 , the diamine 75 was not commercially available but could be synthesized utilizing the orthogonal protecting group strategy used earlier. Alkylation of 74 with benzyl (2-bromoethyl)carbamate and subsequent CBzdeprotection yielded 75, which underwent amide coupling with 37 to provide 76. After Boc-deprotection, freeing of the HCl salt, and alkylation at room temperature, 34 was produced, with the diazepane being slightly more nucleophilic than the



71
31


32

Scheme 4-12. Synthesis of compounds 31-32. Reagents and conditions: (a) Benzyl (2bromoethyl)carbamate, DIPEA, MeCN, rt. (b) $\mathrm{H}_{2}$ (balloon), Pd/C (10 wt. \%), MeOH, rt. (c) HATU, DIPEA, DMF, rt. (d) 4M HCl in 1,4-dioxane, MeOH , rt then $10 \% \mathrm{NaHCO}_{3}, \mathrm{DCM}$, rt. (e) 3-but-3-ynyl-3-(2-iodoethyl)diazirine, $\mathrm{K}_{2} \mathrm{CO}_{3}, \mathrm{DMF}, 50^{\circ} \mathrm{C}$. (f) Sat. $\mathrm{NaHCO}_{3}, \mathrm{DCM}, \mathrm{rt}$.

72
37
73
33


Scheme 4-13. Synthesis of compounds 33-34. Reagents and conditions: (a) HATU, DIPEA, DMF, $50^{\circ} \mathrm{C}$. (b) 4 M HCl in 1,4-dioxane, MeOH , rt then sat. $\mathrm{NaHCO}_{3}$, DCM, rt. (c) 3-but-3-ynyl-3-(2-iodoethyl)diazirine, $\mathrm{K}_{2} \mathrm{CO}_{3}$, DMF, rt - $50^{\circ} \mathrm{C}$. (d) Benzyl (2-bromoethyl)carbamate, DIPEA, MeCN, rt. (e) $\mathrm{H}_{2}$ (balloon), Pd/C (10 wt.\%), MeOH, rt. (f) HATU, DIPEA, DMF, rt. (g) 3-but-3-ynyl-3-(2iodoethyl)diazirine, $\mathrm{K}_{2} \mathrm{CO}_{3}$, DMF, rt.
respective piperazine.

In summary, to participate in target identification studies with BMH-21, a related photoaffinity chemical probe 32 was prepared. SARs again reinforced the heightened sensitivity towards structural change that the target exhibits, with even the most conservative changes to incorporate the photoreactive diazirine group rendering the analogs inactive. Although a chemical probe candidate was generated and a proteomics analysis identified potential target proteins, the modest RPA194 degradation potency exhibited by the photoprobe calls into question whether the probe is working by a similar mechanism to that of BMH-21. To address this, confirmation of Pol I inhibition via qPCR should be carried out. Should a more potent photoprobe be desired, further SAR work will be needed. Although several dozen proteins were identified in the proteomics analysis, none were related to Pol I transcription. This is disappointing given the desire to ideally pulldown some subunit(s) of the Pol I complex and to be able to model the compound binding utilizing the recently reported human Pol I cryo-EM structures ${ }^{11-}$ ${ }^{13}$ to inform future compound design. However, these results may be explained by the photoprobe's modest potency reflecting that the probe may have altered characteristics relative to $\mathrm{BMH}-21$, or that it is possible that the molecule does not interact with a protein interface. The compounds may only interact with DNA and maybe even RNA. Whether the true target is a protein or a nucleic acid, target identification studies are a valuable endeavor to further understand the key pharmacophores and to rationally design optimal compounds.

## Experimental

Synthesis. General Methods. All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on an ISCO CombiFlash Rf or Biotage Isolera using Biotage Flash cartridges with peak detection at 254 nm . Reverse phase purification was accomplished using a Gilson 215 liquid handler equipped with a Phenomenex C18 column ( $150 \mathrm{~mm} \times 20 \mathrm{~mm}$ i.d., $5 \mu \mathrm{~m}$ ). Peak collection was triggered by UV detection at 214 or $254 \mathrm{~nm} .{ }^{1} \mathrm{H}$ NMR spectra were recorded on a Bruker 400 instrument operating at 400 MHz with tetramethylsilane or residual protonated solvent used as a reference. Analytical LC/MS was performed using an Agilent 1260 equipped with autosampler (Agilent Poroshell 120 C18 column (50 $\mathrm{mm} \times 4.6 \mathrm{~mm}$ i.d., $3.5 \mu \mathrm{~m})$; $0.05 \%$ TFA in water/acetonitrile gradient; UV detection at 215 and 254 nm ) and electrospray ionization. All final compounds showed purity greater than $95 \%$ at 215 and 254 nm using this method.

12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961. To a 250 mL round-bottom flask charged with a magnetic stir bar and fitted with a reflux condenser was added 2-chloronicotinic acid ( $8417 \mathrm{mg}, 53.42 \mathrm{mmol}$ ), 3-amino-2-naphthoic acid ( $5000 \mathrm{mg}, 26.71 \mathrm{mmol}$ ), Ethanol ( 45 mL ), and hydrochloric acid (Concentrated) ( $6.69 \mathrm{~mL}, 80.13 \mathrm{mmol}$ ). The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 5 days. The reaction mixture was allowed to cool to room temperature, vacuum filtered, washed with EtOH, and air dried overnight to give 12-oxo-12H-
benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (7.71 g, $26.56 \mathrm{mmol}, 99 \%$ yield) as a yellow solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d ${ }_{6}$ ) $\delta$ ppm $9.15(\mathrm{~s}, 1 \mathrm{H}), 9.03(\mathrm{dd}, J=7.3,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.63(\mathrm{dd}, J=7.0,1.6$ $\mathrm{Hz}, 1 \mathrm{H}), 8.48(\mathrm{~s}, 1 \mathrm{H}), 8.39(\mathrm{dd}, J=7.3,2.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.18$ $(\mathrm{d}, \mathrm{J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.00-7.94(\mathrm{~m}, 1 \mathrm{H}), 7.79-7.72(\mathrm{~m}, 1 \mathrm{H}), 7.67-7.60(\mathrm{~m}, 1 \mathrm{H})$, $7.18(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 6.69(\mathrm{dd}, \mathrm{J}=7.2,6.3 \mathrm{~Hz}, 1 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{17} \mathrm{H}_{10} \mathrm{~N}_{2} \mathrm{O}_{3}$, 290.1; found $291.6[\mathrm{M}+\mathrm{H}]^{+}$.

1-(2-aminoethyl)piperidine-4,4-diol hydrochloride (42). This compound was synthesized as described by ACS Med. Chem. Lett. 2022, 13, 608-614.

7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxylic acid. This compound was synthesized as described by ACS Med. Chem. Lett. 2022, 13, 608-614.

N-(2-(dimethylamino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide (1). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961.

12-oxo-N-(2-(pyrrolidin-1-yl)ethyl)-12H-benzo[g]pyrido[2, 1-b]quinazoline-4carboxamide (2). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961.

12-oxo-N-(2-(piperidin-1-yl)ethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide (3). This compound was synthesized as described by J. Med. Chem. 2014, 57, 4950-4961.

N-(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (4). This compound was synthesized as described by ACS Med. Chem. Lett. 2022, 13, 608-614.

Method A: General amide coupling procedure. To a 20 mL vial charged with a magnetic stir bar was added carboxylic acid ( $0.0345 \mathrm{mmol}, 1 \mathrm{eq}$.), amide coupling reagent ( $0.0379 \mathrm{mmol}, 1.1 \mathrm{eq}$. ), DIPEA ( $1.034 \mathrm{mmol}, 3 \mathrm{eq}$. ), and DMF ( 5 mL ). To the resulting mixture was added amine ( $0.517 \mathrm{mmol}, 1.5 \mathrm{eq}$.) and the reaction mixture was stirred at room temperature for 1 hour then diluted with 15 mL of water and vacuum filtered. The resulting residue was purified via automated NPLC.

Method B: General HCl salt formation procedure. To a 20 mL vial charged with a magnetic stir bar was added amide ( $0.0341 \mathrm{mmol}, 1 \mathrm{eq}$.), DCM ( 1 mL ), and 2 M HCl in $\mathrm{Et}_{2} \mathrm{O}$ ( $0.0682 \mathrm{mmol}, 2 \mathrm{eq}$.). The resulting mixture was stirred at room temperature for 15 minutes then concentrated to dryness.

Method C: General diazirine synthesis procedure. To a 250 mL round-bottom flask charged with a magnetic stir bar was added ketone ( $25.094 \mathrm{mmol}, 1 \mathrm{eq}$.) and 7 N $\mathrm{NH}_{3}$ in MeOH ( $175.66 \mathrm{mmol}, 7$ eq.). The resulting mixture was stirred at room temperature for 4 hours then cooled to $-40{ }^{\circ} \mathrm{C}$. Hydroxylamine-O-sulfonic acid ( 37.641 mmol, 1.5 eq.) was added slowly, and the reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was vacuum filtered through a pad of Celite. The filtrate was collected and concentrated in vacuo. The resulting residue was taken up in $\mathrm{MeOH}(24 \mathrm{~mL})$ and triethylamine ( $52.698 \mathrm{mmol}, 2.1$ eq.) was added. Iodine ( $26.086 \mathrm{mmol}, 1.04 \mathrm{eq}$. ) was added
portionwise slowly until the red color persisted, and the resulting mixture was stirred at room temperature for 1 hour then concentrated in vacuo. The resulting residue was taken up in DCM and washed $1 x$ with $1 \mathrm{M} \mathrm{Na} 2 \mathrm{~S}_{2} \mathrm{O}_{3}$. The organic layer was dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC.

Method D. General diamine alkylation procedure. To a 250 mL round-bottom flask charged with a magnetic stir bar was added amine ( $6.775 \mathrm{mmol}, 1 \mathrm{eq}$.), MeCN (14 mL ), DIPEA ( $27.098 \mathrm{mmol}, 4 \mathrm{eq}$. ), and bromide ( $10.162 \mathrm{mmol}, 1.5 \mathrm{eq}$.). The resulting mixture was stirred at room temperature overnight then concentrated in vacuo. Crude material was purified via automated NPLC.

Method E. General Boc-deprotection procedure. To a 250 mL round-bottom flask charged with a magnetic stir bar was added Boc-protected amine (16 mmol, 1 eq.), $\mathrm{MeOH}(16 \mathrm{~mL})$, and 4 M HCl in 1,4-dioxane (16 mmol, 4 eq .). The resulting mixture was stirred at room temperature overnight then concentrated to dryness and used without further purification.

Method F. General CBz-deprotection procedure. To a 250 mL round-bottom flask charged with a magnetic stir bar was added CBz-protected amine ( $9.602 \mathrm{mmol}, 1$ eq.) and $\mathrm{MeOH}(24 \mathrm{~mL})$. A stream of $\mathrm{N}_{2}$ was blown over the neck of the flask while adding $10 \%$ palladium on carbon ( $0.48 \mathrm{mmol}, 0.05 \mathrm{eq}$.). The flask was capped with a rubber septum, evacuated, refilled with $\mathrm{N}_{2}$, evacuated again, and a balloon containing $\mathrm{H}_{2}$ was inserted by needle. The resulting mixture was stirred vigorously at room temperature overnight. The reaction mixture was vacuum filtered through
a pad of Celite. The filtrate was collected and concentrated in vacuo and used without further purification.

Method G. General amine alkylation procedure. To an 8 mL vial charged with a magnetic stir bar was added amine ( $0.241 \mathrm{mmol}, 1 \mathrm{eq}$. ), DMF ( 2.2 mL ), $\mathrm{K}_{2} \mathrm{CO}_{3}$ ( $0.722 \mathrm{mmol}, 3 \mathrm{eq}$. ), and iodide ( $0.241 \mathrm{mmol}, 1 \mathrm{eq}$.). The resulting mixture was stirred at room temperature for 4 days then diluted with EtOAc and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with $10 \% \mathrm{LiCl}, 1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Crude material was purified via automated NPLC.

Method H. General tert-butyl ester hydrolysis procedure. To a 250 mL roundbottom flask charged with a magnetic stir bar was added tert-butyl ester (2.098 mmol, 1 eq.), DCM ( 21 mL ), and 2,2,2-trifluoroacetic acid ( $91.475 \mathrm{mmol}, 43.6 \mathrm{eq}$.$) .$ The resulting mixture was stirred at room temperature for 1 hour then concentrated in vacuo. The resulting residue was taken up in DCM, concentrated to dryness, repeated $5 x$, and used without further purification.

N-(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-3-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxamide hydrochloride (5). To a 250 mL round-bottom flask charged with a magnetic stir bar was added 2-Chloronicotinic acid (35) (4559 mg, 28.93 mmol ), 2-amino-4-bromo-benzoic acid (38) (5000.mg, 23.14mmol), EtOH (47 mL), and Hydrochloric acid (Concentrated) (1.96mL, 23.84 mmol ). The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 3 days. Added an additional 0.25 eq . 2-

Chloronicotinic acid and heated at $80^{\circ} \mathrm{C}$ for 16 hours. Added an additional 0.5 eq. 2-Chloronicotinic acid and heated at $80^{\circ} \mathrm{C}$ for 3 days. The reaction mixture was allowed to cool to room temperature, vacuum filtered, and washed with EtOH to give 3-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (39) (7.56 g, 23.69 mmol ) as a yellow solid, used without further purification. LCMS (ESI): m/z calcd for $\mathrm{C}_{13} \mathrm{H}_{7} \mathrm{BrN}_{2} \mathrm{O}_{3}, 318.0$; found $319.5[\mathrm{M}+\mathrm{H}]^{+}$.

