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Structural Optimization of a Retrograde Trafficking Inhibitor that Protects Cells from Infections by Human Polyoma- and Papillomaviruses

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Abstract

Human polyoma- and papillomaviruses are non-enveloped DNA viruses that cause severe pathologies and mortalities. Under circumstances of immunosuppression, JC polyomavirus causes a fatal demyelinating disease called progressive multifocal leukoencephalopathy (PML) and the BK polyomavirus is the etiological agent of polyomavirus-induced nephropathy and hemorrhagic cystitis. Human papillomavirus type 16, another non-enveloped DNA virus, is associated with the development of cancers in tissues like the uterine cervix and oropharynx. Currently, there are no approved drugs or vaccines to treat or prevent polyomavirus infections. We recently discovered that the small molecule Retro-2^{cycl}, an inhibitor of host retrograde trafficking, blocked infection by several human and monkey polyomaviruses. Here, we report diversity-oriented syntheses of Retro-2^{cycl} and evaluation of the resulting analogs using an assay of human cell infections by JC polyomavirus. We defined structure-activity relationships and also discovered analogs with significantly improved potency as suppressors of human polyoma- and papillomavirus infection *in vitro*. Our findings represent an advance in the development of drug candidates that can broadly protect humans from non-enveloped DNA viruses and toxins that exploit retrograde trafficking as a means for cell entry.

Keywords

Polyomavirus; Papillomavirus; Retrograde Trafficking; Dihydroquinazolinone; SAR

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1. Introduction

Polyomaviruses are small, non-enveloped, viruses with an icosahedral capsid that contains a double-stranded DNA genome.¹ These viruses have established latent infections in the vast majority of the human population.^{1,2} Primary infection often occurs early in life, and it is estimated that as much as 90% of the adult population is seropositive for BK polyomavirus (BKPyV) and as much as 40% of adults are seropositive for JC polyomavirus (JCPyV).² Polyomaviruses establish an asymptomatic infection in humans, with polyomavirus-associated disease seen only in the context of immunosuppression, such as in AIDS patients or during immunomodulatory therapy. During circumstances of reduced immune function, increased replication and dissemination of JCPyV can lead to the development of the neurodegenerative disease progressive multifocal leukoencephalopathy (PML), which affects 3–5% of AIDS patients.^{3,4} BKPyV-associated disease is most often observed during immunomodulatory therapy, and can lead to the development of hemorrhagic cystitis and polyomavirus-associated nephropathy in as many as 10% of transplant patients.^{1,5} Human papillomaviruses (HPVs) are also non-enveloped, DNA viruses.⁶ HPV infection and replication is limited to the squamous epithelial tissue⁷ and is believed to be associated with as much as 5% of all cancers, most notable of which are cancer of the cervix, other anogenital tissue, and the oropharynx.^{6,8} Prophylactic vaccination against certain types of HPV has been successful. However, HPV related diseases will remain a significant human health problem for at least several decades for individuals who refuse vaccination or who become infected before being vaccinated. Currently, there are no approved small-molecule therapeutics for the treatment or prevention of PyV and HPV infection. Therapeutic strategies that help to manage the spread of these viruses would have a significant benefit to the health community.

The development of antiviral agents is often guided by consideration of viral life cycles. Many non-enveloped viruses, including PyV and HPV, are unable to access the host cytoplasm directly from the cell surface or from endosomes after endocytosis.^{7,9,10} They therefore tend to exploit host vesicular trafficking *en route* to the Golgi apparatus or the endoplasmic reticulum, from which they are released into the cytoplasm before reaching the nucleus for replication.^{9–12} The movement of virus particles, macromolecules, and metabolites from the cell surface to the endoplasmic reticulum via the Golgi is known as retrograde trafficking. This phenomenon is an important intracellular transport mechanism wherein protein, lipids, and small molecules are transported from endosomes to the trans-Golgi network, Golgi-membranes.¹² Retrograde trafficking is the primary mechanism for recycling chaperones, receptors, and other cargo molecules that are targeted to the cell membrane from the Golgi. In principle, small molecule modulation of host intracellular trafficking could serve as a useful strategy for the prevention of infections by non-enveloped viruses. Indeed, we recently reported that Retro-2^{cycl}, a dihydroquinazolinone (DHQZ) inhibitor of retrograde trafficking^{13,14}, blocked the infection of cells by human and monkey polyomaviruses¹⁵ as well as by human papillomaviruses.¹⁶

Retro-2^{cycl} apparently blocks toxin and viral retrograde transport without significantly affecting endogenous trafficking.^{13–16} However, the specific host cellular factor that is targeted by Retro-2^{cycl} is not yet known. Here, we have used a diversity-oriented synthetic

strategy to prepare DHQZs that are structurally related to Retro-2^{cycl}. The capacities of the compounds to inhibit the infection of human cells by JCPyV were assessed systematically. The experiments revealed critical structure activity-relationships (SAR) and led to the discovery of Retro-2^{cycl} analogs with enhanced capacities to prevent infections both JCPyV and HPV infections.

2. Results

2.1. Structure-Activity Relationship (SAR) Analysis of the Dihydroquinazolinone Retro-2^{cycl}

The suppression of virus infection by Retro-2^{cycl} encouraged us to pursue a SAR analysis to define critical structural elements for bioactivity. We anticipated that such an analysis would also yield compounds with superior potency as retrograde trafficking inhibitors. For this analysis, we divided the Retro-2^{cycl} structure into three distinct elements (Scheme 1)- a heterocycle moiety, an amide moiety, and a benzo moiety. We therefore carried out compound diversification in consecutive phases wherein one moiety was varied independently of the other two. After each phase, the most active compound served as a new lead structure for the subsequent diversification phase. The activities of the compounds prepared in each stage were systematically assessed in assays wherein human HeLaM cells were infected with JCPyV.

Two routes were used for the diversity-oriented synthesis of DHQZs (Scheme 1). In one route, primary amines were coupled to 2-nitrobenzoic acid (**1**) using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine; subsequently, the nitro group was reduced by transfer hydrogenation with ammonium formate and 10% palladium on carbon in methanol to afford anthranilamides (**2**). The anthranilamide intermediates were condensed with aromatic aldehydes in the presence of scandium (III) triflate under microwave irradiation to afford the desired DHQZs. Alternatively, DHQZs were prepared in a one-pot, tandem reaction sequence comprised of the decarboxylative condensation of an isatoic anhydride (**3**) and primary amines in THF, followed by the scandium (III) triflate-catalyzed condensation of the resulting amides with aromatic aldehydes. Preparation of DHQZs from isatoic anhydrides (**3**) was the preferred route. However, in certain instances, the availability of starting materials or the weak nucleophilicity of primary amines (like anilines) necessitated preparation of DHQZs from 2-nitrobenzoic acids (**1**).

The first moiety to be analyzed was the heterocycle substituent of the dihydroquinazolinone aminal carbon (Table 1). In particular, three structural aspects of the heterocycle moiety were investigated: the identity of the heteroatom, the ring substitution pattern, and the identity of the ring substituent. Compounds **4-6**, which are substituted with an unsubstituted thiophene, a pyrrole, or a furan, all exhibited significantly attenuated anti-JCPyV activity relative to Retro-2^{cycl}. Interestingly, compound **7**, bearing a 5-methylfuran moiety, had similar activity to that of Retro-2^{cycl}, which itself bears a 5-methylthiophene moiety. Although the 5-methylthiophene moiety confers greater potency, it is apparent that a 5-methylfuran moiety can also be tolerated. The pattern and identity of the thiophene substituent also proved to be significant with respect to bioactivity. Compound **8**, bearing a methyl group at the thiophene 4 position, had nearly equal activity to that of Retro-2^{cycl}. On the other hand, the activity of compound **9**, bearing a methyl group at the 3 position of the

thiophene, was attenuated to the same extent as Retro-2^{cycl} analogs with unsubstituted heterocycle moieties. An analog that had a 5-ethyl thiophene (compound **10**) in place of the parent compound's 5-methyl thiophene had markedly improved potency. Interestingly, activity is severely compromised when the heterocycle is a benzothiophene ring (compound **11**). Therefore, the 5-ethylthiophene moiety was held constant throughout the remainder of the SAR analysis.

We next turned our attention to the amide moiety (Table 2) and the effects of various aliphatic and aromatic amide groups on JCPyV infectivity. Most of the compounds with substituents on the amide nitrogen had activities that were similar those of compound **10** and compound **12**, which has only a hydrogen substituent. Dramatic improvements in activity were observed in compounds wherein the phenyl group of Retro2^{cycl} was replaced by either benzyl (compound **17**) or methylnaphthyl (compound **19**) groups. In the context of SAR analysis, our finding that the incorporation of a benzyl group onto the amide moiety improved potency was fortuitous because many substituted benzylamines are commercially available. Using readily available building blocks, we were able to prepare many DHQZs with structurally diverse benzyl substituents on the amide moiety. We investigated the effects of ring substituents, including methoxy group(s) (compounds **20-23**), a fluorine atom (compounds **24-25**), and nitro groups (compounds **26-27**), on the bioactivity of the compounds. In most cases, the addition of substituents on to the benzyl group was tolerated, aromatic nitro groups being a particular exception. A compound with a *para*-fluorobenzylamide (compound **24**) was more potent than one with an unsubstituted benzyl group at the same position (compound **17**).

In DHQZ syntheses that use achiral building blocks, the ring-forming reaction yields racemic products. In two cases, chiral α -methylbenzylamines (compounds **28-29**) were used to generate the amide moieties with the hopes of achieving stereochemical induction at the DHQZ aminal stereocenter.¹⁷ The DHQZ-forming reactions of substrates prepared from chiral α -methylbenzylamines yielded configurationally stable products as single diastereomers. The compounds with chiral α -methylbenzylamide groups were more than four-fold less active than the compound with the unsubstituted benzylamide (compound **17**). Interestingly, the DHQZ analog with the (*R*)- α -methylbenzylamide was much less active than its diastereomer derived from (*S*)- α -methylbenzylamine.

In the third phase of SAR analysis, the benzo moiety was varied while the heterocycle and amide moieties were held constant as a 5-ethylthiophene group and a 4-fluorobenzyl group, respectively (Table 3). We first examined the effect of methylation at each of the four otherwise unsubstituted aromatic carbons of the benzo moiety. Methyl groups were tolerated at C-5 (**30**) and C-6 (**31**). However, compounds with methyl substituents at C-7 (**32**) and C-8 (**33**) had severely attenuated activity. A chlorine atom could likewise be tolerated at C-6 (**34**), but not a C-7 (**35**). Interestingly, compounds with fluorine atoms at either C-6 (**36**) or C-7 exhibited potent bioactivity (**37**). Although the former was more active, the finding that a compounds with a fluorine, and not a methyl or chlorosubstituent, at C-7 is tolerated suggests that there substituent size at this atom of the benzo moiety is a critical factor for binding to the cellular target.

Since the SAR analysis of the benzo moiety suggested that C-5 and C-6 can be structurally elaborated without loss in potency, we sought to explore this possibility. We envisioned that aromatic substitution or cross-coupling reactions of the halogenated species (compounds **34-37**) could enable elaboration of the dihydroquinazolinone substructure into new chemical space. As a proof of principle, *n*-butylamino analog **40** was prepared (Scheme 2). A S_NAr reaction of 5-fluoro-2-nitroamide **38** with *n*-butylamine provided 5-(*n*-butylamino)-2-nitroamide **39**. Reduction of the 2-nitro group followed by condensation/cyclization with 5-ethyl-2-thiophenecarboxaldehyde provided DHQZ **40**. Unfortunately, incorporation of the *n*-butylamino group at C-6 of the benzo moiety resulted in a severe attenuation of activity (*i.e.*, infectivity at 25 μ M: 71.0 ± 8.28 % DMSO Control).

