Bioorganic & Medicinal Chemistry 21 (2013) 1880–1897

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Novel inhibitors of bacterial virulence: Development of 5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-ones for the inhibition of group A streptococcal streptokinase expression

Bryan D. Yestrepsky [a,†], Yuanxi Xu [c,†], Meghan E. Breen [a], Xiaoqin Li [b], Walajapet G. Rajeswaran [a], Jenny G. Ryu [a], Roderick J. Sorenson [a], Yasuhiro Tsume [b], Michael W. Wilson [a], Wenpeng Zhang [b], Duxin Sun [b], Hongmin Sun [c,*], Scott D. Larsen [a,*]

[a] *Vahlteich Medicinal Chemistry Core, Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, United States*
[b] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, United States*
[c] *School of Medicine, Department of Internal Medicine, University of Missouri, Columbia, MO 65212, United States*

ARTICLE INFO

*Article history:*
Received 30 November 2012
Revised 8 January 2013
Accepted 15 January 2013
Available online 1 February 2013

*Keywords:*
Group A Streptococcus
Virulence inhibitor
Antibiotic
Streptokinase
Antibiotic resistance
Metabolic oxidation
Biofilm
Antivirulence

ABSTRACT

Resistance to antibiotics is an increasingly dire threat to human health that warrants the development of new modes of treating infection. We recently identified **1** (CCG-2979) as an inhibitor of the expression of streptokinase, a critical virulence factor in Group A Streptococcus that endows blood-borne bacteria with fibrinolytic capabilities. In this report, we describe the synthesis and biological evaluation of a series of novel 5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one analogs of **1** undertaken with the goal of improving the modest potency of the lead. In addition to achieving an over 35-fold increase in potency, we identified structural modifications that improve the solubility and metabolic stability of the scaffold. The efficacy of two new compounds **12c** (CCG-203592) and **12k** (CCG-205363) against biofilm formation in *Staphylococcus aureus* represents a promising additional mode of action for this novel class of compounds.



## 1. Introduction

Bacterial infection remains one of the most critical threats to human health, especially in third world countries and in children. *Streptococcus pyogenes,* or Group A Streptococcus (GAS), represents one aspect of this global threat. GAS is a ubiquitous human pathogen responsible for up to 700 million infections per year worldwide.[1] While most commonly associated with upper respiratory infection ('strep throat') and superficial skin infections, systemic infections with GAS such as streptococcal toxic shock syndrome, rheumatoid fever, and necrotizing fasciitis are very often fatal. Cases of systemic GAS infection are thought to approach 500,000 cases annually, with a mortality rate of 15–35%.[2,3]

The rapid emergence of bacterial resistance to antibiotic treatment is an alarming aspect of systemic infection, which can rapidly progress in the absence of effective treatment. As dangerous resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) become increasingly prevalent, especially in the hospital setting,[4,5] the necessity for antibiotics with novel mechanisms of action grows. Virulence factors are among the targets currently being evaluated for antibiotic activity.[6] By inhibiting the mechanisms bacteria use to spread and thrive in a host organism, virulence inhibitors are intended to retain highly infective pathogenic bacteria in a relatively benign state, allowing additional time for the host's immune system or a course of antibiotics to clear the infection. Since inhibitors of virulence do not necessarily affect the reproduction of bacterial cells, they may represent an avenue of antibiotic treatment that

*Abbreviations:* GAS, Group A Streptococcus (*Streptococcus pyogenes)*; SK, streptokinase; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; HTS, high-throughput screening; SAR, Structure–activity relationship; MLM, mouse liver microsomal extract; CID, collision-induced dissociation; ESI, electrospray ionization; EPI, enhanced product ion scan; MRM, multiple reaction monitoring; EMS, enhanced mass spectrometry; DMEM, Dulbecco's modified Eagle's medium; THY, Todd-Hewitt media with 0.2% yeast extract.

* Corresponding authors. Tel.: +1 573 882 2296 (H.S.); tel.: +1 734 615 0454 (S.D.L.).

*E-mail addresses:* sunh@health.missouri.edu (H. Sun), sdlarsen@umich.edu (S.D. Larsen).

† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.01.046

does not encourage the development of resistance. A number of virulence mechanisms have been targeted for inhibition by small molecules in the literature, including bacterial toxins,[7] adhesion factors,[8] quorum-sensing systems,[9,10] and virulence-inducing signaling pathways.[11] Though no anti-virulence drugs have yet progressed to use in the clinic, inhibitors of anthrax toxin cell permeability,[12] *N*-acyl homoserine lactone-mediated quorum sensing,[13] and QseC histidine sensor kinase activation[11] have demonstrated efficacy in murine models of infection.

Sun et al. previously identified the protein streptokinase (SK) as a potent virulence factor in GAS.[14] SK is an activator of human plasminogen, which generates the fibrinolytic plasmin and allows the bacteria to circumvent the host's defense mechanism of clotting around foci of infection. We recently reported a high-throughput screening (HTS) campaign that identified lead compound **1** (CCG-2979, Fig. 1) and analog **6b** (CCG-102487) as capable of reducing the expression of streptokinase at the transcriptional level, confirmed by Western blot and RNA microarray analysis. This compound was also found to elicit a significant protective effect in a transgenic mouse model of GAS infection.[15] These findings prompted the initiation of a structure-activity relationship (SAR) study based on **1** with the intent of increasing its modest potency (% of plasmin activity at 50 μM = 48 ± 28%) and improving the predicted pharmacokinetic properties of the scaffold. In particular, we were concerned about its high lipophilicity (*c*Log*P* = 7.1), which can be associated with poor solubility and metabolic stability. Ultimately we would also like to identify the macromolecular target for **1**, which is currently unknown due to the phenotypic nature of the HTS and biological assay. To design affinity probes for this purpose, we needed to perform SAR in order to both improve potency and to identify areas on the molecule amenable to substitution without loss of activity.

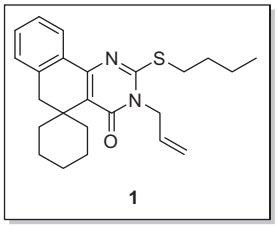
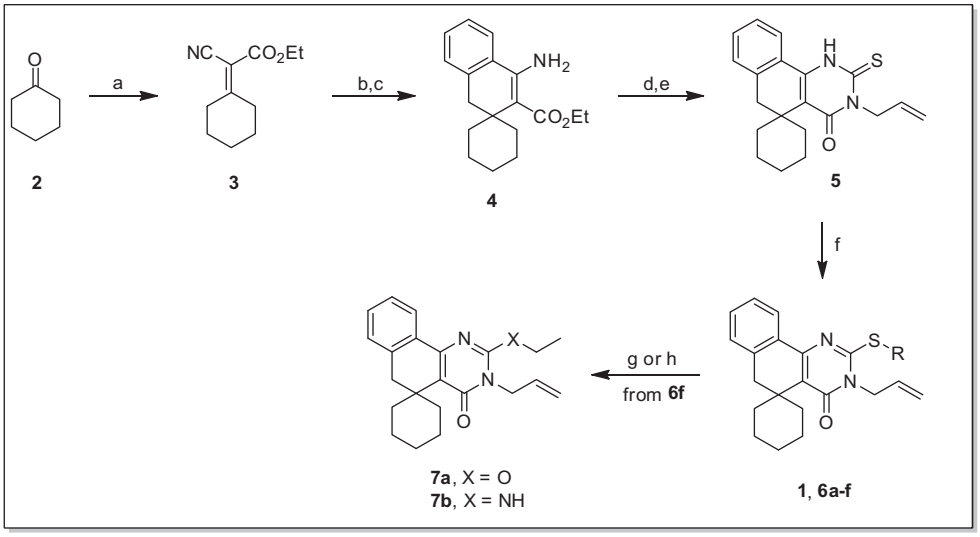



**Figure 1.** Structure of HTS lead compound **1**.

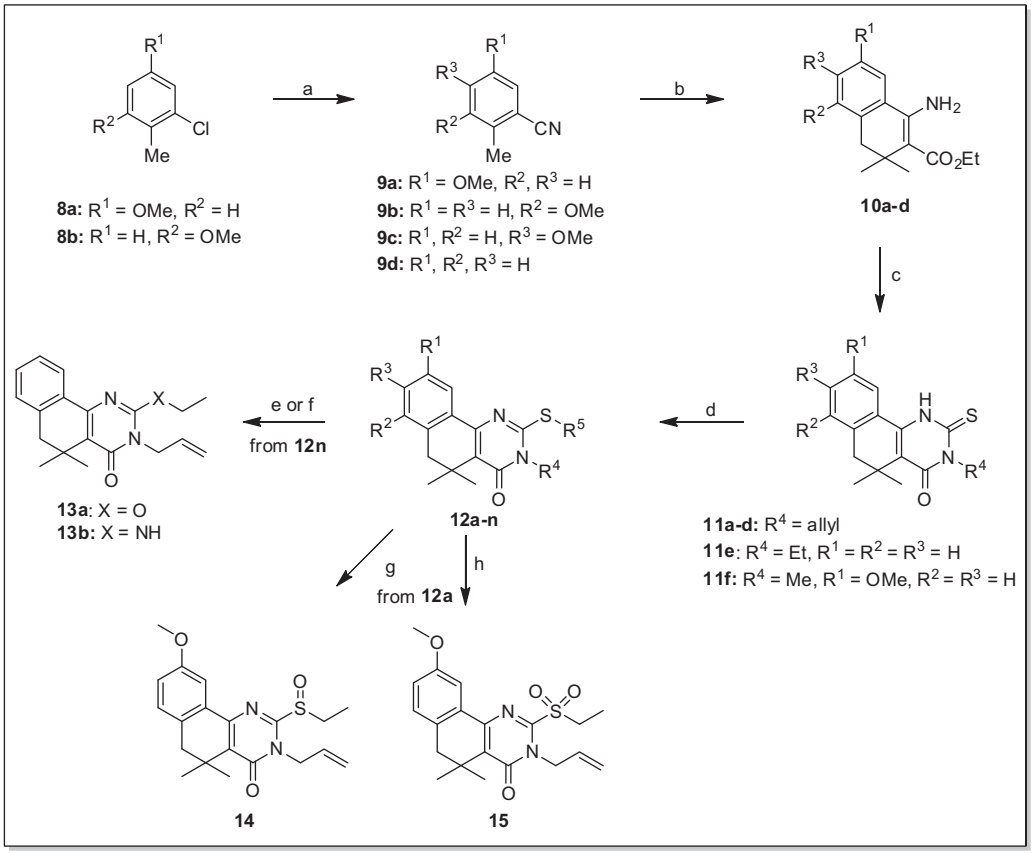
## 2. Chemistry

Access to the core structure of **1** was accomplished with a known 5-step synthetic pathway (Scheme 1). β-aminoester **4** was synthesized from cyclohexanone via a Knoevenagel condensation with ethyl cyanoacetate followed by Michael addition of benzylmagnesium chloride, and finally acid-mediated cyclization.[16] The 2-thioxo-dihydropyrimidinone ring could then be installed via nucleophilic addition of **4** to allyl isothiocyanate, followed by base-catalyzed cyclization.[17] Finally, sulfur alkylation under basic conditions gave access to **1** and S-substituted analogs **6a–f**. Additional analogs that replace the sulfide with ether (**7a**) and amine (**7b**) linkages were accessed via the *S*-methyl derivative **6f**, either by direct nucleophilic substitution with sodium ethoxide, or further functionalization to the methyl sulfone and displacement with ethylamine under basic conditions.[18]

Given the relative ease with which the original spirocyclohexyl-substituted analogs were synthesized, we were surprised to find that we were not able to generate gem-dimethyl analogs (e.g., **12**, Scheme 2) using this scheme. While Michael addition of benzylmagnesium chloride into ethyl 2-cyano-3-methylbut-2-enoate proceeded as desired, no set of conditions was successful in generating the gem-dimethyl analog of **4**. It was finally determined that the β-aminoesters **10a–d** could be accessed in one step beginning from the corresponding *o*-tolunitriles **9a–d**, available commercially or by $SN_{Ar}$ addition of cyanide into the corresponding chlorotoluenes **8a,b**[19] (Scheme 2). In the presence of LDA, smooth addition of **9a–d** to the β-position of ethyl 3,3-dimethyl acrylate afforded an enol ester intermediate that readily cyclized in the presence of $ZnI_2$, providing amino esters **10a–d**.[20] Modified conditions for condensation with alkyl isothiocyanates were required to efficiently produce the 2-thioxo-dihydropyrimidinone intermediates **11a–f**. Subsequent alkylation at sulfur ultimately allowed the synthesis of gem-dimethyl analogs **12a–n** in a total of 3 to 4 steps (Scheme 2). Further elaboration of these compounds to generate ether and amino derivatives **13a,b** was achieved in the same manner as for **7a**,**b**. Chemoselective sulfur oxidation of **12a** via treatment with 1.1 eq of Oxone®[21] or an excess of *m*CPBA delivered



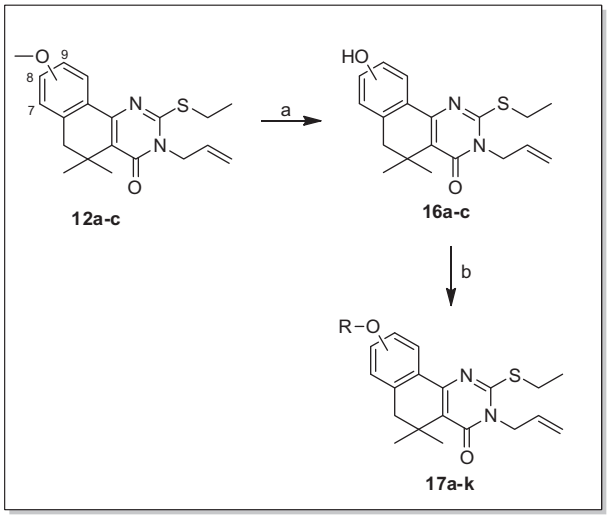

**Scheme 1.** Preparation of spirocyclohexyl analogs of **1**. Reagents and conditions: (a) ethyl cyanoacetate, AcOH, $NH_4OAc$, toluene, 150 °C, 5 h; (b) BnMgCl, $Et_2O$, RT, 72 h; (c) $H_2SO_4$, 0 °C to rt, 7 h; (d) allyl isothiocyanate, EtOH, reflux, 10 h; (e) KOH, EtOH/$H_2O$ (1:1), reflux, 10 h; (f) R-X, base, EtOH or MEK, 0–70 °C, 10 min–16 h; (g) NaOEt, EtOH, 40 °C, 48 h; (h) (i) *m*CPBA, DCM, rt, 16 h, (ii) $EtNH_2$, $K_2CO_3$, THF/DMF (1:1), rt, 5 h.



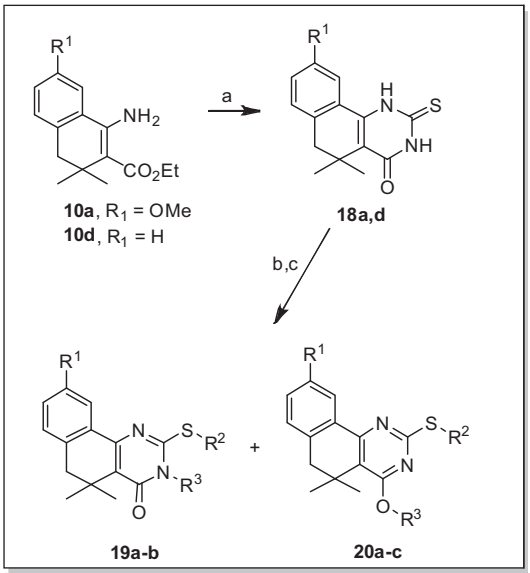

**Scheme 2.** Synthesis of gem-dimethyl substituted analogs of **1**. Reagents and conditions: (a) $NiBr_2$, Zn(dust), $PPh_3$, then KCN, THF, 50–60 °C, 24 h; (b) LDA, diglyme, −78 °C, 1 h, then ethyl 3,3-dimethyl acrylate, $ZnI_2$, −78 °C to rt, 2 h; (c) $R^4$-NCS, AcOH, EtOH, 70 °C, 16 h; (d) $R^5$-X, $Cs_2CO_3$, MEK or DMF, 70 °C, 16 h; (e) NaOEt, EtOH, 40 °C, 48 h; f) (i) *m*CPBA, DCM, 0 °C to RT, 16 h, (ii) $EtNH_2$, $K_2CO_3$, THF/DMF (1:1), rt, 5 h; (g) 1.1 equiv Oxone, THF/$H_2O$ (1:1), 0 °C to rt, 16 h; (h) *m*CPBA, DCM, rt, 12 h.

sulfoxide and sulfone-modified analogs **14** and **15**, respectively. No epoxidation of the allyl group was observed under these mild oxidation conditions.

Small changes or additions to the core procedure allowed access to a number of other substitution patterns. Beginning from the methoxy-substituted aryl ethers **12a–c**, treatment with $BBr_3$ revealed phenols **16a–c**, which could be elaborated into a diverse set of aryl ethers **17a–k** (Scheme 3). Exploring diversity at the N- and O-positions of the pyrimidinone required assembling the unsubstituted thioxo-dihydropyrimidinone ring via treatment of **10a** or **10d** with benzoyl isothiocyanate, followed by ring closure and hydrolysis of the resulting *N*-benzoyl group with aqueous KOH to generate **18a** and **18d**[22] (Scheme 4). Selective alkylation




**Scheme 3.** Synthesis of aryl ethers **17a–k**. .Reagents and conditions: (a) $BBr_3$, DCM, 0 °C to rt, 16 h; (b) R-X, $Cs_2CO_3$, MEK or DMF, rt −70 °C, 16 h.




**Scheme 4.** Generation of N- and O-alkylated substitution pairs **19a–b** and **20a–c**. Reagents and conditions: (a) (i) benzoyl isothiocyanate, EtOH, 75 °C, 5 h, (ii) KOH, EtOH/$H_2O$ (2:1) 70 °C, 2 h; (b) $R^2$-X, $NaHCO_3$, DMF, rt −70 °C, 30 min–16 h; (c) $R^3$-X, base, EtOH or DMF, 70–80 °C, 16–24 h.

of the sulfur was achieved under neutral conditions, while subsequent base-catalyzed alkylation of the amide generally gave some mixture of N- and O-alkylated analogs **19a–b** and **20a–c**.

Finally, carboxylic acid derivatives at the 8-position (Scheme 5) were accessed via lithiation and carboxylation of 4-bromo-2-methylbenzonitrile **21**, followed by cyclization and esterification to generate **23**. Sensitivity to the standard acid-catalyzed cyclization with allyl isothiocyanate necessitated the use of the stepwise procedure seen for the conversion of **10** to **18** (Scheme 4). Subsequent alkylations at sulfur and nitrogen delivered the 8-methylcarboxylate analog **25**, which could be saponified to acid **26**. Amides **27a** and **27b** were accessed via amide coupling conditions using HOBt and EDC.