To a 40 mL vial charged with a magnetic stir bar was added 3-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (39) (1596 mg, 5.001 mmol ), TBTU (2409 mg, 7.503 mmol$)$, DMF ( 15 mL ), DIPEA ( $2.05 \mathrm{~mL}, 15 \mathrm{mmol}$ ), and EtOH ( $0.58 \mathrm{~mL}, 10 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 23 hours. The reaction mixture was diluted with EtOAc and washed $1 x$ with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with $10 \% \mathrm{LiCl}, 1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Prepared dry load by dissolving crude material in excess DCM. Purified via automated NPLC (10-50\% EtOAc/heptane, 120 g silica cartridge) to give ethyl 3-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6carboxylate ( $800 \mathrm{mg}, 2.304 \mathrm{mmol}, 46 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO-d6) $\delta$ ppm $8.88(\mathrm{dd}, J=7.3,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.22(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{dd}$, $J=6.8,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.90(\mathrm{~d}, J=1.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.69(\mathrm{dd}, J=8.6,1.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.12$ (t, $J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.40(\mathrm{q}, ~ J=7.1 \mathrm{~Hz}, 2 \mathrm{H}), 1.36(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{15} \mathrm{H}_{11} \mathrm{BrN}_{2} \mathrm{O}_{3}, 346.0$; found $346.5[\mathrm{M}+\mathrm{H}]^{+}$.

To a 10-20 mL microwave vial charged with a magnetic stir bar was added ethyl 3-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylate (1100 mg, $3.169 \mathrm{mmol})$, DMF ( 16 mL ), ethynyl(triisopropyl)silane ( $1.39 \mathrm{~mL}, 6.337 \mathrm{mmol}$ ), and DIPEA ( $1.73 \mathrm{~mL}, 12.674 \mathrm{mmol})$. The resulting mixture was bubbled with $\mathrm{N}_{2}$ for 5 minutes before adding $\mathrm{Pd}(\mathrm{amphos})_{2} \mathrm{Cl}_{2}(113 \mathrm{mg}, 0.16 \mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $120^{\circ} \mathrm{C}$ for 1.5 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and vacuum filtered through a pad of Celite. The filtrate was washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with $10 \%$ LiCL, $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated NPLC (10-50\% EtOAc/heptane, 120 g silica cartridge) to give ethyl 11-oxo-3-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylate (40) ( $990 \mathrm{mg}, 2.207 \mathrm{mmol}, 70 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta \mathrm{ppm} 8.95(\mathrm{dd}, J=7.4,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.35(\mathrm{~d}, J=$ $8.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.96(\mathrm{~d}, J=1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.84(\mathrm{dd}, J=6.7,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.53(\mathrm{dd}, J=$ 8.3, 1.5 Hz, 1H), $6.87(\mathrm{dd}, J=9.5,4.6 \mathrm{~Hz}, 2 \mathrm{H}), 4.51(\mathrm{q}, J=7.1 \mathrm{~Hz}, 2 \mathrm{H}), 1.46(\mathrm{t}, J$ $=7.1 \mathrm{~Hz}, 3 \mathrm{H}), 1.15(\mathrm{~d}, \mathrm{~J}=2.6 \mathrm{~Hz}, 21 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{26} \mathrm{H}_{32} \mathrm{~N}_{2} \mathrm{O}_{3} \mathrm{Si}$, 448.2; found $448.8[\mathrm{M}+\mathrm{H}]^{+}$.

To a 40 mL vial charged with a magnetic stir bar was added ethyl 11-oxo-3-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylate (40) (990 $\mathrm{mg}, 2.207 \mathrm{mmol})$ and $6 \mathrm{M} \mathrm{HCl}(20 \mathrm{~mL}, 120 \mathrm{mmol})$. The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 13 hours. The reaction mixture was allowed to cool to room temperature, diluted with DCM, and washed $1 x$ with sat. brine. The aqueous layer
was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated NPLC (0-25\% EtOAc/DCM, 40 g silica cartridge) to give 11-oxo-3-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid ( 780 mg , $1.855 \mathrm{mmol}, 84 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H} \operatorname{NMR}\left(400 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta \mathrm{ppm} 16.37$ (s, 1H), $9.08(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.83-8.76(\mathrm{~m}, 1 \mathrm{H}), 8.39(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}$, $1 \mathrm{H}), 7.89(\mathrm{~d}, \mathrm{~J}=1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.61(\mathrm{dd}, J=8.3,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.21-7.09(\mathrm{~m}, 2 \mathrm{H})$, $1.19-1.15(\mathrm{~m}, 30 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{28} \mathrm{~N}_{2} \mathrm{O}_{3} \mathrm{Si}, 420.2$; found 420.8 $[\mathrm{M}+\mathrm{H}]^{+}$.

To a 40 mL vial charged with a magnetic stir bar was added 11-oxo-3-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (780 mg, $1.855 \mathrm{mmol})$, DCM ( 10 mL ), and TBAF ( 1 M in THF) ( $3.89 \mathrm{~mL}, 3.89 \mathrm{mmol}$ ). The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with DCM then sat. brine and 1 M HCl were added. A precipitate formed. The aqueous layer was extracted 10+ times with dichloromethane (until precipitate dissolved). Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, vacuum filtered through a pad of $\mathrm{Na}_{2} \mathrm{SO}_{4}$, and concentrated in vacuo. Dry load prepared by dissolving crude material in excess DCM and adding dry silica gel then concentrating to dryness. Purified via automated NPLC (5-30\% EtOAc/DCM, 40 g silica cartridge) to give 3-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid ( $110 \mathrm{mg}, 0.416 \mathrm{mmol}, 22 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 16.38(\mathrm{~s}, 1 \mathrm{H}), 9.06(\mathrm{dd}, J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H})$, $8.65(\mathrm{~d}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.07(\mathrm{~d}, J=1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.62$
(dd, $J=8.3,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.68(\mathrm{~s}, 1 \mathrm{H}) . \operatorname{LCMS}(E S I): m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{8} \mathrm{~N}_{2} \mathrm{O}_{3}$, 264.1; found $265.0[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 3-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (103 mg, 0.399 mmol ), TBTU ( $188 \mathrm{mg}, 0.586 \mathrm{mmol}$ ), DMF ( 2 mL ), and DIPEA (2 eq.). The resulting mixture was stirred at room temperature for 5 minutes. In a separate vial, 1-(2-aminoethyl)piperidine-4,4-diol hydrochloride ( $115 \mathrm{mg}, 0.585 \mathrm{mmol}$ ) was dissolved in DMF ( 2 mL ) and DIPEA (2 eq.) then added dropwise to the reaction mixture and stirred at room temperature for 23 hours. The reaction mixture was diluted with ~ 16 mL water and stirred at room temperature for 30 minutes then vacuum filtered. Product found in residue and filtrate. Filtrate diluted with EtOAc and aqueous layer pH adjusted from $\sim 6$ to $\sim 9$ with $10 \% \mathrm{NaHCO}_{3}$. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with $10 \% \mathrm{LiCl}, 1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Attempted to purify several times with $0-5 \% \mathrm{MeOH} / \mathrm{DCM}$ and $0-20 \% \mathrm{MeOH} / \mathrm{DCM}$ gradients but no separation achieved. Acidified with 0.15 mL 2 N HCl , dissolved in MeOH , and purified via automated RPLC (10-50\% MeCN/water 30x150 LUNA). Collected fractions were concentrated in vacuo and azeotroped $3 x$ with MeCN to dryness. Took up in DCM, added 1 mL 2 M HCl in $\mathrm{Et}_{2} \mathrm{O}$, and stirred at room temperature for 15 minutes then concentrated to dryness and azeotroped TFA with $\mathrm{Et}_{2} \mathrm{O}$ to give N -(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-3-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxamide hydrochloride (5) (108 mg, $0.244 \mathrm{mmol}, 63 \%$ yield) as a yellow-brown solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.28 (s, 1H), 11.05
$(\mathrm{t}, J=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 9.02(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.66(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H})$, $8.34(\mathrm{~d}, J=1.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.29(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.58(\mathrm{dd}, J=8.3,1.6 \mathrm{~Hz}, 1 \mathrm{H})$, $7.24(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.67(\mathrm{~s}, 1 \mathrm{H}), 3.96(\mathrm{q}, \mathrm{J}=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.85(\mathrm{~s}, 2 \mathrm{H}), 3.51-$ $3.47(\mathrm{~m}, 4 \mathrm{H}), 2.97-2.85(\mathrm{~m}, 2 \mathrm{H}), 2.55(\mathrm{~s}, 1 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{22} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{4}, 406.2$; found $406.7[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-2-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxamide hydrochloride (6). To a 250 mL round-bottom flask charged with a magnetic stir bar was added 2-Chloronicotinic acid (35) (4559 mg, 28.937 mmol ), 2-amino-5-bromo-benzoic acid (43) (5000 mg, 23.145 mmol ), EtOH $(47 \mathrm{~mL})$, and Hydrochloric acid (Concentrated) ( $1.96 \mathrm{~mL}, 23.839 \mathrm{mmol}$ ). The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 2 days. The reaction mixture was allowed to cool to room temperature, vacuum filtered, and washed with EtOH to give 2-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (44) (3.89 g, 12.190 mmol, $53 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz, DMSO-d6) $\delta \mathrm{ppm} 16.32$ (s, $1 \mathrm{H}), 9.07(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.69-8.62(\mathrm{~m}, 1 \mathrm{H}), 8.44(\mathrm{~d}, J=2.5 \mathrm{~Hz}, 1 \mathrm{H})$, $8.14(\mathrm{dd}, J=8.8,2.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.91(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.31(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{13} \mathrm{H}_{7} \mathrm{BrN}_{2} \mathrm{O}_{3}, 318.0$; found $318.5[\mathrm{M}+\mathrm{H}]^{+}$.

To a 250 round-bottom flask charged with a magnetic stir bar was added 2-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (44) (1500 mg, $4.701 \mathrm{mmol})$, DCM ( 24 mL ), oxalyl chloride ( $0.49 \mathrm{~mL}, 5.641 \mathrm{mmol}$ ), and 1M DMF in DCM ( $0.47 \mathrm{~mL}, 0.47 \mathrm{mmol}$ ) (dropwise). The resulting mixture was stirred at room temperature for 30 minutes then concentrated in vacuo. The residue was taken up
in DCM ( 24 mL ) and EtOH ( $0.41 \mathrm{~mL}, 7.051 \mathrm{mmol}$ ) was added dropwise. The reaction mixture was stirred at room temperature for 30 minutes then concentrated to dryness to give ethyl 2-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6carboxylate ( $1.94 \mathrm{~g}, 5.588 \mathrm{mmol}$ ) as a yellow solid, used without further purification. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 8.90(\mathrm{dd}, J=7.4,1.6 \mathrm{~Hz}, 1 \mathrm{H})$, $8.40(\mathrm{~d}, \mathrm{~J}=2.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.08-8.00(\mathrm{~m}, 2 \mathrm{H}), 7.68(\mathrm{~d}, \mathrm{~J}=8.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.13(\mathrm{t}, \mathrm{J}=$ $7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.40(\mathrm{q}, J=7.1 \mathrm{~Hz}, 2 \mathrm{H}), 1.36(\mathrm{t}, J=7.1 \mathrm{~Hz}, 3 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{11} \mathrm{BrN}_{2} \mathrm{O}_{3}, 346.0$; found $347.6[\mathrm{M}+\mathrm{H}]^{+}$.

To a 10-20 mL microwave vial charged with a magnetic stir bar was added ethyl 2-bromo-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylate (1.5 g, 4.321 $\mathrm{mmol})$, DMF ( 11 mL ), ethynyl(triisopropyl)silane ( $1.9 \mathrm{~mL}, 8.642 \mathrm{mmol}$ ), and DIPEA ( $2.36 \mathrm{~mL}, 17.283 \mathrm{mmol}$ ). The resulting mixture was bubbled with $\mathrm{N}_{2}$ for 5 minutes before adding $\mathrm{Pd}(\text { amphos })_{2} \mathrm{Cl}_{2}(153 \mathrm{mg}, 0.216 \mathrm{mmol})$. The reaction mixture was capped, sealed, and heated at $120^{\circ} \mathrm{C}$ for 2 hours. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc, and vacuum filtered through a pad of Celite. The filtrate was washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with EtOAc. Combined organic layers were washed 1 x with $10 \% \mathrm{LiCl}, 1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated NPLC (5-50\% EtOAc/heptane, 330 g silica cartridge) to give ethyl 11-oxo-2-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylate (45) ( $920 \mathrm{mg}, 2.051 \mathrm{mmol}, 47 \%$ yield) as a greenishgold solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta \mathrm{ppm} 8.89(\mathrm{dd}, \mathrm{J}=7.3,1.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.30$ (d, $J=2.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{dd}, J=6.7,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.90(\mathrm{dd}, J=8.6,2.0 \mathrm{~Hz}, 1 \mathrm{H})$,
$7.68(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.12(\mathrm{t}, J=7.0 \mathrm{~Hz}, 1 \mathrm{H}), 4.40(\mathrm{q}, J=7.1 \mathrm{~Hz}, 2 \mathrm{H}), 1.36(\mathrm{t}$, $J=7.1 \mathrm{~Hz}, 2 \mathrm{H}), 1.13(\mathrm{~d}, J=3.3 \mathrm{~Hz}, 21 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{26} \mathrm{H}_{32} \mathrm{~N}_{2} \mathrm{O}_{3} \mathrm{Si}$, 448.2; found $448.8[\mathrm{M}+\mathrm{H}]^{+}$.