Two final analogs were synthesized in order to probe SAR around the DHQZ aromatic amine (Scheme 3). First, we examined the significance of the heterocycle's oxidation state by preparing and evaluating the quinazolinone form of compound **24**. The quinazolinone species (**41**) has a more planar geometry than the corresponding DHQZ (**24**). Also, **41** lacks the *N*-1 amino hydrogen, a potentially important hydrogen bond donor. Interestingly, quinazolinone **41** was much less active than DHQZ **24**. We also prepared an *N*-methyl-DHQZ (**43**) and found that this compound was similarly less active than DHQZ **24**. From these observations, one could predict that the *N*-1 amine is acting as hydrogen bond donor in the molecule's interaction with its cellular target.

The most potent Retro-2^{cycl} analog discovered in this study was DHQZ **36**. In order to determine the fold improvement in potency of our optimized compound over Retro-2^{cycl}, dose response curves were generated for both compounds against JCPyV and HPV16 pseudovirus, from which IC₅₀ for inhibition of infectivity were determined (Figure 1). While JCPyV appeared to be more susceptible to the DHQZs, there was an overall 6.7 fold improvement in potency from Retro-2^{cycl} to our optimized compound, DHQZ **36** in the case of both viruses. This is consistent with the compounds targeting a *host* cellular factor as opposed to viral proteins.

2.2. Optimized dihydroquinazolinone inhibitor interferes with retrograde trafficking

In order to confirm that the optimized DHQZ (**36**) inhibited retrograde trafficking, we conducted a proximity ligation assay, which detects co-localization between the viral capsid proteins and localized host cell marker proteins (Figure 2). Marker proteins were chosen from known intracellular sites where the virions are delivered immediately downstream from their trafficking through the retrograde pathway. For JCPyV, we immunostained for the viral capsid protein, VP1, and the ER protein, PDI (protein disulfide isomerase) in SVGA cells. For HPV16, we immunostained for viral capsid protein, L1, and Golgi protein, TGN46, in HeLaM cells. In the proximity ligation assay, co-localization is indicated by a fluorescent signal, which is detected only when the target proteins are within 40 nm of each other. Cells were pretreated with Retro-2^{cycl}, DHQZ **36**, or DMSO then inoculated with virus. Cells were fixed and immunostained after an 8 hour infection period for JCPyV or a 16 hour period for HPV16. In DMSOtreated cells, there was strong co-localization signal for both JCPyV and HPV16. In Retro-2^{cycl} and DHQZ **36** treated cells there is a clear reduction in co-localization signal for both JCPyV and HPV16. Furthermore, in the case JCPyV,

DHQZ **36** appears to inhibit co-localization more potently than Retro-2^{cycl}. This trend is less clear in the case of HPV16. In any case, we can conclude that the optimized DHQZ **36** inhibits retrograde transport in a similar manner as Retro-2^{cycl}.

3. Discussion

Most anti-infective therapeutic strategies are based on targeting the pathogen with a small molecule that critically perturbs its physiology. Since some pathogens use the host cell machinery for infection or for delivery of toxins and other virulence factors, one could also envision therapeutic agents that subtly perturb a host's physiology in ways that make it unsusceptible to pathogens and/or their toxins. This phenomenon is compellingly illustrated by the dihydroquinazolinone inhibitors of retrograde trafficking described here and elsewhere.^{13–16} Remarkably, one of these molecules can protect cells from polyomaviruses, papillomaviruses, ricin, and Shiga-like toxins, due to their shared reliance on intracellular trafficking.^{13,16} We suspect that the lethality of other pathogens and toxins could be suppressed by DHQZ retrograde trafficking inhibitors as well. Beyond their potentially broad applications in medicine, compounds that target the host cellular machinery rather than the pathogen may have the added advantage of being less prone to resistance-conferring mutations in the pathogen.

Herein, we define structure-activity relationships associated with DHQZ inhibitors of retrograde trafficking. Through our investigations, we have identified new analogs of Retro-2^{cycl} with significantly improved potency. We employed a whole-cell based polyomavirus infectivity assay to assess the efficacies of the compounds as inhibitors of retrograde trafficking. The strength of this assay is that it precludes the use of deadly toxins (*e.g.*, ricin, Shiga toxin) or cancer-causing viruses (*e.g.*, HPV). Since the non-enveloped viruses, ricin, and the Shiga toxins exploit the same retrograde trafficking mechanism, we believe that the compounds identified in this assay will have broad utility. This is evidenced by the fact that a small molecule optimized to inhibit polyomavirus infection exhibited similarly improved activity against human papillomavirus as compared to Retro-2^{cycl}. The drawbacks of our PyV infectivity assay are its requirement of multiple replicates for reliability and its incompatibility with high-throughput screening technologies. Currently, we are working to address these shortcomings.

The key DHQZ structural features that led to improvement in activity were the incorporation of a 5-ethylthiophene as the heterocycle moiety, incorporation of a *p*-fluorobenzyl group as the amide moiety, and addition of a fluorine atom onto C-6 of the benzo moiety. During the late stages of our experiments, two Retro-2^{cycl} SAR papers focused on inhibition of Shiga-like toxin trafficking were published by Cintrat and co-workers.^{20,21} While our optimized DHQZ **36** differs in structure from the optimized compounds reported by Cintrat, many of our general structure activity relationships are in agreement. The combined SAR data sets suggest additional analogs that may surpass the potency of both our own and Cintrat's optimized inhibitors. While our findings concerning dihydroquinazolinone SAR analyses largely agree with those that have been published, the only point of incongruence is the consequences of methylation of the dihydroquinazolinone *N*-1 amino nitrogen. We observed a severe attenuation of activity resulting from methylation of the DHQZ *N*-1 amino nitrogen,

while the Cintrat and co-workers reported that *N*-methyldihydroquinazolinones are highly potent inhibitors of ricin intoxication. The only issue addressed by Cintrat and co-workers in their studies²¹ that cannot be addressed in our work concerns the configuration of the stereocenter in the bioactive dihydroquinazolinone structure. After chiral separation, they were able to evaluate the two DHQZ enantiomers and found that the (*S*)-*N*-methyldihydroquinazolinones were significantly more active than the corresponding (*R*)-*N*-methyldihydroquinazolinones.²¹ We are unable to address the issue of configuration with DHQZ **36** because the *N*-des-methyl-DHQZs are known to be configurationally unstable.²²

While there are obvious opportunities for additional structural optimization, identification of the cellular target of the retrograde trafficking inhibitors is the next crucial step. The SAR studies described in this report, as well as by Cintrat and co-workers, will guide the design of affinity reagents that can be used to identify of the sub-cellular target of the DHQZ retrograde trafficking inhibitors. The preparation and use of these affinity reagents is currently underway in these laboratories.

4. Experimental Procedures

4.1. Cells, Viruses, Plasmids, and Antibodies

SVG-A cells were maintained in MEM complete media (minimal essential media containing 10% fetal bovine serum, 1% penicillin, 1% streptomycin) and have been previously described.²³ HeLaM cells were maintained in DMEM complete media (Dulbecco's modification of minimal essential media containing 10% fetal bovine serum, 1% penicillin, 1% streptomycin, 10mM Hepes pH 7.5). HeLaM cells, a subclone of HeLaS3 cells, were a kind gift from Walther Mothes (Yale University). The Mad1-SVE strain of JCPyV used in these experiments has previously been described.²⁴ Stocks of JCPyV were generated according to previously published studies²⁵. HPV16 pseudovirus expressing the reporter HcRed was generated from the p16sheLL plasmid, which expresses HPV L1 and L2, and pCAG-HcRed were obtained from Addgene. HPV pseudovirus stocks were generated according to previously published methods.¹⁶ The antibodies to PDI, SV40 VP1, and TGN46 were purchased from Abcam. Polyclonal sera to HPV L1 was a kind gift from Pat Day (National Institute of Health). The PAB597 anti-VP1 hybridoma was a kind gift from Ed Harlow.

4.2. Inhibition of JCPyV infection by Retro-2^{cycl} analogs

SVG-A cells were seeded in 12 well plates at a density of 1×10^5 cells per well and incubated overnight at 37°C. Cells were then pre-incubated for 0.5 h with 25 μ M of Retro-2^{cycl}, the indicated Retro-2^{cycl} analog, or the vehicle control. The final DMSO concentration was 0.04%. JCPyV was then added at an MOI of 0.25 and allowed to infect in the presence of the indicated compound for 72 h. Samples were then processed for flow cytometry. An infected culture that was not treated with (~20% total infected cells) was then normalized to 100% and any reduction in infection in the DHQZ treated samples were compared to this untreated control. Three independent experiments were performed and used to calculate standard deviations.

For the IC₅₀ analysis, 1:2 serial dilutions of a 2500x stock of Retro-2^{cycl} or DHQZ **36** were performed in DMSO. These dilutions were then added to complete media, such that the media contained a 1x concentration of the indicated concentration of compound in a final DMSO concentration of 0.04%. Samples were pretreated with the indicated compound and challenged with JCPyV or HPV16 as above. Three independent experiments were performed and used to calculate standard deviations.

4.3. Flow cytometric scoring of viral infection

JCPyV-infected SVG-A cells were detached from 12-well plates by aspirating the growth media, washing once with phosphate buffered saline (PBS), and detaching with Trypsin-EDTA. These cells were then transferred to flow cytometry tubes and pelleted by centrifugation at 600 × g for 5 min, washed with PBS and fixed in 0.5 mL 4% paraformaldehyde (PFA) for 10 min. Samples were pelleted and washed with PBS, and permeabilized with 0.5 mL PBS containing 1% Triton X-100 for 10 min at 21°C. Cells were then pelleted and resuspended in 0.1 mL PBS containing 3% BSA and an Alexa Fluor-labeled monoclonal antibody to VP1 (PAB 597-AF488). After incubation for 1 h at 21°C, cells were washed once with PBS and infected cells were scored by flow cytometry (BD FACSCalibur).

HPV16-infected HeLaM cells were detached from 12-well plates by aspirating the growth media, washing once with phosphate buffered saline (PBS), and detaching with Trypsin-EDTA. These cells were then transferred to flow cytometry tubes and pelleted by centrifugation at 600 × g for 5 min, washed with PBS and fixed in 0.5 mL 4% paraformaldehyde (PFA) for 10 min. Infected cells were then scored flow cytometry for HcRed expression.

4.4. Proximity ligation assays

Colocalization experiments between virus and ER or Golgi markers was performed using proximity ligation assays, as previously described (Bethyl Labs) (24222489, 23569269). The primary antibodies used for PLA were rabbit anti-VP1 and mouse anti-PDI in the case of JCPyV, and rabbit anti-L1 and mouse anti-TGN46 in the case of HPV16. Cells were pretreated with Retro-2^{cycl}, DHQZ36, or vehicle control for 0.5 h. SVGA cells (JCPyV) or HeLaM cells (HPV16) were then inoculated with virus at an MOI of 100 for 1 h at 37°C. Virus was removed by aspiration and any unbound virus was removed by washing with media. Fresh media was added containing either 0.1 mM Retro-2^{cycl} or 0.05 mM DHQZ **36**, or a vehicle control. Cells were then incubated at 37°C for 8 h (JCPyV) or 16 h (HPV) prior to fixation with 4% PFA. Cells were permeabilized with PBS containing 0.5% Triton X-100 for 0.5 h, washed three times in PBS, then blocked in 5% Donkey Serum for 1 h at 37°C. For JCPyV, cells were then stained for VP1 (1:1,000 dilution) and PDI (1:100 dilution) by overnight incubation at 4°C. For HPV16, cells were stained with L1 (1:1,000 dilution) and TGN46 (1:200 dilution) by incubation for 2h at 37°C. These cells were then immunostained using the proximity ligation assay, following manufacturer's instructions. Cells were washed and the cell nuclei were counterstained using DAPI. Fluorescence micrographs were collected by confocal microscopy and maximal z-projections were displayed.