## 3. Results and discussion

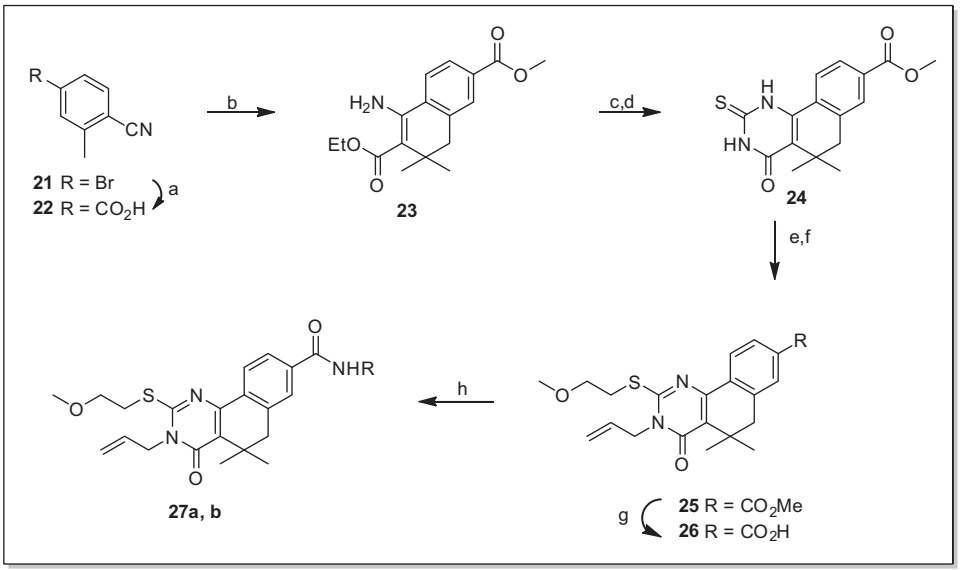
### 3.1. SK expression assay

All new compounds were evaluated for their ability to suppress SK expression via a chromogenic assay of plasmin activity. Two identical cultures of GAS were grown in the presence of the desired concentration of test compound or DMSO and allowed to grow to $OD_{600}$ ≈1.0. After centrifugation, an aliquot of supernatant was combined with human plasma and synthetic plasmin substrate S-2403 (see Section 5.1.1). Decreased expression of SK induced by the test compound lowers the amount of activated plasmin, in turn reducing the rate of cleavage of S-2403 to colored product *p*-nitroaniline. The concentration of *p*-nitroaniline was measured by absorbance at 405 nm ($A_{405}$) and compared to the DMSO control. Activity data is reported as the ratio of $A_{405}$(test) divided by $A_{405}$(control) (*T*/*C*). To provide an approximate readout of potency, the assay was run initially at both 5 and 50 μM of test compound. An optical density reading at 600 nm ($OD_{600}$) gave an estimate of cell density and thus overall bacterial growth inhibition; this data is also reported for the higher concentration of compound (50 μM) as a ratio of the $OD_{600}$ of the test culture divided by the $OD_{600}$ of the DMSO control culture. Selected compounds that inhibited SK activity by more than 50% at 50 μM were subjected to full dose–response titrations to determine approximate $IC_{50}$ values using the same assay.

During the course of evaluating the new analogs, it was observed that there was a relatively high level of inter-assay variability in the activity assay. There are a number of likely contributing factors, most notably the variability inherent to working in a live bacterial system. For example, it has been noted that minimum inhibitory concentration (MIC) values for determining bacteriotoxicity tend to vary by up to three-fold from assay to assay.[23] Furthermore, the currently unknown macromolecular target of these compounds likely affects a pathway including a transcription factor, which are known to be low-abundance proteins[24] that exhibit significant temporal expression changes, potentially contributing to the variability. It was noted early into the SAR effort that **12h** was both moderately active and particularly consistent from assay to assay. In an effort to reduce the number of anomalous data points, **12h** was included in each assay as a positive control. Assays in which **12h** exhibited activity deviating from the mean activity at 5 and 50 μM by more than one standard deviation (calculated from a total of 35 assays) were discarded.

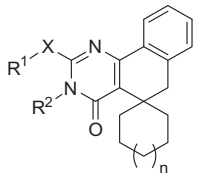
### 3.2. SAR development

We first examined a series of spirocycloalkyl substituted compounds with varying substitution patterns at the 2-position of the pyrimidinone ring system (Table 1). Aromatic substitution at $R^1$ (**6a–c**) did not appreciably increase potency over **1,** but methyl substitution (**6f**) conferred a modest increase in activity ($IC_{50}$ = 19 μM vs >50 μM). Interestingly, polar groups were tolerated as part of smaller alkyl groups at –$XR^1$ (e.g., **6d** and **7a**). The replacement of sulfur with nitrogen (**7b**) led to an unacceptable decrease in growth. Similarly, basic amine functionality appended to sulfur (**6e**) led to growth inhibition in some trials. We defined acceptable growth inhibition to be no greater than 15% at 50 μM of test compound. The favorable potency of commercially available spirocyclopentyl analog **28** relative to closely related spirocyclohexyl analog **6b** led us to hypothesize that the size of the geminal substitution on the central ring could be reduced, allowing




**Scheme 5.** Synthesis of carboxylic acid derivatives 24–26. Reagents and conditions: (a) *n*BuLi, then $CO_2$, THF, −78 °C to rt, 1 h; (b) (i) LDA, diglyme, −78 °C, 20 min, then ethyl 3,3-dimethyl acrylate, $ZnI_2$, −78 °C to rt, 3 h, (ii) TMS-$CHN_2$, MeOH/toluene (1:5), rt, 30 min; (c) benzoyl isothiocyanate, EtOH, reflux, 2.5 h; (d) NaOMe, MeOH, 70 °C, 2 h; (e) 2-methoxyethyl-4-methylbenzenesulfonate, $Cs_2CO_3$, DMF, 40 °C to rt, 16 h; (f) allyl bromide, NaOMe, MeOH, 65 °C, 2 h; (g) NaOH, MeOH, rt, 16 h; (h) $NH_2R$, HOBt, EDC, THF, rt, 16 h.

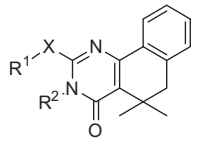
**Table 1**
Inhibition of SK activity by spirocycloalkyl analogs



| No. | $R^1$ | $R^2$ | X | *n* | 5 μM SK *T*/*C* [a] | 50 μM SK *T*/*C* [a] | 50 μM Growth *T*/*C*[b] | $IC_{50}$ (μM) | MLM $t_{1/2}$[c] (min) | Aq Sol.[d] (μM) |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | *n*-Bu | Allyl | S | 1 | 0.84 ± 0.14 | 0.48 ± 0.28 | 0.95 ± 0.08 | >50 | 37.2 | 9 ± 2 |
| **6a** | 4-$MeOPhCH_2$ | Allyl | S | 1 | 1.00 ± 0.33 | 0.82 ± 0.44 | 1.05 ± 0.06 | | | |
| **6b** | $PhCH_2$ | Allyl | S | 1 | 0.74 ± 0.16 | 0.57 ± 0.21 | 0.96 ± 0.06 | >50 | 0.6 | 9 ± 2 |
| **6c** | 4-$AcNHPhCH_2$ | Allyl | S | 1 | 0.96 ± 0.46 | 0.74 ± 0.28 | 0.96 ± 0.14 | | | |
| **6d** | $MeOCH_2CH_2$ | Allyl | S | 1 | 0.47 ± 0.28 | 0.29 ± 0.15 | 0.99 ± 0.10 | | 6.4 | 9 ± 2 |
| **6e** | $Me_2NCH_2CH_2$ | Allyl | S | 1 | 0.72 ± 0.12 | 0.52 ± 0.37 | 0.88 ± 0.20 | | | |
| **6f** | Me | Allyl | S | 1 | 0.60 ± 0.19 | 0.42 ± 0.03 | 0.98 ± 0.03 | 19 ± 6.0 | 13.5 | 22 ± 5 |
| **7a** | Et | Allyl | O | 1 | 0.53 ± 0.23 | 0.14 ± 0.05 | 1.02 ± 0.09 | | | 14.5 ± 3.5 |
| **7b** | Et | Allyl | NH | 1 | 0.78 ± 0.47 | 0.56 ± 0.70 | 0.32 ± 0.18 | | | 9 ± 2 |
| **28** | $PhCH_2$ | Et | S | 0 | 0.44 ± 0.06 | 0.23 ± 0.16 | 1.07 ± 0.04 | | | |

[a] Ratio of $A_{405}$ of SK-cleaved substrate in GAS culture treated with the indicated concentration of test compound divided by $A_{405}$ of DMSO control (see Section 5.1.1). Values are the mean of at least three experiments ± standard deviation.
[b] Ratio of OD (600 nm) for growth of GAS in the presence of test compound divided by DMSO control. Values are the mean of at least three experiments ± standard deviation.
[c] Half-life of parent compound during incubation with mouse liver microsomes.
[d] Kinetic solubility of compound in aqueous Todd Hewitt bacterial media.

**Table 2**
Inhibition of SK activity by gem-dimethyl analogs



| No. | $R^1$ | $R^2$ | X | 5 μM SK *T*/*C* [a] | 50 μM SK *T*/*C*[a] | 50 μM Growth *T*/*C*[b] | $IC_{50}$ (μM) | MLM $t_{1/2}$[c] (min) | Aq Sol.[d] (μM) |
|---|---|---|---|---|---|---|---|---|---|
| **12d** | $H_2NCOCH_2$ | Allyl | S | 0.79 ± 0.17 | 0.64 ± 0.16 | 1.00 ± 0.02 | | <2 | |
| **12e** | 1-pyridyl$CH_2$ | Allyl | S | 0.40 ± 0.09 | 0.13 ± 0.07 | 1.05 ± 0.02 | | | |
| **12f** | $HOCH_2CH_2$ | Allyl | S | 0.48 ± 0.17 | — | 0.06 ± 0.03 | | | |
| **12g** | $NCCH_2$ | Allyl | S | 0.37 ± 0.17 | 0.23 ± 0.06 | 1.00 ± 0.03 | | 20.6 | 34.5 ± 7.5 |
| **12h** | $MeOCH_2CH_2$ | Allyl | S | 0.35 ± 0.11 | 0.13 ± 0.07 | 0.86 ± 0.10 | 3.2 ± 0.6 | 1.7 | 22.5 ± 4.5 |
| **12i** | $MeOCH_2CH_2$ | Et | S | 0.41 ± 0.23 | — | 0.39 ± 0.15 | | 0.8 | |
| **13a** | Et | Allyl | O | 0.34 ± 0.08 | 0.23 ± 0.10 | 0.50 ± 0.23 | | 0.9 | |
| **13b** | Et | Allyl | NH | 0.48 ± 0.19 | — | 0.06 ± 0.03 | | 1.1 | |
| **19a** | $MeOCH_2CH_2$ | 4-$MeOPhCH_2CH_2$ | S | 0.71 ± 0.40 | 0.61 ± 0.32 | 0.97 ± 0.07 | | 9.4 | |
| **29** | $PhCH_2$ | Allyl | S | 0.65 ± 0.25 | 0.35 ± 0.14 | 1.03 ± 0.03 | 10.8 ± 3.0 | | 9 ± 2 |
| **30** | Et | Allyl | S | 0.24 ± 0.07 | 0.17 ± 0.08 | 0.89 ± 0.05 | 2.0 ± 0.5 | | |
| **31** | $EtO_2CCH_2$ | Allyl | S | 0.54 ± 0.10 | 0.27 ± 0.12 | 1.01 ± 0.02 | | | |

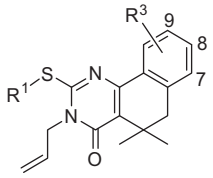
[a] Ratio of $A_{405}$ of SK-cleaved substrate in GAS culture treated with the indicated concentration of test compound divided by $A_{405}$ of DMSO control (see Section 5.1.1). Values are the mean of at least three experiments ± standard deviation.
[b] Ratio of OD (600 nm) for growth of GAS in the presence of test compound divided by DMSO control. Values are the mean of at least three experiments ± standard deviation.
[c] Half-life of parent compound during incubation with mouse liver microsomes.
[d] Kinetic solubility of compound in aqueous Todd Hewitt bacterial media.

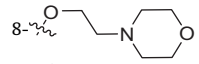
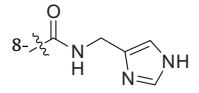
reduction of both the lipophilicity and the molecular weight of future analogs.

Exploration of analogs with gem-dimethyl substitution of the central ring (Table 2) began with the assessment of commercially available analogs **29**, **30**, and **31**, all of which showed an increase in potency in comparison to **1**. Polar substitution at the $R^1$ position was generally well-tolerated (**12e, 12g, 12h, 31**) except for primary amide **12d**. Introducing an alcohol on the sidechain (**12f**) to improve solubility led to unacceptable bacteriotoxicity. This toxicity was also observed with heteroatomic analogs **13a** and **13b** versus sulfur analog **30**, leading us to conclude that retention of the sulfur was necessary to avoid inhibition of bacterial growth. Altering the substitution of the *N*-position of the pyrimidinone was found to lead to bacterial toxicity (**12i)** or attenuation of activity (**19a**) compared to *N*-allyl (**12h**). Overall, the *S*-Et and *S*-$CH_2CH_2OMe$ analogs **30** and **12h** were the optimum compounds from this series (Table 2).

Our attention then turned to exploring substitution of the aromatic ring (Table 3) at 3 different positions. Assessment of methyl ether analogs **12a–c** revealed that substitution at the 8- and 9- positions conferred a moderate increase in potency over **30**, while substitution of the 7-position did not. The installation of larger and/or more polar ethers (**17a–d, 17h–k**) generally led to the attenuation of these gains, though a few compounds retained activity similar

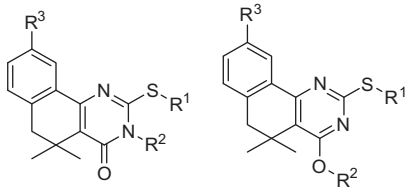
**Table 3**
Inhibition of SK activity by analogs with 7-, 8-, and 9-position substitution



| No. | $R^1$ | $R^3$ | 5 μM SK $T/C$[a] | 50 μM SK $T/C$[a] | 50 μM Growth $T/C$[b] | $IC_{50}$ (μM) | MLM $t_{1/2}$[c] (min) | Aq Sol.[d] (μM) |
|---|---|---|---|---|---|---|---|---|
| **12a** | Et | 9-OMe | 0.10 ± 0.06 | 0.07 ± 0.02 | 0.73 ± 0.07 | 1.3 ± 0.6 | 2.3 | 9 ± 2 |
| **12b** | Et | 7-OMe | 0.26 ± 0.07 | 0.29 ± 0.18 | 0.28 ± 0.26 | | | |
| **12c** | Et | 8-OMe | 0.18 ± 0.09 | 0.07 ± 0.01 | 0.87 ± 0.19 | 3.1 ± 0.7 | 0.8 | 14.5 ± 3.5 |
| **12j** | $MeOCH_2CH_2$ | 8-OMe | 0.47 ± 0.25 | 0.25 ± 0.18 | 0.89 ± 0.16 | | | |
| **12k** | Allyl | 8-OMe | 0.33 ± 0.15 | 0.28 ± 0.16 | 0.92 ± 0.16 | 6.9 ± 0.3 | 0.6 | 14.5 ± 3.5 |
| **17a** | Et | 9-$MeOCH_2CH_2O$ | 0.58 ± 0.24 | 0.43 ± 0.15 | 1.02 ± 0.10 | | | |
| **17b** | Et | 7-$MeOCH_2CH_2O$ | 0.84 ± 0.23 | 0.40 ± 0.21 | 0.94 ± 0.08 | | | |
| **17c** | Et | 8-$MeOCH_2CH_2O$ | 0.46 ± 0.35 | 0.36 ± 0.41 | 0.85 ± 0.22 | | | |
| **17d** | Et | 8-$EtO_2CCH_2$ | 0.64 ± 0.67 | 0.47 ± 0.48 | 1.02 ± 0.10 | | | |
| **17e** | Et |  | 0.53[e] | 0.08[e] | 0.41[e] | | | |
| **17f** | Et | 8-O-*i*Pr | 0.29 ± 0.19 | 0.13 ± 0.12 | 0.87 ± 0.23 | 4.1 ± 0.7 | | 14.5 ± 3.5 |
| **17g** | Et | 8-$NCCH_2O$ | 0.45 ± 0.25 | 0.20 ± 0.17 | 0.94 ± 0.15 | | | |
| **17h** | Et | 8-*n*-BuO | 0.48 ± 0.26 | 0.35 ± 0.29 | 0.87 ± 0.10 | | | |
| **17i** | Et | 8-$PhCH_2CH_2O$ | 0.68 ± 0.34 | 0.59 ± 0.55 | 1.01 ± 0.03 | | | |
| **17j** | Et | 8-$CH_3(CH_2)_6O$ | 1.03 ± 0.18 | 0.87 ± 0.12 | 1.05 ± 0.03 | | | |
| **17k** | Et | 8-$HO_2CCH_2O$ | 0.96 ± 0.21 | 0.44 ± 0.12 | 0.86 ± 0.07 | | | |
| **25** | $MeOCH_2CH_2$ | 8-$CO_2Me$ | 0.71 ± 0.30 | 0.58 ± 0.19 | 1.01 ± 0.04 | | | |
| **26** | $MeOCH_2CH_2$ | 8-$CO_2H$ | 0.75 ± 0.36 | 0.70 ± 0.52 | 0.86 ± 0.11 | | | |
| **27a** | $MeOCH_2CH_2$ | 8-CONHBn | 1.14 ± 0.24 | 0.91 ± 0.18 | 1.07 ± 0.02 | | | |
| **27b** | $MeOCH_2CH_2$ |  | 0.84 ± 0.10 | 0.77 ± 0.27 | 0.99 ± 0.02 | | | |

[a] Ratio of $A_{405}$ of SK-cleaved substrate in GAS culture treated with the indicated concentration of test compound divided by $A_{405}$ of DMSO control (see Section 5.1.1). Values are the mean of at least three experiments ± standard deviation.
[b] Ratio of OD (600 nm) for growth of GAS in the presence of test compound divided by DMSO control. Values are the mean of at least three experiments ± standard deviation.
[c] Half-life of parent compound during incubation with mouse liver microsomes.
[d] Kinetic solubility of compound in aqueous Todd Hewitt bacterial media.
[e] Values derived from only 1 experiment due to toxicity.