To a 40 mL vial charged with a magnetic stir bar was added ethyl 11-oxo-2-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylate (45) (920 $\mathrm{mg}, 2.051 \mathrm{mmol})$ and $6 \mathrm{M} \mathrm{HCl}(18.46 \mathrm{~mL}, 110.74 \mathrm{mmol})$. The resulting mixture was heated at $80^{\circ} \mathrm{C}$ for 4 hours. The reaction mixture was allowed to cool to room temperature, diluted with DCM, and washed 1 x with sat. brine. The aqueous layer was extracted $3 x$ with DCM. Combined organic layers were washed $1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Purified via automated NPLC (0-25\% EtOAc/DCM, 80 g silica cartridge) to give 11-oxo-2-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid ( 490 mg , $1.165 \mathrm{mmol}, 57 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm}$ $16.38(\mathrm{~s}, 1 \mathrm{H}), 9.08(\mathrm{dd}, J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.67(\mathrm{dd}, J=7.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.32$ (d, $J=2.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.99(\mathrm{dd}, J=8.6,1.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.91(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.31$ (dd, $J=9.5,4.7 \mathrm{~Hz}, 1 \mathrm{H}), 1.13(\mathrm{~d}, J=3.6 \mathrm{~Hz}, 21 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{28} \mathrm{~N}_{2} \mathrm{O}_{3} \mathrm{Si}, 420.2$; found $420.8[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 11-oxo-2-((triisopropylsilyl)ethynyl)-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (467 mg, $1.110 \mathrm{mmol})$, $\mathrm{DCM}(6 \mathrm{~mL})$, and $\operatorname{TBAF}(1 \mathrm{M}$ in THF) $(2.33 \mathrm{~mL}, 2.33 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 26 hours. The reaction mixture was diluted with DCM and washed 1 x with sat. brine. The aqueous layer
was extracted $3 x$ with DCM. Combined organic layers were washed 1 x with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Prepared dry load by dissolving crude material in excess DCM and adding dry silica gel then concentrating to dryness. Purified via automated NPLC (0-25\% EtOAc/DCM, 20 g silica cartridge) to give 2-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6carboxylic acid (46) ( $250 \mathrm{mg}, 0.946 \mathrm{mmol}, 85 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 16.40 (s, 1H), 9.07 (dd, $J=7.2,1.6 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.67 (dd, $J=7.0,1.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.36(\mathrm{~d}, J=2.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.01(\mathrm{dd}, J=8.6,2.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.91$ (d, $J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.32(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.45(\mathrm{~s}, 1 \mathrm{H}) . \operatorname{LCMS}(\mathrm{ESI}): m / z$ calcd for $\mathrm{C}_{15} \mathrm{H}_{8} \mathrm{~N}_{2} \mathrm{O}_{3}$, 264.1; found $264.6[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added 2-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxylic acid (46) (50 mg, 0.189 mmol ), TBTU (92 mg, 0.287 mmol ), DMF ( 2 mL ), and DIPEA (2 eq.). The resulting mixture was stirred at room temperature for 5 minutes. In a separate vial, 1-(2-aminoethyl)piperidine-4,4-diol hydrochloride ( $56 \mathrm{mg}, 0.285 \mathrm{mmol}$ ) was dissolved in DMF ( 2 mL ) and DIPEA (2 eq.) was added. This solution was added to the reaction mixture and was stirred at room temperature for 20 hours. By LCMS, a small amount of unreacted SM remained. Added 0.5 eq. additional TBTU and stirred at room temperature for 24 hours. No change observed by LCMS. The reaction mixture was diluted with $\sim 16 \mathrm{~mL}$ water and vacuum filtered. Product found in filtrate by LCMS. The filtrate was extracted $3 x$ with EtOAc. Combined organic layers were washed $1 x$ with $10 \% \mathrm{LiCl}, 1 x$ with sat. brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated in vacuo. Attempted to purify via automated NPLC (10-

60\% EtOAc/DCM, 12 g silica cartridge). Switched solvent system to 5\% $\mathrm{MeOH} / \mathrm{DCM}$ to elute N -(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-2-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6-carboxamide ( $16 \mathrm{mg}, 0.039 \mathrm{mmol}$ ) as a yellow film. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{22} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{4}, 406.2$; found $406.8[\mathrm{M}+\mathrm{H}]^{+}$.