4.5. Chemical synthesis-general

All commercially available reagents were used without further purification. Reactions were carried out in oven dried glassware, with dry solvent, and under ambient atmosphere. All spectra were referenced to residual solvent signals in DMSO-d₆ (2.50 ppm for ¹H, 39.51 ppm for ¹³C).

4.6. Nitrobenzamide synthesis general procedure

Dicyclohexylcarbodiimide (5.00 mmol) and a nitrobenzoic acid (5.55 mmol) were dissolved in DCM (20 mL) and allowed to stir for 5 minutes before the addition of a primary amine (5.55 mmol) and dimethylaminopyridine (0.055 mmol). The coupling reaction was allowed to proceed for 16 hours, after which the DCM was removed *in vacuo*. The solid residue was resuspended in ethyl acetate and filtered through a silica gel plug to remove the dicyclohexylurea byproduct. The filtrate was then concentrated and the desired nitrobenzamide isolated by silica gel flash chromatography using a hexanes/ethyl acetate solvent gradient.

4.6.1. *N*-(4-fluorobenzyl)-2-methyl-6-nitrobenzamide—From 2-nitrobenzoic acid, Yield 1.15g, 95%. HRMS (FAB) [C₁₃H₁₅N₂O₃ + Na]⁺ Predicted: 265.0589 Found: 265.0589. ¹H NMR (400MHz, DMSO-d₆) δ = 10.65 (s, 1 H), 8.15 (d, *J* = 8.3 Hz, 1 H), 7.91 - 7.83 (m, 1 H), 7.80 - 7.73 (m, 2 H), 7.67 (d, *J* = 8.0 Hz, 2 H), 7.36 (t, *J* = 8.0 Hz, 2 H), 7.16 - 7.09 (m, 1 H). ¹³C NMR (151MHz, DMSO-d₆) δ = 164.1, 146.4, 138.8, 134.1, 132.7, 130.9, 129.3, 128.8, 124.2, 123.9, 119.6.

4.6.2. 2-methyl-6-nitro-*N*-phenylbenzamide—(2 mmol scale) From 2-methyl-6-nitrobenzoic acid, Yield: 510 mg, 89%. HRMS (FAB) [C₁₅H₁₃FN₂O₃ + Na]⁺ Predicted: 311.0808, Found: 311.0816. ¹H NMR (400MHz, DMSO-d₆) δ = 9.15 (t, *J* = 5.9 Hz, 1 H), 7.96 (d, *J* = 8.1 Hz, 1 H), 7.51 - 7.46 (m, 1 H), 7.46 - 7.43 (m, 1 H), 7.40 (dd, *J* = 5.7, 8.5 Hz, 2 H), 7.22 - 7.14 (m, 2 H), 4.43 (d, *J* = 5.8 Hz, 2 H), 2.43 (s, 3 H). ¹³C NMR (101MHz, DMSO-d₆) δ = 165.8, 162.5, 160.1, 144.8, 144.6, 135.1, 135.1, 132.7, 130.9, 129.5, 129.4, 129.3, 124.3, 115.2, 115.0, 41.9, 20.8.

4.6.3. *N*-(4-fluorobenzyl)-5-methyl-2-nitrobenzamide—(2 mmol scale) From 5-methyl-2-nitrobenzoic acid, Yield: 561 mg, 97%. HRMS (FAB) [C₁₅H₁₃FN₂O₃ + Na]⁺ Predicted: 311.0808, Found: 311.0820. ¹H NMR (400MHz, DMSO-d₆) δ = 9.16 (t, *J* = 5.8 Hz, 1 H), 7.97 (d, *J* = 8.3 Hz, 1 H), 7.49 (dd, *J* = 1.0, 8.3 Hz, 1 H), 7.46 - 7.43 (m, 1 H), 7.43 - 7.37 (m, 2 H), 7.23 - 7.14 (m, 2 H), 4.43 (d, *J* = 6.1 Hz, 2 H), 2.43 (s, 3 H). ¹³C NMR (101MHz, DMSO-d₆) δ = 165.8, 162.5, 160.1, 144.8, 144.6, 135.1, 135.1, 132.7, 130.9, 129.5, 129.4, 129.3, 124.3, 115.2, 115.0, 41.9, 20.8.

4.6.4. *N*-(4-fluorobenzyl)-4-methyl-2-nitrobenzamide—(2 mmol scale) From 4-methyl-2-nitrobenzoic acid, Yield: 413 mg, 72%. HRMS (FAB) [C₁₅H₁₃FN₂O₃ + Na]⁺ Predicted: 311.0808, Found: 311.0805. ¹H NMR (400MHz, DMSO-d₆) δ = 9.18 (t, *J* = 5.8 Hz, 1 H), 7.86 (s, 1 H), 7.67 - 7.57 (m, 1 H), 7.57 - 7.51 (m, 1 H), 7.39 (dd, *J* = 5.8, 8.3 Hz, 2 H), 7.18 (t, *J* = 8.8 Hz, 2 H), 4.42 (d, *J* = 5.8 Hz, 2 H), 2.43 (s, 3 H). ¹³C NMR (101MHz,

DMSO- d_6) δ = 165.5, 162.5, 160.1, 147.4, 141.4, 135.2, 133.8, 129.5, 128.9, 124.2, 115.2, 115.0, 41.9, 20.5.

4.6.5. *N*-(4-fluorobenzyl)-3-methyl-2-nitrobenzamide—(2 mmol scale) From 3-methyl-2-nitrobenzoic acid, Yield: 458 mg, 80%. HRMS (FAB) $[C_{15}H_{13}FN_2O_3 + Na]^+$ Predicted: 311.0808, Found: 311.0801. 1H NMR (400MHz, DMSO- d_6) δ = 9.33 (t, J = 5.7 Hz, 1 H), 7.59 (s, 3 H), 7.46 - 7.29 (m, 2 H), 7.26 - 7.05 (m, 2 H), 4.40 (d, J = 5.8 Hz, 2 H), 2.30 (s, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 164.6, 162.5, 160.1, 149.1, 135.2, 135.2, 133.7, 130.7, 130.3, 129.6, 129.2, 129.2, 126.3, 115.2, 115.0, 41.9, 16.8.

4.6.6. 5-fluoro-*N*-(4-fluorobenzyl)-2-nitrobenzamide (38)—(2 mmol scale) From 5-fluoro-2-nitrobenzoic acid, Yield: 477mg, 82%. HRMS (FAB) $[C_{14}H_{10}F_2N_2O_3 + Na]^+$ Predicted: 315.0557, Found: 311.0549. 1H NMR (400MHz, DMSO- d_6) δ = 9.25 (t, J = 5.4 Hz, 1 H), 8.18 (dd, J = 4.8, 8.6 Hz, 1 H), 7.67 - 7.49 (m, 2 H), 7.41 (dd, J = 5.6, 8.3 Hz, 2 H), 7.28 - 7.09 (m, 2 H), 4.44 (d, J = 5.8 Hz, 2 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 165.2, 164.3, 162.7, 162.6, 160.1, 143.2, 135.5, 135.4, 134.8, 134.8, 129.5, 129.4, 127.6, 127.5, 117.7, 117.4, 116.6, 116.4, 115.2, 115.0, 42.0.

4.6.7. 4-fluoro-*N*-(4-fluorobenzyl)-2-nitrobenzamide—(2 mmol scale) From 4-fluoro-2-nitrobenzoic acid, Yield: 330 mg, 57%. HRMS (FAB) $[C_{14}H_{10}F_2N_2O_3 + Na]^+$ Predicted: 315.0557, Found: 311.0565. 1H NMR (400MHz, DMSO- d_6) δ = 9.26 (t, J = 5.4 Hz, 1 H), 8.03 (dd, J = 2.3, 8.6 Hz, 1 H), 7.81 - 7.65 (m, 2 H), 7.39 (dd, J = 5.7, 8.2 Hz, 2 H), 7.27 - 7.10 (m, 2 H), 4.43 (d, J = 6.1 Hz, 2 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 164.6, 163.0, 162.5, 160.5, 160.1, 148.4, 148.3, 135.0, 135.0, 131.4, 131.3, 129.4, 129.3, 128.6, 128.6, 120.5, 120.3, 115.2, 115.0, 112.3, 112.0, 42.0.

4.7. Dihydroquinazolinone synthesis

4.7.1 General procedure A—The nitrobenzamide intermediate (0.5 mmol) was dissolved in methanol (2.5 mL). To the solution was added 10% Pd/C (75 mg) and ammonium formate (32 mg, 0.5 mmol). After 1 hour the reaction was filtered, rinsing with methanol. The filtrate was adjusted to a volume 1mL in methanol and then treated with an aldehyde (0.55 mmol) and scandium (III) triflate (0.05) mmol. The reaction was microwave irradiated at 100°C for 1 hour, after which the solvent was removed *in vacuo*. The product dihydroquinazolinones were isolated by silica gel flash chromatography with a hexanes/ethyl acetate solvent gradient. The chromatographed products were subsequently purified by recrystallization.

4.7.2 General procedure B—An isatoic anhydride (1.20 mmol) was added to THF (6 mL) and heated to 60°C. To the hot solution of isatoic anhydride was added a primary amine or aqueous ammonia (1.00 mmol), which was allowed to react for 1–2 hours. Once the amine had been completely consumed, an aldehyde (1.20 mmol) and scandium triflate (0.1 mmol) were added and allowed to react at 60°C for an additional 3–5 hours, after which the solvent was removed. The product dihydroquinazolinones were isolated by silica gel flash chromatography with a hexanes/ethyl acetate solvent gradient. The chromatographed products were subsequently purified by recrystallization.

4.7.3. 3-phenyl-2-(thiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (4)—Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (1 mmol scale), Yield: 181 mg, 59%. HRMS (FAB) $[C_{18}H_{14}N_2O_2 + Na]^+$ Predicted: 313.0953 Found: 313.0945. 1H NMR (400MHz, DMSO- d_6) δ = 7.72 (d, J = 7.3 Hz, 1 H), 7.62 (d, J = 3.0 Hz, 1 H), 7.58 (dd, J = 0.8, 1.9 Hz, 1 H), 7.43 - 7.35 (m, 2 H), 7.35 - 7.28 (m, 3 H), 7.28 - 7.23 (m, 1 H), 6.83 (d, J = 8.0 Hz, 1 H), 6.79 - 6.70 (m, J = 1.0, 7.5, 7.5 Hz, 1 H), 6.33 (dd, J = 1.9, 3.4 Hz, 1 H), 6.25 (t, J = 3.3 Hz, 2 H). ^{13}C NMR (75MHz, DMSO- d_6) δ = 161.8, 152.9, 146.5, 143.1, 140.6, 133.7, 128.7, 127.9, 126.3, 117.8, 115.5, 114.9, 110.4, 108.4, 67.3.