**Table 4**
Effect of N- versus O-alkylation on GAS-SK inhibition and metabolic stability



**19** **20**

| No. | $R^1$ | $R^2$ | $R^3$ | 5 μM SK $T/C$[a] | 50 μM SK $T/C$[a] | 50 μM Growth $T/C$[b] | $IC_{50}$ (μM) | MLM $t_{1/2}$ (min)[c] |
|---|---|---|---|---|---|---|---|---|
| **19a** | $MeOCH_2CH_2$ | 4-$MeOPhCH_2CH_2$ | H | 0.71 ± 0.40 | 0.61 ± 0.32 | 0.97 ± 0.07 | | 9.4 |
| **20a** | $MeOCH_2CH_2$ | 4-$MeOPhCH_2CH_2$ | H | 0.46 ± 0.37 | 0.24 ± 0.15 | 1.00 ± 0.09 | 5.5 ± 0.7 | 45.1 |
| **19b** | Et | $CF_3CH_2$ | MeO | 0.78 ± 0.36 | 0.49 ± 0.17 | 1.01 ± 0.04 | | 3.0 |
| **20b** | Et | $CF_3CH_2$ | MeO | 0.51 ± 0.20 | 0.38 ± 0.23 | 0.89 ± 0.02 | | 45.5 |
| **12a** | Et | Allyl | MeO | 0.10 ± 0.06 | 0.07 ± 0.02 | 0.73 ± 0.07 | 1.3 ± 0.6 | 2.3 |
| **20c** | Et | Allyl | MeO | 0.89 ± 0.33 | 0.68 ± 0.39 | 0.96 ± 0.02 | | 8.3 |

[a] Ratio of $A_{405}$ of SK-cleaved substrate in GAS culture treated with the indicated concentration of test compound divided by $A_{405}$ of DMSO control (see Section 5.1.1). Values are the mean of at least three experiments ± standard deviation.
[b] Ratio of OD (600 nm) for growth of GAS in the presence of test compound divided by DMSO control. Values are the mean of at least three experiments ± standard deviation.
[c] Half-life of parent compound during incubation with mouse liver microsomes.

to **30** (**17f, g).** Incorporation of a basic amine (**17e**) introduced significant growth inhibition. Benzoic acid-derived analogs **25–27** also displayed reduced potency compared to **30**. Ultimately, 9-methoxy analog **12a** was found to be the optimal compound from this series, exhibiting a 90% reduction in streptokinase activity at 5 μM and an $IC_{50}$ of 1.3 μM, representing a greater than 35-fold improvement

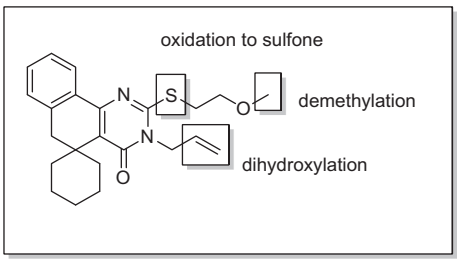



**Figure 2.** Potential routes of metabolism identified by MLM incubation and MS analysis. No oxidation of the left-hand side of the molecule was observed.

over **1**, although some modest inhibition of bacterial growth at high concentrations was introduced.

The final analogs for the SAR effort were designed to assess the effect of alkylation at the pyrimidinone oxygen in comparison to identical substitution at nitrogen (Table 4). No general efficacy advantage for N- or O-alkylation was observed; O-alkylated **20a** was somewhat more potent than **19a**, while **12a** remained much more potent than its O-alkylated counterpart **20c**. Compounds **19b** and **20b** displayed similarly weak activity.

### 3.3. Microsomal stability and metabolite identification

In our previous publication identifying **1** and **6b** as inhibitors of SK expression, it was determined that **1** displayed efficacy in mouse models of GAS infection while **6b** did not, despite having similar activity in bacterial assays.[15] We hypothesized that a difference in susceptibility to oxidative metabolism might be playing a role in the differential activity in mammalian systems. Incubation of each of these compounds in mouse liver microsomal extract (MLM) supported this hypothesis, with **1** displaying more than 60-fold higher stability to oxidation than **6b** ($t_{1/2}$ = 37.2 min vs 0.6 min, respectively). A survey of several more compounds in the series (Tables 1–3) revealed that most were highly unstable ($t_{1/2}$ <5 min), despite several structural modifications that would potentially reduce their propensity to oxidation, including lowered lipophilicity, fewer unsubstituted aliphatic/olefinic/aryl carbons, and replacement of sulfur with oxygen or nitrogen. Interestingly, we also noted that the spirocyclohexyl compounds were generally more stable than the corresponding gem-dimethyl compounds (e.g., **6d** $t_{1/2}$ = 6.4 min vs **12h** $t_{1/2}$ = 1.7 min), despite their higher lipophilicity.

A metabolite ID study was performed on compound **6d** to elucidate the most metabolically labile sites of the scaffold. Possible metabolites were identified by LC–MS analysis, then fragmented via collision-induced dissociation (CID). Subsequent MS analysis of the resulting metabolite fragments allowed us to deduce the structure of each metabolite. This study indicated that the most metabolically labile portions of the molecule were the substitutions on the pyrimidinone ring (Fig. 2). No oxidation was indicated on the spirocyclohexane ring or any of the four unsubstituted aromatic carbons. Based on the findings of the metabolite ID study, we prepared five analogs of our optimum compound **12a** that deactivated this portion of the scaffold to oxidation (Table 5); however, these were also found to be quickly metabolized. Interestingly, metabolic assessment of the O-alkylated compounds **20a–c** (Table 4) showed that they were consistently more stable (4 to 15-fold) than their N-alkylated counterparts. This observation, paired with the generally greater stability of the hindered spirocyclohexyl analogs versus the corresponding gem-dimethyl analogs, suggests that hydrolysis of the pyrimidinone amide may play a key role in the metabolic breakdown of these compounds. Compound **20a**, despite being four-fold less potent than **12a** ($IC_{50}$ = 5.5 μM vs 1.3 μM), demonstrates the potential viability of O-alkylated analogs as more metabolically stable alternatives to the N-alkylated compounds.

### 3.4. Solubility

In addition to microsomal stability, the aqueous solubility of small molecules has been shown to be a critical physicochemical property for predicting the efficacy of compounds in living systems. Lipinski suggests that to achieve oral bioavailability at a dose of 1 mg/kg, a compound minimally needs to achieve an aqueous solubility of 52 μg/mL,[25] corresponding to a solubility of ~150 μM for small analogs such as **12a**. Therefore, several key compounds from the SAR library were assessed for kinetic solubility in Todd Hewitt (THY) bacterial media. The solubility of all compounds tested (Tables 1–3) was equal to or less than 25 μM, with the exception of nitrile **12g** (aq sol. = 34.5 ± 7.5 μM). It was found that the solubility of these compounds was considerably lower in phosphate-buffered saline (PBS) than in THY media (e.g., 2 ± 1 vs 9 ± 2 μM [PBS:THY] for **1**, 3 ± 1 vs 34.5 ± 7.5 μM [PBS:THY] for **12g**), suggesting that protein binding may increase the effective aqueous solubility of this compound class. Preliminary activity studies that included human serum albumin (HSA) in the reaction mixture also suggested some compounds in this series may bind to protein.[‡] The measured solubility has a weak negative correlation to the *c*Log*P* ($R^2$ = 0.533), but the most soluble compound **12g** does indeed have the lowest *c*Log*P* (3.55). Further decreases in *c*Log*P* should logically result in greater aqueous solubility; however, nearly all efforts to significantly decrease lipophilicity, including replacement of the fused phenyl ring with pyridyl and replacement of the central ring with lactones or lactams (unpublished data), have thus far led to the reduction or loss of activity.

### 3.5. Mammalian cytotoxicity

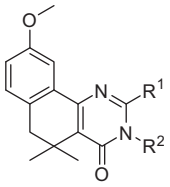
A representative subset of compounds (**1**, **12a**, **12c**, **12h**, **19a**, **20a**, **29**) was assessed for toxic effect in HeLa cells using a standard tetrazolium-formazan assay of cell viability. It was found that none of the compounds inhibited growth by more than 35% up to 100 μM, the highest concentration tested.

### 3.6. Biofilm inhibition

As reported previously, a gene expression microarray assay of GAS mRNA levels after incubation with **6b** confirmed the down-regulation of several virulence genes, including streptokinase, antiphagocytic factors, and cytolytic toxins. Reduced transcription of genes related to cell adhesion and biofilm formation, such as laminin- and fibronectin-binding proteins and collagen-like surface protein, was also observed.[15] Biofilm formation is critical for bacterial colonization of solid substrates in the body and serves to mechanically sequester bacteria from the effects of the immune system and antibiotics.[26] Diminishing the ability of bacteria to form biofilms represents a new mode of action by which compounds in this series can exert anti-virulence activity, and more importantly, expands the range of susceptible bacterial species to clinically important strains such as *S. aureus*. The compounds produced for the SK effort were assessed for their ability to inhibit biofilm formation in this strain using a previously developed plate-based OD assay.[27] These assays identified compounds **12c** (CCG-203592) and **12k** (CCG-205363) as potent *S. aureus* biofilm inhibitors; these results are reported in greater detail elsewhere.[28]

[‡] Compound **1** showed attenuated efficacy in the presence of 10% HSA compared to the standard assay conditions. This effect was less pronounced for the more hydrophilic compound **12g** (*data not shown*).

**Table 5**
Analogs of **12a** with potentially reduced metabolic liability



| No. | $R^1$ | $R^2$ | 5 μM SK $T/C$[a] | 50 μM SK $T/C$[a] | 50 μM Growth $T/C$[b] | MLM $t_{1/2}$[c] (min) |
|---|---|---|---|---|---|---|
| **12a** | S-Et | Allyl | 0.10 ± 0.06 | 0.07 ± 0.02 | 0.73 ± 0.07 | 2.3 |
| **12l** | S-$CH_2CF_3$ | Allyl | 0.45 ± 0.19 | 0.22 ± 0.08 | 0.86 ± 0.07 | 4.8 |
| **12m** | S-Et | Me | 0.66 ± 0.20 | 0.20 ± 0.14 | 0.83 ± 0.15 | 1.7 |
| **14** | (SO)Et | Allyl | 1.00 ± 0.13 | 0.51 ± 0.31 | 1.01 ± 0.05 | 2.8 |
| **15** | ($SO_2$)Et | Allyl | 0.83 ± 0.31 | 0.70 ± 0.13 | 1.03 ± 0.03 | 3.8 |
| **19b** | S-Et | $CF_3CH_2$ | 0.78 ± 0.36 | 0.49 ± 0.17 | 1.01 ± 0.04 | 3.0 |

[a] Ratio of $A_{405}$ of SK-cleaved substrate in GAS culture treated with the indicated concentration of test compound divided by $A_{405}$ of DMSO control (see Section 5.1.1). Values are the mean of at least three experiments ± standard deviation.
[b] Ratio of OD (600 nm) for growth of GAS in the presence of test compound divided by DMSO control. Values are the mean of at least three experiments ± standard deviation.
[c] Half-life of parent compound during incubation with mouse liver microsomes.

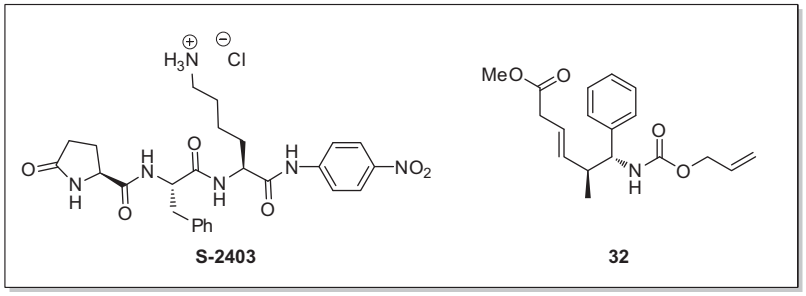



**Figure 3.** Structure of chromogenic plasmin substrate S-2403 and microsomal stability assay internal standard 32.

## 4. Conclusions

We report the preparation and biological evaluation of 45 analogs of **1**, resulting in the achievement of a greater than 35-fold improvement in activity ($IC_{50}$ >50 μM to 1.3 μM) over compound **1** with optimum analog **12a**. During the optimization process, we observed a general instability of new analogs to oxidative metabolism. Through careful structure metabolism relationship analysis, we have developed a hypothesis that a primary route of metabolism for this class of compounds is through hydrolysis of the pyrimidinone amide, which should guide the design of new analogs with improved potential for activity in vivo. Murine infection studies are in fact currently planned for the most stable active analogs from this work (**6f, 12g, 20a, 20b**) and will be reported in due course. Concurrently, a more focused effort toward optimizing the biofilm inhibition activity of this class of compounds is underway.

## 5. Experimental section

### 5.1. Biological/physicochemical characterization

#### 5.1.1. SK expression assay

The GAS strain UMAA2616 used in this study was derived from the GAS M type 1 strain MGAS166.[29] UMAA2616 was originally designated as UMAA2392 and contains mutations in the CovR/S system to generate increased expression of SK and other virulence factors.[30]

Extracellular GAS-SK activity was measured by a previously described chromogenic assay.[15] Briefly, a single colony of UMAA2616 was inoculated into Todd-Hewitt broth containing 0.2% yeast extract (THY) (Difco, Detroit, MI) supplemented with 100 μg/mL streptomycin and grown overnight at 37 °C. Vials of THY medium (4 mL) containing different concentrations of test compound in a final concentration of 0.1% DMSO were inoculated with 4 μl of the UMAA2616 overnight culture. The cultures were grown in triplicate at 37 °C to an $OD_{600}$ ≈1.0. Each sample was then centrifuged at 11,000*g* for 8 min. An aliquot (20 μL) of supernatant was mixed with 100 μl PBS, 10 μl human plasma (Innovative Research, Novi, MI), and 10 μl of 1 mg/ml S-2403 solution (Fig. 3; Diapharma Group Inc., West Chester, OH), then incubated at 37 °C for 2 h. Activity was reported as the ratio of SK activity as measured by absorbance at 405 nm of the test cultures compared to that of a culture treated with only DMSO. A GAS strain deficient in SK activity, UMAA2641,[31] was used as a blank. The SK activity was calculated based on a standard curve made with serial dilutions of control UMAA2616 cultures grown under the same conditions with 0.1% DMSO. The numbers were then corrected for inhibition of growth by each test compound versus DMSO control, as measured by $OD_{600}$. The experiments were performed in triplicate to obtain mean and standard deviation values for each test concentration.

#### 5.1.2. Aqueous solubility assay

Compounds were dissolved in DMSO to a stock concentration of 10 mM. 100 μL of each stock was diluted with DMSO through a series of twelve 35% dilutions, resulting in working solutions with concentrations ranging from 10 mM to 0.0875 mM. 2 μL of each working solution was added to one well of a clear plastic, round-bottom 96-well plate containing 200 μL of standard-strength Todd-Hewitt broth or phosphate-buffered saline solution warmed to 37 °C, resulting in final testing concentrations of 0.87 to 99 μM. After addition of compound to all test wells, the plate was loaded into a Molecular Devices SpectraMax Plus UV/Vis spectrophotometer, shaken for 15 seconds, and incubated for 5 min at 37 °C. Measurement of the $OD_{600}$ resulted in a series of curves. The concentration at which the average ($n = 3$ for each concentration and test compound) $OD_{600}$ reading rose above background ($OD_{600} \geqslant 0.005$ AU) was noted, and the aqueous solubility is reported as the mean concentration between this point and the previous point (± the concentration difference between the two points *0.5).

#### 5.1.3. Microsomal stability assay

Stock solutions of test compounds were generated via the dilution of 100 mM DMSO stocks 1000-fold with phosphate-buffered saline containing up to 10% MeOH as a co-solvent. To 366 μL of 100 mM phosphate buffer containing 3.3 mM $MgCl_2$ was added 10 μL of 20 mg/mL mouse liver microsomal extract (XenoTech, Lenexa, KS) and 4 μL of test compound solution (100 μM). Enzymatic oxidation was initiated by adding 15 μL of 16.7 mg/mL NADPH in 100 mM phosphate buffer containing 3.3 mM $MgCl_2$ (final concentrations of mouse liver microsomes and test compound = 0.5 mg/mL and 1 μM, respectively). The reactions were carried out at 37 °C for 60 min. At each time point (0, 1, 3, 5, 10, 30, and 60 min), an aliquot of the reaction mixture (30 μL) was removed and added to 90 μL of cold acetonitrile containing a known concentration of internal standard compound (**32**, Fig. 3) to quench the reaction. The quenched sample mixtures were centrifuged at 16,000*g* for 10 min. The supernatant was then analyzed via an LC–MS/MS system equipped with a reverse-phase column.

LC–MS/MS analysis of test compounds was performed on an LC–MS/MS-3200 system (AB Sciex, Framingham, MA) equipped with an electrospray ionization (ESI) source. The AB Sciex LC-MS/MS 2800 system consisting of Agilent 1200 series (Agilent, Santa Clara, CA) with a Zorbax Extend C-18 column (5 μm, 50 × 2.1 mm) was used for the separation and the effluent from the column was directly fed into the ionization source. The system was controlled by Analyst software (version 1.4.2) to collect and process data. The mobile phase consisted of water containing 0.1% formic acid (Solvent A) and acetonitrile containing 0.1% formic acid (Solvent B) for all test compounds with the solvent B gradient changing from 10–95% during a 15 min run. The LC–MS/MS was operated at a flow rate of 0.4 mL/min. Metabolic half-lives were derived from the rate of disappearance of the parent compound MS/MS readout as determined by comparison to the internal standard.

#### 5.1.4. Metabolite identification study

Metabolic oxidation and HPLC separation were performed in the same manner as in the metabolic half-life determination assays. Samples from the metabolic oxidation of **6d** were injected into the HPLC and subjected to enhanced product ion (EPI), MS/MS ($MS^2$), and $MS^3$ scanning to obtain $MS^2$ and $MS^3$ fragmentation spectra of each potential metabolite derived from the M+H ion of **6d**. Samples of **6d** incubated with inactivated (boiled) MLM or omitting the addition of NADPH to the reaction mixture served as negative controls. Potential metabolites were identified using multiple reaction monitoring (MRM), enhanced mass spectrometry (EMS) full scan, and precursor scanning detection modes. Only the ions detected in the test sample and absent in both control samples were regarded as possible metabolites. Based on the $MS^2$ and $MS^3$ spectra of the possible metabolites and the proposed fragmentation pathways of **6d**, the likely structures of the metabolites were deduced.