To a 20 mL vial charged with a magnetic stir bar was added N -(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-2-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline-6carboxamide (16 mg, 0.039 mmol$)$, $\mathrm{DCM}(1 \mathrm{~mL})$, and 4 M HCl in dioxane ( 0.02 mL , $0.08 \mathrm{mmol})$. The resulting mixture was stirred at room temperature for 1.5 hours then concentrated to dryness. Purified via automated RPLC (10-50\% MeCN/water $50 \times 21.2 \mathrm{~mm}$ LUNA), loaded in methanol. Collected fractions were concentrated in vacuo and azeotroped with MeCN to dryness. Taken up in 2 mL DCM and added 1 mL 2 M HCl in $\mathrm{Et}_{2} \mathrm{O}$. Stirred at room temperature for 15 minutes then concentrated to dryness. Azeotroped remaining TFA with 1,4-dioxane and dried to give N -(2-(4,4-dihydroxypiperidin-1-yl)ethyl)-2-ethynyl-11-oxo-11H-pyrido[2,1-b]quinazoline6 -carboxamide hydrochloride (6) (6 mg, $0.014 \mathrm{mmol}, 36 \%$ yield) as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.03 (d, $\left.J=6.1 \mathrm{~Hz}, 1 \mathrm{H}\right), 10.47(\mathrm{~s}, 1 \mathrm{H}), 9.08$ - $9.01(\mathrm{~m}, 1 \mathrm{H}), 8.67(\mathrm{~d}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.36(\mathrm{~d}, J=2.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.12(\mathrm{~d}, J=8.6$ $\mathrm{Hz}, 1 \mathrm{H}), 8.07-8.00(\mathrm{~m}, 1 \mathrm{H}), 7.27(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.44(\mathrm{~s}, 1 \mathrm{H}), 3.93(\mathrm{~d}, \mathrm{~J}=6.0$ $\mathrm{Hz}, 1 \mathrm{H}$ ), 3.86 (s, 5H), $2.80(\mathrm{~s}, 2 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{22} \mathrm{H}_{22} \mathrm{~N}_{4} \mathrm{O}_{4}, 406.2$; found $406.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(1,2,5-triazaspiro[2.4]hept-1-en-5-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (7). This compound was synthesized
from 1-N-Boc-3-pyrrolidinone (47a) (5000 mg, 26.995 mmol ) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide (1158 mg, 5.167 mmol ) according to Method D, Bocdeprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol ) using HATU (145 mg, 0.381 mmol ) at $50^{\circ} \mathrm{C}$ according to Method A , and HCl salt formation according to Method B to give $18 \mathrm{mg}, 0.040 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta \mathrm{ppm} 11.23$ (s, 1H), 10.79 (s, 1H), 9.14 (s, 1H), 8.96 (dd, $J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.61-8.54(\mathrm{~m}, 2 \mathrm{H}), 8.33(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.11$ (d, $J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.72(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}$, 1H), 4.00 (s, 1H), 3.89 (s, 2H), 3.60 (s, 2H), 1.80 (s, 1H). LCMS (ESI): m/z calcd for $\mathrm{C}_{23} \mathrm{H}_{20} \mathrm{~N}_{6} \mathrm{O}_{2}, 412.2$; found $412.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(1,2,5-triazaspiro[2.5]oct-1-en-5-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (8). This compound was synthesized from 1-N-Boc-3-piperidinone (47b) (5000 mg, 25.094 mmol$)$ according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide ( $6407 \mathrm{mg}, 28.6 \mathrm{mmol}$ ) according to Method D, Bocdeprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol ) using HATU (145 mg, 0.381 mmol ) according to Method A , and HCl salt formation according to Method B to give $21 \mathrm{mg}, 0.045 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta$ ppm 11.14 (t, J = $5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.96$ (s, 1H), 9.12 (s, 1H), 8.95 (dd, $J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.59(\mathrm{~s}, 1 \mathrm{H}), 8.54(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}$,
$J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.58(\mathrm{~m}, 1 \mathrm{H})$, $7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.91(\mathrm{q}, J=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.75(\mathrm{~d}, J=12.5 \mathrm{~Hz}, 1 \mathrm{H}), 3.67-$ $3.58(\mathrm{~m}, 1 \mathrm{H}), 3.50(\mathrm{~s}, 1 \mathrm{H}), 3.23(\mathrm{~d}, J=10.7 \mathrm{~Hz}, 1 \mathrm{H}), 2.91-2.85(\mathrm{~m}, 1 \mathrm{H}), 2.13(\mathrm{~d}$, $J=13.3 \mathrm{~Hz}, 1 \mathrm{H}), 2.03-1.91(\mathrm{~m}, 2 \mathrm{H}), 0.87(\mathrm{~d}, J=14.2 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{22} \mathrm{~N}_{6} \mathrm{O}_{2}, 426.2$; found $426.5[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(1,2,6-triazaspiro[2.5]oct-1-en-6-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (9). This compound was synthesized from piperidin-4-one hydrochloride (3328 mg, 24.545 mmol ) and 2-(Bocamino)ethyl bromide ( $5000 \mathrm{mg}, 22.311 \mathrm{mmol}$ ) according to Method D, followed by diazirine formation according to Method C, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol$)$ using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50{ }^{\circ} \mathrm{C}$ according to Method $A$, and HCl salt formation according to Method $B$ to give 30 $\mathrm{mg}, 0.065 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.20$ $(\mathrm{s}, 1 \mathrm{H}), 11.02(\mathrm{~s}, 1 \mathrm{H}), 9.11(\mathrm{~s}, 1 \mathrm{H}), 8.98-8.92(\mathrm{~m}, 1 \mathrm{H}), 8.66(\mathrm{~s}, 1 \mathrm{H}), 8.59-8.53$ $(\mathrm{m}, 1 \mathrm{H}), 8.31(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.77-7.71(\mathrm{~m}, 1 \mathrm{H})$, $7.65-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.12-7.05(\mathrm{~m}, 1 \mathrm{H}), 3.99(\mathrm{q}, J=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.74(\mathrm{~d}, J=12.0$ $\mathrm{Hz}, 2 \mathrm{H}), 3.53(\mathrm{q}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.44(\mathrm{q}, J=10.5 \mathrm{~Hz}, 2 \mathrm{H}), 2.48-2.39(\mathrm{~m}, 2 \mathrm{H})$, 0.98 (d, $J=15.0 \mathrm{~Hz}, 2 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{24} \mathrm{H}_{22} \mathrm{~N}_{6} \mathrm{O}_{2}$, 426.2; found $426.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(1-(1,2,5-triazaspiro[2.5]oct-1-en-5-yl)propan-2-yl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (10). This
compound was synthesized 1-N-Boc-3-piperidinone (47b) (5000 mg, 25.094 mmol) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with tert-butyl $N$-(2-bromo-1-methyl-ethyl)carbamate (2420 $\mathrm{mg}, 10.163 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol$)$ using HATU (145 mg, 0.381 mmol ) at $50{ }^{\circ} \mathrm{C}$ according to Method $A$, and HCl salt formation according to Method $B$ to give 18 $\mathrm{mg}, 0.038 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d6) $\delta \mathrm{ppm} 11.28$ (d, $J=15.4 \mathrm{~Hz}, 1 \mathrm{H}), 10.02(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.98(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.55$ $(\mathrm{s}, 1 \mathrm{H}), 8.48(\mathrm{~s}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.17(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.62(\mathrm{dd}, J$ $=8.5,6.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.10(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.64(\mathrm{~s}, 1 \mathrm{H}), 4.54(\mathrm{~s}, 1 \mathrm{H}), 3.07(\mathrm{~d}, \mathrm{~J}=$ $13.4 \mathrm{~Hz}, 1 \mathrm{H}), 2.15-1.79(\mathrm{~m}, 3 \mathrm{H}), 1.47(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{6} \mathrm{O}_{2}, 440.2$; found $441.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(1-(1,2,6-triazaspiro[2.5]oct-1-en-6-yl)propan-2-yl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (11). This compound was synthesized from 1-Boc-4-piperidinone (47c) (5000 mg, 25.094 mmol) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with tert-butyl $N$-(2-bromo-1-methyl-ethyl)carbamate (2420 $\mathrm{mg}, 10.163 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol$)$ using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50{ }^{\circ} \mathrm{C}$ according to Method A , and HCl salt formation according to Method B to give 18 $\mathrm{mg}, 0.037 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6$ ) $\delta \mathrm{ppm} 11.33(\mathrm{~d}$,
$J=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 9.70(\mathrm{~s}, 1 \mathrm{H}), 9.15(\mathrm{~s}, 1 \mathrm{H}), 9.00-8.95(\mathrm{~m}, 1 \mathrm{H}), 8.59-8.54(\mathrm{~m}$, $1 \mathrm{H}), 8.51(\mathrm{~s}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.18(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.76(\mathrm{dd}, J=$ 8.4, 6.7 Hz, 1H), $7.66-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.13-7.07(\mathrm{~m}, 1 \mathrm{H}), 4.68(\mathrm{~s}, 1 \mathrm{H}), 3.92(\mathrm{~s}$, $1 \mathrm{H}), 3.43(\mathrm{~s}, 3 \mathrm{H}), 2.20(\mathrm{~d}, \mathrm{~J}=39.9 \mathrm{~Hz}, 2 \mathrm{H}), 1.51(\mathrm{~d}, \mathrm{~J}=6.6 \mathrm{~Hz}, 3 \mathrm{H}), 1.16(\mathrm{~d}, \mathrm{~J}=$ $13.8 \mathrm{~Hz}, 1 \mathrm{H}$ ), $1.08(\mathrm{~d}, J=15.4 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{6} \mathrm{O}_{2}$, 440.2; found $440.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(5-methyl-1,2,6-triazaspiro[2.5]oct-1-en-6-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (12). This compound was synthesized from tert-butyl 2-methyl-4-oxo-piperidine-1carboxylate ( $\mathbf{4 7 d}$ ) ( $2000 \mathrm{mg}, 9.378 \mathrm{mmol}$ ) according to Method C, followed by Bocdeprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide ( $2039 \mathrm{mg}, 9.098 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (215 mg, 0.741 mmol$)$ using HATU (310 mg, 0.815 mmol ) at $50^{\circ} \mathrm{C}$ according to Method A , and HCl salt formation according to Method B to give $32 \mathrm{mg}, 0.067 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d 6 ) $\delta$ ppm 11.29 - $11.19(\mathrm{~m}, 1 \mathrm{H}), 10.61(\mathrm{~s}, 1 \mathrm{H}), 9.13(\mathrm{~s}, 1 \mathrm{H}), 8.99-8.92$ (m, 1H), $8.66(\mathrm{~d}, J=3.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.59-8.53(\mathrm{~m}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.3 \mathrm{~Hz}, 1 \mathrm{H})$, $8.11(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.11-7.04(\mathrm{~m}$, $1 \mathrm{H}), 4.02-3.92(\mathrm{~m}, 3 \mathrm{H}), 3.87-3.53(\mathrm{~m}, 2 \mathrm{H}), 3.53-3.46(\mathrm{~m}, 1 \mathrm{H}), 2.33-2.23(\mathrm{~m}$, $1 \mathrm{H}), 2.18-2.03(\mathrm{~m}, 1 \mathrm{H}), 1.44(\mathrm{dd}, \mathrm{J}=20.8,6.6 \mathrm{~Hz}, 3 \mathrm{H}), 1.25-1.06(\mathrm{~m}, 1 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{6} \mathrm{O}_{2}$, 440.2 ; found $440.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(1,2,5-triazaspiro[2.5]oct-1-en-5-yl)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-bJquinazoline-12-carboxamide hydrochloride (13). This compound was synthesized from 1-N-Boc-3-piperidinone (47b) ( $5000 \mathrm{mg}, 25.094 \mathrm{mmol}$ ) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide ( $6407 \mathrm{mg}, 28.6 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxylic acid ( $100 \mathrm{mg}, 0.345$ mmol) using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) according to Method A, and HCl salt formation according to Method $B$ to give $11 \mathrm{mg}, 0.024 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 10.86(\mathrm{~s}, 1 \mathrm{H}), 10.64(\mathrm{t}, \mathrm{J}=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 9.18$ (dd, $J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.84-8.78(\mathrm{~m}, 1 \mathrm{H}), 8.59(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.22$ (d, $J=8.8 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.16-8.10(\mathrm{~m}, 1 \mathrm{H}), 7.97(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.93-7.86(\mathrm{~m}$, $2 \mathrm{H}), 7.38(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.00(\mathrm{q}, J=6.5 \mathrm{~Hz}, 2 \mathrm{H}), 3.76(\mathrm{~d}, J=12.5 \mathrm{~Hz}, 1 \mathrm{H})$, $3.66-3.42(\mathrm{~m}, 2 \mathrm{H}), 3.24(\mathrm{~d}, J=10.8 \mathrm{~Hz}, 1 \mathrm{H}), 2.85(\mathrm{~d}, J=13.4 \mathrm{~Hz}, 1 \mathrm{H}), 2.14-$ $1.87(\mathrm{~m}, 1 \mathrm{H}), 0.88(\mathrm{~d}, \mathrm{~J}=14.5 \mathrm{~Hz}, 1 \mathrm{H})$. LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{24} \mathrm{H}_{22} \mathrm{~N}_{6} \mathrm{O}_{2}$, 426.2; found $426.9[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(1,2,6-triazaspiro[2.5]oct-1-en-6-yl)ethyl)-7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12-carboxamide hydrochloride (14). This compound was synthesized from piperidin-4-one hydrochloride ( $3328 \mathrm{mg}, 24.545 \mathrm{mmol}$ ) and 2-(Boc-amino)ethyl bromide ( $5000 \mathrm{mg}, 22.311 \mathrm{mmol}$ ) according to Method D, followed by diazirine formation according to Method C, Boc-deprotection according to Method E, amide coupling with 7-oxo-7H-benzo[h]pyrido[2,1-b]quinazoline-12carboxylic acid ( $100 \mathrm{mg}, 0.345 \mathrm{mmol}$ ) using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ )
according to Method A , and HCl salt formation according to Method $B$ to give 21 $\mathrm{mg}, 0.045 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.00$ (s, $1 \mathrm{H}), 10.68(\mathrm{t}, J=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 9.17(\mathrm{dd}, J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.87-8.81(\mathrm{~m}, 1 \mathrm{H})$, 8.60 (dd, $J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.21(\mathrm{~d}, J=8.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.12(\mathrm{dd}, J=7.9,1.4 \mathrm{~Hz}$, $1 \mathrm{H}), 7.99-7.86(\mathrm{~m}, 3 \mathrm{H}), 7.38(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.09(\mathrm{q}, J=6.5 \mathrm{~Hz}, 2 \mathrm{H}), 3.73(\mathrm{~d}$, $J=12.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.56(\mathrm{q}, J=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.44(\mathrm{q}, J=11.2 \mathrm{~Hz}, 2 \mathrm{H}), 2.45-2.35$ (m, 2H), 0.97 (d, $J=15.1 \mathrm{~Hz}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{24} \mathrm{H}_{22} \mathrm{~N}_{6} \mathrm{O}_{2}, 426.2$; found $426.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(methyl((3-methyl-3H-diazirin-3-yl)methyl)amino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (15). This compound was synthesized from tert-butyl N -acetonyl- N -methyl-carbamate (54a) ( $2000 \mathrm{mg}, 10.681 \mathrm{mmol}$ ) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide (1860 $\mathrm{mg}, 8.3 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol$)$ using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50{ }^{\circ} \mathrm{C}$ according to Method $A$, and HCl salt formation according to Method $B$ to give 20 $\mathrm{mg}, 0.044 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta$ ppm 11.24 (t, $J=5.8 \mathrm{~Hz}, 1 \mathrm{H}), 10.38(\mathrm{~s}, 1 \mathrm{H}), 9.13(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.61(\mathrm{~s}$, $1 \mathrm{H}), 8.55(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.4 \mathrm{~Hz}$, $1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.93(\mathrm{~d}, J=$ $6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.44(\mathrm{~s}, 1 \mathrm{H}), 3.16(\mathrm{~s}, 1 \mathrm{H}), 3.05-3.01(\mathrm{~m}, 3 \mathrm{H}), 1.26(\mathrm{~s}, 3 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{23} \mathrm{H}_{22} \mathrm{~N}_{6} \mathrm{O}_{2}$, 414.2 ; found $414.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(3-(3-methyl-3H-diazirin-3-yl)pyrrolidin-1-yl)ethyl)-12-oxo-12H-
benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (16). This compound was synthesized from tert-butyl 3-acetylpyrrolidine-1-carboxylate (54b) (1000 mg, 4.689 mmol ) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide (1581 $\mathrm{mg}, 7.055 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (549 mg, 1.891 mmol$)$ using HATU ( $791 \mathrm{mg}, 2.080 \mathrm{mmol}$ ) according to Method A , and HCl salt formation according to Method $B$ to give $8 \mathrm{mg}, 0.017 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.23(\mathrm{~d}, J=6.6 \mathrm{~Hz}, 1 \mathrm{H})$, $10.22(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{~d}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.64(\mathrm{~d}, J=3.6 \mathrm{~Hz}, 1 \mathrm{H}), 8.59$ - $8.54(\mathrm{~m}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}, J=7.7$ Hz, 1H), 7.62 (t, J = 7.7 Hz, 1H), 7.12 - $7.05(\mathrm{~m}, 1 \mathrm{H}), 3.86(\mathrm{~d}, J=5.7 \mathrm{~Hz}, 2 \mathrm{H}), 3.71$ - $3.63(\mathrm{~m}, 1 \mathrm{H}), 3.54-3.34(\mathrm{~m}, 3 \mathrm{H}), 3.11(\mathrm{~s}, 1 \mathrm{H}), 2.86(\mathrm{q}, \mathrm{J}=10.4 \mathrm{~Hz}, 1 \mathrm{H}), 2.35$ $(\mathrm{d}, J=10.5 \mathrm{~Hz}, 1 \mathrm{H}), 2.10-1.86(\mathrm{~m}, 1 \mathrm{H}), 1.63(\mathrm{~s}, 1 \mathrm{H}), 1.52-1.39(\mathrm{~m}, 1 \mathrm{H}), 1.04$ (s, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{25} \mathrm{H}_{24} \mathrm{~N}_{6} \mathrm{O}_{2}$, 440.2; found $441.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(3-(3-methyl-3H-diazirin-3-yl)piperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (17). This compound was synthesized from tert-butyl 3-acetylpiperidine-1-carboxylate (54c) (1000 mg, 4.40 mmol ) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide (511 $\mathrm{mg}, 2.280 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic
acid (37) (248 mg, 0.854 mmol$)$ using HATU ( $357 \mathrm{mg}, 0.939 \mathrm{mmol}$ ) according to Method A , and HCl salt formation according to Method $B$ to give $70 \mathrm{mg}, 0.143$ mmol as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-d_{6}$ ) $\delta \mathrm{ppm} 11.25(\mathrm{~s}, 1 \mathrm{H}), 10.00$ (s, 1H), 9.13 (s, 1H), $8.99-8.94(\mathrm{~m}, 1 \mathrm{H}), 8.66(\mathrm{~d}, J=2.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.57(\mathrm{~d}, \mathrm{~J}=6.8$ $\mathrm{Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H})$, $7.62(\mathrm{t}, \mathrm{J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.12-7.05(\mathrm{~m}, 1 \mathrm{H}), 3.95(\mathrm{~d}, \mathrm{~J}=6.6 \mathrm{~Hz}, 2 \mathrm{H}), 3.68(\mathrm{~d}, \mathrm{~J}=$ $11.9 \mathrm{~Hz}, 1 \mathrm{H}), 3.60(\mathrm{~d}, \mathrm{~J}=11.9 \mathrm{~Hz}, 1 \mathrm{H}), 3.42-3.39(\mathrm{~m}, 2 \mathrm{H}), 2.92-2.84(\mathrm{~m}, 1 \mathrm{H})$, $2.77(\mathrm{q}, J=11.4 \mathrm{~Hz}, 1 \mathrm{H}), 1.95(\mathrm{t}, J=12.4 \mathrm{~Hz}, 1 \mathrm{H}), 1.83(\mathrm{~d}, J=14.2 \mathrm{~Hz}, 1 \mathrm{H}), 1.72$ $(\mathrm{t}, J=14.1 \mathrm{~Hz}, 1 \mathrm{H}), 1.54(\mathrm{~d}, J=13.1 \mathrm{~Hz}, 1 \mathrm{H}), 0.97(\mathrm{~d}, J=2.0 \mathrm{~Hz}, 3 \mathrm{H}), 0.88(\mathrm{q}, J$ $=12.8 \mathrm{~Hz}, 1 \mathrm{H}) . \mathrm{LCMS}(\mathrm{ESI}): \mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{26} \mathrm{H}_{26} \mathrm{~N}_{6} \mathrm{O}_{2}, 454.2$; found $454.9[\mathrm{M}+\mathrm{H}]^{+}$. N-(2-(4-(3-methyl-3H-diazirin-3-yl)piperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (18). This compound was synthesized from tert-butyl 4-acetylpiperidine-1-carboxylate (54d) (1000 mg, 4.40 mmol ) according to Method C, followed by Boc-deprotection according to Method E, diamine formation with 2-(Boc-amino)ethyl bromide (1276 $\mathrm{mg}, 5.694 \mathrm{mmol}$ ) according to Method D, Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol$)$ using HATU (145 mg, 0.381 mmol ) according to Method A , and HCl salt formation according to Method $B$ to give $5 \mathrm{mg}, 0.010 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO-d6) $\delta \mathrm{ppm} 11.21(\mathrm{~s}, 1 \mathrm{H}), 9.38(\mathrm{~s}, 1 \mathrm{H})$, $9.14(\mathrm{~s}, 1 \mathrm{H}), 8.96(\mathrm{~d}, J=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.60-8.52(\mathrm{~m}, 2 \mathrm{H}), 8.33(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H})$, $8.11(\mathrm{~d}, \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.76(\mathrm{t}, \mathrm{J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.63(\mathrm{t}, \mathrm{J}=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.08(\mathrm{t}, \mathrm{J}$ $=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.89(\mathrm{~d}, J=6.6 \mathrm{~Hz}, 2 \mathrm{H}), 3.68(\mathrm{~d}, J=12.2 \mathrm{~Hz}, 2 \mathrm{H}), 3.36(\mathrm{~d}, J=5.9$
$\mathrm{Hz}, 2 \mathrm{H}), 2.98(\mathrm{q}, J=11.7 \mathrm{~Hz}, 2 \mathrm{H}), 1.73(\mathrm{~d}, J=13.0 \mathrm{~Hz}, 3 \mathrm{H}), 1.35-1.09(\mathrm{~m}, 4 \mathrm{H})$, 0.98 (s, 3H). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{26} \mathrm{H}_{26} \mathrm{~N}_{6} \mathrm{O} 2$, 454.2 ; found $454.9[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4,4-dimethylpiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxamide hydrochloride (19). This compound was synthesized from 4,4-dimethylpiperidine hydrochloride ( 57 a ) ( $1000 \mathrm{mg}, 6.682 \mathrm{mmol}$ ) and 2-(Boc-amino)ethyl bromide ( $2995 \mathrm{mg}, 13.365 \mathrm{mmol}$ ) according to Method D, followed by Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol ) using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A, and HCl salt formation according to Method $B$ to give $30 \mathrm{mg}, 0.065 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d} 6$ ) $\delta \mathrm{ppm} 11.24(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 9.70(\mathrm{~s}, 1 \mathrm{H})$, 9.13 (s, 1H), 8.95 (dd, $J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.61(\mathrm{~s}, 1 \mathrm{H}), 8.55(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}$, $1 \mathrm{H}), 8.35-8.30(\mathrm{~m}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, \mathrm{J}=7.1$ $\mathrm{Hz}, 1 \mathrm{H}), 3.93(\mathrm{q}, J=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.52(\mathrm{~d}, J=12.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.44(\mathrm{q}, J=6.1 \mathrm{~Hz}$, 2 H ), $3.22-3.11(\mathrm{~m}, 2 \mathrm{H}), 1.75-1.65(\mathrm{~m}, 2 \mathrm{H}), 1.53(\mathrm{~d}, \mathrm{~J}=14.1 \mathrm{~Hz}, 2 \mathrm{H}), 1.03(\mathrm{~s}$, 3 H ), 0.96 ( $\mathrm{s}, 3 \mathrm{H}$ ). LCMS (ESI): m/z calcd for $\mathrm{C}_{26} \mathrm{H}_{28} \mathrm{~N}_{4} \mathrm{O}_{2}$, 428.2; found 428.9 $[\mathrm{M}+\mathrm{H}]^{+}$.