4.7.4. 3-phenyl-2-(1H-pyrrol-2-yl)-2,3-dihydroquinazolin-4(1H)-one (5)—Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (0.25 mmol scale), Yield: 29 mg, 40%. HRMS (FAB) $[C_{18}H_{15}N_3O + Na]^+$ Predicted: 312.1113 Found: 312.1118. 1H NMR (400MHz, DMSO- d_6) δ = 10.72 (br. s., 1 H), 7.72 (dd, J = 1.5, 7.8 Hz, 1 H), 7.34 - 7.26 (m, 3 H), 7.26 - 7.21 (m, 3 H), 7.21 - 7.15 (m, 1 H), 6.79 (dd, J = 0.5, 8.3 Hz, 1 H), 6.75 (ddd, J = 1.0, 7.1, 7.5 Hz, 1 H), 6.63 (dt, J = 1.5, 2.6 Hz, 1 H), 6.18 (d, J = 2.3 Hz, 1 H), 5.89 (t, J = 3.5 Hz, 1 H), 5.81 (q, J = 2.7 Hz, 1 H). ^{13}C NMR (75MHz, DMSO- d_6) δ = 162.4, 146.8, 140.7, 133.4, 130.0, 128.5, 127.9, 126.6, 126.1, 118.2, 117.8, 115.9, 115.1, 107.6, 107.1, 68.1.

4.7.5. 2-(furan-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (6)—Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (0.25 mmol scale), Yield: 61 mg, 84%. HRMS (FAB) $C_{18}H_{14}N_2O_2Na^+$ Predicted: 313.0953 Found: 313.0945. 1H NMR (400MHz, DMSO- d_6) δ = 7.72 (d, J = 7.3 Hz, 1 H), 7.62 (d, J = 3.0 Hz, 1 H), 7.58 (dd, J = 0.8, 1.9 Hz, 1 H), 7.43 - 7.35 (m, 2 H), 7.35 - 7.28 (m, 3 H), 7.28 - 7.23 (m, 1 H), 6.83 (d, J = 8.0 Hz, 1 H), 6.79 - 6.70 (m, J = 1.0, 7.5, 7.5 Hz, 1 H), 6.33 (dd, J = 1.9, 3.4 Hz, 1 H), 6.25 (t, J = 3.3 Hz, 2 H). ^{13}C NMR (75MHz, DMSO- d_6) δ = 161.8, 152.9, 146.5, 143.1, 140.6, 133.7, 128.7, 127.9, 126.3, 117.8, 115.5, 114.9, 110.4, 108.4, 67.3

4.7.6. 2-(5-methylfuran-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (7)—Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (0.25 mmol scale), Yield: 42 mg, 55%. HRMS (FAB) $C_{19}H_{16}N_2O_2Na^+$ Predicted: 327.1109 Found: 327.1125. 1H NMR (400MHz, DMSO- d_6) δ = 7.72 (dd, J = 1.0, 7.8 Hz, 1 H), 7.62 (d, J = 3.0 Hz, 1 H), 7.42 - 7.33 (m, 4 H), 7.30 (ddd, J = 1.8, 7.0, 8.3 Hz, 1 H), 7.24 (tt, J = 1.8, 7.3 Hz, 1 H), 6.83 (d, J = 8.0 Hz, 1 H), 6.78 - 6.72 (m, 1 H), 6.17 (d, J = 3.3 Hz, 1 H), 6.11 (d, J = 3.0 Hz, 1 H), 5.93 (dd, J = 1.0, 3.0 Hz, 1 H), 2.16 (s, 3 H). ^{13}C NMR (75MHz, DMSO- d_6) δ = 161.8, 151.7, 150.8, 146.4, 140.6, 133.6, 128.7, 127.9, 126.3, 117.7, 115.5, 115.0, 109.4, 106.5, 67.3, 13.3.

4.7.7. 2-(4-methylthiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (8)—Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (0.87 mmol scale), Yield: 145 mg, 52%. HRMS (FAB) $C_{19}H_{16}N_2OSNa^+$ Predicted: 343.0881, Found: 343.0872. 1H NMR (400MHz, DMSO- d_6) δ = 7.73 (dd, J = 1.5, 7.8 Hz, 1 H), 7.62 (d, J = 2.8 Hz, 1 H), 7.42 - 7.29 (m, 5 H), 7.27 - 7.22 (m, 1 H), 6.94 - 6.90 (m, 1 H), 6.82 (d, J = 8.0 Hz, 1 H), 6.80 - 6.74 (m, 2 H), 6.44 (d, J = 2.8 Hz, 1 H), 2.07 (d, J = 0.8 Hz, 3 H). ^{13}C NMR

(101MHz, DMSO- d_6) δ = 161.5, 146.3, 144.6, 140.5, 136.2, 133.8, 128.7, 128.3, 128.0, 126.3, 126.3, 120.9, 118.0, 115.6, 115.3, 69.4, 15.3.

4.7.8. 2-(3-methylthiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (9)

—Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (0.87 mmol scale), Yield: 119 mg, 43%. HRMS (FAB) $C_{19}H_{16}N_2OSNa^+$ Predicted: 343.0881, Found: 343.0885. 1H NMR (400MHz, DMSO- d_6) δ = 7.74 (dd, J = 1.5, 7.8 Hz, 1 H), 7.48 (d, J = 2.0 Hz, 1 H), 7.37 - 7.29 (m, 3 H), 7.25 - 7.17 (m, 4 H), 6.83 - 6.75 (m, 2 H), 6.67 (d, J = 5.0 Hz, 1 H), 6.54 (d, J = 2.0 Hz, 1 H), 1.91 (s, 3 H). ^{13}C NMR (75MHz, DMSO- d_6) δ = 161.8, 146.6, 140.2, 137.0, 135.1, 133.8, 129.5, 128.6, 127.9, 127.5, 126.7, 124.2, 117.8, 115.0, 114.9, 67.9, 13.3.

4.7.9. 2-(5-ethylthiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (10)—

Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (1 mmol scale), Yield: 125 mg, 37%. HRMS (FAB) $C_{20}H_{18}N_2OSNa^+$ Predicted: 357.1038 Found: 357.1028. 1H NMR (400MHz, DMSO- d_6) δ = 7.74 (dd, J = 1.5, 7.8 Hz, 1 H), 7.63 (d, J = 2.8 Hz, 1 H), 7.41 - 7.34 (m, 2 H), 7.34 - 7.29 (m, 3 H), 7.27 - 7.21 (m, 1 H), 6.83 (d, J = 8.0 Hz, 1 H), 6.80 - 6.76 (m, 1 H), 6.75 (d, J = 3.5 Hz, 1 H), 6.58 (td, J = 1.1, 3.3 Hz, 1 H), 6.42 (d, J = 2.8 Hz, 1 H), 2.67 (q, J = 7.4 Hz, 2 H), 1.13 (t, J = 7.4 Hz, 3 H). ^{13}C NMR (75MHz, DMSO- d_6) δ = 161.5, 146.7, 146.3, 141.7, 140.5, 133.8, 128.7, 128.0, 126.4, 126.3, 126.1, 122.7, 118.0, 115.5, 115.2, 69.7, 22.7, 15.6

4.7.10. 2-(benzo[β]thiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (11)—

Prepared via procedure (A) from 2-nitro-*N*-phenylbenzamide (1 mmol scale), Yield: 168 mg, 47%. HRMS (FAB) $C_{22}H_{16}N_2OSNa^+$ Predicted: 379.0881, Found: 379.0866. 1H NMR (400MHz, DMSO- d_6) δ = 7.88 - 7.82 (m, 1 H), 7.79 - 7.71 (m, 3 H), 7.42 - 7.37 (m, 4 H), 7.37 - 7.33 (m, 1 H), 7.33 - 7.28 (m, 3 H), 7.28 - 7.22 (m, 1 H), 6.86 (d, J = 8.0 Hz, 1 H), 6.80 (dt, J = 1.0, 7.5 Hz, 1 H), 6.64 (d, J = 3.3 Hz, 1 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.4, 146.2, 145.3, 144.8, 140.4, 138.3, 134.0, 128.8, 128.0, 126.5, 124.9, 124.5, 123.8, 123.0, 122.6, 118.2, 115.6, 115.3, 107.6, 69.9.

4.7.11. 2-(5-ethylthiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (12)—

Prepared via procedure B (1 mmol scale), yield: 148 mg, 57%. HRMS (FAB) $[C_{14}H_{14}N_2OS + Na]^+$ Predicted: 281.0725 Found: 287.0730. 1H NMR (400MHz, DMSO- d_6) δ = 8.39 (br. s, 1 H), 7.60 (dd, J = 1.5, 7.8 Hz, 1 H), 7.28 - 7.22 (m, 1 H), 7.21 (br. s, 1 H), 6.91 (d, J = 3.3 Hz, 1 H), 6.74 (d, J = 8.1 Hz, 1 H), 6.72 - 6.66 (m, 2 H), 5.92 (t, J = 1.8 Hz, 1 H), 2.74 (dq, J = 0.8, 7.6 Hz, 2 H), 1.18 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (151MHz, DMSO- d_6) δ = 163.0, 147.2, 146.7, 143.4, 133.3, 127.2, 125.3, 122.7, 117.4, 115.0, 114.6, 62.8, 22.8, 15.8.

4.7.12. 2-(5-ethylthiophen-2-yl)-3-propyl-2,3-dihydroquinazolin-4(1H)-one (13)

—Prepared via procedure B (1 mmol scale), Yield: 108 mg, 36%. HRMS (FAB) $[C_{17}H_{20}N_2OS + Na]^+$ Predicted: 323.1194 Found: 323.1188. 1H NMR (400MHz, DMSO- d_6) δ = 7.68 - 7.61 (m, 1 H), 7.36 (br. s., 1 H), 7.24 (dt, J = 1.5, 7.7 Hz, 1 H), 6.88 (d, J = 3.5 Hz, 1 H), 6.74 - 6.67 (m, 2 H), 6.63 (d, J = 3.5 Hz, 1 H), 6.01 (d, J = 2.3 Hz, 1 H), 3.88 - 3.74 (m, 1 H), 2.81 (ddd, J = 5.7, 8.1, 13.5 Hz, 1 H), 2.67 (q, J = 7.6 Hz, 2 H), 1.67 - 1.55

(m, 1 H), 1.55 - 1.42 (m, 1 H), 1.13 (t, $J = 7.6$ Hz, 3 H), 0.85 (t, $J = 7.5$ Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) $\delta = 161.6, 146.4, 146.1, 142.1, 133.2, 127.4, 125.7, 122.6, 117.5, 115.2, 114.7, 66.8, 45.8, 22.7, 20.8, 15.7, 11.2$.

4.7.13. 2-(5-ethylthiophen-2-yl)-3-isopropyl-2,3-dihydroquinazolin-4(1H)-one (14)—Prepared via procedure B (1 mmol scale), yield: 142 mg, 47%. HRMS (FAB)

$[\text{C}_{17}\text{H}_{20}\text{N}_2\text{OS} + \text{Na}]^+$ Predicted: 323.1194 Found: 323.1180. ^1H NMR (600MHz, DMSO- d_6) $\delta = 7.66$ (dd, $J = 1.1, 7.7$ Hz, 1 H), 7.26 (d, $J = 2.9$ Hz, 1 H), 7.22 (ddd, $J = 1.5, 7.3, 8.2$ Hz, 1 H), 6.87 (d, $J = 3.7$ Hz, 1 H), 6.71 (t, $J = 7.5$ Hz, 1 H), 6.68 (d, $J = 8.1$ Hz, 1 H), 6.59 (d, $J = 3.7$ Hz, 1 H), 6.06 (d, $J = 2.9$ Hz, 1 H), 4.55 (spt, $J = 6.8$ Hz, 1 H), 2.64 (q, $J = 7.5$ Hz, 2 H), 1.23 (d, $J = 7.0$ Hz, 3 H), 1.11 (t, $J = 7.5$ Hz, 3 H), 1.04 (d, $J = 7.0$ Hz, 3 H). ^{13}C NMR (151MHz, DMSO- d_6) $\delta = 161.2, 145.8, 145.7, 143.8, 132.9, 127.5, 125.2, 122.3, 117.6, 116.3, 114.8, 63.0, 45.6, 22.6, 20.3, 20.1, 15.5$.