#### 5.1.5. Mammalian cytotoxicity assay

HeLa cells (ATCC, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1% L-glutamine at 37 °C under 5% $CO_2$ atmosphere. Cell suspensions (200 μL, $2.5 \times 10^4$ cells/well) with different concentrations (0.39 μM to 100 μM) of test compounds or DMSO were plated in 96-well plates and cultured for 24 h in triplicate. CellTiter 96 Aqueous One Solution reagent (Promega, Madison, WI) (20 μL) was added to each sample and incubated for 2 h. Viability was reported as the ratio of absorbance at 490 nm of the test compound wells in comparison to DMSO control. The experiment was repeated three times to obtain mean and standard error values.

### 5.2. Chemistry procedures

*General information:* Chemical names follow CAS nomenclature. Starting materials were purchased from Fisher, Sigma–Aldrich Lancaster, Fluka or TCI-America and were used as supplied unless otherwise indicated. All reaction solvents were purchased from Fisher and used as received. Reactions were monitored by TLC using precoated silica gel 60 F254 plates. All anhydrous reactions were run under an atmosphere of dry nitrogen. Silica gel chromatography was performed with silica gel (220–240 mesh) obtained from Silicycle. Solvent abbreviations used: $CDCl_3$, deutero-chloroform; DCM, dichloromethane; DMSO, dimethyl sulfoxide; EtOH, ethanol; MEK, methyl ethyl ketone; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; EtOAc, ethyl acetate; hex, hexanes. Reagent abbreviations used: $Cs_2CO_3$, cesium carbonate; $Na_2SO_4$, sodium sulfate; $MgSO_4$, magnesium sulfate; *m*CPBA, *meta*-chloro peroxybenzoic acid; KOH, potassium hydroxide; LDA, lithium diisopropylamide; $NaHCO_3$, sodium bicarbonate; NaOMe, sodium methoxide; TMS, trimethylsilyl; HOBt 1-hydroxybenzotriazole; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; $ZnI_2$, zinc iodide.

NMR spectra were recorded on a Bruker 400 MHz, Bruker 500 MHz, Varian 400 MHz, or Varian 500 MHz spectrometer. Chemical shifts are reported in $\delta$ (parts per million), by reference to the hydrogen residues of deuterated solvent as internal standard $CDCl_3$: $\delta = 7.28$ ($^1$H NMR), or in reference to the hydrogen peaks of tetramethylsilane, $\delta = 0.00$ ($^1$H NMR). Mass spectra were recorded on a Micromass LCT time-of-flight instrument utilizing electrospray ionization operating in positive-ion (ESI+) or negative-ion (ESI−) modes where indicated. Melting points were measured on a MEL-TEMP melting point apparatus and are uncorrected. The purity of the compounds was assessed via analytical rpHPLC with one of three gradient methods. 'Method A': 10% B to 90% B over 6 min, hold at 90% B for 7 additional minutes; 'Method B': 50% B to 90% B, hold at 90% B for 7 additional minutes; 'Method C': 90% B over 12 min (solvent A $H_2O$, solvent B acetonitrile, C18 column, 3.5 um, 4.6 × 100 mm, 254 nm μ).

#### 5.2.1. Ethyl 2-cyano-2-cyclohexylideneacetate (3)

To a solution of cyclohexanone (1.50 ml, 14.47 mmol) in toluene (24.12 ml) was added ethyl cyanoacetate (1.556 ml, 14.62 mmol), acetic acid (0.166 ml, 2.89 mmol), and ammonium acetate (0.112 g, 1.447 mmol). The mixture was heated to reflux at 150 °C in a Dean-Stark apparatus. After 5 h, the reaction was cooled and washed with water and saturated $NaHCO_3$ solution. The organics were dried over $Na_2SO_4$, filtered, and concentrated.

The resulting residue (2.48 g, 89% yield) was used without further purification. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 4.29 (q, $J$ = 7.2 Hz, 2H), 3.00 (t, $J$ = 6.0 Hz, 2H), 2.68 (t, $J$ = 6.1 Hz, 2H), 1.82 (p, J = 6.0 Hz, 2H), 1.75 (p, $J$ = 6.1 Hz, 2H), 1.70–1.65 (m, 2H), 1.37 (t, $J$ = 7.2 Hz, 3H).

#### 5.2.2. Ethyl 4′-amino-1′H-spiro[cyclohexane-1,2-naphthalene]-3′-carboxylate (4)

1 M benzylmagnesium chloride in diethyl ether (18.92 ml) was added dropwise to a solution of 3 (1.828 g, 9.46 mmol) in diethyl ether (6.31 ml) at room temperature. The mixture was stirred at room temperature for 3 days, then 10% hydrochloric acid (7.83 ml, 255 mmol) was added dropwise at 0 °C while stirring. The organic layer was separated, washed with water, dried over $Na_2SO_4$, filtered, and concentrated to dryness in vacuo. Concentrated $H_2SO_4$ (4.59 ml) was added dropwise to the crude solid at 0 °C. The mixture was stirred at room temperature for 7 h, then ice water was added to the mixture resulting in a precipitate. The precipitate was dissolved in $Et_2O$ and washed with 28% $NH_3$ solution. The organic layer was washed with water, dried over $Na_2SO_4$, filtered, and concentrated to an orange oil (1.66 g, 68% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.42 (d, $J$ = 6.8 Hz, 1H), 7.36–7.25 (m, 2H), 7.22 (d, $J$ = 6.8 Hz, 1H), 6.04 (br s, 2H), 4.25 (q, $J$ = 7.0 Hz, 2H), 2.87 (s, 2H), 2.24–2.05 (m, 2H), 1.76–1.20 (m, 9H).

#### 5.2.3. 3-Allyl-2-thioxo-2,3-dihydro-1*H*-spiro[benzo[*h*]quinazoline-5,1′-cyclohexan]-4(6*H*)-one (5)

Intermediate 4 (1.66 g, 5.82 mmol) and allyl isothiocyanate (0.600 ml, 6.13 mmol) were dissolved in ethanol (9.69 ml) and refluxed at 85 °C. for 10 h. A solution of KOH (0.653 g, 11.63 mmol) in water (9.69 ml) was then added and the reaction mixture was refluxed for 3 h. The cooled reaction mixture was acidified to pH 3.0–3.5 resulting in precipitation. The precipitate was collected via vacuum filtration, washed with water, and recrystallized from butanol. Recovered as pale yellow crystals (587 mg, 29.8% yield). TLC $R_f$ = 0.25 (10% EtOAc/hex). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 9.28 (s, 1H), 7.50–7.35 (m, 3H), 7.32 (d, $J$ = 7.4 Hz, 1H), 6.00 (ddt, $J$ = 16.2, 11.3, 5.8 Hz, 1H), 5.37 (dd, 1H), 5.27 (dd, 1H), 5.06 (d, $J$ = 5.8 Hz, 2H), 3.03 (s, 2H), 2.48 (td, $J$ = 13.3, 4.4 Hz, 2H), 1.72 (d, $J$ = 13.3 Hz, 1H), 1.62–1.44 (m, 4H), 1.33 (d, $J$ = 14.3 Hz, 3H).

#### 5.2.4. General Method A for generating compounds 1, 6a–e

Compound **5** (100 mg, 0.295 mmol) was combined with base (0.443 mmol) and alkylating agent (0.325 mmol) in ethanol or MEK (1.75 mL). The suspension was warmed to 70 °C and allowed to stir for 16 h. The suspension was subsequently diluted with EtOAc and washed with water and brine. The isolated organic layer was then dried over $MgSO_4$, filtered, and concentrated in vacuo. Further purification via flash chromatography (0% to 10% EtOAc/hex) delivered the desired compounds in 72–83% yield.

#### 5.2.5. 3-Allyl-2-(butylthio)-3*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclohexan]-4(6*H*)-one (CCG-2979) (1)

Prepared from **5** according to General Method A, using 1-iodobutane as the alkylating agent, $Cs_2CO_3$ as the base, and MEK as the solvent. Isolated after flash chromatography (0–5% EtOAc/hex) as white crystals (87 mg, 74% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.08 (d, $J$ = 6.7 Hz, 1H), 7.38–7.28 (m, 2H), 7.20 (d, $J$ = 6.8 Hz, 1H), 5.99–5.87 (m, 1H), 5.33–5.22 (m, 2H), 4.68 (d, $J$ = 5.2 Hz, 2H), 3.31 (t, $J$ = 7.3 Hz, 2H), 3.03 (s, 2H), 2.67–2.52 (m, 2H), 1.80 (p, $J$ = 7.4 Hz, 2H), 1.71 (d, $J$ = 11.3 Hz, 1H), 1.63–1.45 (m, 6H), 1.44–1.31 (m, 3H), 0.98 (t, $J$ = 7.4 Hz, 3H). ESI+MS $m/z$ = 395.2 ($M+H^+$), 417.2 ($M+Na^+$). HPLC (Method C, $t_R$ = 7.44 min), purity >95%.

#### 5.2.6. 3-Allyl-2-((4-methoxybenzyl)thio)-3*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclohexan]-4(6*H*)-one (6a)

Prepared from **5** according to General Method A, using 4-methoxybenzyl bromide as the alkylating agent, KOH as the base, and ethanol as the solvent. Isolated as a clear oil (97 mg, 72% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.17 (d, $J$ = 7.6 Hz, 1H), 7.41–7.36 (m, 3H), 7.34 (t, $J$ = 6.8 Hz, 1H), 7.24 (d, $J$ = 7.2 Hz, 1H), 6.89 (d, $J$ = 8.6 Hz, 2H), 5.94 (ddt, $J$ = 17.1, 10.7, 5.6 Hz, 1H), 5.37–5.18 (m, 2H), 4.67 (d, $J$ = 5.6 Hz, 2H), 4.57 (s, 2H), 3.82 (s, 3H), 3.07 (s, 2H), 2.72–2.51 (m, 2H), 1.74 (d, $J$ = 12.8 Hz, 1H), 1.66–1.50 (m, 4H), 1.49–1.32 (m, 3H). ESI+MS $m/z$ = 481.2 ($M+Na^+$). HPLC (Method C, $t_R$ = 5.11 min), purity >95%.

#### 5.2.7. 3-Allyl-2-(benzylthio)-3*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclohexan]-4(6*H*)-one (CCG-102487) (6b)

Prepared from **5** according to General Method A, using benzyl bromide as the alkylating agent, KOH as the base, and ethanol as the solvent. Isolated as a clear oil (95 mg, 75% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.11 (d, $J$ = 7.4 Hz, 1H), 7.44 (d, $J$ = 7.2 Hz, 2H), 7.39–7.27 (m, 5H), 7.21 (d, $J$ = 7.2 Hz, 1H), 5.92 (ddt, $J$ = 15.8, 10.7, 5.5 Hz, 1H), 5.32–5.21 (m, 2H), 4.66 (d, $J$ = 5.5 Hz, 2H), 4.59 (s, 2H), 3.04 (s, 2H), 2.65–2.53 (m, 2H), 1.71 (d, $J$ = 12.7 Hz, 1H), 1.61–1.52 (m, 4H), 1.43–1.32 (m, 3H). ESI+MS $m/z$ = 429.2 ($M+H^+$), 451.2 ($M+Na^+$). HPLC (Method C, $t_R$ = 4.68 min), purity >95%.

#### 5.2.8. *N*-(4-(((3-Allyl-4-oxo-4,6-dihydro-3*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclohexan]-2-yl)thio)methyl)phenyl)acetamide (6c)

Prepared from **5** according to General Method A, using *N*-(4-(chloromethyl)phenyl)acetamide as the alkylating agent, $Cs_2CO_3$ as the base, and MEK as the solvent. Isolated as a white solid (71 mg, 83% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.11 (d, $J$ = 7.2 Hz, 1H), 7.46 (d, $J$ = 8.5 Hz, 2H), 7.39 (d, $J$ = 8.5 Hz, 2H), 7.35 (t, $J$ = 7.3 Hz, 1H), 7.31 (t, $J$ = 6.9 Hz, 1H), 7.22 (d, $J$ = 7.3 Hz, 1H), 7.16 (s, 1H), 5.91 (ddt, $J$ = 15.9, 10.5, 5.6 Hz, 1H), 5.35–5.11 (m, 2H), 4.65 (d, $J$ = 5.6 Hz, 2H), 4.55 (s, 2H), 3.49 (d, $J$ = 5.3 Hz, 1H), 3.04 (s, 2H), 2.65–2.51 (m, 2H), 2.18 (s, 3H), 1.71 (d, $J$ = 13.4 Hz, 1H), 1.60–1.51 (m, 4H), 1.45–1.32 (m, 3H). ESI+MS $m/z$ = 508.2 ($M+Na^+$). HPLC (Method C, $t_R$ = 2.75 min), purity >95%.

#### 5.2.9. 3-Allyl-2-((2-methoxyethyl)thio)-3*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclohexan]-4(6*H*)-one (6d)

Prepared from **5** according to General Method A, using 2-methoxyethyl *p*-toluenesulfonate as the alkylating agent, KOH as the base, and ethanol as the solvent. Isolated as a yellow oil (88 mg, 75% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.08 (d, $J$ = 7.5 Hz, 1H), 7.38 (t, $J$ = 7.3 Hz, 1H), 7.33 (t, $J$ = 6.8 Hz, 1H), 7.24 (d, $J$ = 7.2 Hz, 1H), 5.96 (ddt, $J$ = 15.9, 11.0, 5.6 Hz, 1H), 5.35–5.26 (m, 2H), 4.71 (d, $J$ = 5.6 Hz, 2H), 3.79 (t, $J$ = 6.2 Hz, 2H), 3.56 (t, $J$ = 6.2 Hz, 2H), 3.44 (s, 3H), 3.06 (s, 2H), 2.66–2.56 (m, 2H), 1.73 (d, $J$ = 13.0 Hz, 1H), 1.62–1.54 (m, 4H), 1.44–1.35 (m, 3H). ESI+MS $m/z$ = 397.2 ($M+H^+$), 419.2 ($M+Na^+$). HPLC (Method C, $t_R$ = 3.54 min), purity >95%.

#### 5.2.10. 3-Allyl-2-((2-(dimethylamino)ethyl)thio)-3*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclohexan]-4(6*H*)-one (6e)

Prepared from **5** according to General Method A, using β-dimethylaminoethyl bromide hydrobromide as the alkylating agent, KOH as the base, and MEK as the solvent. Isolated as white crystals (88 mg, 75% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.10 (d, $J$ = 7.6 Hz, 1H), 7.35 (t, $J$ = 7.3 Hz, 1H), 7.30 (t, $J$ = 7.4 Hz, 1H), 7.21 (d, $J$ = 7.1 Hz, 1H), 5.93 (ddt, $J$ = 15.9, 10.4, 5.5 Hz, 1H), 5.32–5.23 (m, 2H), 4.69 (d, $J$ = 5.5 Hz, 2H), 3.46 (t, $J$ = 7.0 Hz, 2H), 3.04 (s, 2H), 2.73 (t, $J$ = 7.0 Hz, 2H), 2.64–2.53 (m, 2H), 2.34 (s, 6H),

1.71 (d, $J$ = 12.6 Hz, 1H), 1.59–1.51 (m, 4H), 1.42–1.33 (m, 3H). ESI+MS $m/z$ = 410.3 ($M+H^+$). HPLC (Method A, $t_R$ = 6.29 min), purity >95%.

#### 5.2.11. 3-Allyl-2-(methylthio)-3*H*-spiro[benzo[*h*]quinazoline-5,1′-cyclohexan]-4(6*H*)-one (6f)

Compound **5** (150 mg, 0.443 mmol) was dissolved in absolute EtOH at 0 °C (2.61 mL) to which KOH (37 mg, 0.665 mmol) and methyl iodide (33 μL, 0.532 mmol) were added. The solution was allowed to stir for 10 min, resulting in the precipitation of white crystals. The suspension was diluted with $H_2O$ and vacuum filtered to collect the precipitate. The precipitate was washed with water and dried in vacuo. Recovered 150 mg (95% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.14 (d, $J$ = 7.4 Hz, 1H), 7.35 (t, $J$ = 7.3 Hz, 1H), 7.31 (t, $J$ = 6.8 Hz, 1H), 7.21 (d, $J$ = 7.1 Hz, 1H), 5.94 (ddt, $J$ = 15.9, 10.8, 5.6 Hz, 1H), 5.32–5.24 (m, 2H), 4.68 (d, $J$ = 5.6 Hz, 2H), 3.04 (s, 2H), 2.68 (s, 3H), 2.64–2.54 (m, 2H), 1.71 (d, $J$ = 12.8 Hz, 1H), 1.60–1.51 (m, 4H), 1.42–1.33 (m, 3H). ESI+MS $m/z$ = 353.2 ($M+H^+$), 375.2 ($M+Na^+$). HPLC (Method C, $t_R$ = 4.19 min), purity >95%.

#### 5.2.12. General Method B for generating 7a and 13a

Metallic sodium (78 mg, 3.40 mmol) was added to absolute ethanol (1.67 mL) and allowed to stir at room temperature for 30 min. Once all solid had dissolved, the corresponding 2-methylthio 5,6-dihydrobenzo[$h$]quinazolin-4(3$H$)-one (**6f** or **12n**, 0.284 mmol) was added. The reaction was warmed to 40 °C and allowed to stir for 48 h. After the completion of the reaction, the solution was diluted with $H_2O$ and extracted with 3 portions of ethyl acetate. The organic layers were combined, washed with water and brine, then isolated, dried over $MgSO_4$, vacuum filtered, and concentrated to a yellow solid that was further purified via silica flash chromatography (15% EtOAc/hex), 69–73% yield.

#### 5.2.13. 3-Allyl-2-ethoxy-3*H*-spiro[benzo[*h*]quinazoline-5,1′-cyclohexan]-4(6*H*)-one (7a)

Prepared according to General Method B from **6f**. Isolated as a light yellow crystalline solid (73 mg, 73% yield). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.07 (d, $J$ = 8.0 Hz, 1H), 7.38–7.25 (m, 2H), 7.20 (d, $J$ = 6.8 Hz, 1H), 5.99–5.85 (m, 1H), 5.27–5.15 (m, 2H), 4.61 (d, $J$ = 5.8 Hz, 3H), 4.56 (q, $J$ = 7.2 Hz, 2H), 3.03 (s, 2H), 2.66–2.53 (m, 2H), 1.70 (d, $J$ = 12.1 Hz, 1H), 1.60–1.50 (m, 4H), 1.45 (t, $J$ = 7.2 Hz, 3H), 1.41–1.33 (m, 3H). ESI+MS $m/z$ = 351.2 ($M+H^+$), 373.2 ($M+Na^+$) HPLC (Method B, $t_R$ = 9.75 min), purity >95%.