N -(2-(6-azaspiro[2.5]octan-6-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (20). This compound was synthesized from 6-azaspiro[2.5]octane hydrochloride (57b) ( $2000 \mathrm{mg}, 13.546 \mathrm{mmol}$ ) and 2-(Boc-amino)ethyl bromide ( $4554 \mathrm{mg}, 20.321 \mathrm{mmol}$ ) according to Method D, followed by Boc-deprotection according to Method E, amide coupling with 12-oxo-

12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (200 mg, 0.689 mmol ) using HATU ( $289 \mathrm{mg}, 0.760 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A, and HCl salt formation according to Method $B$ to give $27 \mathrm{mg}, 0.058 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $\left.500 \mathrm{MHz}, ~ D M S O-d_{6}\right) \delta \mathrm{ppm} 11.25(\mathrm{t}, \mathrm{J}=5.9 \mathrm{~Hz}, 1 \mathrm{H}), 10.00(\mathrm{~s}, 1 \mathrm{H})$, 9.13 (s, 1H), 8.96 (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.62(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}$, $1 \mathrm{H}), 8.33(\mathrm{~d}, \mathrm{~J}=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-$ $7.59(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.96(\mathrm{q}, J=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.65(\mathrm{~d}, J=12.5$ $\mathrm{Hz}, 3 \mathrm{H}), 3.46(\mathrm{q}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.18-3.08(\mathrm{~m}, 2 \mathrm{H}), 2.17(\mathrm{t}, J=12.7 \mathrm{~Hz}, 2 \mathrm{H})$, $1.15(\mathrm{~d}, J=14.0 \mathrm{~Hz}, 3 \mathrm{H}), 0.44(\mathrm{dd}, J=8.3,5.1 \mathrm{~Hz}, 2 \mathrm{H}), 0.41-0.34(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{26} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}$, 426.2; found $427.0[\mathrm{M}+\mathrm{H}]^{+}$.

12-oxo-N-(1-(piperidin-1-yl)propan-2-yl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride (21). This compound was synthesized from 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (200 mg, 0.689 mmol ) and 1-(1-piperidyl)propan-2-amine (59a) ( $148 \mathrm{mg}, 1.040 \mathrm{mmol}$ ) using HATU ( $289 \mathrm{mg}, 0.760 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A followed by HCl salt formation according to Method $B$ to give $15 \mathrm{mg}, 0.033 \mathrm{mmol}$ as a yellow-brown solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.29(\mathrm{~d}, J=8.2 \mathrm{~Hz}, 1 \mathrm{H}), 9.15(\mathrm{~s}, 1 \mathrm{H})$, 8.97 (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.56$ (dd, $J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.50(\mathrm{~s}, 1 \mathrm{H}), 8.33$ (d, $J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.17(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 7.79-7.72(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.59(\mathrm{~m}, 1 \mathrm{H})$, $7.09(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.65(\mathrm{~s}, 1 \mathrm{H}), 3.79(\mathrm{~d}, J=12.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.54-3.46(\mathrm{~m}$, 2 H ), $3.42-3.32(\mathrm{~m}, 1 \mathrm{H}), 3.06-2.96(\mathrm{~m}, 1 \mathrm{H}), 1.93-1.61(\mathrm{~m}, 5 \mathrm{H}), 1.48(\mathrm{~d}, \mathrm{~J}=6.7$ $\mathrm{Hz}, 3 \mathrm{H}$ ). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{25} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}, 414.2$; found $414.9[\mathrm{M}+\mathrm{H}]^{+}$.

12-oxo-N-(2-(piperidin-1-yl)propyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride (22). This compound was synthesized from 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol ) and 2-(1-piperidyl)propan-1-amine (59b) ( $74 \mathrm{mg}, 0.520 \mathrm{mmol}$ ) using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A followed by HCl salt formation according to Method $B$ to give $30 \mathrm{mg}, 0.067 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO-d 6 ) $\delta$ ppm 11.25 (t, $J=6.1 \mathrm{~Hz}, 1 \mathrm{H}$ ), 9.87 (s, 1H), 9.13 (s, 1H), 8.95 (dd, J = 7.3, 1.7 Hz, 1H), $8.54(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.52(\mathrm{~s}, 1 \mathrm{H})$, $8.35-8.30(\mathrm{~m}, 1 \mathrm{H}), 8.14-8.09(\mathrm{~m}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.58(\mathrm{~m}, 1 \mathrm{H})$, $7.08(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.04-3.95(\mathrm{~m}, 1 \mathrm{H}), 3.86-3.77(\mathrm{~m}, 1 \mathrm{H}), 3.66(\mathrm{~d}, J=8.3$ $\mathrm{Hz}, 1 \mathrm{H}), 3.52(\mathrm{~d}, \mathrm{~J}=11.8 \mathrm{~Hz}, 2 \mathrm{H}), 3.18-3.06(\mathrm{~m}, 2 \mathrm{H}), 1.92-1.81(\mathrm{~m}, 5 \mathrm{H}), 1.72$ (d, $J=13.2 \mathrm{~Hz}, 1 \mathrm{H}$ ), 1.45 ( $\mathrm{d}, \mathrm{J}=6.8 \mathrm{~Hz}, 3 \mathrm{H}$ ). LCMS (ESI): m/z calcd for $\mathrm{C}_{25} \mathrm{H}_{26} \mathrm{~N}_{4} \mathrm{O}_{2}, 414.2$; found $414.9[\mathrm{M}+\mathrm{H}]^{+}$.

12-oxo-N-(2-(pyrrolidin-1-yl)propyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxamide hydrochloride (23). This compound was synthesized from 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol ) and 2-pyrrolidin-1-ylpropan-1-amine (59c) ( $67 \mathrm{mg}, 0.522 \mathrm{mmol}$ ) using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A followed by HCl salt formation according to Method $B$ to give $30 \mathrm{mg}, 0.069 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.22(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 1 \mathrm{H}), 10.48(\mathrm{~s}, 1 \mathrm{H}), 9.13$ (s, 1H), 8.96 (dd, $J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.54(\mathrm{dd}, J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.51(\mathrm{~s}, 1 \mathrm{H})$, $7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{t}, \mathrm{J}=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.88(\mathrm{t}, \mathrm{J}=6.1$ $\mathrm{Hz}, 2 \mathrm{H}), 3.82-3.52(\mathrm{~m}, 1 \mathrm{H}), 3.28-3.12(\mathrm{~m}, 2 \mathrm{H}), 2.09-1.96(\mathrm{~m}, 1 \mathrm{H}), 1.89(\mathrm{t}, \mathrm{J}=$
$7.6 \mathrm{~Hz}, 2 \mathrm{H}$ ), 1.48 (d, $J=6.6 \mathrm{~Hz}, 3 \mathrm{H}$ ). LCMS (ESI): $\mathrm{m} / \mathrm{z}$ calcd for $\mathrm{C}_{24} \mathrm{H}_{24} \mathrm{~N}_{4} \mathrm{O}_{2}, 400.2$; found $400.9[\mathrm{M}+\mathrm{H}]^{+}$.

## N-(2-(4-(tert-buty))piperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-

b]quinazoline-4-carboxamide hydrochloride (24). This compound was synthesized from 4-tert-butylpiperidine hydrochloride (57c) ( $1000 \mathrm{mg}, 5.627 \mathrm{mmol}$ ) and 2-(Bocamino)ethyl bromide ( $2523 \mathrm{mg}, 11.258 \mathrm{mmol}$ ) according to Method D, followed by Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) ( $100 \mathrm{mg}, 0.345 \mathrm{mmol}$ ) using HATU ( $145 \mathrm{mg}, 0.381 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A, and HCl salt formation according to Method $B$ to give $37 \mathrm{mg}, 0.075 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO-d6) $\delta$ ppm 11.21 (t, $J=5.8 \mathrm{~Hz}, 1 \mathrm{H}), 9.73(\mathrm{~s}, 1 \mathrm{H}), 9.13$ (s, 1 H ), 8.96 (dd, $J=7.2,1.7 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.62 (s, 1H), 8.55 (dd, $J=7.1,1.7 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.33 (d, $J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.10(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.72(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.59(\mathrm{~m}$, $1 \mathrm{H}), 7.08(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.93(\mathrm{q}, J=6.3 \mathrm{~Hz}, 2 \mathrm{H}), 3.70(\mathrm{~d}, J=11.8 \mathrm{~Hz}, 2 \mathrm{H})$, $3.38(\mathrm{q}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.00(\mathrm{q}, J=11.6 \mathrm{~Hz}, 2 \mathrm{H}), 1.83(\mathrm{~d}, J=13.7 \mathrm{~Hz}, 2 \mathrm{H}), 1.62$ - $1.51(\mathrm{~m}, 2 \mathrm{H}), 1.29(\mathrm{t}, \mathrm{J}=12.3 \mathrm{~Hz}, 1 \mathrm{H}), 0.84(\mathrm{~s}, 9 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{28} \mathrm{H}_{32} \mathrm{~N}_{4} \mathrm{O}_{2}, 456.3$; found $457.0[\mathrm{M}+\mathrm{H}]^{+}$.