4.7.14. 3-butyl-2-(5-ethylthiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (15)—

Prepared via procedure B (1 mmol scale), yield: 167 mg, 53 %. HRMS (FAB) $[\text{C}_{18}\text{H}_{22}\text{N}_2\text{OS} + \text{Na}]^+$ Predicted: 337.1351 Found: 337.1366. ^1H NMR (600MHz, DMSO- d_6) $\delta = 7.64$ (dd, $J = 1.5, 8.1$ Hz, 1 H), 7.33 (d, $J = 2.6$ Hz, 1 H), 7.27 - 7.21 (m, 1 H), 6.88 (d, $J = 3.7$ Hz, 1 H), 6.73 - 6.67 (m, 2 H), 6.64 (td, $J = 1.0, 3.6$ Hz, 1 H), 6.00 (d, $J = 2.6$ Hz, 1 H), 3.86 (ddd, $J = 6.6, 8.4, 13.6$ Hz, 1 H), 2.83 (ddd, $J = 5.5, 8.3, 13.7$ Hz, 1 H), 2.68 (dq, $J = 1.1, 7.5$ Hz, 2 H), 1.59 - 1.51 (m, 1 H), 1.51 - 1.43 (m, 1 H), 1.34 - 1.21 (m, 2 H), 1.13 (t, $J = 7.5$ Hz, 3 H), 0.87 (t, $J = 7.3$ Hz, 3 H). ^{13}C NMR (151MHz, DMSO- d_6) $\delta = 161.5, 146.4, 146.0, 142.0, 133.1, 127.4, 125.7, 122.5, 117.5, 115.2, 114.6, 66.7, 43.7, 29.5, 22.6, 19.5, 15.6, 13.6$.

4.7.15. 3-cyclohexyl-2-(5-ethylthiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (16)—Prepared via procedure B (1 mmol scale), yield: 120 mg, 35%. HRMS (FAB)

$[\text{C}_{20}\text{H}_{24}\text{N}_2\text{OS} + \text{Na}]^+$ Predicted: 363.1507 Found: 363.1522. ^1H NMR (400MHz, DMSO- d_6) $\delta = 7.65$ (dd, $J = 1.2, 7.7$ Hz, 1 H), 7.28 (d, $J = 2.4$ Hz, 1 H), 7.21 (ddd, $J = 1.5, 6.8, 8.6$ Hz, 1 H), 6.85 (d, $J = 3.7$ Hz, 1 H), 6.70 (t, $J = 7.5$ Hz, 1 H), 6.66 (d, $J = 8.1$ Hz, 163 H), 6.58 (d, $J = 3.4$ Hz, 1 H), 6.08 (d, $J = 2.9$ Hz, 1 H), 4.23 (t, $J = 11.9$ Hz, 1 H), 2.64 (q, $J = 7.4$ Hz, 2 H), 1.76 (d, $J = 10.8$ Hz, 2 H), 1.68 (br. s., 1 H), 1.57 (br. s., 3 H), 1.39 - 1.15 (m, 4 H), 1.11 (t, $J = 7.5$ Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) $\delta = 161.2, 145.7, 145.7, 144.0, 133.0, 127.6, 125.2, 122.4, 117.6, 116.4, 114.9, 63.0, 53.4, 30.3, 30.3, 25.7, 25.6, 24.9, 22.6, 15.5$.

4.7.16. 3-benzyl-2-(5-ethylthiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (17)

—Prepared via procedure B, HRMS (FAB) $[\text{C}_{21}\text{H}_{20}\text{N}_2\text{OS} + \text{Na}]^+$ Predicted: 371.1194 Found: 371.1186. ^1H NMR (600MHz, DMSO- d_6) $\delta = 7.70$ (dd, $J = 1.8, 7.7$ Hz, 1 H), 7.37 (d, $J = 2.6$ Hz, 1 H), 7.36 - 7.33 (m, 2 H), 7.32 - 7.30 (m, 2 H), 7.30 - 7.25 (m, 2 H), 6.87 (d, $J = 3.7$ Hz, 1 H), 6.74 (dt, $J = 1.1, 7.5$ Hz, 1 H), 6.71 (d, $J = 8.1$ Hz, 1 H), 6.65 (td, $J = 1.1, 3.3$ Hz, 1 H), 5.90 (d, $J = 2.6$ Hz, 1 H), 5.26 (d, $J = 15.4$ Hz, 1 H), 3.92 (d, $J = 15.4$ Hz, 1 H), 2.69 (dq, $J = 1.1, 7.5$ Hz, 2 H), 1.14 (t, $J = 7.5$ Hz, 3 H). ^{13}C NMR (151MHz, DMSO- d_6) $\delta = 161.7, 146.6, 146.1, 141.2, 137.5, 133.4, 128.4, 127.6, 127.4, 127.1, 126.0, 122.6, 117.7, 114.8, 114.7, 66.5, 46.7, 22.7, 15.6$.

4.7.17. 2-(5-ethylthiophen-2-yl)-3-phenethyl-2,3-dihydroquinazolin-4(1H)-one

(18)—Prepared via procedure B (1 mmol scale), yield: 261 mg, 72%. HRMS (FAB) $[C_{22}H_{22}N_2OS + Na]^+$ Predicted: 385.1351, Found: 385.1369. 1H NMR (400MHz, DMSO- d_6) δ = 7.65 (d, J = 7.8 Hz, 1 H), 7.37 (s, 1 H), 7.33 - 7.25 (m, 3 H), 7.25 - 7.17 (m, 3 H), 6.92 (d, J = 3.5 Hz, 1 H), 6.77 - 6.68 (m, 2 H), 6.68 - 6.62 (m, 1 H), 6.04 (d, J = 2.3 Hz, 1 H), 4.09 - 3.93 (m, 1 H), 3.14 - 3.01 (m, 1 H), 2.98 - 2.84 (m, 1 H), 2.81 - 2.71 (m, J = 5.1, 9.0, 9.0 Hz, 1 H), 2.68 (q, J = 7.3 Hz, 2 H), 1.13 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.7, 146.7, 146.2, 141.8, 139.1, 133.3, 128.6, 128.4, 127.4, 126.2, 125.9, 122.6, 117.5, 114.9, 114.6, 67.0, 45.9, 33.6, 22.7, 15.7.

4.7.18. 2-(5-ethylthiophen-2-yl)-3-(naphthalen-1-ylmethyl)-2,3-

dihydroquinazolin-4(1H)-one (19)—Prepared via procedure B (1 mmol scale), yield: 221 mg, 56%. HRMS (FAB) $[C_{25}H_{22}N_2OS + Na]^+$ Predicted: 421.1351 Found: 421.1359. 1H NMR (400MHz, DMSO- d_6) δ = 8.13 - 8.06 (m, 1 H), 8.00 - 7.94 (m, 1 H), 7.91 (d, J = 7.8 Hz, 1 H), 7.76 (d, J = 7.8 Hz, 1 H), 7.58 - 7.46 (m, 4 H), 7.34 (d, J = 2.3 Hz, 1 H), 7.32 - 7.26 (m, 1 H), 6.91 (d, J = 3.5 Hz, 1 H), 6.80 - 6.74 (m, 1 H), 6.68 (d, J = 8.3 Hz, 1 H), 6.67 - 6.64 (m, 1 H), 5.89 (d, J = 15.4 Hz, 1 H), 5.80 (d, J = 2.5 Hz, 1 H), 4.22 (d, J = 15.7 Hz, 1 H), 2.69 (q, J = 7.5 Hz, 2 H), 1.14 (dt, J = 1.3, 7.4 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.6, 146.6, 146.0, 140.8, 133.7, 133.5, 132.2, 131.1, 128.6, 128.2, 127.8, 126.5, 126.2, 126.0, 125.5, 123.6, 122.7, 117.8, 114.9, 114.7, 65.8, 44.4, 22.7, 15.7.

4.7.19. 2-(5-ethylthiophen-2-yl)-3-(4-methoxybenzyl)-2,3-

dihydroquinazolin-4(1H)-one (20)—Prepared via procedure B (1 mmol scale), yield: 201 mg, 53%. HRMS (FAB) $[C_{22}H_{22}N_2O_2S + Na]^+$ Predicted: 401.1300 Found: 401.1285. 1H NMR (400MHz, DMSO- d_6) δ = 7.69 (dd, J = 1.3, 7.6 Hz, 1 H), 7.34 (d, J = 2.8 Hz, 1 H), 7.32 - 7.19 (m, 3 H), 6.90 (d, J = 8.6 Hz, 2 H), 6.86 (d, J = 3.5 Hz, 1 H), 6.73 (t, J = 7.5 Hz, 1 H), 6.69 (d, J = 7.8 Hz, 1 H), 6.65 (d, J = 3.5 Hz, 1 H), 5.84 (d, J = 2.5 Hz, 1 H), 5.22 (d, J = 14.9 Hz, 1 H), 3.80 (d, J = 14.9 Hz, 1 H), 3.73 (s, 3 H), 2.68 (q, J = 7.5 Hz, 2 H), 1.14 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.6, 158.6, 146.6, 146.1, 141.3, 133.5, 129.3, 129.1, 127.6, 126.0, 122.7, 117.7, 114.8, 114.7, 113.9, 66.2, 55.1, 46.0, 22.7, 15.7.

4.7.20. 2-(5-ethylthiophen-2-yl)-3-(3-methoxybenzyl)-2,3-

dihydroquinazolin-4(1H)-one (21)—Prepared via procedure B (1 mmol scale), yield: 187 mg, 49%. HRMS (FAB) $[C_{22}H_{22}N_2O_2S + Na]^+$ Predicted: 401.1300 Found: 401.1282. 1H NMR (400MHz, DMSO- d_6) δ = 7.69 (dd, J = 1.4, 7.7 Hz, 1 H), 7.39 (d, J = 2.5 Hz, 1 H), 7.32 - 7.22 (m, 2 H), 6.92 - 6.82 (m, 4 H), 6.78 - 6.69 (m, 2 H), 6.64 (d, J = 3.3 Hz, 1 H), 5.90 (d, J = 2.8 Hz, 1 H), 5.23 (d, J = 15.4 Hz, 1 H), 3.90 (d, J = 15.4 Hz, 1 H), 3.72 (s, 3 H), 2.68 (q, J = 7.5 Hz, 2 H), 1.14 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 165.2, 159.4, 146.6, 141.3, 139.1, 133.5, 129.6, 127.6, 126.0, 122.6, 119.6, 117.7, 114.8, 113.2, 112.4, 66.6, 55.0, 46.7, 22.7, 15.7.