#### 5.2.14. General Method C for generating 7b and 13b

Compound **6f** or **12n** (1.28 mmol) was dissolved in DCM (19.3 mL), then $m$CPBA (70 wt%, 787 mg, 3.19 mmol) was added and the reaction mixture allowed to stir over the course of 16 h at room temperature. At this time the reaction mixture was diluted with DCM, washed with saturated aqueous $NaHCO_3$ solution, water, and brine. The organic layer was dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Purification via flash chromatography isolated the sulfone intermediate which was then dissolved in a 1:1 mixture of THF:DMF (2 mL). Potassium carbonate (165 mg, 1.19 mmol) and 2 M ethylamine solution in THF (1.00 mL, 2.00 mmol) were added, then the reaction vessel was tightly capped and allowed to stir 5 h at RT. The reaction mixture was diluted with diethyl ether, then washed with 3 portions of $H_2O$ followed by brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. The residue was further purified by flash chromatography (10–33% EtOAc/hex) and isolated in 41–53% yield over 2 steps.

#### 5.2.15. 3-Allyl-2-(ethylamino)-3*H*-spiro[benzo[*h*]quinazoline-5,1′-cyclohexan]-4(6*H*)-one (7b)

Prepared from **6f** according to General Method C. Isolated as a white crystalline solid, 53% yield over 2 steps. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.13 (d, $J$ = 7.4 Hz, 1H), 7.34–7.27 (m, 2H), 7.18 (d, $J$ = 6.6 Hz, 1H), 5.92 (ddt, $J$ = 17.3, 10.5, 5.3 Hz, 1H), 5.36–5.26 (m, 2H), 4.66 (d, $J$ = 5.2 Hz, 2H), 4.56 (d, $J$ = 5.0 Hz, 1H), 3.57 (qd, $J$ = 7.2, 5.0 Hz, 2H), 3.01 (s, 2H), 2.64–2.54 (m, 2H), 1.70 (d, $J$ = 12.6 Hz, 1H), 1.62–1.48 (m, 5H), 1.38 (d, $J$ = 12.8 Hz, 3H), 1.28 (t, $J$ = 7.2 Hz, 3H). ESI+MS $m/z$ = 350.2 ($M+H^+$), 372.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.18 min), purity = 94%.

#### 5.2.16. General Method D for generating 9a and 9b

To anhydrous THF (6.4 mL) in a dry round-bottom flask was added nickel(II)bromide (279 mg, 1.28 mmol), zinc powder (250 mg, 3.83 mmol), and triphenylphosphine (1.68 g, 6.39 mmol). The mixture was heated to 50 °C and stirred for 30 min. The selected $o$-chlorotoluene (**8a** or **8b**, 12.78 mmol) was added, the temperature raised to 60 °C, and the reaction was tightly capped and allowed to stir 30 min, then potassium cyanide (1.66 g, 25.5 mmol) was added over the course of 5 h in 2 equal portions. The mixture was allowed to stir an additional 16 h. Water was added to quench the reaction, and the suspension extracted 3× with ether. The resulting organic layer was washed with $H_2O$ and brine, dried over $MgSO_4$, filtered, and concentrated to a heterogeneous mixture of white crystals and clear oil. The residue was diluted with 10 mL of toluene, then methyl iodide (997 mg, 7.02 mmol) was added and allowed to stir 16 h at room temperature. The resulting white crystals were removed via vacuum filtration. The filtrate was concentrated in vacuo to a clear oil. Flash chromatography with 2% EtOAc/hex delivered the pure product in 68–90% yield.

#### 5.2.17. 5-Methoxy-2-methylbenzonitrile (9a)

Synthesized from **8a** according to General Method D. Isolated in 90% yield. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.21 (d, $J$ = 8.5 Hz, 1H), 7.08 (d, $J$ = 2.8 Hz, 1H), 7.03 (dd, $J$ = 8.5, 2.8 Hz, 1H), 3.81 (s, 3H), 2.47 (s, 3H).

#### 5.2.18. 3-Methoxy-2-methylbenzonitrile (9b)

Synthesized from **8b** according to General Method D. Isolated as a clear oil, 68% yield. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.24 (t, $J$ = 7.9 Hz, 1H), 7.19 (d, $J$ = 7.7 Hz, 1H), 7.03 (d, $J$ = 8.1 Hz, 1H), 3.86 (s, 3H), 2.42 (s, 3H).

#### 5.2.19. General Method E for synthesizing 10a–10d, 23

A dry round bottom flask was charged with anhydrous diglyme (24 mL) and diisopropylamine (2.91 mL, 20.4 mmol), then cooled to −78 °C in a dry ice/acetone bath. To this solution was added $n$-butyllithium (2.5 M in hexanes, 8.15 mL, 20.4 mmol). The reaction was removed from the dry ice/acetone bath for 10 min, then re-cooled to −78 °C. The desired $o$-tolunitrile **9a–d** (6.79 mmol), dissolved in anhydrous diglyme (2 mL), was then added slowly dropwise then allowed to stir at -78 °C for 45 min.

A separate dry flask was charged with anhydrous diglyme (8.0 mL) and zinc powder (1.11 g, 17.0 mmol). Molecular iodine (3.45 g, 13.6 mmol) was added portionwise over the course of 10 min. The suspension was subsequently heated via heat gun in 30-s intervals until a silver precipitate of $ZnI_2$ had formed and all iodine color had disappeared (caution: exothermic).

Ethyl-3,3-dimethyl acrylate (1.42 mL, 10.2 mmol) was added to the first flask dropwise over 10 min. The flask containing the $ZnI_2$ suspension was then added to the reaction vessel and the resulting suspension allowed to stir for an additional 2 h, slowly warming to room temperature. The reaction was quenched by the addition of saturated ammonium chloride solution and the resulting biphasic suspension was extracted with diethyl ether (3 × 30 mL), then

washed with water (4 × 50 mL) and brine (1 × 50 mL). The organic extract was dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (silica gel, 5% EtOAc/hex) delivered the desired **10a–d** in 33–59% yields.

#### 5.2.20. Ethyl 1-amino-7-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carboxylate (10a)

Prepared according to General Method E from **9a**. Isolated as a pale yellow oil (623 mg, 33% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.11 (d, $J$ = 8.1 Hz, 1H), 6.96 (s, 1H), 6.88 (d, $J$ = 8.1 Hz, 1H), 6.27 (s, 2H), 4.27 (q, $J$ = 7.1 Hz, 2H), 3.85 (s, 3H), 2.60 (s, 2H), 1.37 (t, $J$ = 7.1 Hz, 3H), 1.20 (s, 6H).

#### 5.2.21. Ethyl 1-amino-5-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carboxylate (10b)

Prepared according to General Method E from **9b**. Isolated as a yellow oily solid (636 mg, 35% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.24 (t, $J$ = 8.1 Hz, 2H), 7.04 (d, $J$ = 8.1 Hz, 1H), 6.92 (d, $J$ = 8.1 Hz, 1H), 6.31 (s, 2H), 4.25 (q, $J$ = 7.1 Hz, 2H), 3.85 (s, 3H), 2.66 (s, 2H), 1.34 (t, $J$ = 7.1 Hz, 3H), 1.20 (s, 6H).

#### 5.2.22. Ethyl 1-amino-6-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carboxylate (10c)

Prepared according to General Method E from **9c**. Isolated as pale yellow crystals (1.108 g, 59% yield). TLC $R_f$ = 0.14 (10% EtOAc/hex). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.35 (d, $J$ = 8.5 Hz, 1H), 6.81 (dd, $J$ = 8.5, 2.4 Hz, 1H), 6.73 (d, $J$ = 2.4 Hz, 1H), 6.37 (s, 1H), 4.26 (q, $J$ = 7.1 Hz, 2H), 3.86 (s, 3H), 2.64 (s, 2H), 1.36 (t, $J$ = 7.1 Hz, 3H), 1.22 (s, 6H).

#### 5.2.23. Ethyl 1-amino-3,3-dimethyl-3,4-dihydronaphthalene-2-carboxylate (10d)

Prepared according to General Method E from **9d**. Isolated as a pale yellow oil (860 mg, 41% yield). TLC $R_f$ = 0.30 (10% EtOAc/hex). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.42 (d, $J$ = 8.6 Hz, 1H), 7.36–7.29 (m, 2H), 7.20 (d, $J$ = 7.3 Hz, 1H), 6.35 (s, 1H), 4.28 (q, $J$ = 7.1 Hz, 2H), 2.68 (s, 2H), 1.37 (t, $J$ = 7.1 Hz, 3H), 1.22 (s, 6H).

#### 5.2.24. General Method F for synthesizing compounds 11a–f

Glacial acetic acid (147 μL, 2.56 mmol) and an alkyl isothiocyanate (2.56 mmol) were combined with the selected intermediate **10a–d** (1.28 mmol) in absolute ethanol (1.7 mL). The solution was allowed to stir at reflux under nitrogen atmosphere for 1 h. Additional allyl isothiocyanate (373 μL, 3.84 mmol) was added in equal portions over the course of 3 h. The reaction was allowed to stir at reflux 16 additional hours, then diluted with ethyl acetate. The organic mixture was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Trituration or flash chromatography delivered compounds **11a–f** in 21–59% yield.

#### 5.2.25. 3-Allyl-9-methoxy-5,5-dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (11a)

Prepared according to General Method F from **10a** and allyl isothiocyanate. Purified via trituration with hexanes and diethyl ether (125 mg, 21% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 9.29 (s, 1H), 7.35 (d, $J$ = 8.0 Hz, 1H), 7.06 (d, $J$ = 8.0 Hz, 1H), 7.00 (s, 1H), 6.08 (ddt, $J$ = 15.8, 10.9, 5.5 Hz, 1H), 5.45–5.30 (m, 2H), 5.18 (d, $J$ = 5.5 Hz, 2H), 3.85 (s, 3H), 2.73 (s, 2H), 1.34 (s, 6H).

#### 5.2.26. 3-Allyl-7-methoxy-5,5-dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (11b)

Prepared according to General Method F from **10c** and allyl isothiocyanate. Purified via trituration of the crude organic isolate with hexanes and diethyl ether (133 mg, 24% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 9.29 (s, 1H), 7.35 (t, $J$ = 8.0 Hz, 1H), 7.08–7.01 (m, 2H), 6.00 (ddt, $J$ = 16.8, 10.8, 5.8 Hz, 1H), 5.37 (d, $J$ = 16.8 Hz, 1H), 5.27 (d, $J$ = 10.8 Hz, 1H), 5.06 (d, $J$ = 5.8 Hz, 2H), 3.89 (s, 3H), 2.79 (s, 2H), 1.34 (s, 6H).

#### 5.2.27. 3-allLyl-8-methoxy-5,5-dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (11c)

Prepared according to General Method F from **10c** and allyl isothiocyanate. Purified via trituration of the crude organic isolate with hexanes and diethyl ether; isolated as tan crystals (252 mg, 59% yield). TLC $R_f$ = 0.11 (10% EtOAc/hex). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 9.27 (s, 1H), 7.38 (d, $J$ = 8.6 Hz, 1H), 6.88 (dd, $J$ = 8.6, 2.5 Hz, 1H), 6.80 (d, $J$ = 2.5 Hz, 1H), 5.99 (ddt, $J$ = 16.6, 10.4, 5.7 Hz, 1H), 5.36 (dd, $J$ = 16.6, 1.3 Hz, 2H), 5.26 (dd, $J$ = 10.4, 1.3 Hz, 1H), 5.06 (d, $J$ = 5.7 Hz, 2H), 3.87 (s, 3H), 2.74 (s, 2H), 1.33 (s, 6H). ESI-MS $m/z$ = 327 ($M-H^+$).

#### 5.2.28. 3-Allyl-5,5-dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (11d)

Prepared according to General Method F from **10d** and allyl isothiocyanate. Purified via trituration of the crude organic isolate with hexanes and diethyl ether; recovered in 24% yield. $R_f$ = 0.34 (10% EtOAc/hex). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 9.28 (s, 1H), 7.50–7.37 (m, 3H), 7.29 (d, $J$ = 7.4 Hz, 1H), 6.00 (ddt, $J$ = 17.1, 10.3, 5.8 Hz, 1H), 5.37 (d, $J$ = 17.1 Hz, 1H), 5.27 (d, $J$ = 10.3 Hz, 1H), 5.07 (d, $J$ = 5.8 Hz, 2H), 2.79 (s, 2H), 1.34 (s, 6H).

#### 5.2.29. 3-Ethyl-5,5-dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (11e)

Prepared according to General Method F from **10d** and ethyl isothiocyanate. Purified via flash chromatography (0% to 10% EtOAc/hex); recovered in 31% yield. TLC $R_f$ = 0.20 (10% EtOAc/hex). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 9.24 (s, 1H), 7.49–7.35 (m, 3H), 7.29 (d, $J$ = 7.4 Hz, 1H), 4.49 (q, $J$ = 7.0 Hz, 2H), 2.79 (s, 2H), 1.35 (t, $J$ = 7.0 Hz, 3H), 1.34 (s, 6H).

#### 5.2.30. 9-Methoxy-3,5,5-trimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (11f)

Prepared according to General Method F from ethyl **10a** and methyl isothiocyanate. Purified via flash chromatography (0–10% EtOAc/hex) and recovered in 27% yield. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 9.35 (s, 1H), 7.20 (d, $J$ = 8.3 Hz, 1H), 6.99 (dd, $J$ = 8.3, 2.3 Hz, 1H), 6.94 (d, $J$ = 2.3 Hz, 1H), 3.87 (s, 3H), 3.74 (s, 3H), 2.71 (s, 2H), 1.33 (s, 6H).

#### 5.2.31. General Method G for the generation of compounds 12a–m

The selected intermediate **11a–f** (0.101 mmol) was dissolved in MEK (0.591 mL), to which $Cs_2CO_3$ (66 mg, 0.201 mmol) and alkylating agent (0.151 mmol) were added. The reaction was heated to 70 °C and allowed to stir 16 h. The reaction mixture was diluted with $H_2O$ and extracted 2× with EtOAc. The combined organic layers were washed with water and brine, then isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography in an appropriate solvent system delivered the desired *S*-alkylated compounds in 24–88% yield.

#### 5.2.32. 3-Allyl-2-(ethylthio)-9-methoxy-5,5-dimethyl-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (12a)

Prepared according to General Method G from intermediate **11a**, using iodoethane as the alkylating agent. Isolated via flash chromatography as a white solid (74 mg, 71% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.70 (s, 1H), 7.10 (d, $J$ = 8.2 Hz, 1H), 6.91 (d, $J$ = 8.2 Hz, 1H), 5.93 (ddt, $J$ = 15.8, 10.9, 5.5 Hz, 1H), 5.33–5.21 (m, 2H), 4.68 (d, $J$ = 5.5 Hz, 2H), 3.85 (s, 3H), 3.31 (q, $J$ = 7.3 Hz, 2H), 2.72 (s, 2H), 1.49 (t, $J$ = 7.3 Hz, 3H), 1.37 (s, 6H).

ESI+MS $m/z$ = 357.2 ($M+H^+$), 379.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 8.53 min) purity >95%.

#### 5.2.33. 3-Allyl-2-(ethylthio)-7-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12b)

Prepared according to General Method G from intermediate **11b**, using iodoethane as the alkylating agent. Isolated via flash chromatography (75 mg, 69% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.80 (d, $J$ = 7.8 Hz, 1H), 7.30 (t, $J$ = 8.0 Hz, 1H), 6.97 (d, $J$ = 8.1 Hz, 1H), 5.95 (ddt, $J$ = 16.0, 10.6, 5.6 Hz, 1H), 5.34–5.25 (m, 2H), 4.70 (d, $J$ = 5.5 Hz, 2H), 3.90 (s, 3H), 3.33 (q, $J$ = 7.3 Hz, 2H), 2.82 (s, 2H), 1.49 (t, $J$ = 7.3 Hz, 3H), 1.41 (s, 6H). ESI+MS $m/z$ = 357.2 ($M+H^+$), 379.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 8.52 min), purity >95%.

#### 5.2.34. 3-Allyl-2-(ethylthio)-8-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (CCG-203592) (12c)

Prepared according to General Method G from intermediate **11c**, using iodoethane as the alkylating agent. Isolated as white crystals via flash chromatography (105 mg, 88% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.05 (d, $J$ = 8.6 Hz, 1H), 6.84 (dd, $J$ = 8.6, 2.4 Hz, 1H), 6.71 (d, $J$ = 2.1 Hz, 1H), 5.93 (ddt, $J$ = 15.8, 10.8, 5.5 Hz, 1H), 5.30–5.22 (m, 2H), 4.67 (d, $J$ = 5.4 Hz, 2H), 3.86 (s, 3H), 3.30 (q, $J$ = 7.3 Hz, 2H), 2.75 (s, 2H), 1.47 (t, $J$ = 7.3 Hz, 3H), 1.38 (s, 6H). ESI+MS $m/z$ = 357.2 ($M+H^+$), 379.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 8.26 min), purity >95%.

#### 5.2.35. 2-((3-Allyl-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazolin-2-yl)thio)acetamide (12d)

Prepared according to General Method G from intermediate **11d**, using 2-bromoacetamide as the alkylating agent. Further purification via flash chromatography (2:1 EtOAc/hex) delivered the desired product as a white crystalline solid (26 mg, 74% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.07 (d, $J$ = 7.1 Hz, 1H), 7.41–7.31 (m, 2H), 7.20 (d, $J$ = 7.9 Hz, 1H), 6.83 (s, 1H), 5.93 (ddt, $J$ = 17.3, 10.1, 5.6 Hz, 1H), 5.39 (s, 1H), 5.34–5.27 (m, 2H), 4.70 (d, $J$ = 5.6 Hz, 2H), 3.96 (s, 2H), 2.80 (s, 2H), 1.38 (s, 6H). ESI+MS $m/z$ = 378.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 2.77 min), purity >95%.

#### 5.2.36. 3-Allyl-5,5-dimethyl-2-((pyridin-2-ylmethyl)thio)-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12e)

Prepared according to General Method G from intermediate **11d**, using 2-(bromomethyl)pyridine hydrobromide as the alkylating agent, as well as an additional equivalent of $Cs_2CO_3$ to neutralize the acid. Isolated via flash chromatography (2:1 EtOAc/hex) as a white crystalline solid (34 mg, 87% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.62 (d, $J$ = 5.5 Hz, 1H), 8.11 (d, $J$ = 7.7 Hz, 1H), 7.66 (t, $J$ = 7.7 Hz, 1H), 7.52 (d, $J$ = 7.8 Hz, 1H), 7.41–7.30 (m, 2H), 7.26–7.18 (m, 2H), 5.96 (ddt, $J$ = 16.0, 10.5, 5.6 Hz, 1H), 5.35–5.26 (m, 2H), 4.76 (s, 2H), 4.73 (d, $J$ = 5.6 Hz, 2H), 2.81 (s, 2H), 1.40 (s, 6H). ESI+MS $m/z$ = 390.1 ($M+H^+$), 412.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 2.36 min), purity >95%.