12-oxo-N-(2-(4-phenylpiperidin-1-yl)ethyl)-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (25). This compound was synthesized from 4phenylpiperidine ( $\mathbf{5 7 d}$ ) ( $1000 \mathrm{mg}, 6.202 \mathrm{mmol}$ ) and 2-(Boc-amino)ethyl bromide $(1390 \mathrm{mg}, 6.203 \mathrm{mmol})$ according to Method D, followed by Boc-deprotection according to Method E, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxylic acid (37) ( $100 \mathrm{mg}, 0.345 \mathrm{mmol}$ ) using HATU ( 145 mg , 0.381 mmol ) according to Method A , and HCl salt formation according to Method B to give $10 \mathrm{mg}, 0.019 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR $\left(500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}\right) \delta$ ppm $11.27(\mathrm{~s}, 1 \mathrm{H}), 9.72(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.97(\mathrm{~d}, \mathrm{~J}=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.63(\mathrm{~s}, 1 \mathrm{H})$, 8.58 (d, $J=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}$, $J=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.62(\mathrm{t}, J=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.34(\mathrm{t}, J=7.9 \mathrm{~Hz}, 2 \mathrm{H}), 7.24(\mathrm{~d}, J=7.5$ $\mathrm{Hz}, 3 \mathrm{H}), 7.09(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.97(\mathrm{~d}, J=6.6 \mathrm{~Hz}, 2 \mathrm{H}), 3.80(\mathrm{~d}, J=12.2 \mathrm{~Hz}, 2 \mathrm{H})$, $3.21(\mathrm{~d}, \mathrm{~J}=11.7 \mathrm{~Hz}, 2 \mathrm{H}), 2.91-2.81(\mathrm{~m}, 1 \mathrm{H}), 2.09-1.92(\mathrm{~m}, 3 \mathrm{H}), 1.30-1.11(\mathrm{~m}$, $2 H$ ). LCMS (ESI): $m / z$ calcd for $\mathrm{C}_{3} 0 \mathrm{H}_{28} \mathrm{~N}_{4} \mathrm{O}_{2}, 476.2$; found $477.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-aminopiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (26). This compound was synthesized from 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) ( $775 \mathrm{mg}, 2.67 \mathrm{mmol}$ ) and tert-butyl $N$-[1-(2-aminoethyl)-4-piperidyl]carbamate ( $\mathbf{6 0}$ ) ( $975 \mathrm{mg}, 4.01 \mathrm{mmol}$ ) using HATU ( $1117 \mathrm{mg}, 2.94 \mathrm{mmol}$ ) at $50^{\circ} \mathrm{C}$ according to Method A followed by Boc-deprotection according to Method E to give $810 \mathrm{mg}, 1.79 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.17$ (t, $J=5.9 \mathrm{~Hz}, 1 \mathrm{H}$ ), 10.81 (s, 1H), 9.12 (d, J = $3.4 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.95(\mathrm{dd}, J=7.3,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.67(\mathrm{~s}, 1 \mathrm{H}), 8.59-8.52$ (m, 1H), 8.39 (d, J = $5.4 \mathrm{~Hz}, 3 \mathrm{H}), 8.35-8.29(\mathrm{~m}, 1 \mathrm{H}), 8.18-8.10(\mathrm{~m}, 1 \mathrm{H}), 7.78-$ $7.70(\mathrm{~m}, 1 \mathrm{H}), 7.65-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{t}, J=7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.95(\mathrm{q}, J=6.4 \mathrm{~Hz}, 2 \mathrm{H})$, $3.77-3.69(\mathrm{~m}, 2 \mathrm{H}), 3.37(\mathrm{q}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.16(\mathrm{q}, J=11.6 \mathrm{~Hz}, 2 \mathrm{H}), 2.13(\mathrm{~d}, J$ $=13.0 \mathrm{~Hz}, 2 \mathrm{H}$ ), 2.09-1.98 (m, 2H). LCMS (ESI): m/z calcd for $\mathrm{C}_{24} \mathrm{H}_{25} \mathrm{~N}_{5} \mathrm{O}_{2}, 415.2$; found $415.8[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(hept-6-yn-1-yl(methyl)amino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-
b]quinazoline-4-carboxamide hydrochloride (27). This compound was synthesized from 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (1000 $\mathrm{mg}, 3.445 \mathrm{mmol}$ ) and tert-Butyl-(2-aminoethyl)methylcarbamate (63) (901 mg, $5.171 \mathrm{mmol})$ using TBTU ( $1660 \mathrm{mg}, 5.170 \mathrm{mmol}$ ) according to Method A, followed by Boc-deprotection according to Method E, washing 1x. with sat. $\mathrm{NaHCO}_{3}$, alkylation with 7-iodohept-1-yne (62) (prepared according to Krasiński et al. ${ }^{31}$ ) (170 $\mathrm{mg}, 0.766 \mathrm{mmol}$ ) according to Method G , and HCl salt formation according to Method B to give $49 \mathrm{mg}, 0.103 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR (400 MHz, DMSO-d6) $\delta$ ppm 11.23 - $11.15(\mathrm{~m}, 1 \mathrm{H}), 10.32(\mathrm{~s}, 1 \mathrm{H}), 9.12(\mathrm{~s}, 1 \mathrm{H}), 8.95(\mathrm{dd}, J=$ 7.3, 1.7 Hz, 1H), $8.65(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{dd}, J=6.9,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.32(\mathrm{~d}, J=8.4 \mathrm{~Hz}$, $1 \mathrm{H}), 8.11(\mathrm{~d}, J=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.58(\mathrm{~m}, 1 \mathrm{H}), 7.08(\mathrm{t}, J=$ $7.1 \mathrm{~Hz}, 1 \mathrm{H}), 3.97-3.87(\mathrm{~m}, 2 \mathrm{H}), 3.54-3.42(\mathrm{~m}, 1 \mathrm{H}), 3.42-3.31(\mathrm{~m}, 1 \mathrm{H}), 3.30-$ $3.19(\mathrm{~m}, 1 \mathrm{H}), 3.16-3.03(\mathrm{~m}, 1 \mathrm{H}), 2.88(\mathrm{~d}, J=4.9 \mathrm{~Hz}, 3 \mathrm{H}), 2.74(\mathrm{t}, J=2.6 \mathrm{~Hz}, 1 \mathrm{H})$, $2.15-2.05(\mathrm{~m}, 2 \mathrm{H}), 1.78-1.65(\mathrm{~m}, 2 \mathrm{H}), 1.50-1.29(\mathrm{~m}, 3 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{27} \mathrm{H}_{28} \mathrm{~N}_{4} \mathrm{O}_{2}$, 440.2; found $441.6[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-(hept-6-yn-1-ylcarbamoyl)piperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (28). This compound was synthesized from tert-butyl piperidine-4-carboxylate hydrochloride (65) (473 mg, 2.133 mmol$)$ and benzyl $N$-(2-bromoethyl)carbamate ( $500 \mathrm{mg}, 1.937$ mmol ) according to Method D, followed by CBz-deprotection according to Method F, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (100 mg, 0.345 mmol$)$ using TBTU (166 mg, 0.517 mmol$)$ according to

Method A, tert-butyl ester hydrolysis according to Method H, amide coupling with hept-6-yn-1-amine ( $34 \mathrm{mg}, 0.306 \mathrm{mmol}$ ) using TBTU ( $98 \mathrm{mg}, 0.305 \mathrm{mmol}$ ) according to Method A , and HCl salt formation according to Method B to give 86 $\mathrm{mg}, 0.15 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.16$ (s, 1H), 10.29 (s, 1H), 9.11 (s, 1H), 8.95 (dd, $J=7.3,1.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.64$ (d, $J=11.5 \mathrm{~Hz}$, 1 H ), 8.57 (dd, $J=6.8,1.7 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.31(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}$ ), $8.10(\mathrm{~d}, J=8.6 \mathrm{~Hz}$, $1 \mathrm{H}), 7.98(\mathrm{t}, \mathrm{J}=5.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.77-7.70(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.57(\mathrm{~m}, 1 \mathrm{H}), 7.09(\mathrm{t}, J=$ $7.1 \mathrm{~Hz}, 1 \mathrm{H}), 4.00-3.88(\mathrm{~m}, 2 \mathrm{H}), 3.70(\mathrm{~d}, \mathrm{~J}=11.8 \mathrm{~Hz}, 2 \mathrm{H}), 3.47-3.34(\mathrm{~m}, 1 \mathrm{H})$, $3.11-2.97(\mathrm{~m}, 2 \mathrm{H}), 2.76(\mathrm{t}, \mathrm{J}=2.6 \mathrm{~Hz}, 1 \mathrm{H}), 2.41-2.30(\mathrm{~m}, 1 \mathrm{H}), 2.19-2.07(\mathrm{~m}$, 2 H ), $1.96-1.84(\mathrm{~m}, 3 \mathrm{H}), 1.47-1.25(\mathrm{~m}, 7 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{32} \mathrm{H}_{3} \mathrm{~N}_{5} \mathrm{O}_{3}$, 537.3 ; found $537.9[\mathrm{M}+\mathrm{H}]^{+}$.
$N-(2-((2-(3-(b u t-3-y n-1-y /)-3 H-d i a z i r i n-3-y l)$ ethyl)(methyl)amino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (29). This compound was synthesized from N -(2-(methylamino)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide (64) (prepared as an intermediate in the synthesis of 27) ( $100 \mathrm{mg}, 0.289 \mathrm{mmol}$ ) and 3-but-3-ynyl-3-(2iodoethyl)diazirine ( $72 \mathrm{mg}, 0.290 \mathrm{mmol}$ ) according to Method G followed by HCl salt formation according to Method $B$ to give $64 \mathrm{mg}, 0.127 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.22(\mathrm{~s}, 1 \mathrm{H}), 10.02(\mathrm{~s}, 1 \mathrm{H}), 9.13$ (s, $1 \mathrm{H}), 8.99-8.93(\mathrm{~m}, 1 \mathrm{H}), 8.63(\mathrm{~s}, 1 \mathrm{H}), 8.59-8.53(\mathrm{~m}, 1 \mathrm{H}), 8.33(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}$, 1 H ), 8.11 (d, $J=8.5 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.75 (dd, $J=8.5,6.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.62$ (dd, $J=8.3,6.8$ $\mathrm{Hz}, 1 \mathrm{H}), 7.11-7.05(\mathrm{~m}, 1 \mathrm{H}), 3.90(\mathrm{~d}, J=6.4 \mathrm{~Hz}, 2 \mathrm{H}), 3.46(\mathrm{dd}, J=12.7,6.2 \mathrm{~Hz}$, $1 \mathrm{H}), 3.34$ (dd, $J=13.0,6.4 \mathrm{~Hz}, 1 \mathrm{H}), 3.13(\mathrm{q}, J=9.6 \mathrm{~Hz}, 1 \mathrm{H}), 2.89-2.82(\mathrm{~m}, 4 \mathrm{H})$,
$2.02-1.95(\mathrm{~m}, 2 \mathrm{H}), 1.85(\mathrm{t}, \mathrm{J}=8.6 \mathrm{~Hz}, 2 \mathrm{H}), 1.69-1.55(\mathrm{~m}, 2 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{27} \mathrm{H}_{26} \mathrm{~N}_{6} \mathrm{O}_{2}$, 466.2; found $466.9[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-((2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)carbamoyl)piperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (30). This compound was synthesized from tert-butyl piperidine-4carboxylate hydrochloride (65) (2000 mg, 9.020 mmol ) and 2-(Boc-amino)ethyl bromide ( $3033 \mathrm{mg}, 13.534 \mathrm{mmol}$ ) according to Method D, followed by Bocdeprotection using 4 M HCl in 1,4-dioxane (20 eq.) at $0^{\circ} \mathrm{C}$ - room temperature according to Han et al., ${ }^{30}$ amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (1100 mg, 3.789 mmol$)$ using HATU (1585 mg, 4.169 mmol ) at $50^{\circ} \mathrm{C}$ according to Method A, tert-butyl ester hydrolysis according to Method H, amide coupling with 2-(3-but-3-ynyldiazirin-3-yl)ethanamine ( 51 mg , 0.371 mmol ) using HATU ( $104 \mathrm{mg}, 0.274 \mathrm{mmol}$ ) according to Method A , and HCl salt formation according to Method B to give $23 \mathrm{mg}, 0.038 \mathrm{mmol}$ as a yellow solid. ${ }^{1} \mathrm{H}$ NMR ( 500 MHz , DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.22(\mathrm{q}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 9.76(\mathrm{~s}, 1 \mathrm{H}), 9.13$ $(\mathrm{d}, J=3.1 \mathrm{~Hz}, 1 \mathrm{H}), 8.99-8.93(\mathrm{~m}, 1 \mathrm{H}), 8.63(\mathrm{~d}, J=15.3 \mathrm{~Hz}, 1 \mathrm{H}), 8.57(\mathrm{dd}, J=$ $7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.36-8.30(\mathrm{~m}, 1 \mathrm{H}), 8.15-8.08(\mathrm{~m}, 1 \mathrm{H}), 8.00(\mathrm{t}, J=5.5 \mathrm{~Hz}, 1 \mathrm{H})$, $7.78-7.71(\mathrm{~m}, 1 \mathrm{H}), 7.66-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.12-7.05(\mathrm{~m}, 1 \mathrm{H}), 3.93(\mathrm{q}, \mathrm{J}=6.4 \mathrm{~Hz}$, $2 H), 3.73(\mathrm{~d}, J=11.9 \mathrm{~Hz}, 2 \mathrm{H}), 3.40(\mathrm{q}, J=5.8 \mathrm{~Hz}, 2 \mathrm{H}), 3.06(\mathrm{q}, J=11.5 \mathrm{~Hz}, 2 \mathrm{H})$, $2.94(\mathrm{q}, J=6.8 \mathrm{~Hz}, 2 \mathrm{H}), 2.84(\mathrm{t}, J=2.6 \mathrm{~Hz}, 1 \mathrm{H}), 2.44-2.33(\mathrm{~m}, 1 \mathrm{H}), 2.01-1.83$ (m, 5H), $1.57(\mathrm{t}, J=7.4 \mathrm{~Hz}, 2 \mathrm{H}), 1.51(\mathrm{t}, J=6.9 \mathrm{~Hz}, 2 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{32} \mathrm{H}_{33} \mathrm{~N}_{7} \mathrm{O}_{3}, 563.3$; found $564.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(3-((2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)amino)pyrrolidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (31). This compound was synthesized from tert-butyl $N$-pyrrolidin-3-ylcarbamate (69) (3000 mg, 16.107 mmol ) and benzyl N -(2-bromoethyl)carbamate ( 6237 mg , 24.164 mmol according to Method D, followed by CBz-deprotection according to Method F, amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxylic acid (37) (1000 mg, 3.445 mmol$)$ using HATU (1441 mg, 3.79 mmol ) according to Method A, Boc-deprotection according to Method E, washing 1 x with sat. $\mathrm{NaHCO}_{3}$, alkylation with 3-but-3-ynyl-3-(2-iodoethyl)diazirine ( $62 \mathrm{mg}, 0.25$ mmol ) at $50^{\circ} \mathrm{C}$ according to Method G , and HCl salt formation according to Method B to give $68 \mathrm{mg}, 0.122 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta$ ppm $11.26(\mathrm{~s}, 1 \mathrm{H}), 9.14(\mathrm{~s}, 1 \mathrm{H}), 8.95(\mathrm{~s}, 1 \mathrm{H}), 8.56(\mathrm{~d}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=$ $8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.13(\mathrm{~d}, \mathrm{~J}=8.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.75(\mathrm{t}, \mathrm{J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.62(\mathrm{t}, \mathrm{J}=7.6 \mathrm{~Hz}$, $1 \mathrm{H}), 7.07(\mathrm{~s}, 1 \mathrm{H}), 3.91(\mathrm{~s}, 1 \mathrm{H}), 3.65(\mathrm{~s}, 1 \mathrm{H}), 2.88(\mathrm{~s}, 1 \mathrm{H}), 1.97(\mathrm{~s}, 2 \mathrm{H}), 1.64(\mathrm{~s}, 2 \mathrm{H})$, $1.58(\mathrm{t}, J=7.3 \mathrm{~Hz}, 2 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{30} \mathrm{H}_{31} \mathrm{~N}_{7} \mathrm{O}_{2}, 521.3$; found 522.0 $[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-((2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)amino)piperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (32). This compound was synthesized from N -(2-(4-aminopiperidin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (26) (171 mg, 0.378 mmol ) after washing 1 x with $10 \% \mathrm{NaHCO}_{3}$, alkylation with 3-but-3-ynyl-3-(2iodoethyl)diazirine ( $60 \mathrm{mg}, 0.242 \mathrm{mmol}$ ) according to Method G , and HCl salt formation according to Method B to give $65 \mathrm{mg}, 0.114 \mathrm{mmol}$ as an orange solid.
${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMSO-d ) $\delta \mathrm{ppm} 11.21$ (s, 1H), 9.13 (s, 1H), 8.95 (s, 1H), 8.56 (d, J = $6.9 \mathrm{~Hz}, 1 \mathrm{H}$ ), 8.38 (s, 1H), 8.33 (d, J = $8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.13$ (d, J = $8.5 \mathrm{~Hz}, 1 \mathrm{H}$ ), $7.74(\mathrm{t}, \mathrm{J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.62(\mathrm{t}, \mathrm{J}=7.5 \mathrm{~Hz}, 1 \mathrm{H}), 7.07(\mathrm{~s}, 1 \mathrm{H}), 3.93(\mathrm{~s}, 1 \mathrm{H}), 3.60(\mathrm{~s}$, 1H), 3.16 (s, 1H), 2.89 (d, J = $2.7 \mathrm{~Hz}, 1 \mathrm{H}), 2.71$ (s, 1H), 2.13 (s, 1H), 2.01 (s, 3H), $1.76-1.48(m, 3 H)$.