4.7.21. 2-(5-ethylthiophen-2-yl)-3-(2-methoxybenzyl)-2,3-

dihydroquinazolin-4(1H)-one (22)—Prepared via procedure B (1 mmol scale), yield:

234 mg, 62%. HRMS (FAB) $[C_{22}H_{22}N_2O_2S + Na]^+$ Predicted: 401.1300, Found: 401.1289. 1H NMR (400MHz, DMSO- d_6) δ = 7.67 (dd, J = 1.4, 7.7 Hz, 1 H), 7.41 (d, J = 2.8 Hz, 1 H), 7.32 - 7.24 (m, 2 H), 7.22 (d, J = 7.3 Hz, 1 H), 7.02 (d, J = 7.8 Hz, 1 H), 6.93 (t, J = 7.5 Hz, 1 H), 6.85 (d, J = 3.5 Hz, 1 H), 6.77 - 6.69 (m, 2 H), 6.67 - 6.63 (m, 1 H), 5.92 (d, J = 2.3 Hz, 1 H), 5.11 (d, J = 15.9 Hz, 1 H), 3.96 (d, J = 15.9 Hz, 1 H), 3.81 (s, 3 H), 2.69 (q, J = 7.6 Hz, 2 H), 1.14 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 157.0, 146.2, 141.5, 133.5, 128.4, 127.7, 127.6, 125.9, 122.7, 120.4, 117.7, 114.9, 114.8, 110.7, 66.8, 55.4, 42.1, 22.7, 15.7.

4.7.22. 3-(2,4-dimethoxybenzyl)-2-(5-ethylthiophen-2-yl)-2,3-

dihydroquinazolin-4(1H)-one (23)—Prepared via procedure B (1 mmol scale), yield: 202 mg, 50%. HRMS (FAB) $C_{23}H_{24}N_2O_3SNa^+$ Predicted: 431.1405 Found: 431.1405. 1H NMR (400MHz, DMSO- d_6) δ = 7.69 (dd, J = 1.4, 7.7 Hz, 1 H), 7.36 (d, J = 2.8 Hz, 1 H), 7.30 - 7.23 (m, 1 H), 7.14 (d, J = 8.3 Hz, 1 H), 6.84 (d, J = 3.5 Hz, 1 H), 6.76 - 6.68 (m, 2 H), 6.65 (d, J = 3.5 Hz, 1 H), 6.59 (d, J = 2.5 Hz, 1 H), 6.52 (dd, J = 2.4, 8.5 Hz, 1 H), 5.87 (d, J = 2.5 Hz, 1 H), 5.08 (d, J = 15.4 Hz, 1 H), 3.89 (d, J = 15.4 Hz, 1 H), 3.79 (s, 3 H), 3.75 (s, 3 H), 2.69 (q, J = 7.5 Hz, 2 H), 1.14 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.6, 160.0, 158.1, 146.5, 146.0, 141.6, 133.4, 129.2, 127.6, 125.7, 122.7, 117.6, 117.0, 115.0, 114.7, 104.7, 98.4, 66.5, 55.5, 55.2, 41.5, 22.7, 15.7.

4.7.23. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-2,3-dihydroquinazolin-4(1H)-one (24)—Prepared via procedure B (1 mmol scale) Yield 130 mg, 36%. HRMS (FAB)

$[C_{21}H_{19}FN_2OS + Na]^+$ Predicted: 389.1100 Found: 389.1109. 1H NMR (400MHz, DMSO- d_6) δ = 7.69 (dd, J = 1.6, 7.7 Hz, 1 H), 7.40 (d, J = 2.8 Hz, 1 H), 7.38 - 7.32 (m, 2 H), 7.31 - 7.25 (m, 1 H), 7.19 - 7.12 (m, 2 H), 6.87 (d, J = 3.5 Hz, 1 H), 6.79 - 6.69 (m, 2 H), 6.64 (td, J = 1.0, 3.5 Hz, 1 H), 5.94 (d, J = 2.5 Hz, 1 H), 5.16 (d, J = 15.4 Hz, 1 H), 3.98 (d, J = 15.4 Hz, 1 H), 2.68 (dq, J = 0.9, 7.5 Hz, 2 H), 1.13 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.8, 146.7, 146.2, 141.3, 133.8, 133.8, 133.5, 129.6, 129.5, 127.6, 127.4, 126.1, 122.6, 117.7, 115.3, 115.1, 114.8, 114.8, 66.7, 46.3, 22.7, 15.7.

4.7.24. 2-(5-ethylthiophen-2-yl)-3-(3-fluorobenzyl)-2,3-dihydroquinazolin-4(1H)-one (25)—Prepared via procedure B (1 mmol scale), yield: 172 mg, 47%. HRMS (FAB)

$[C_{21}H_{19}FN_2OS + Na]^+$ Predicted: 389.1100, Found: 389.1082. 1H NMR (400MHz, Acetone) δ = 7.70 (dd, J = 1.1, 8.0 Hz, 1 H), 7.44 (d, J = 2.5 Hz, 1 H), 7.41 - 7.33 (m, 1 H), 7.29 (dt, J = 1.6, 7.6 Hz, 1 H), 7.16 (d, J = 7.6 Hz, 1 H), 7.14 - 7.05 (m, 2 H), 6.88 (d, J = 3.3 Hz, 1 H), 6.78 - 6.71 (m, 2 H), 6.67 - 6.62 (m, 1 H), 6.00 (d, J = 2.5 Hz, 1 H), 5.16 (d, J = 15.7 Hz, 1 H), 4.06 (d, J = 15.7 Hz, 1 H), 2.68 (q, J = 7.5 Hz, 2 H), 1.13 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (101MHz, Acetone) δ = 163.5, 161.9, 161.0, 146.7, 146.3, 141.2, 140.8, 140.7, 133.6, 130.4, 130.3, 127.6, 126.2, 123.4, 123.4, 122.6, 117.8, 114.9, 114.7, 114.2, 114.0, 113.7, 67.0, 46.7, 22.7, 15.7.

4.7.25. 2-(5-ethylthiophen-2-yl)-3-(4-nitrobenzyl)-2,3-dihydroquinazolin-4(1H)-one (26)—Prepared via procedure B (1 mmol scale), yield: 230 mg, 58%. HRMS (FAB)

$[C_{21}H_{19}N_3O_3S + Na]^+$ Predicted: 416.1045, Found: 416.1061. 1H NMR (400MHz, DMSO- d_6) δ = 8.27 - 8.12 (m, 2 H), 7.69 (dd, J = 1.5, 8.1 Hz, 1 H), 7.55 (d, J = 8.8 Hz, 2 H), 7.49

(d, $J = 2.3$ Hz, 1 H), 7.36 - 7.25 (m, 1 H), 6.89 (d, $J = 3.5$ Hz, 1 H), 6.82 - 6.71 (m, 2 H), 6.63 (td, $J = 0.9, 3.5$ Hz, 1 H), 6.07 (d, $J = 2.5$ Hz, 1 H), 5.15 (d, $J = 16.2$ Hz, 1 H), 4.29 (d, $J = 16.2$ Hz, 1 H), 2.67 (q, $J = 7.4$ Hz, 2 H), 1.12 (t, $J = 7.5$ Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) $\delta = 162.1, 146.9, 146.6, 146.4, 146.0, 141.1, 133.7, 128.4, 127.6, 126.4, 123.5, 122.6, 117.8, 114.9, 114.6, 67.4, 47.0, 22.7, 15.8$.

4.7.26. 2-(5-ethylthiophen-2-yl)-3-(3-nitrobenzyl)-2,3-dihydroquinazolin-4(1H)-one (27)—Prepared via procedure B (1 mmol scale), yield: 181 mg, 46%. HRMS (FAB) $[\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_3\text{S} + \text{Na}]^+$ Predicted: 416.1045, Found: 416.1038. ^1H NMR (400MHz, DMSO- d_6) $\delta = 8.17 - 8.04$ (m, 2 H), 7.76 (d, $J = 7.8$ Hz, 1 H), 7.74 - 7.67 (m, 1 H), 7.67 - 7.56 (m, 1 H), 7.48 (d, $J = 2.3$ Hz, 1 H), 7.30 (dt, $J = 1.6, 7.6$ Hz, 1 H), 6.88 (d, $J = 3.5$ Hz, 1 H), 6.83 - 6.71 (m, 2 H), 6.66 - 6.58 (m, 1 H), 6.13 (d, $J = 2.5$ Hz, 1 H), 5.07 (d, $J = 15.4$ Hz, 1 H), 4.39 (d, $J = 15.7$ Hz, 1 H), 2.65 (q, $J = 7.5$ Hz, 2 H), 1.10 (t, $J = 7.6$ Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) $\delta = 162.2, 147.7, 146.8, 146.4, 141.3, 140.4, 134.3, 133.7, 129.9, 127.6, 126.4, 122.6, 122.1, 117.8, 114.9, 114.6, 67.4, 46.9, 22.7, 15.7$.

4.7.27. 2-(5-ethylthiophen-2-yl)-3-((S)-1-phenylethyl)-2,3-dihydroquinazolin-4(1H)-one (28)—Prepared via procedure B (1 mmol scale), yield: 256 mg, 71%. HRMS (FAB) $[\text{C}_{22}\text{H}_{22}\text{N}_2\text{OS} + \text{Na}]^+$ Predicted: 385.135, Found: 385.1342. ^1H NMR (400MHz, DMSO- d_6) $\delta = 7.71$ (d, $J = 7.8$ Hz, 1 H), 7.43 - 7.35 (m, 4 H), 7.34 - 7.28 (m, 1 H), 7.28 - 7.20 (m, 2 H), 6.81 (d, $J = 3.3$ Hz, 1 H), 6.74 (t, $J = 7.5$ Hz, 1 H), 6.65 (d, $J = 8.1$ Hz, 1 H), 6.61 (d, $J = 3.3$ Hz, 1 H), 5.90 (q, $J = 7.1$ Hz, 1 H), 5.78 (d, $J = 3.0$ Hz, 1 H), 2.66 (q, $J = 7.7$ Hz, 2 H), 1.36 (d, $J = 7.3$ Hz, 3 H), 1.12 (t, $J = 7.6$ Hz, 3 H). ^{13}C NMR (101MHz, CHLOROFORM- d) $\delta = 161.6, 146.0, 145.8, 143.6, 141.5, 133.3, 128.5, 127.8, 127.3, 126.8, 125.3, 122.5, 117.8, 115.9, 115.0, 63.0, 51.0, 22.6, 17.5, 15.6$.

4.7.28. 2-(5-ethylthiophen-2-yl)-3-((R)-1-phenylethyl)-2,3-dihydroquinazolin-4(1H)-one (29)—Prepared via procedure B (1 mmol scale), yield: 212 mg, 59%. HRMS (FAB) $[\text{C}_{22}\text{H}_{22}\text{N}_2\text{OS} + \text{Na}]^+$ Predicted: 385.1351 Found: 385.1335. ^1H NMR (400MHz, DMSO- d_6) $\delta = 7.71$ (dd, $J = 1.5, 7.8$ Hz, 1 H), 7.42 - 7.35 (m, 4 H), 7.34 - 7.28 (m, 1 H), 7.28 - 7.22 (m, 2 H), 6.81 (d, $J = 3.5$ Hz, 1 H), 6.78 - 6.70 (m, 1 H), 6.65 (d, $J = 8.1$ Hz, 1 H), 6.61 (d, $J = 3.5$ Hz, 1 H), 5.90 (q, $J = 7.2$ Hz, 1 H), 5.78 (d, $J = 3.0$ Hz, 1 H), 2.66 (q, $J = 7.5$ Hz, 2 H), 1.36 (d, $J = 7.3$ Hz, 3 H), 1.12 (t, $J = 7.6$ Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) $\delta = 161.5, 146.0, 145.8, 143.6, 141.5, 133.3, 128.5, 127.8, 127.2, 126.8, 125.3, 122.5, 117.8, 115.9, 115.0, 63.0, 51.0, 22.6, 17.5, 15.6$.