#### 5.2.37. 3-Allyl-2-((2-hydroxyethyl)thio)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12f)

Prepared according to General Method G from intermediate **11d**, using 2-bromoethanol as the alkylating agent. Isolated via flash chromatography (5% EtOAc/hex) as a white crystalline solid (27 mg, 78% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.04 (d, $J$ = 7.2 Hz, 1H), 7.39–7.29 (m, 2H), 7.19 (d, $J$ = 6.7 Hz, 1H), 5.94 (ddt, $J$ = 15.9, 10.7, 5.6 Hz, 1H), 5.34–5.26 (m, 2H), 4.72 (d, $J$ = 5.6 Hz, 2H), 4.04 (t, $J$ = 5.3 Hz, 2H), 3.55 (t, $J$ = 5.6 Hz, 2H), 2.93 (s, 1H), 2.78 (s, 2H), 1.37 (s, 6H). ESI+MS $m/z$ = 343.1 ($M+H^+$), 365.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 4.71 min), purity >95%.

#### 5.2.38. 2-((3-Allyl-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazolin-2-yl)thio)acetonitrile (12g)

Prepared according to General Method G from intermediate **11d**, using α-chloroacetonitrile as the alkylating agent. Isolated via flash chromatography (5% EtOAc/hex) as a white crystalline solid (92 mg, 81% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.16 (d, $J$ = 7.0 Hz, 1H), 7.41–7.32 (m, 2H), 7.19 (d, $J$ = 6.3 Hz, 1H), 5.91 (ddt, $J$ = 16.1, 10.6, 5.6 Hz, 1H), 5.34–5.26 (m, 2H), 4.65 (d, $J$ = 5.6 Hz, 2H), 4.06 (s, 2H), 2.80 (s, 2H), 1.39 (s, 6H). ESI+MS $m/z$ = 338.1 ($M+H^+$), 360.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 5.57 min), purity >95%.

#### 5.2.39. 3-Allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12h)

Prepared according to General Method G from intermediate **11d**, using 2-methoxyethyl *p*-toluenesulfonate as the alkylating agent. Isolated via flash chromatography (5% EtOAc/hex) as a white crystalline solid (86 mg, 72% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.08 (d, $J$ = 7.4 Hz, 1H), 7.35 (t, $J$ = 7.3 Hz, 1H), 7.31 (t, $J$ = 7.3 Hz, 1H), 7.19 (d, $J$ = 7.1 Hz, 1H), 5.93 (ddt, $J$ = 15.9, 10.8, 5.6 Hz, 1H), 5.32–5.24 (m, 2H), 4.70 (d, $J$ = 5.6 Hz, 2H), 3.76 (t, $J$ = 6.2 Hz, 2H), 3.54 (t, $J$ = 6.2 Hz, 2H), 3.42 (s, 3H), 2.79 (s, 2H), 1.38 (s, 6H). ESI+MS $m/z$ = 357.1 ($M+H^+$), 379.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.47 min), purity = 94%.

#### 5.2.40. 3-Ethyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12i)

Prepared according to General Method G from intermediate **11e**, using 2-methoxyethyl *p*-toluenesulfonate as the alkylating agent. Isolated as a clear oil after flash chromatography (66 mg, 71% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.09 (dd, $J$ = 7.5, 1.3 Hz, 1H), 7.37 (td, $J$ = 7.3, 1.6 Hz, 1H), 7.33 (td, $J$ = 7.5, 1.4 Hz, 1H), 7.21 (d, $J$ = 6.9 Hz, 1H), 4.14 (q, $J$ = 7.1 Hz, 2H), 3.80 (t, $J$ = 6.2 Hz, 2H), 3.57 (t, $J$ = 6.2 Hz, 2H), 3.46 (s, 3H), 2.81 (s, 2H), 1.43–1.36 (m, 9H). ESI+MS $m/z$ = 345.2 ($M+H^+$), 367.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.53 min), purity >95%.

#### 5.2.41. 3-Allyl-8-methoxy-2-((2-methoxyethyl)thio)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12j)

Prepared according to General Method G from intermediate **11c**, using 2-methoxyethyl *p*-toluenesulfonate as the alkylating agent. Isolated as a crystalline white solid after flash chromatography (67 mg, 76% yield). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.01 (d, $J$ = 8.6 Hz, 1H), 6.83 (d, $J$ = 8.6 Hz, 1H), 6.71 (s, 1H), 5.92 (ddt, $J$ = 16.1, 10.6, 5.5 Hz, 1H), 5.31–5.23 (m, 2H), 4.69 (d, $J$ = 5.5 Hz, 2H), 3.86 (s, 3H), 3.75 (t, $J$ = 6.3 Hz, 2H), 3.52 (t, $J$ = 6.3 Hz, 2H), 3.42 (s, 3H), 2.75 (s, 2H), 1.38 (s, 6H). ESI+MS $m/z$ = 387.2 ($M+H^+$), 409.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.05 min), purity >95%.

#### 5.2.42. 3-Allyl-2-(allylthio)-8-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (CCG-205363) (12k)

Prepared using a method similar to General Method G from intermediate **11c**, using allyl bromide as the alkylating agent, DMF as the solvent, and stirring for 18 h at room temperature. Flash chromatography (hexanes/diethyl ether 3:2) afforded the product (22 mg, 78% yield) as a white solid; mp 75–78 °C. $^1H$ NMR (400 MHz, dmso) $\delta$ (ppm) 8.03 (d, $J$ = 8.6, 1H), 6.92 (dd, $J$ = 8.6, 2.5, 1H), 6.86 (d, $J$ = 2.3, 1H), 6.01 (dd, $J$ = 16.9, 10.1, 1H), 5.94–5.78 (m, 1H), 5.39 (d, $J$ = 17.0, 1H), 5.27–5.07 (m, 3H), 4.60 (d, $J$ = 5.1,2H), 4.00 (d, $J$ = 6.8, 2H), 3.82 (s, 3H), 2.75 (s, 2H), 1.29 (s, 3H), 1.24 (s, 3H). ESI+MS $m/z$ = 369 ($M+H^+$), 391 ($M+Na^+$). HPLC (Method A $t_R$ = 9.40 min), purity = 96%.

#### 5.2.43. 3-Allyl-9-methoxy-5,5-dimethyl-2-((2,2,2-trifluoroethyl)thio)-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12l)

Prepared according to General Method G from intermediate **11a**, using 2,2,2-trifluoro-1-iodoethane as the alkylating agent. Reaction was heated to 50 °C to avoid evaporation of the alkylating agent. Flash chromatography (5–10% EtOAc/hex) delivered the product (7 mg, 24% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.61 (s, 1H), 7.10 (d, $J$ = 8.1 Hz, 1H), 6.93 (d, $J$ = 8.1 Hz, 1H), 5.93 (ddt, $J$ = 16.5, 11.1, 5.6 Hz, 1H), 5.34–5.23 (m, 2H), 4.71 (d, $J$ = 5.6 Hz, 2H), 4.16 (q, $J$ = 9.7 Hz, 2H), 3.84 (s, 3H), 2.73 (s, 2H), 1.37 (s, 6H). ESI+MS $m/z$ = 411.2 (M+H$^+$), HPLC (Method B, $t_R$ = 7.98 min), purity = 88%.

#### 5.2.44. 2-(Ethylthio)-9-methoxy-3,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12m)

Prepared according to General Method G from intermediate **11f**, using iodoethane as the alkylating agent. Isolated as a colorless oil (20 mg, 55% yield) after flash chromatography. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.70 (s, 1H), 7.10 (d, $J$ = 8.2 Hz, 1H), 6.90 (d, $J$ = 8.2 Hz, 1H), 3.85 (s, 3H), 3.50 (s, 3H), 3.32 (q, $J$ = 7.3 Hz, 2H), 2.72 (s, 2H), 1.50 (t, $J$ = 7.3 Hz, 3H), 1.38 (s, 6H). ESI+MS $m/z$ = 331.2 (M+H$^+$), 353.2 (M+Na$^+$). HPLC (Method A, $t_R$ = 9.25 min), purity >95%.

#### 5.2.45. 3-Allyl-5,5-dimethyl-2-(methylthio)-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (12n)

Intermediate **11d** (116 mg, 0.39 mmol) was dissolved in EtOH (2.3 mL), to which KOH (38 mg, 0.58 mmol) and methyl iodide (27 μL, 0.43 mmol) were added. The solution was stirred for 30 min, precipitating a crystalline white solid. The solution was diluted with ethyl acetate and water. The aqueous layer was extracted with additional EtOAc, then the combined organic layers were washed with water and brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. The recovered crystalline material (116 mg, 96% yield) was found to be pure by NMR and used without further purification. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.15 (d, $J$ = 7.3, 1H), 7.38–7.28 (m, 2H), 7.18 (d, $J$ = 6.9, 1H), 6.01–5.75 (m, 1H), 5.33–5.23 (m, 2H), 4.69 (d, $J$ = 5.5, 2H), 2.79 (s, 2H), 2.69 (s, 3H), 1.39 (s, 6H). HPLC (Method B, $t_R$ = 7.57 min), purity >95%.

#### 5.2.46. 3-Allyl-2-ethoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (13a)

Prepared according to General Method B from intermediate **12n**. Isolated as a light yellow solid (22 mg, 69% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.09 (d, $J$ = 7.3 Hz, 1H), 7.37–7.27 (m, 2H), 7.18 (d, $J$ = 7.5 Hz, 1H), 5.92 (ddt, $J$ = 16.0, 10.3, 5.8 Hz, 1H), 5.25–5.16 (m, 2H), 4.62 (d, $J$ = 5.8 Hz, 2H), 4.57 (q, $J$ = 7.1 Hz, 2H), 2.78 (s, 2H), 1.45 (t, $J$ = 7.1 Hz, 3H), 1.37 (s, 6H). ESI+MS $m/z$ = 311.1 (M+H$^+$), 333.1 (M+Na$^+$). HPLC (Method B, $t_R$ = 7.94 min), purity >95%.

#### 5.2.47. 3-Allyl-2-(ethylamino)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (13b)

Prepared according to General Method C from intermediate **12n**. Isolated as a white crystalline solid (23 mg, 41% yield over 2 steps). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.15 (d, $J$ = 7.3 Hz, 1H), 7.36–7.26 (m, 2H), 7.16 (d, $J$ = 7.4 Hz, 1H), 5.92 (ddt, $J$ = 17.3, 10.5, 5.3 Hz, 1H), 5.36–5.26 (m, 2H), 4.68 (d, $J$ = 5.2 Hz, 2H), 4.58 (t, $J$ = 4.9 Hz, 1H), 3.57 (qd, $J$ = 7.2, 5.2 Hz, 2H), 2.77 (s, 2H), 1.37 (s, 6H), 1.28 (t, 3H). ESI+MS $m/z$ = 310.1 (M+H$^+$), 332.1 (M+Na$^+$). HPLC (Method B, $t_R$ = 5.47 min), purity >95%.

#### 5.2.48. 3-Allyl-2-(ethylsulfinyl)-9-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (14)

Compound **12a** (20 mg, 0.056 mmol) was added to a 3.5:1 THF/water mixture (0.617 mL), and the reaction then cooled to 0 °C. A solution of Oxone (34 mg, 0.056 mmol) in water (0.411 mL) was added slowly dropwise, and the reaction was allowed to stir 3.5 h at 0 °C, then allowed to warm to room temperature over 16 h. At this point the reaction was partitioned between EtOAc and water. The organic layer was washed with water and brine, then isolated, dried over $MgSO_4$, vacuum filtered, and concentrated under reduced pressure. The resulting residue was purified via flash chromatography (10–33% EtOAc/hex) to deliver the product as light yellow crystals (13 mg, 62% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.66 (d, $J$ = 2.7 Hz, 1H), 7.12 (d, $J$ = 8.2 Hz, 1H), 6.93 (dd, $J$ = 8.2, 2.7 Hz, 1H), 6.56–5.61 (m, 1H), 5.30 (d, $J$ = 10.3 Hz, 1H), 5.24 (d, $J$ = 17.2 Hz, 1H), 5.13 (ab with 5.00; ddt, $J$ = 1.6, 5.0, 15.8 Hz, 1H), 5.00 (ab with 5.13; dd, $J$ = 5.0, 15.8 Hz, 1H), 3.86 (s, 3H), 3.46–3.27 (m, 2H), 2.75 (s, 2H), 1.43 (t, $J$ = 7.5 Hz, 3H), 1.39 (s, 3H), 1.38 (s, 3H). ESI+MS $m/z$ = 395.2 (M+H$^+$). HPLC (Method A, $t_R$ = 7.39 min), purity >95%.

#### 5.2.49. 3-Allyl-2-(ethylsulfonyl)-9-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (15)

Compound **12a** (28 mg, 0.79 mmol) and *m*CPBA (70 wt%, 48 mg, 0.196 mmol) were combined in DCM (1.2 mL) at 0 °C. The reaction mixture was kept at 0 °C for 1 h, then allowed to warm to RT over the course of 16 h. The reaction mixture was then diluted with more DCM and washed with saturated sodium bicarbonate solution, water, and brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (5–10% EtOAc/hex) delivered the desired product as a light yellow solid (19 mg, 62% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.43 (s, 1H), 7.14 (d, $J$ = 8.2 Hz, 1H), 6.94 (d, $J$ = 8.2 Hz, 1H), 6.04 (ddt, $J$ = 16.8, 10.3, 5.5 Hz, 1H), 5.40 (d, $J$ = 16.8 Hz, 1H), 5.30 (d, $J$ = 10.3 Hz, 1H), 5.02 (d, $J$ = 5.5 Hz, 2H), 3.84 (s, 3H), 3.81 (q, $J$ = 7.3 Hz, 2H), 2.75 (s, 2H), 1.62 (t, $J$ = 7.3 Hz, 3H), 1.38 (s, 6H). ESI+MS $m/z$ = 389.2 (M+H$^+$), 411.2 (M+Na$^+$). HPLC (Method A, $t_R$ = 8.64 min), purity >95%.

#### 5.2.50. General Method H for preparation of phenols 16a–c

Boron tribromide solution (1 M in dichloromethane, 4.42 mL) was added gradually to a stirred solution of the selected intermediate **12a–c** (2.1 mmol) in dichloromethane (14 mL) at room temperature under $N_2$. The mixture was heated to reflux for 6 h, then cooled to room temperature. Water (10 mL) was then added dropwise and the mixture was partitioned between DCM and water. The layers were separated and the organic layer washed with water, saturated aqueous $NaHCO_3$, and saturated brine, then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was triturated in ethyl acetate, filtered, and dried under high vacuum. The resulting crystalline phenols **16a–c** (43–62% isolated yield) were used without further purification.

#### 5.2.51. 3-Allyl-2-(ethylthio)-9-hydroxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (16a)

Prepared according to General Method H from compound **12a.** Isolated as a tan solid (60 mg, 62% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.91 (s, 1H), 7.05 (d, $J$ = 8.2 Hz, 1H), 6.98 (d, $J$ = 8.2 Hz, 1H), 5.90 (ddt, $J$ = 16.3, 10.7, 5.6 Hz, 1H), 5.44–5.36 (m, 2H), 4.83 (d, $J$ = 5.6 Hz, 2H), 3.95 (q, $J$ = 6.8 Hz, 2H), 2.75 (s, 2H), 1.52 (t, $J$ = 6.8 Hz, 3H), 1.36 (s, 6H).

#### 5.2.52. 3-Allyl-2-(ethylthio)-7-hydroxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (16b)

Prepared according to General Method H from compound **12b.** Isolated as a tan solid (43 mg, 45% yield). $^1$H NMR (500 MHz,

$CDCl_3$) $\delta$ (ppm) 7.76 (d, $J$ = 7.8 Hz, 1H), 7.18 (t, $J$ = 7.8 Hz, 1H), 6.89 (d, $J$ = 7.8 Hz, 1H), 5.99–5.87 (m, 1H), 5.31–5.24 (m, 2H), 5.18 (s, 1H), 4.68 (d, $J$ = 5.5 Hz, 2H), 3.31 (q, $J$ = 7.4 Hz, 2H), 2.78 (s, 2H), 1.47 (t, $J$ = 7.4 Hz, 3H), 1.41 (s, 6H).

#### 5.2.53. 3-Allyl-2-(ethylthio)-8-hydroxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (16c)

Prepared according to General Method H from compound **12c.** Isolated as tan crystals (310 mg, 43% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.62 (d, $J$ = 8.2 Hz, 1H), 6.85–6.79 (m, 2H), 5.88 (ddt, $J$ = 16.3, 10.8, 5.9 Hz, 1H), 5.48–5.40 (m, 2H), 4.86 (d, $J$ = 5.9 Hz, 2H), 4.22 (q, $J$ = 7.3 Hz, 2H), 2.74 (s, 2H), 1.59 (t, $J$ = 7.3 Hz, 3H), 1.31 (s, 6H).

#### 5.2.54. General Method I to generate compounds 17a–j

The selected phenol intermediate **12a–c** (0.622 mmol) was dissolved in DMF (3.66 mL), to which $Cs_2CO_3$ (304 mg, 0.933 mmol) and an alkylating agent (0.716 mmol) were added. The resulting suspension was stirred at 25 °C to 70 °C for 30 min to 16 h until the completion of the reaction. Compounds were purified as indicated in 35–92% yield.

#### 5.2.55. 3-Allyl-2-(ethylthio)-9-(2-methoxyethoxy)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17a)

Prepared using General Method I from **12a** and 2-methoxyethyl *p*-toluenesulfonic ester**,** heated to 70 °C for 16 h**.** Reaction mixture was partitioned between ethyl acetate and water, then the organic layer washed with water and brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (0–10% EtOAc/hex) delivered the desired product (38 mg, 65% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.71 (s, 1H), 7.09 (d, $J$ = 8.2 Hz, 1H), 6.94 (d, $J$ = 8.2 Hz, 1H), 5.93 (ddt, $J$ = 16.0, 10.7, 5.6 Hz, 1H), 5.31–5.23 (m, 2H), 4.68 (d, $J$ = 5.5 Hz, 2H), 3.81–3.75 (m, 2H), 3.61–3.55 (m, 2H), 3.47 (s, 3H), 3.31 (q, $J$ = 7.3 Hz, 2H), 2.72 (s, 2H), 1.48 (t, $J$ = 7.3 Hz, 3H), 1.37 (s, 6H). ESI+MS $m/z$ = 401.2 ($M+H^+$), 423.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.83 min), purity >95%.