N-(2-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)piperazin-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxamide hydrochloride (33). This compound was synthesized from 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4carboxylic acid (37) (2000 mg, 6.89 mmol$)$ and tert-butyl 4-(2-aminoethyl)piperazine-1-carboxylate (72) ( $2370 \mathrm{mg}, 10.34 \mathrm{mmol}$ ) using HATU (2882 mg, 7.58 mmol ) at $50^{\circ} \mathrm{C}$ according to Method A, followed by Bocdeprotection according to Method E, washing 1 x with sat. $\mathrm{NaHCO}_{3}$, alkylation with 3-but-3-ynyl-3-(2-iodoethyl)diazirine ( $62 \mathrm{mg}, 0.25 \mathrm{mmol}$ ) according to Method G, and HCl salt formation according to Method $B$ to give $21 \mathrm{mg}, 0.038 \mathrm{mmol}$ as an orange solid. ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMSO}-\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.21$ ( $\mathrm{d}, \mathrm{J}=33.7 \mathrm{~Hz}, 1 \mathrm{H}$ ), 9.13 (s, 1H), 8.95 (s, 1H), 8.55 (dd, $J=7.0,1.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.33(\mathrm{~d}, J=8.4 \mathrm{~Hz}, 1 \mathrm{H}), 8.13$ (s, 1H), $7.75(\mathrm{t}, \mathrm{J}=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.66-7.59(\mathrm{~m}, 1 \mathrm{H}), 7.07(\mathrm{t}, \mathrm{J}=7.2 \mathrm{~Hz}, 1 \mathrm{H}), 3.92$ (s, 1H), 3.64 (s, 1H), 3.46 (s, 2H), 3.16 (s, 5H), 3.04 (s, 3H), 2.86 (s, 1H), 2.75 (s, 1H), 2.21 (s, 1H), 2.00 (s, 2H), 1.80 (s, 1H), 1.59 (s, 3H). LCMS (ESI): m/z calcd for $\mathrm{C}_{30} \mathrm{H}_{31} \mathrm{~N}_{7} \mathrm{O}_{2}$, 521.3 ; found $522.0[\mathrm{M}+\mathrm{H}]^{+}$.

N-(2-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-1,4-diazepan-1-yl)ethyl)-12-oxo-12H-benzo[g]pyrido[2, 1-b]quinazoline-4-carboxamide hydrochloride (34).

This compound was synthesized from 1-Boc-homopiperazine (74) (3000 mg, $14.979 \mathrm{mmol})$ and benzyl N -(2-bromoethyl)carbamate ( $5800 \mathrm{mg}, 22.471 \mathrm{mmol}$ ) according to Method D , followed by CBz-deprotection according to Method F , amide coupling with 12-oxo-12H-benzo[g]pyrido[2,1-b]quinazoline-4-carboxylic acid (37) (2000 mg, 6.89 mmol$)$ using HATU ( $2882 \mathrm{mg}, 7.58 \mathrm{mmol}$ ) according to Method A, Boc-deprotection according to Method E, washing 1 x with sat. $\mathrm{NaHCO}_{3}$, alkylation with 3-but-3-ynyl-3-(2-iodoethyl)diazirine ( $60 \mathrm{mg}, 0.242 \mathrm{mmol}$ ) according to Method G, and HCl salt formation according to Method B to give $65 \mathrm{mg}, 0.114$ mmol as an orange solid. ${ }^{1} \mathrm{H}$ NMR (500 MHz, DMSO- $\mathrm{d}_{6}$ ) $\delta \mathrm{ppm} 11.19$ (d, J = 6.5 $\mathrm{Hz}, 1 \mathrm{H}), 10.07(\mathrm{~s}, 1 \mathrm{H}), 9.12(\mathrm{~d}, J=5.2 \mathrm{~Hz}, 1 \mathrm{H}), 8.95(\mathrm{~d}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}), 8.55(\mathrm{~d}$, $J=6.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.47(\mathrm{~s}, 1 \mathrm{H}), 8.32(\mathrm{t}, \mathrm{J}=6.8 \mathrm{~Hz}, 1 \mathrm{H}), 8.14(\mathrm{~s}, 1 \mathrm{H}), 7.75(\mathrm{t}, J=7.3$ $\mathrm{Hz}, 1 \mathrm{H}), 7.62(\mathrm{t}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.07(\mathrm{q}, J=6.7 \mathrm{~Hz}, 1 \mathrm{H}), 3.72(\mathrm{~s}, 2 \mathrm{H}), 3.13(\mathrm{~d}, J$ $=27.5 \mathrm{~Hz}, 10 \mathrm{H}), 2.85(\mathrm{~s}, 1 \mathrm{H}), 2.72(\mathrm{~s}, 2 \mathrm{H}), 1.98(\mathrm{~s}, 4 \mathrm{H}), 1.65(\mathrm{~s}, 2 \mathrm{H}), 1.54(\mathrm{~s}, 2 \mathrm{H})$. LCMS (ESI): m/z calcd for $\mathrm{C}_{31} \mathrm{H}_{33} \mathrm{~N}_{7} \mathrm{O}_{2}$, 535.3; found $536.0[\mathrm{M}+\mathrm{H}]^{+}$.

Cell culture. Performed by Daming Chen. A375 melanoma cells were maintained at $37{ }^{\circ} \mathrm{C}$ in a humidified atmosphere containing $5 \% \mathrm{CO}_{2}$. A375 cells were cultured in DMEM supplemented with $10 \%$ fetal bovine serum (FBS) and 4 mM glutamine.

RPA194 Degradation Assay. Performed by Daming Chen. A375 cells were seeded on 96-well plates (PerkinElmer ViewPlate-96 Black, catalog \# 6005182) and treated with the compounds at $0.01,0.03,0.1,0.3,1,3,10$, and $30 \mu \mathrm{M}$ or treated with vehicle (DMSO) for 4 h . After treatment, cells were washed with phosphate-buffered saline (PBS), fixed in 3.5-4\% paraformaldehyde,
permeabilized with 0.1-0.5\% NP-40, and blocked with 1-3\% bovine serum albumin (BSA). Cells were incubated with primary antibody, anti-RPA194 (C1) [sc-48385, Santa Cruz Biotechnology], for 2 h at $37^{\circ} \mathrm{C}$ and washed three times with PBS. Cells were incubated with secondary antibody, Alexa 594-conjugated anti-mouse (A11005, Invitrogen) or Alexa 488-conjugated anti-mouse (A11001, Thermo Fisher), for 1 h at $37^{\circ} \mathrm{C}$, washed three times with PBS, and DNA was stained with Hoechst 33342 (H-21492, Invitrogen). Images were acquired using a Molecular Devices ImageXpress Micro XLS High Content Imager (20X objective, 9 fields/well) and processed using MetaXpress High Content Software-6. The fold change to control was determined. IC $5_{50}$ was determined using GraphPad Prism for Windows (version 6.01) using a three or four-parameter fit.

Cell Viability Assay. Performed by Daming Chen. A375 cells were seeded on 96well plates and incubated for 3 days with the compounds at $37{ }^{\circ} \mathrm{C}$ in $5 \% \mathrm{CO}_{2}$. Viability was determined using CellTiter-Glo® Luminescent Cell Viability Assay (Promega).

Diazirine Crosslinking and Affinity Purification. This protocol was adapted from Head and Liu. ${ }^{20}$ A375 cells were seeded in three 6 cm dishes and treated with DMSO only (D), Probe ( $5 \mu \mathrm{M}$ ) only (P), or probe $(5 \mu \mathrm{M})+$ competitor $(1 \mu \mathrm{M})(\mathrm{C})$ for 15 minutes. The dishes were placed on ice and washed gently with ice-cold PBS ( pH 7.4 ) to remove excess probe, then recovered with ice-cold PBS. The dishes were placed on top of an ice pack and centered 3 cm under the UV lamp (365 nm, Spectroline FC100) then irradiated for 3 minutes to induce crosslinking. After
irradiation, the PBS was aspirated from the cells and ice-cold PBS ( pH 7.4 ) with protease inhibitors was added to each plate. Cells were detached from the plate using a rubber scraper and transferred to pre-labeled microcentrifuge tubes on ice. SDS was added to a final concentration of $0.4 \%$. Cells were lysed by sonicating the suspension for 10 pulses (output 1, duty cycle $30 \%$, Branson Sonifier 250), incubated on ice for 1 minute, then sonicated again for 10 pulses. The samples were boiled on a hot plate set to $95^{\circ} \mathrm{C}$ for 5 minutes to complete cell lysis and denature all the proteins. The protein concentration in each sample was measured using the DC Protein Assay Kit II (Bio-Rad catalog \# 5000112) according to the manufacturer's instructions. Protein concentrations in each sample were normalized to the lowest measured protein concentration by adding PBS ( pH 7.4 ) $+0.4 \%$ SDS. The same volumes of lysates were used across all samples ( $250 \mu \mathrm{~L}$ ). The lysates were pre-cleared by adding to $50 \mu \mathrm{~L}$ high-capacity streptavidin agarose beads, pre-washed $2 x$ with PBS (pH 7.4) and incubated at $4^{\circ} \mathrm{C}$ for 1 hour with rotation. The beads were pelleted by centrifugation at $1,000 \times \mathrm{g}$ for 3 minutes. The supernatant was removed and placed into a new microcentrifuge tube on ice and the beads were discarded. Biotin-azide, TCEP, and TBTA were added and mixed by vortexing, then $\mathrm{CuSO}_{4} \cdot 5 \mathrm{H}_{2} \mathrm{O}$ was added, vortexed briefly, and incubated at room temperature for 30 minutes. To each sample was added 4 sample volumes of acetone chilled to $-20^{\circ} \mathrm{C}$. The samples were vortexed and incubated overnight at $-80^{\circ} \mathrm{C}$ to completely precipitate the proteins and remove unreacted biotin-azide. The samples were centrifuged at $17,000 \times \mathrm{g}$ for 15 minutes at $4{ }^{\circ} \mathrm{C}$ to pellet the precipitated proteins. The supernatant was aspirated completely, and the proteins
were resolubilized by sonication in PBS ( pH 7.4 ) $+1 \%$ SDS. PBS ( pH 7.4 ) was added to dilute the concentration of SDS to $0.2 \%$. The samples were added to 30 $\mu \mathrm{L}$ of pre-washed high-capacity streptavidin agarose beads and incubated at $4{ }^{\circ} \mathrm{C}$ for 1 hour with rotation. The beads were pelleted by centrifugation at $1,000 \times \mathrm{g}$ for 3 minutes. The supernatant containing unbound proteins was aspirated and discarded. Wash buffer ( $400 \mathrm{mM} \mathrm{NaCl}, 50 \mathrm{mM}$ Tris, $0.2 \%$ SDS, pH 7.4 ) was added to the beads and the samples were incubated at room temperature for 5 minutes with rotation. The centrifugation, aspiration, and washing steps were repeated 3 times. The wash buffer was aspirated completely from the beads and $2 x$ SDS sample buffer was added to the samples. The samples were incubated for 5 minutes on a $95^{\circ} \mathrm{C}$ heat block to release the proteins from the beads. The samples were centrifuged at $13,000 \times \mathrm{g}$ for 1 minute at room temperature. The sample buffer containing the proteins off of the beads was carefully pipetted into new labeled microcentrifuge tubes and stored at $-20^{\circ} \mathrm{C}$.

Proteomics Sample Preparation. Performed under the guidance of Ahmed Warshanna. This part of the protocol was adapted from the Protifi S-Trap mini use 4.1 protocol. To each sample was added a solution of dithiothreitol (DTT) in tetraethylammonium bromide (TEAB) (prepared using No Weigh DTT tubes) to a final DTT concentration of 20 nM . The samples were heated at $95^{\circ} \mathrm{C}$ for 5 minutes then allowed to cool to room temperature. To each sample was added a solution of iodoacetamide in TEAB to a final iodoacetamide concentration of 40 mM . The samples were incubated in the dark for 30 minutes. Acidifier ( $12 \%$ phosphoric acid) was added to each sample, and the samples were vortexed. Binding/wash buffer
( 100 mM TEAB (final) in $90 \% \mathrm{MeOH}$ ) was added to each sample, and the samples were vortexed. The samples were transferred to the S-Trap columns placed in a 2 mL receiver tube for waste flow through and centrifuged at $4,000 \times \mathrm{g}$ for 1 minute. Binding/wash buffer was added to the samples, and the samples were centrifuged at $4,000 \times \mathrm{g}$ for 1 minute. This was repeated 3 x . After the final centrifuge, the S Trap column was transferred to a new 2 mL receiver tube and digestion buffer (50 mM TEAB containing $5 \mu \mathrm{~g}$ of trypsin) was added to each sample, and the samples were incubated at $37{ }^{\circ} \mathrm{C}$ for 3 hours. Elution buffer 1 ( 50 mM TEAB in water) was added to the samples, and the samples were centrifuged at $1,500 \times \mathrm{g}$ for 1 minute. Elution buffer 2 ( $0.2 \%$ formic acid in water) was added to the samples, and the samples were centrifuged at $1,500 \mathrm{xg}$ for 1 minute. Elution buffer 3 ( $50 \% \mathrm{MeCN}$ in water) was added to the samples, and the samples were centrifuged at $1,500 \mathrm{x}$ g for 1 minute. Pooled eluted peptides were dried using a speed vac.