4.7.29. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-5-methyl-2,3-dihydroquinazolin-4(1H)-one (30)—Prepared via procedure A from *N*-(4-fluorobenzyl)-2-methyl-6-nitrobenzamide (0.5 mmol scale), yield: 136 mg, 72 %. HRMS (FAB) $[\text{C}_{22}\text{H}_{21}\text{FN}_2\text{OS} + \text{Na}]^+$ Predicted: 403.1256 Found: 403.1262. ^1H NMR (400MHz, DMSO- d_6) $\delta = 7.50$ (s, 1 H), 7.34 (dd, $J = 5.6, 8.6$ Hz, 2 H), 7.21 (d, $J = 2.5$ Hz, 1 H), 7.19 - 7.13 (m, 2 H), 7.11 (dd, $J = 2.0, 8.1$ Hz, 1 H), 6.85 (d, $J = 3.5$ Hz, 1 H), 6.67 - 6.60 (m, 2 H), 5.89 (d, $J = 2.5$ Hz, 1 H), 5.16 (d, $J = 15.2$ Hz, 1 H), 3.97 (d, $J = 15.4$ Hz, 1 H), 2.67 (q, $J = 7.5$ Hz, 2 H), 2.21 (s, 3 H), 1.13 (t, $J = 7.5$ Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) $\delta =$

161.9, 146.6, 144.0, 141.4, 134.4, 129.6, 129.5, 127.5, 126.4, 126.1, 122.6, 115.3, 115.1, 115.0, 114.8, 66.8, 46.3, 22.7, 20.2, 15.8.

4.7.30. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-6-methyl-2,3-

dihydroquinazolin-4(1H)-one (31)—Prepared via procedure A from *N*-(4-fluorobenzyl)-5-methyl-2-nitrobenzamide (0.5 mmol scale), yield: 88 mg 46%. HRMS (FAB) $C_{22}H_{21}FN_2OSNa^+$ Predicted: 403.1256 Found: 403.1250. 1H NMR (400MHz, Acetone) δ = 7.50 (s, 1 H), 7.34 (dd, J = 5.6, 8.1 Hz, 2 H), 7.23 - 7.07 (m, 4 H), 6.85 (d, J = 3.5 Hz, 1 H), 6.71 - 6.54 (m, 2 H), 5.90 (d, J = 2.3 Hz, 1 H), 5.16 (d, J = 15.4 Hz, 1 H), 3.97 (d, J = 15.4 Hz, 1 H), 2.67 (q, J = 7.4 Hz, 2 H), 2.21 (s, 3 H), 1.13 (t, J = 7.3 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.9, 146.6, 144.0, 141.4, 134.4, 129.6, 129.5, 127.5, 126.4, 126.1, 122.6, 115.3, 115.1, 115.0, 114.8, 66.8, 46.3, 22.7, 20.1, 15.8.

4.7.31. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-7-methyl-2,3-

dihydroquinazolin-4(1H)-one (32)—Prepared via procedure A from *N*-(4-fluorobenzyl)-4-methyl-2-nitrobenzamide (0.5 mmol scale), yield: 157 mg, 82 %. HRMS (FAB): $C_{22}H_{21}FN_2OSNa^+$ Predicted: 403.1256 Found: 403.1241. 1H NMR (400MHz, DMSO- d_6) δ = 7.57 (d, J = 7.8 Hz, 1 H), 7.37 - 7.29 (m, 3 H), 7.19 - 7.11 (m, 2 H), 6.85 (d, J = 3.5 Hz, 1 H), 6.63 (td, J = 0.9, 3.4 Hz, 1 H), 6.56 (dd, J = 1.0, 8.1 Hz, 1 H), 6.51 (s, 1 H), 5.90 (d, J = 2.5 Hz, 1 H), 5.15 (d, J = 15.2 Hz, 1 H), 3.95 (d, J = 15.4 Hz, 1 H), 2.68 (q, J = 7.6 Hz, 2 H), 1.16 - 1.11 (m, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 162.1, 146.4, 143.9, 142.0, 134.1, 134.0, 133.9, 129.6, 129.6, 125.5, 125.5, 122.9, 122.7, 117.5, 115.4, 115.2, 115.2, 66.1, 46.5, 22.7, 16.8, 15.6

4.7.32. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-8-methyl-2,3-

dihydroquinazolin-4(1H)-one (33)—Prepared via procedure A from *N*-(4-fluorobenzyl)-3-methyl-2-nitrobenzamide (0.5 mmol scale), yield: 93 mg, 50%. HRMS (FAB) $[C_{22}H_{21}FN_2OS + Na]^+$ Predicted: 403.1256 Found: 403.1265. 1H NMR (400MHz, DMSO- d_6) δ = 7.57 (d, J = 8.1 Hz, 1 H), 7.45 - 7.32 (m, 2 H), 7.18 (t, J = 7.8 Hz, 3 H), 6.97 (d, J = 3.5 Hz, 1 H), 6.81 (d, J = 3.3 Hz, 1 H), 6.71 - 6.65 (m, 1 H), 6.62 (d, J = 3.3 Hz, 1 H), 5.90 (d, J = 3.8 Hz, 1 H), 5.27 (d, J = 15.9 Hz, 1 H), 4.04 (d, J = 14.9 Hz, 1 H), 2.67 (q, J = 7.6 Hz, 2 H), 2.07 (s, 3 H), 1.13 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 162.1, 146.4, 142.0, 134.1, 129.7, 129.6, 125.5, 125.5, 122.9, 122.8, 117.6, 115.4, 115.2, 66.1, 46.5, 22.7, 16.8, 15.7.

4.7.33. 6-chloro-2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-2,3-

dihydroquinazolin-4(1H)-one (34)—Prepared via procedure B (1 mmol scale), yield: 335 mg, 84%). HRMS (FAB) $[C_{21}H_{18}FN_2OS + Na]^+$ Predicted: 423.0710 Found: 423.0725. 1H NMR (400MHz, DMSO- d_6) δ = 7.63 (dd, J = 2.5, 5.3 Hz, 2 H), 7.42 - 7.29 (m, 3 H), 7.20 - 7.12 (m, 2 H), 6.88 (d, J = 3.3 Hz, 1 H), 6.76 (d, J = 8.8 Hz, 1 H), 6.67 - 6.63 (m, 1 H), 6.01 (d, J = 2.5 Hz, 1 H), 5.13 (d, J = 15.2 Hz, 1 H), 4.01 (d, J = 15.2 Hz, 1 H), 2.68 (q, J = 7.4 Hz, 2 H), 1.14 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 160.7, 146.9, 145.0, 140.9, 133.4, 129.6, 129.6, 126.6, 126.3, 122.7, 121.4, 116.9, 115.8, 115.3, 115.1, 66.6, 46.4, 22.7, 15.7.

4.7.34. 7-chloro-2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-2,3-

dihydroquinazolin-4(1H)-one (35)—Prepared via procedure B (1 mmol scale), yield: 172 mg, 43%). HRMS (FAB) $[C_{21}H_{18}FN_2OS + Na]^+$ Predicted: 423.0710 Found: 423.0718. 1H NMR (400MHz, DMSO- d_6) δ = 7.71 - 7.65 (m, 2 H), 7.33 (dd, J = 5.7, 8.5 Hz, 2 H), 7.15 (t, J = 8.8 Hz, 2 H), 6.89 (d, J = 3.5 Hz, 1 H), 6.79 - 6.73 (m, 2 H), 6.68 - 6.63 (m, 1 H), 6.02 (d, J = 2.5 Hz, 1 H), 5.12 (d, J = 15.4 Hz, 1 H), 3.99 (d, J = 15.4 Hz, 1 H), 2.69 (q, J = 7.5 Hz, 2 H), 1.14 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (101MHz, DMSO- d_6) δ = 161.1, 160.2, 147.2, 147.0, 140.9, 138.1, 133.6, 133.6, 129.7, 129.6, 129.5, 126.4, 122.8, 117.8, 115.3, 115.1, 113.9, 113.4, 66.7, 46.3, 22.7, 15.7.

4.7.35. 2-(5-ethylthiophen-2-yl)-6-fluoro-3-(4-fluorobenzyl)-2,3-

dihydroquinazolin-4(1H)-one (36)—Prepared via procedure A from 5-fluoro-*N*-(4-fluorobenzyl)-2-nitrobenzamide (0.5 mmol scale), yield: 187 mg, 97%. HRMS (FAB) $[C_{21}H_{18}F_2N_2OS + Na]^+$ Predicted: 407.1006 Found: 407.1018. 1H NMR (400MHz, DMSO- d_6) δ = 7.43 - 7.37 (m, 2 H), 7.34 (dd, J = 5.6, 8.3 Hz, 2 H), 7.23 - 7.12 (m, 3 H), 6.87 (d, J = 3.5 Hz, 1 H), 6.76 (dd, J = 4.5, 8.8 Hz, 1 H), 6.66 - 6.62 (m, 1 H), 5.97 (d, J = 2.5 Hz, 1 H), 5.12 (d, J = 15.4 Hz, 1 H), 4.02 (d, J = 15.2 Hz, 1 H), 2.68 (q, J = 7.6 Hz, 2 H), 1.13 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (151MHz, DMSO- d_6) δ = 162.2, 161.0, 160.6, 155.8, 154.2, 146.7, 142.8, 140.9, 133.6, 133.6, 129.6, 129.5, 126.2, 122.6, 121.1, 120.9, 116.7, 116.6, 115.5, 115.5, 115.2, 115.1, 112.8, 112.6, 66.8, 46.4, 22.7, 15.6.

4.7.36. 2-(5-ethylthiophen-2-yl)-7-fluoro-3-(4-fluorobenzyl)-2,3-

dihydroquinazolin-4(1H)-one (37)—Prepared via procedure A from 4-fluoro-*N*-(4-fluorobenzyl)-2-nitrobenzamide (0.5 mmol scale), yield: 167 mg, 88%. HRMS (FAB) $[C_{21}H_{18}F_2N_2OS + Na]^+$ Predicted: 407.1006 Found: 407.1025. 1H NMR (600MHz, DMSO- d_6) δ = 7.74 (dd, J = 6.6, 8.8 Hz, 1 H), 7.66 (d, J = 2.6 Hz, 1 H), 7.37 - 7.30 (m, 2 H), 7.18 - 7.12 (m, 2 H), 6.89 (d, J = 3.7 Hz, 1 H), 6.65 (td, J = 1.0, 3.6 Hz, 1 H), 6.54 (dt, J = 2.4, 8.7 Hz, 1 H), 6.49 (dd, J = 2.2, 10.6 Hz, 1 H), 5.99 (d, J = 2.6 Hz, 1 H), 5.13 (d, J = 15.4 Hz, 1 H), 3.98 (d, J = 15.4 Hz, 1 H), 2.69 (q, J = 7.3 Hz, 2 H), 1.14 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (151MHz, DMSO- d_6) δ = 165.4, 162.7, 161.6, 148.7, 147.4, 141.5, 134.1, 134.1, 131.2, 131.1, 130.1, 130.0, 126.7, 123.2, 115.7, 115.6, 111.9, 105.7, 105.6, 101.1, 100.9, 67.2, 46.7, 23.2, 16.2.