#### 5.2.56. 3-Allyl-2-(ethylthio)-7-(2-methoxyethoxy)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17b)

Prepared using General Method I from phenol intermediate **12b** and 2-methoxyethyl *p*-toluenesulfonic ester**,** heated to 70 °C for 16 h**.** Reaction mixture was partitioned between ethyl acetate and water, then the organic layer washed with water and brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (0–10% EtOAc/hex) delivered the desired product (36 mg, 68% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 7.78 (d, $J$ = 7.8 Hz, 1H), 7.25 (t, $J$ = 8.0 Hz, 1H), 6.96 (d, $J$ = 8.1 Hz, 1H), 5.99–5.87 (m, 1H), 5.31–5.23 (m, 2H), 4.68 (d, $J$ = 5.5 Hz, 2H), 4.17 (dd, $J$ = 5.5, 4.2 Hz, 2H), 3.80 (dd, $J$ = 5.5, 4.2 Hz, 2H), 3.48 (s, 3H), 3.30 (q, $J$ = 7.4 Hz, 2H), 2.83 (s, 2H), 1.47 (t, $J$ = 7.4 Hz, 3H), 1.39 (s, 6H). ESI+MS $m/z$ = 401.2 ($M+H^+$), 423.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.91 min), purity >95%.

#### 5.2.57. 3-Allyl-2-(ethylthio)-8-(2-methoxyethoxy)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17c)

Prepared using General Method I from phenol intermediate **12c** and 2-methoxyethyl *p*-toluenesulfonic ester**,** heated to 70 °C for 16 h**.** Reaction mixture was partitioned between ethyl acetate and water, then the organic layer washed with water and brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (0–10% EtOAc/hex) delivered the desired product (39 mg, 71% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ (ppm) 8.04 (d, $J$ = 8.6 Hz, 1H), 6.86 (dd, $J$ = 8.6, 2.4 Hz, 1H), 6.74 (d, $J$ = 2.2 Hz, 1H), 5.92 (ddt, $J$ = 15.9, 10.6, 5.4 Hz, 1H), 5.30–5.22 (m, 2H), 4.67 (d, $J$ = 5.5 Hz, 2H), 4.18 (t, $J$ = 5.0 Hz, 2H), 3.78 (t, $J$ = 5.0 Hz, 2H), 3.47 (s, 3H), 3.30 (q, $J$ = 7.4 Hz, 2H), 2.74 (s, 2H), 1.47 (t, $J$ = 7.3 Hz, 3H), 1.37 (s, 6H). ESI+MS $m/z$ = 401.1 ($M+H^+$), 423.1 ($M+Na^+$). HPLC (Method B, $t_R$ = 7.56 min), purity >95%.

#### 5.2.58. Ethyl 2-((3-allyl-2-(ethylthio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydro-benzo[*h*]-quinazolin-8-yl)oxy)acetate (17d)

Prepared using General Method I from phenol intermediate **12c** and ethyl bromoacetate**,** stirred at room temperature for 2.5 h**.** The mixture was poured into 40 mL of water, then the precipitate was filtered off, rinsed with water 3 times and dried to afford the product (54 mg, 86% yield) as a white powder. $^1$H NMR (400 MHz, dmso) $\delta$ (ppm) 8.01 (d, $J$ = 8.6, 1H), 6.90 (dd, 1H), 6.86 (s, 1H), 5.94–5.83 (m, 1H), 5.22 (d, $J$ = 10.2, 1H), 5.13 (d, $J$ = 17.2, 1H), 4.85 (s, 2H), 4.59 (d, $J$ = 4.3, 2H), 4.19 (q, 2H), 3.29 (q, 2H), 2.75 (s, 2H), 1.39 (t, $J$ = 7.2, 3H), 1.29 (s, 6H), 1.23 (t, $J$ = 7.1, 3H). ESI+MS $m/z$ = 429 ($M+H^+$), 451 ($M+Na^+$). HPLC (Method A, $t_R$ = 10.21 min), purity = 92%.

#### 5.2.59. 3-Allyl-2-(ethylthio)-5,5-dimethyl-8-(2-morpholinoethoxy)-5,6-dihydrobenzo[*h*]-quinazolin-4(3*H*)-one (17e)

Prepared using General Method I from phenol intermediate **12c** and 4-(2-chloroethyl)-morpholine hydrochloride, stirred at 80 °C for 4 h**.** The mixture was poured into water and extracted with ethyl acetate three times, then the combined extracts were washed with water and saturated brine, dried over $MgSO_4$, and concentrated in vacuo to a tan solid (0.035 g, 69% yield), mp 91–92 °C. $^1$H NMR (400 MHz, dmso) $\delta$ (ppm) 8.06 (d, 1H), 6.98 (dd, 1H), 6.92 (d, 1H), 5.93 (ddt, 1H), 5.23 (d, 1H), 5.19 (d, 1H), 4.64 (d, 2H), 4.21 (t, 2H), 3.65 (m, 4H), 3.34 (q, 2H), 2.80 (m, 4H), 2.53 (m, 2H), 1.44 (t, 3H), 1.35 (s, 6H). ESI+MS $m/z$ = 456 ($M+H^+$). HPLC (Method A, $t_R$ = 10.23 min), purity = 91%.

#### 5.2.60. 3-Allyl-2-(ethylthio)-8-isopropoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17f)

Prepared using General Method I from phenol intermediate **12c,** using 2-bromopropane as the alkylating agent and stirring at room temperature for 4 h. The reaction mixture was poured into water, then extracted into ethyl acetate. The organic layer was washed with water and brine, then isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo delivering the final product as a sticky yellow oil (34 mg, 92% yield).. $^1$H NMR (400 MHz, dmso) $\delta$ (ppm) 8.04 (d, 1H), 6.94 (dd, 1H), 6.87 (d, 1H), 5.92 (m, 1H), 5.27 (dd, 1H), 5.18 (m, 1H), 4.82–4.69 (m, 1H), 4.64 (d, 2H), 3.31–3.39 (m, 2H), 2.79 (s, 2H), 1.44(t, 3H), 1.35 (m, 12H). ESI+MS $m/z$ = 385 ($M+H^+$), 407 $M+Na^+$). HPLC (Method A, $t_R$ = 9.25 min), purity = 93%.

#### 5.2.61. 2-((3-Allyl-2-(ethylthio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazolin-8-yl)oxy)-acetonitrile (17g)

Prepared using General Method I from phenol intermediate **12c** using bromoacetonitrile as the alkylating agent and stirring at room temperature for 4 h. The reaction mixture was partitioned between ethyl acetate and water, then the organic layer was washed with water and brine. After drying over $MgSO_4$, filtration, and concentration in vacuo, recrystallization of the residue from isopropanol gave the pure product (13 mg, 39% yield); mp 130–131 °C. $^1$H NMR (400 MHz, dmso) $\delta$ (ppm) 8.03 (d, 1H), 7.01 (dd, 1H),6.95 (d, 1H), 5.91–5.74 (m, 1H), 5.19 (m, 3H), 5.09 (d, 1H), 4.55 (d, 2H), 3.26 (q, 2H), 2.74 (s, 2H), 1.35 (t, 3H), 1.26 (s, 6H). ESI+MS $m/z$ = 382 ($M+H^+$), 404 ($M+Na^+$). HPLC (Method A, $t_R$ = 8.69 min), purity = 89%.

#### 5.2.62. 3-Allyl-8-butoxy-2-(ethylthio)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17h)

Prepared according to General Method I from intermediate **12c** and 1-bromopentane. Isolated in 75% yield. $^1$H NMR (500 MHz, DMSO) $\delta$ 8.00 (d, 1H), 6.90 (d, 1H), 6.84 (s, 1H), 5.94–5.77 (m, 1H), 5.21 (d, 1H), 5.13 (d, 1H), 4.59 (d, 2H), 4.03 (t, 2H), 3.28 (q, 2H), 2.74 (s, 2H), 1.71 (m, 2H), 1.48 (m, 2H), 1.41 (t, 3H), 1.29 (s, 6H), 0.95 (t, 3H). ESI+MS $m/z$ = 399 ($M+H^+$), 421 ($M+Na^+$). HPLC (Method C, $t_R$ = 5.45 min), purity = 94%.

#### 5.2.63. 3-Allyl-2-(ethylthio)-5,5-dimethyl-8-phenethoxy-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17i)

Prepared according to General Method I from intermediate **12c** and (2-bromoethyl)benzene. Isolated in 77% yield. $^1$H NMR (500 MHz, DMSO) $\delta$ 8.05 (d, 1H), 7.5–7.29 (m, 5H), 6.97 (d, 1H), 6.91 (s, 1H), 6.09–5.81 (m, 1H), 5.27 (d, 1H), 5.18 (d, 1H), 4.64 (d, 2H), 4.31 (t, 2H), 3.33 (q, 2H), 3.12 (t, 2H), 2.79 (s, 2H), 1.45 (t, 3H), 1.33 (s, 6H). ESI+MS $m/z$ = 447 ($M+H^+$), 469 ($M+Na^+$). HPLC (Method A, $t_R$ = 8.89 min), purity = 93%.

#### 5.2.64. 3-Allyl-2-(ethylthio)-8-(heptyloxy)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (17j)

Prepared according to General Method I from intermediate **12c** and 1-bromoheptane. Isolated in 35% yield. $^1$H NMR (500 MHz, DMSO) $\delta$ 7.95 (d, 1H), 6.85 (d, 1H), 6.80 (s, 1H), 5.89–5.78 (m, 1H), 5.18 (d, 1H), 5.09 (d, 1H), 4.54 (d, 2H), 3.98 (t, 2H), 3.23 (q, 2H), 2.70 (s, 2H), 1.69 (m, 2H), 1.45–1.12 (m, 17H), 0.84 (t, 3H). ESI+MS $m/z$ = 441 ($M+H^+$), 463 ($M+Na^+$). HPLC (Method C, $t_R$ = 11.67 min), purity >95%.

#### 5.2.65. 2-((3-Allyl-2-(ethylthio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazolin-8-yl)oxy)acetic acid (17k)

Compound **17d** (40 mg, 0.93 mmol) was added to MeOH (0.5 mL), and KOH solution (1 M, 0.75 mL) was added. The mixture was stirred and heated at 50 °C for 90 min then cooled, poured into 20 mL of water, stirred and acidified with 2 N HCl. After 2 h the mixture was extracted twice with ethyl acetate. The combined extracts were washed with water then saturated brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue recrystallized from acetonitrile to afford the product (10 mg, 27% yield) as a white solid; mp 167–168 °C. $^1$H NMR (400 MHz, dmso) $\delta$ 13.03 (s, 1H), 7.97 (d, 1H), 6.85 (dd, 1H), 6.82 (d, 1H), 5.91–5.74 (m, 1H), 5.19 (dd, 1H), 5.09 (d, 1H), 4.70 (d, 2H), 4.54 (d, 2H), 3.26–3.19 (m, 2H), 2.71 (s, 2H), 1.34 (t, 3H), 1.25 (s, 6H). ESI+MS $m/z$ = 401 ($M+H^+$), 423 ($M+Na^+$); ESI-MS $m/z$ = 399 ($M-H^+$). HPLC (Method A $t_R$ = 7.69 min), purity = 93%.

#### 5.2.66. General Method J for generating unsubstituted 2-thioxopyrimidinone intermediates 18a, 18d, 24

To a solution of the selected β-aminoester intermediate (**10a, 10d, 23**)(12.64 mmol) in absolute EtOH (15 mL) was added benzoyl isothiocyanate (1.7 mL, 12.64 mmol), and the reaction mixture was warmed to reflux for 30 min. Additional benzoyl isothiocyanate (400 μL, 2.7 mmol) was added in two equal portions over the course of 1 hour, then the reaction was allowed to cool. The thiourea adduct was isolated via crystallization from ethanol or via flash chromatography, then added to a solution of KOH (1.21 g, 21.57 mmol) in ethanol/water (2:1, 30 mL). The reaction mixture was warmed to reflux and allowed to stir for 1.5 h. The reaction mixture was cooled, then acidified to pH 5–6 with 1 N HCl. The resulting precipitate was isolated via vacuum filtration, washed with EtOH and water and dried under high vacuum. Isolated in 61–83% yield.

#### 5.2.67. 9-Methoxy-5,5-dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (18a)

Generated using General Method J from β-aminoester **10a**. Isolated the desired product as a white solid (650 mg, 61% yield over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 9.32 (s, 2H), 7.20 (d, $J$ = 8.2 Hz, 1H), 7.00 (dd, $J$ = 8.2, 2.4 Hz, 1H), 6.97 (d, $J$ = 2.4 Hz, 1H), 3.87 (s, 3H), 2.71 (s, 2H), 1.32 (s, 6H).

#### 5.2.68. 5,5-Dimethyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*h*]quinazolin-4(1*H*)-one (18d)

Generated using General Method J from β-aminoester **10d**. Isolated the desired product as a white solid (2.7 g, 83% yield over 2 steps); $R_f$: 0.56 (2:3, EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ (ppm) 9.95 (s, 1H, NH), 9.75 (s, 1H, NH), 7.57 (d, 1H, J = 7.7 Hz), 7.46 (t, 1H, J = 7.4 Hz), 7.39 (t, 1H, J = 7.5 Hz), 7.28 (d, 1H, J = 7.5 Hz), 2.78 (s, 2H), 1.33 (s, 6H).

#### 5.2.69. 2-((2-Methoxyethyl)thio)-3-(4-methoxyphenethyl)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quin-azolin-4(3*H*)-one, (19a) and 2-((2-Methoxyethyl)thio)-4-(4-methoxyphenethoxy)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazoline, (20a)

A solution of compound **18d** (2.7 g, 10.45 mmole) and KOH (590 mg, 10.45 mmole) in absolute EtOH (60 mL) was refluxed for 30 min. Then 2-methoxyethyl toluenesulfonic ester (2.41 g, 10.45 mmole) was added in EtOH (3 mL). The reaction was allowed to reflux for 15 h, then allowed to cool to room temperature. The crystallized solid was filtered, washed with EtOH (3 mL) and water (75 mL), dried under suction and then under high vacuum. A portion of the resulting *S*-alkylated compound (25 mg, 0.079 mmol) in DMF (1 mL) was treated with lithium carbonate (17 mg, 0.23 mmol) and 1-(2-bromoethyl)-4-methoxybenzene (18 mg, 0.084 mmol). The reaction was warmed to 80 °C and allowed to stir 24 h, at which point additional lithium carbonate (17 mg, 0.23 mmol) and 1-(2-bromoethyl)-4-methoxybenzene (13 mg, 0.061 mmol) were added. After 24 additional h at reflux, the solvent was removed in vacuo and the resulting mixture of *N* and *O*-alkylated products were separated via flash chromatography (4% EtOAc/hex).

#### 5.2.70. 2-((2-Methoxyethyl)thio)-3-(4-methoxyphenethyl)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quin-azolin-4(3*H*)-one, (19a)

Yield: 5 mg (11% over 2 steps); $R_f$: 0.30 (1:9, EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.08 (dd, 1H, J = 1.6 & 7.1 Hz), 7.26–7.37 (m, 4H), 7.19 (d, 1H, J = 7.2 Hz), 6.86 (d, 2H, J = 8.6 Hz), 4.19 (t, 2H, J = 8.3 Hz), 3.80 (s, 3H), 3.77 (t, 2H, J = 6.2 Hz), 3.56 (t, 2H, J = 6.2 Hz), 3.44 (s, 3H), 3.00 (t, 2H, J = 8.3 Hz), 2.79 (s, 2H) & 1.39 (s, 6H); ESI+MS $m/z$ = 451 ($M+H^+$), 473 ($M+Na^+$). HPLC (Method B, $t_R$ = 9.9 min), purity = 98.8%.

#### 5.2.71. 2-((2-Methoxyethyl)thio)-4-(4-methoxyphenethoxy)-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazoline, (20a)

Yield: 24 mg (54% over 2 steps); $R_f$: 0.38 (1:9, EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.20 (dd, 1H, J = 1.7 & 7.2 Hz), 7.29–7.36 (m, 2H), 7.14–7.21 (m, 3H), 6.85 (m, 2H), 4.60 (t, 2H, J = 7.0 Hz), 3.79 (s, 3H), 3.74 (t, 2H, J = 6.9 Hz), 3.41 (m, 5H), 3.06 (t, 2H, J = 7.0 Hz), 2.77 (s, 2H) & 1.25 (s, 6H); ESI+MS $m/z$ = 451 ($M+H^+$), 473 ($M+Na^+$). HPLC (Method B, $t_R$ = 10.1 min), purity = 97.3%.

#### 5.2.72. 2-(Ethylthio)-9-methoxy-5,5-dimethyl-3-(2,2,2-trifluoroethyl)-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (19b) and 2-(ethylthio)-9-methoxy-5,5-dimethyl-4-(2,2,2-trifluoroethoxy)-5,6-dihydrobenzo[*h*]quinazoline (20b)

The 2-thioxo intermediate **18a** (118 mg, 0.409 mmol) was dissolved in EtOH (2.4 mL), to which KOH (23 mg, 0.409 mmol) and

iodoethane (64 mg, 0.409 mmol) were added. The reaction was warmed to 75 °C and allowed to stir for 3 h. The reaction mixture was diluted with 1 N HCl and the resulting precipitate was collected via sintered glass funnel, washed with water, and dried under high vacuum. A portion of the S-alkylated adduct (27 mg, 0.085 mmol) was combined with 1,1,1-trifluoro-2-iodoethane (27 mg, 0.128 mmol) and $Cs_2CO_3$ (33 mg, 0.102 mmol) in DMF (500 μL). The reaction was tightly capped and heated to 70 °C for 16 h. The reaction mixture was partitioned between ethyl acetate and water, then the organic layer was washed with water and brine. The organic layer was concentrated in vacuo and purified via flash chromatography (0–3% EtOAc/hex) to yield compounds **19b** (6 mg, 13% yield over 2 steps) and **20b** (17 mg, 36% yield over 2 steps) as crystalline white solids.

#### 5.2.73. 2-(Ethylthio)-9-methoxy-5,5-dimethyl-3-(2,2,2-trifluoroethyl)-5,6-dihydrobenzo[*h*]quinazolin-4(3*H*)-one (19b)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.68 (d, $J$ = 2.8 Hz, 1H), 7.11 (d, $J$ = 8.2 Hz, 1H), 6.93 (dd, $J$ = 8.2, 2.8 Hz, 1H), 4.78 (q, $J$ = 8.0 Hz, 2H), 3.85 (s, 3H), 3.35 (q, $J$ = 7.3 Hz, 2H), 2.74 (s, 2H), 1.50 (t, $J$ = 7.3 Hz, 3H), 1.36 (s, 6H). ESI+MS $m/z$ = 399.2 ($M+H^+$), 421.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 8.49 min), purity >95%.