This part of the protocol was adapted from the Pierce Peptide Desalting Spin Columns User Guide, ThermoFisher Scientific. The white tip was removed from the bottom of the spin columns and discarded. Spin columns were placed into a 2 mL microcentrifuge tube. The spin columns were centrifuged at $5,000 \times \mathrm{g}$ for 1 minute to remove the solution and pack the resin material. The eluted liquid was discarded. The screw cap located at the top of the spin column was removed and MeCN was loaded into the spin column. The caps were replaced, the spin columns were placed back into 2 mL microcentrifuge tubes and were centrifuged at $5,000 \mathrm{x}$ g for 1 minute. The eluted MeCN was discarded, and the wash step was repeated. The spin columns were washed twice with $0.1 \%$ TFA in water, as described above.

Digested samples were resuspended in $0.1 \%$ TFA in water. The spin columns were placed into new 2 mL microcentrifuge tubes. Samples were loaded onto the spin columns, the top caps were replaced, and the spin columns were centrifuged at $3,000 \times \mathrm{g}$ for 1 minute. The flowthrough was discarded. The spin columns were placed into new 2 mL microcentrifuge tubes and $0.1 \%$ TFA was loaded onto the spin columns and centrifuged at $3,000 \times g$ for 1 minute to collect the wash. The eluate was discarded, and the wash step was repeated 2 more times for a total of 3 washes. The spin columns were placed into new 2 mL microcentrifuge tubes and $50 \% \mathrm{MeCN}+0.1 \%$ TFA was loaded onto the spin column and centrifuged at 3,000 $x \mathrm{~g}$ for 1 minute to collect the eluate. Another addition of the $50 \% \mathrm{MeCN}+0.1 \%$ TFA solution was made, and the spin columns were centrifuged at $3,000 \times \mathrm{g}$ for 1 minute, collecting the eluate into the same microcentrifuge tubes. The liquid contents of each sample tube were evaporated to dryness using vacuum centrifugation. The dry samples were resuspended with $0.1 \%$ formic acid at a concentration of $100 \mathrm{ng} / \mu \mathrm{L}$.

## References

(1) Peltonen, K.; Colis, L.; Liu, H.; Jäämaa, S.; Moore, H. M.; Enbäck, J.; Laakkonen, P.; Vaahtokari, A.; Jones, R. J.; af Hällström, T. M.; Laiho, M. Identification of Novel P53 Pathway Activating Small-Molecule Compounds Reveals Unexpected Similarities with Known Therapeutic Agents. PLoS ONE 2010, 5 (9), e12996. https://doi.org/10.1371/journal.pone.0012996.
(2) Peltonen, K.; Colis, L.; Liu, H.; Trivedi, R.; Moubarek, M. S.; Moore, H. M.; Bai, B.; Rudek, M. A.; Bieberich, C. J.; Laiho, M. A Targeting Modality for Destruction of RNA Polymerase I That Possesses Anticancer Activity. Cancer Cell 2014, 25 (1), 77-90. https://doi.org/10.1016/j.ccr.2013.12.009.
(3) Adams, A.; Guss, J. M.; Collyer, C. A.; Denny, W. A.; Wakelin, L. P. G. Crystal Structure of the Topoisomerase II Poison 9-Amino-[ N -(2-Dimethylamino)Ethyl]Acridine-4-Carboxamide Bound to the DNA Hexanucleotide d(CGTACG) 2 †. Biochemistry 1999, 38 (29), 9221-9233. https://doi.org/10.1021/bi990352m.
(4) Jancarik, J.; Kim, S.-H. Sparse Matrix Sampling: A Screening Method for Crystallization of Proteins. J. Appl. Crystallogr. 1991, 24 (4), 409-411. https://doi.org/10.1107/S0021889891004430.
(5) Adams, A.; Guss, J. M.; Collyer, C. A.; Denny, W. A.; Wakelin, L. P. G. A Novel Form of Intercalation Involving Four DNA Duplexes in an Acridine-4Carboxamide Complex of d(CGTACG)2. Nucleic Acids Res. 2000, 28 (21), 4244-4253. https://doi.org/10.1093/NAR/28.21.4244.
(6) Lapinsky, D. J. Tandem Photoaffinity Labeling-Bioorthogonal Conjugation in Medicinal Chemistry. Bioorg. Med. Chem. 2012, 20 (21), 6237-6247. https://doi.org/10.1016/j.bmc.2012.09.010.
(7) Murale, D. P.; Hong, S. C.; Haque, M. M.; Lee, J. S. Photo-Affinity Labeling (PAL) in Chemical Proteomics: A Handy Tool to Investigate Protein-Protein Interactions (PPIs). Proteome Sci. 2017, 15 (14). https://doi.org/10.1186/s12953-017-0123-3.
(8) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. Angew. Chem. Int. Ed. 2002, 41 (14), 2596-2599. https://doi.org/10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4.
(9) Salisbury, C. M.; Cravatt, B. F. Click Chemistry-Led Advances in High Content Functional Proteomics. QSAR Comb. Sci. 2007, 26 (11-12), 12291238. https://doi.org/10.1002/qsar. 200740090.
(10) Rix, U.; Superti-Furga, G. Target Profiling of Small Molecules by Chemical Proteomics. Nat. Chem. Biol. 2009, 5 (9), 616-624. https://doi.org/10.1038/nchembio.216.
(11) Misiaszek, A. D.; Girbig, M.; Grötsch, H.; Baudin, F.; Murciano, B.; Lafita, A.; Müller, C. W. Cryo-EM Structures of Human RNA Polymerase I. Nat. Struct. Mol. Biol. 2021, 28 (12), 997-1008. https://doi.org/10.1038/s41594-021-00693-4.
(12) Zhao, D.; Liu, W.; Chen, K.; Wu, Z.; Yang, H.; Xu, Y. Structure of the Human RNA Polymerase I Elongation Complex. Cell Discov. 2021, 7 (97), 1-12. https://doi.org/10.1038/s41421-021-00335-5.
(13) Daiß, J. L.; Pilsl, M.; Straub, K.; Bleckmann, A.; Höcherl, M.; Heiss, F. B.; Abascal-Palacios, G.; Ramsay, E. P.; Tlučková, K.; Mars, J. C.; Fürtges, T.; Bruckmann, A.; Rudack, T.; Bernecky, C.; Lamour, V.; Panov, K.; Vannini, A.; Moss, T.; Engel, C. The Human RNA Polymerase I Structure Reveals an HMG-like Docking Domain Specific to Metazoans. Life Sci. Alliance 2022, 5 (11). https://doi.org/10.26508/LSA. 202201568.
(14) Hill, J. R.; Robertson, A. A. B. Fishing for Drug Targets: A Focus on Diazirine Photoaffinity Probe Synthesis. J. Med. Chem. 2018, 61 (16), 6945-6963. https://doi.org/10.1021/acs.jmedchem.7b01561.
(15) Speers, A. E.; Cravatt, B. F. Profiling Enzyme Activities In Vivo Using Click Chemistry Methods. Chem. Biol. 2004, 11 (4), 535-546. https://doi.org/10.1016/J.CHEMBIOL.2004.03.012.
(16) Parker, C. G.; Pratt, M. R. Click Chemistry in Proteomic Investigations. Cell 2020, 180 (4), 605-632. https://doi.org/10.1016/j.cell.2020.01.025.
(17) Colis, L.; Ernst, G.; Sanders, S.; Liu, H.; Sirajuddin, P.; Peltonen, K.; DePasquale, M.; Barrow, J. C.; Laiho, M. Design, Synthesis, and Structure-Activity Relationships of Pyridoquinazolinecarboxamides as RNA Polymerase I Inhibitors. J. Med. Chem. 2014, 57 (11), 4950-4961. https://doi.org/10.1021/jm5004842.
(18) Dorado, T. E.; de León, P.; Begum, A.; Liu, H.; Chen, D.; Rajeshkumar, N. V.; Rey-Rodriguez, R.; Hoareau-Aveilla, C.; Alcouffe, C.; Laiho, M.; Barrow, J. C. Discovery and Evaluation of Novel Angular Fused Pyridoquinazolinonecarboxamides as RNA Polymerase I Inhibitors. ACS Med. Chem. Lett. 2022, 13 (4), 608-614. https://doi.org/10.1021/acsmedchemlett.1c00660.
(19) Geurink, P. P.; Prely, L. M.; van der Marel, G. A.; Bischoff, R.; Overkleeft, H. S. Photoaffinity Labeling in Activity-Based Protein Profiling. In ActivityBased Protein Profiling; Sieber, S. A., Ed.; Topics in Current Chemistry; Springer: Berlin, Heidelberg, 2012; pp 85-113. https://doi.org/10.1007/128_2011_286.
(20) Head, S. A.; Liu, J. O. Identification of Small Molecule-Binding Proteins in a Native Cellular Environment by Live-Cell Photoaffinity Labeling. J. Vis. Exp. 2016, No. 115, e54529. https://doi.org/doi:10.3791/54529.
(21) Bush, J. T.; Walport, L. J.; McGouran, J. F.; Leung, I. K. H.; Berridge, G.; Berkel, S. S. van; Basak, A.; Kessler, B. M.; Schofield, C. J. The Ugi FourComponent Reaction Enables Expedient Synthesis and Comparison of Photoaffinity Probes. Chem. Sci. 2013, 4 (11), 4115-4120. https://doi.org/10.1039/C3SC51708J.
(22) Colis, L.; Peltonen, K.; Sirajuddin, P.; Liu, H.; Sanders, S.; Ernst, G.; Barrow, J. C.; Laiho, M. DNA Intercalator BMH-21 Inhibits RNA Polymerase I Independent of DNA Damage Response. Oncotarget 2014, 5 (12), 43614369. https://doi.org/10.18632/oncotarget. 2020.
(23) Velagapudi, S. P.; Li, Y.; Disney, M. D. A Cross-Linking Approach to Map Small Molecule-RNA Binding Sites in Cells. Bioorg. Med. Chem. Lett. 2019, 29. https://doi.org/10.1016/j.bmcl.2019.04.001.
(24) Kanwal, I.; Mujahid, A.; Rasool, N.; Rizwan, K.; Malik, A.; Ahmad, G.; Shah, S. A. A.; Rashid, U.; Nasir, N. M. Palladium and Copper Catalyzed Sonogashira Cross Coupling Has Been an Excellent Methodology for C-C Bond Formation for 17 Years: A Review. Catalysts 2020, 10 (4), 443. https://doi.org/10.3390/catal10040443.
(25) Karak, M.; Barbosa, L. C. A.; Hargaden, G. C. Recent Mechanistic Developments and next Generation Catalysts for the Sonogashira Coupling Reaction. RSC Adv. 2014, 4 (96), 53442-53466. https://doi.org/10.1039/C4RA09105A.
(26) Mohajer, F.; Heravi, M. M.; Zadsirjan, V.; Poormohammad, N. Copper-Free Sonogashira Cross-Coupling Reactions: An Overview. 2021. https://doi.org/10.1039/dOra10575a.
(27) Guram, A. S.; King, A. O.; Allen, J. G.; Wang, X.; Schenkel, L. B.; Chan, J.; Bunel, E. E.; Faul, M. M.; Larsen, R. D.; Martinelli, M. J.; Reider, P. J. New Air-Stable Catalysts for General and Efficient Suzuki-Miyaura CrossCoupling Reactions of Heteroaryl Chlorides. Org. Lett. 2006, 8 (9), 17871789. https://doi.org/10.1021/ol060268g.
(28) Schilz, M.; Plenio, H. A Guide to Sonogashira Cross-Coupling Reactions: The Influence of Substituents in Aryl Bromides, Acetylenes, and Phosphines. J. Org. Chem. 2012, 77 (6), 2798-2807. https://doi.org/10.1021/jo202644g.
(29) Martyloga, O. V.; Myronenko, A.; Tkachenko, A. M.; Matvienko, V. O.; Kuchkovska, Y. O.; Grygorenko, O. O. Multigram Synthesis of Functionalized Spirocyclic Diazirines. Eur. J. Org. Chem. 2019, 2019 (23), 3744-3750. https://doi.org/10.1002/ejoc.201900485.
(30) Han, G.; Tamaki, M.; Hruby, V. J. Fast, Efficient and Selective Deprotection of the Tert-Butoxycarbonyl (Boc) Group Using HCL/Dioxane (4 M). J. Pept. Res. 2001, 58 (4), 338-341. https://doi.org/10.1034/j.13993011.2001.00935.x.
(31) Krasiński, A.; Radić, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. In Situ Selection of Lead Compounds by Click Chemistry:

Target-Guided Optimization of Acetylcholinesterase Inhibitors. J. Am. Chem. Soc. 2005, 127 (18), 6686-6692. https://doi.org/10.1021/ja043031t.