4.7.37. 5-(butylamino)-*N*-(4-fluorobenzyl)-2-nitrobenzamide (39)—5-fluoro-*N*-(4-

fluorobenzyl)-2-nitrobenzamide (605 mg, 2 mmol) was dissolved in dimethoxy ethane (8 mL) and treated with butylamine (217 μ L, 2.2 mmol) and triethylamine (278 μ L, 2 mmol). The reaction was allowed to proceed for 36 hours, after which the solvent was removed in vacuo and the product was isolated as a bright yellow solid by flash chromatography. Yield: 440 mg, 64%. HRMS (ESI) Predicted $(C_{18}H_{20}FN_3O_3 + H)^+$:346.1567, Found: 346.1565. 1H NMR (400MHz, CHLOROFORM- d) δ = 7.97 (d, J = 9.1 Hz, 1 H), 7.46 - 7.30 (m, 2 H), 7.09 - 6.93 (m, 2 H), 6.54 - 6.37 (m, 2 H), 6.21 (t, J = 5.6 Hz, 1 H), 4.94 (t, J = 5.2 Hz, 1 H), 4.55 (d, J = 5.8 Hz, 2 H), 3.26 - 3.06 (m, 2 H), 1.66 - 1.54 (m, 2 H), 1.40 (qd, J = 7.4, 14.9 Hz, 2 H), 0.95 (t, J = 7.3 Hz, 3 H). ^{13}C NMR (101MHz, CHLOROFORM- d) δ = 167.9, 163.4, 161.0, 153.0, 135.8, 133.9, 133.4, 133.4, 129.8, 129.7, 127.8, 115.6, 115.4, 111.1, 110.9, 43.4, 43.0, 30.9, 20.1, 13.7.

4.7.38. 6-(butylamino)-2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-2,3-dihydroquinazolin-4(1H)-one (40)—

5-(butylamino)-N-(4-fluorobenzyl)-2-nitrobenzamide (368 mg, 1.1 mmol), was dissolved in methanol (5 mL). To the solution was added 10% Pd/C (150 mg) and ammonium formate (64 mg, 1 mmol). After 1 hour the reaction was filtered. The filtrate was adjusted to a volume 2mL in methanol and then treated with 5-ethyl-2-thiophenecarboxaldehyde (138 μ L, 1.1 mmol) and scandium (III) triflate (49 mg, 0.1) mmol. The reaction was microwave irradiated at 100°C for 1 hour, after which the solvent was removed *in vacuo*. The DHQZ (bright yellow solid) product was isolated by silica gel chromatography with a solvent gradient of 30–50 % ethyl acetate in hexanes. The chromatographed product was subsequently purified by recrystallization from ethyl acetate. Yield 236 mg, 54%. HRMS (ESI) Predicted ($C_{25}H_{28}FN_3OS + H$)⁺: 438.2015, Found: 438.2002. ¹H NMR (400MHz, CHLOROFORM-d) δ = 7.34 - 7.26 (m, 4 H), 7.06 - 6.95 (m, 2 H), 6.75 - 6.68 (m, 2 H), 6.58 - 6.52 (m, 2 H), 5.69 (s, 1 H), 5.51 (d, J = 14.8 Hz, 1 H), 4.14 (br. s., 1 H), 3.87 (d, J = 15.3 Hz, 1 H), 3.17 - 3.09 (m, 2 H), 2.73 (dq, J = 1.0, 7.5 Hz, 2 H), 1.67 - 1.56 (m, 2 H), 1.44 (qd, J = 7.3, 14.9 Hz, 2 H), 1.23 (t, J = 7.5 Hz, 3 H), 0.97 (t, J = 7.4 Hz, 3 H). ¹³C NMR (101MHz, CHLOROFORM-d) δ = 163.3, 162.9, 160.9, 148.4, 139.8, 137.2, 132.9, 132.8, 129.6, 129.5, 126.0, 122.3, 120.9, 117.8, 117.1, 115.5, 115.3, 112.5, 67.5, 46.4, 45.3, 31.2, 23.4, 20.2, 15.6, 13.8.

4.7.39. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)quinazolin-4(3H)-one (41)—

Prepared via procedure B (0.29 mmol scale) using ethanol as solvent and CuCl₂ (0.3 mmol) instead of ScOtf₃, yield: 99 mg, 94%. HRMS (FAB) [$C_{21}H_{17}FN_2OS + Na$]⁺ Predicted: 387.0943, Found: 387.0938. ¹H NMR (400MHz, DMSO-d₆) δ = 8.14 (dd, J = 1.3, 8.3 Hz, 1 H), 7.85 (dt, J = 1.4, 7.6 Hz, 1 H), 7.67 (d, J = 7.8 Hz, 1 H), 7.59 - 7.50 (m, 1 H), 7.19 - 7.07 (m, 5 H), 6.83 (dd, J = 0.8, 3.8 Hz, 1 H), 5.45 (s, 2 H), 2.81 (q, J = 7.5 Hz, 2 H), 1.23 (t, J = 7.5 Hz, 3 H). ¹³C NMR (101MHz, DMSO-d₆) δ = 162.5, 161.8, 160.1, 152.0, 150.0, 147.0, 135.0, 134.0, 133.1, 133.1, 129.6, 128.1, 128.0, 127.2, 126.6, 124.7, 119.7, 115.8, 115.5, 48.2, 22.8, 15.8.

4.7.40. 2-(5-ethylthiophen-2-yl)-3-(4-fluorobenzyl)-1-methyl-2,3-dihydroquinazolin-4(1H)-one (43)—

Prepared via procedure B (1 mmol scale), yield: 333 mg, 88%. HRMS (FAB) [$C_{22}H_{21}FN_2OS + Na$]⁺ Predicted: 403.1256 Found: 403.1266. ¹H NMR (400MHz, DMSO-d₆) δ = 7.80 (dd, J = 1.5, 7.6 Hz, 1 H), 7.46 - 7.40 (m, 1 H), 7.40 - 7.33 (m, 2 H), 7.21 - 7.12 (m, 2 H), 6.93 (d, J = 3.5 Hz, 1 H), 6.88 (dt, J = 1.0, 7.5 Hz, 1 H), 6.69 (d, J = 8.1 Hz, 1 H), 6.65 (td, J = 1.0, 3.5 Hz, 1 H), 5.92 (s, 1 H), 5.13 (d, J = 15.4 Hz, 1 H), 3.95 (d, J = 15.4 Hz, 1 H), 2.77 (s, 3 H), 2.68 - 2.60 (m, 2 H), 1.11 (t, J = 7.5 Hz, 3 H). ¹³C NMR (101MHz, DMSO-d₆) δ = 161.3, 147.0, 146.4, 134.1, 133.5, 133.5, 129.6, 129.5, 127.8, 127.7, 122.6, 118.3, 115.3, 115.1, 112.9, 73.3, 46.3, 34.9, 22.6, 15.5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Retro-2^{cycl}JCPyV IC₅₀ = 54 μMHPV16 IC₅₀ = 160 μM **DHQZ 36**JCPyV IC₅₀ = 8.1 μMHPV16 IC₅₀ = 24 μM**Figure 1.**

Inhibition of JCPyV infectivity in SVG-A Cells and HPV16 infectivity in HeLaM Cells:

Cells were pre-incubated with increasing concentrations of Retro-2^{cycl} or DHQZ 36 prior to inoculation with virus.

Figure 2.
HPV16 and JCPyV Proximity Ligation Assay: Cell nuclei are indicated by blue fluorescence. Green fluorescence indicates co-localization of target proteins: JCPyV – capsid protein VP1 and ER protein PDI; HPV16 – capsid protein L1 and Golgi protein TGN 46.

Scheme 1.

Diversity oriented synthesis of Retro-2^{cycl} analogs. a) R₂NH₂, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, dichloromethane, room temperature, 16 hours. b) 10% Pd/C, ammonium formate, methanol. c) R₁CHO, Sc(OTf)₃, methanol, MW 100°C, 1 h. d) R₂NH₂, tetrahydrofuran, reflux, then R₁CHO, Sc(OTf)₃.

Scheme 2.

Synthesis and activity of *n*-butylamino dihydroquinazolinone a) *n*-butylamine, triethylamine, dimethoxyethane, room temperature, 16 h, 64%. b) 10% Pd/C, ammonium formate, methanol, room temperature, 2 h. c) 5-ethylthiophene-2-carboxaldehyde, Sc(OTf)₃, methanol, reflux, 2 h, 54% over 2 steps.

Scheme 3.

Synthesis and activity of quinazolinone and *N*-methyldihydroquinazolinone analogs. a) 4-fluorobenzylamine, ethanol, reflux, then 5-ethylthiophenol, CuCl_2 , 94%. b) 4-fluorobenzylamine, tetrahydrofuran, reflux, then 5-ethylthiophenol, $\text{Sc}(\text{OTf})_3$, 88%.

Table 1Anti-JCPyV Activity of DHQZs at 25 μ M with Varied Heterocycle Moiety^a

Compound	R ₁	Infectivity (% DMSO Control)
Retro-2 ^{cycl}		57.5 $\pm$ 5.9
4		81.1 $\pm$ 16.9
5		92.4 $\pm$ 4.45
6		69.9 $\pm$ 27.8
7		64.4 $\pm$ 12.7
8		55.6 $\pm$ 14.4
9		76.4 $\pm$ 1.1
10		47.8 $\pm$ 3.8
11		95.3 $\pm$ 14.3

^aHeLa M cells were pre-incubated with 25 μ M of the DHQZs prior to inoculation with JC polyomavirus. Infections were scored and normalized to a DMSO-treated control. The data represent the mean of three replicates with indicated standard error.

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Table 2Anti-JCPyV Activity of DHQZs at 25 μ M with Varied Amide Moiety^a

Compound	R ₂	Infectivity (% DMSO Control)
12	H	41.4 $\pm$ 1.4
13		76.4 $\pm$ 1.1
14		69.9 $\pm$ 27.8
15		42.4 $\pm$ 2.6
16		45.7 $\pm$ 10.3
17		13.3 $\pm$ 1.1
18		50.4 $\pm$ 2.3
19		14.5 $\pm$ 0.7
20		21.2 $\pm$ 5.1
21		27.5 $\pm$ 1.8

Compound	R ₂	Infectivity (% DMSO Control)
22		13.7 ± 0.5
23		16.4 ± 3.9
24		7.62 ± 1.0
25		14.0 ± 2.6
26		30.6 ± 3.2
27		44.5 ± 8.8
28		32.4 ± 13.7
29		52.7 ± 1.5

^aHeLa M cells were pre-incubated with 25 μM of the DHQZs prior to inoculation with JC polyomavirus. Infections were scored and normalized to a DMSO-treated control. The data represent the mean of three replicates with indicated standard error.

Table 3Anti-JCPyV Activity of DHQZs at 25 μ M with Varied Benzo Moiety^a

Compound	R ₃	Infectivity (% DMSO Control)
30	5-Methyl	10.2 $\pm$ 5.68
31	6-Methyl	8.34 $\pm$ 2.76
32	7-Methyl	67.1 $\pm$ 9.12
33	8-Methyl	44.5 $\pm$ 2.51
34	6-Chloro	13.7 $\pm$ 16.2
35	7-Chloro	21.3 $\pm$ 2.82
36	6-Fluoro	4.45 $\pm$ 0.613
37	7-Fluoro	8.82 $\pm$ 7.52

^a HeLa M cells were pre-incubated with 25 μ M of the DHQZs prior to inoculation with JC polyomavirus. Infections were scored and normalized to a DMSO-treated control. The data represent the mean of three replicates with indicated standard error.