#### 5.2.74. 2-(Ethylthio)-9-methoxy-5,5-dimethyl-4-(2,2,2-trifluoroethoxy)-5,6-dihydrobenzo[*h*]quinazoline (20b)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.81 (d, $J$ = 2.7 Hz, 1H), 7.10 (d, $J$ = 8.2 Hz, 1H), 6.94 (dd, $J$ = 8.2, 2.7 Hz, 1H), 4.80 (q, $J$ = 8.5 Hz, 2H), 3.87 (s, 3H), 3.21 (q, $J$ = 7.3 Hz, 2H), 2.76 (s, 2H), 1.47 (t, $J$ = 7.3 Hz, 3H), 1.33 (s, 6H). ESI+MS $m/z$ = 399.2 ($M+H^+$). HPLC (Method A, $t_R$ = 9.84 min), purity >95%.

#### 5.2.75. 4-(Allyloxy)-2-(ethylthio)-9-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazoline (20c)

The 2-thioxo intermediate **18a** (118 mg, 0.409 mmol) was dissolved in EtOH (2.4 mL), to which KOH (23 mg, 0.409 mmol) and iodoethane (64 mg, 0.409 mmol) were added. The reaction was warmed to 75 °C and allowed to stir for 3 h. The reaction mixture was diluted with 1 N HCl, and the precipitate (92 mg, 71% yield) was collected via sintered glass funnel, washed with water, and dried under high vacuum. A portion of the *S*-alkylated adduct (30 mg, 0.095 mmol) was combined with $Cs_2CO_3$ (37 mg, 0.114 mmol) and allyl bromide (17 mg, 0.142 mmol) in DMF (558 μL). The reaction mixture was tightly capped and warmed to 75 °C, and allowed to stir for 16 h. The reaction mixture was partitioned between ethyl acetate and water, then the organic layer was washed with water and brine. The organic layer was concentrated in vacuo, and the residue was purified via flash chromatography (0–5% EtOAc/hex) to yield **19e** (14 mg, 30% yield over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.81 (d, $J$ = 2.5 Hz, 1H), 7.08 (d, $J$ = 8.2 Hz, 1H), 6.91 (dd, $J$ = 8.2, 2.5 Hz, 1H), 6.09 (ddt, $J$ = 16.4, 10.6, 5.5 Hz, 1H), 5.40 (d, $J$ = 16.4 Hz, 1H), 5.27 (d, $J$ = 10.6 Hz, 1H), 4.94 (d, $J$ = 5.5 Hz, 2H), 3.87 (s, 3H), 3.19 (q, $J$ = 7.3 Hz, 2H), 2.74 (s, 2H), 1.47 (t, $J$ = 7.3 Hz, 3H), 1.33 (s, 6H). ESI+MS $m/z$ = 357.2 ($M+H^+$), 379.2 ($M+Na^+$). HPLC (Method B, $t_R$ = 10.41 min), purity >95%. *N*-allyl compound **12a** was also isolated (10 mg, 21% yield over 2 steps).

#### 5.2.76. 4-Cyano-3-methylbenzoic acid (22)

A solution of 2.5 M *n*-butyllithium in hexanes(6.7 mL, 16.8 mmol) was added to anhydrous THF (15 mL) at −78 °C. A solution of 4-bromo-2-methylbenzonitrile **21** (3.0 g, 15.3 mmol) in THF (15 mL) was then added dropwise and allowed to stir for 30 min. Dry ice (solid CO2) was added and the solution was allowed to stir 1 h while warming to room temperature. The reaction was then concentrated in vacuo and the resulting residue was triturated in ether and filtered. The solid was dissolved in EtOAc and washed with 2 M HCl and brine. The organic layer was isolated, dried over MgSO4, concentrated in vacuo, then triturated in ether/hexane and dried under high vacuum. Obtained 4-cyano-3-methylbenzoic acid **22** (1.5 g, 60.8% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.18 (s, 1H), 8.02–8.09 (m, 1H), 7.75–7.81 (m, 1H), 2.61 (s, 3H).

#### 5.2.77. 2-Ethyl 6-methyl 1-amino-3,3-dimethyl-3,4-dihydronaphthalene-2,6-dicarboxylate (23)

Prepared according to General Method E from 4-cyano-3-methylbenzoic acid **22** (1.0 g, 6.2 mmol). The impure residue resulting from concentrating the crude organic extract was dissolved in 20% methanolic toluene (20 ml) and treated with trimethylsilyldiazomethane (2.7 ml, 5.4 mmol). The mixture was stirred 30 min at room temperature, then concentrated in vacuo. Purification via flash chromatography (5% to 10% to 20% EtOAc/hex) yielded the desired compound **23** as a yellow solid (0.78 g, 41.5% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 7.98–8.10 (m, 1H), 7.82–7.88 (m, 1H), 7.45–7.51 (m, 1H), 6.24 (br s, 2H), 4.31–4.39 (q, 2H), 3.93 (s, 3H), 2.68 (br s, 2H), 1.38–1.41 (t, 3H), 1.28 (s, 6H). HPLC (Method A, $t_R$ = 8.02 min), purity = 84%.

#### 5.2.78. Methyl 5,5-dimethyl-4-oxo-2-thioxo-1,2,3,4,5,6-hexahydrobenzo[*h*]quinazoline-8-carboxylate (24)

Prepared according to General Method J from β-aminoester **23**. Isolated as a white solid (550 mg, 61% yield over 2 steps) used without further purification. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.11–8.15 (m, 1H), 7.87–7.89 (m, 1H), 7.78–7.81 (m, 1H), 3.89 (s, 3H), 2.82 (br s, 2H), 1.27 (s, 6H). HPLC (Method A, $t_R$ = 6.26 min), purity = 82%.

#### 5.2.79. Methyl 3-allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxylate (25)

To a solution of methyl 5,5-dimethyl-4-oxo-2-thioxo-1,2,3,4,5,6-hexahydrobenzo[*h*]quinazoline-8-carboxylate **24** (850 mg, 2.7 mmol) in DMF (10 mL) was added $Cs_2CO_3$ (1.8 g, 5.4 mmol) followed by 2-methoxyethyl 4-methylbenzenesulfonate (0.74 g, 3.2 mmol). The resulting mixture was stirred at 40 °C for 3 h, then allowed to cool to room temperature and stir an additional 16 h. The reaction mixture was diluted with EtOAc, then washed with water, saturated $NaHCO_3$ solution, and brine, then isolated and dried over MgSO4. The organic extract was concentrated in vacuo and purified by flash chromatography, (5 to 30% EtOAc/hex) to obtain the *S*-2-methoxyethyl adduct as a white solid. A portion of the resulting solid (40 mg, 0.11 mmol) was dissolved in anhydrous methanol (1.5 mL) along with sodium methoxide (10 mg, 0.21 mmol) and allyl bromide (39 mg, 0.32 mmol). The reaction was heated to 65 °C and stirred for 30 min. Additional sodium methoxide (20 mg, 0.42 mmol) and allyl bromide (78 mg, 0.64 mmol) were added in two equal portions over the course of 1 hour and then allowed to stir an additional hour at 65 °C. The mixture was then cooled and concentrated in vacuo. The resulting residue was partitioned between 2 M HCl and EtOAc. The aqueous layer was extracted with additional EtOAc, then the organic extracts were combined, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Purification by flash chromatogaphy (5–20% EtOAc/hex) provided methyl 3-allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxylate **25** (20 mg, 0.05 mmol, 30% yield over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.14–8.16 (m, 1H), 7.98–8.00 (m, 1H), 7.88 (s, 1H), 5.92–5.97 (m, 1H), 5.28–5.33(m, 2H), 3.82 (s, 3H), 3.78- 3.82 (t, 2H), 3.55–3.56 (m, 2H), 3.44 (s, 3H), 2.86 (s, 2H),

1.46 (s, 6H). ESI+MS $m/z$ = 415.1 (M+H $^{+}$). HPLC (Method A, $t_R$ = 9.06 min), purity = 95%.

#### 5.2.80. 3-Allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxylic acid (26)

To a solution of sodium hydroxide (3.1 mg, 0.055 mmol) in methanol (0.5 mL) was added methyl 3-allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxylate **25** (20 mg, 0.06 mmol). The resulting mixture was stirred overnight at room temperature, then concentrated in vacuo and partitioned between 2 M HCl and EtOAc. The aqueous phase was extracted with additional EtOAc, then the organic extracts were combined and washed with brine. The organic layer was isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo to a white powder (19 mg, 0.05 mmol, 86% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.18–8.19 (m, 1H), 8.01–8.05 (m, 1H), 7.95 (br s, 1H), 6.20–6.23 (m, 1H), 5.89–5.94 (m, 1H), 5.26–5.31 (m, 2H), 4.58–4.63 (m, 2H), 3.72–3.78 (m, 4H), 3.49–3.52 (m, 2H), 3.42 (s, 3H), 2.94–2.95 (m, 2H), 2.80 (s, 2H), 1.37 (s, 6H). ESI+MS $m/z$ = 400.49 (M+H$^+$). HPLC (Method A, $t_R$ = 7.5 min), purity = 95%.

#### 5.2.81. 3-Allyl-*N*-benzyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxamide (27a)

To a solution of 3-allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxylic acid **26** (100 mg, 0.25 mmol), EDC (60 mg, 0.30 mmol), and HOBt (50 mg, 0.30 mmol) in dry THF (10 mL) was added benzylamine (30 mg, 0.30 mmol). The reaction was allowed to stir at room temperature for 16 h, then diluted with diethyl ether and water. The organic layer was washed with saturated $Na_2CO_3$ solution, 2 M HCl, water, and brine, then isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (5–20% EtOAc/hex) delivered the desired compound (35 mg, 29% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.12 (d, *J* = *8Hz*, 1H), 7.70 (d,, *J* = *8Hz,* 1H), 7.62 (br s, 1H), 7.32–7.38 (m, 4H), 6.39–4.41 (m, 1H), 5.89–5.93 (m, 1H), 5.26–5.31 (m, 2H), 4.67–4.73 (4H, M), 3.75 (t, *J* = *4Hz,* 2H), 3.53 (t, *J* = *4Hz,* 2H), 3.42 (s, 3H), 2.82 (s, 2H), 1.56 (s, 6H). ESI+MS $m/z$ = 490.3 (M+H$^+$). HPLC (Method A, $t_R$ = 8.33 min), purity = 98.6%.

#### 5.2.82. *N*-(2-(1*H*-imidazol-4-yl)ethyl)-3-allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxamide (27b)

To a solution of 3-allyl-2-((2-methoxyethyl)thio)-5,5-dimethyl-4-oxo-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-8-carboxylic acid **26** (50 mg, 0.13 mmol), EDC (30 mg, 0.15 mmol), and HOBt (20 mg, 0.15 mmol) in dry THF (5 mL) was added 2-(1*H*-imidazol-4-yl)ethanamine hydrochloride (20 mg, 0.15 mmol). The reaction was allowed to stir at room temperature for 16 h, then diluted with diethyl ether and water. The organic layer was washed with saturated $Na_2CO_3$ solution, water, and brine, then isolated, dried over $MgSO_4$, vacuum filtered, and concentrated in vacuo. Further purification via flash chromatography (5–30% EtOAc/hex) delivered the desired compound (13 mg, 21% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) 8.11 (d, *J* = *8Hz*, 1H), 7.73 (d, *J* = *8Hz,* 1H), 7.64 (br s, 2H), 7.22–7.24 (br s, 1H), 7.01 (br s, 1H), 5.87–5.95 (m, 1H), 5.26–5.28 (m, 2H), 4.69–4.70 (2H, M), 4.60–4.61 (2H, m), 3.73 (t, *J* = *4Hz,* 2H), 3.2 (t, *J* = *4Hz,* 2H), 3.41 (s, 3H), 2.82 (s, 2H), 1.38 (s, 6H). (ESI+MS $m/z$ = 494.2 (M+H$^+$). HPLC (Method A, $t_R$ = 5.29 min), purity = 93%.


## Acknowledgements

This work was supported by NIH Grant P01HL573461 to HS, a University of Michigan Life Sciences Institute Innovation Partnership grant to HS and SDL, and NIH Pharmacological Sciences Training Program fellowships (Grant T32 GM007767) for BDY and MEB. We thank Dr. David Ginsburg for his advice regarding optimization of the SK activity assay.


## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.01.046.

## References and notes


1. Carapetis, J. R.; Steer, A. C.; Mulholland, E. K.; Weber, M. *Lancet Infect. Dis.* **2005**, *5*, 685.
2. World Health Organization. The current evidence for the burden of group A streptococcal diseases World Health Organization Discussion Papers on Child, Health, 2005.
3. O'Loughlin, R. E.; Roberson, A.; Cieslak, P. R.; Lynfield, R.; Gershman, K.; Craig, A.; Albanese, B. A.; Farley, M. M.; Barrett, N. L.; Spina, N. L.; Beall, B.; Harrison, L. H.; Reingold, A.; Beneden, C. V.; Team, A. B. C. S. *Clin. Infect. Dis.* **2007**, *45*, 853.
4. D'Agata, E. M. *Infect. Control Hosp. Epidemiol.* **2004**, *25*, 842.
5. Archibald, L.; Phillips, L.; Monnet, D.; McGowan, J. E., Jr.; Tenover, F.; Gaynes, R. *Clin. Infect. Dis.* **1997**, *24*, 211.
6. Rasko, D. A.; Sperandio, V. *Nat. Rev. Drug Disc.* **2010**, *9*, 117.
7. Karginov, V. A.; Nestorovich, E. M.; Yohannes, A.; Robinson, T. M.; Fahmi, N. E.; Schmidtmann, F.; Hecht, S. M.; Bezrukov, S. M. *Antimicrob. Agents Chemother.* **2006**, *50*, 3740.
8. Chorell, E.; Pinkner, J. S.; Bengtsson, C.; Banchelin, T. S. L.; Edvinsson, S.; Linusson, A.; Hultgren, S. J.; Almqvist, F. *Bioorg. Med. Chem.* **2012**, *20*, 3128.
9. Borlee, B. R.; Geske, G. D.; Blackwell, H. E.; Handelsman, J. *Appl. Environ. Microb.* **2010**, *76*, 8255.
10. Marsden, D. M.; Nicholson, R. L.; Skindersoe, M. E.; Galloway, W. R. J. D.; Sore, H. F.; Givskov, M.; Salmond, G. P. C.; Ladlow, M.; Welchd, M.; Spring, D. R. *Org. Biomol. Chem.* **2010**, *8*, 5313.
11. Rasko, D. A.; Moreira, C. G.; Li, D. R.; Reading, N. C.; Ritchie, J. M.; Waldor, M. K.; Williams, N.; Taussig, R.; Wei, S. G.; Roth, M.; Hughes, D. T.; Huntley, J. F.; Fina, M. W.; Falck, J. R.; Sperandio, V. *Science* **2008**, *321*, 1078.
12. Moayeri, M.; Robinson, T. A.; Leppla, S. H.; Karginov, V. A. *Antimicrob. Agents Chemother.* **2008**, *52*, 2239.
13. Wu, H.; Song, Z.; Hentzer, M.; Andersen, J. B.; Molin, S.; Givskov, M.; Hoiby, N. *J. Antimicrob. Chemother.* **2004**, *53*, 1054.
14. Sun, H.; Ringdahl, U.; Homeister, J. W.; Fay, W. P.; Engleberg, N. C.; Yang, A. Y.; Rozek, L. S.; Wang, X.; Sjobring, U.; Ginsburg, D. *Science* **2004**, *305*, 1283.
15. Sun, H.; Xu, Y.; Sitkiewicz, I.; Ma, Y.; Wang, X.; Yestrepsky, B. D.; Huang, Y.; Lapadatescu, M. C.; Larsen, M. J.; Larsen, S. D.; Musser, J. M.; Ginsburg, D. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 3469.
16. Markosyan, A. I.; Kuroyan, R. A.; Lilanyan, S. V. *Khim. Geterotsikl. Soedin.* **1998**, *6*, 820.
17. Markosyan, A. I.; Kuroyan, R. A.; Karapetyan, K. V. *Khim. Geterotsikl. Soedin.* **1999**, *12*, 1655.
18. Boehm, T. L.; Christie, L. C.; Devadas, B.; Madsen, H. M.; Marrufo, L.; Selness, S. Patent WO2006040666, Apr 20, 2006.
19. Chong, J. A.; Abdesaken, F.; Wu, C. C. US Patent 5965,766, Oct 12, 1999.
20. Kobayashi, K.; Uneda, T.; Takada, K.; Tanaka, H.; Kitamura, T.; Morikawa, O.; Konishi, H. *J. Org. Chem.* **1997**, *62*, 664.
21. Peifer, C.; Abadleh, M.; Bischof, J.; Hauser, D.; Schattel, V.; Hirner, H.; Knippschild, U.; Laufer, S. *J. Med. Chem.* **2009**, *52*, 7618.
22. Markosyan, A. I.; Kuroyan, R. A.; Dilanyan, S. V.; Aleksanyan, M. S.; Karapetyan, A. A.; Struchkov, Y. T. *Khim. Geterotsikl. Soedin.* **2000**, *5*, 658.
23. Craig, B. A. *Diagn. Micr. Infec. Dis.* **2000**, *36*, 193.
24. Ghaemmaghami, S.; Huh, W.; Bower, K.; Howson, R. W.; Belle, A.; Dephoure, N.; O'Shea, E. K.; Weissman, J. S. *Nature* **2003**, *425*, 737.
25. Lipinski, C. A. *J. Pharmacol. Toxicol.* **2000**, *44*, 235.
26. Anwar, H.; Strap, J. L.; Costerton, J. W. *Antimicrob. Agents Chemother.* **1992**, *36*, 1347.
27. Christensen, G. D.; Simpson, W. A.; Younger, J. J.; Baddour, L. M.; Barrett, F. F.; Melton, D. M.; Beachey, E. H. *J. Clin. Microbiol.* **1985**, *22*, 996.
28. Ma, Y.; Xu, Y.; Yestrepsky, B. D.; Sorenson, R. J.; Chen, M.; Larsen, S. D.; Sun, H. *PLoS ONE* **2012**, *7*, e47225.
29. Musser, J. M.; Hauser, A. R.; Kim, M. H.; Schlievert, P. M.; Nelson, K.; Selander, R. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2668.
30. Engleberg, N. C.; Heath, A.; Miller, A.; Rivera, C.; DiRita, V. J. *J. Infect. Dis.* **2001**, *183*, 1043.
31. Khil, J.; Im, M.; Heath, A.; Ringdahl, U.; Mundada, L.; Engleberg, N. C.; Fay, W. P. *J. Infect. Dis.* **2003**, *188*, 497.