Optimization of Potent and Selective Quinazolinediones: Inhibitors of Respiratory Syncytial Virus That Block RNA-Dependent RNA-Polymerase Complex Activity

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Supporting Information

ABSTRACT: A quinazolinedione-derived screening hit 2 was discovered with cellular antiviral activity against respiratory syncytial virus (CPE EC₅₀ = 2.1 μM), moderate efficacy in reducing viral progeny (4.2 log at 10 μM), and marginal cytotoxic liability (selectivity index, SI ~ 24). Scaffold optimization delivered analogs with improved potency and selectivity profiles. Most notable were compounds 15 and 19 (EC₅₀ = 300−500 nM, CC₅₀ > 50 μM, SI > 100), which significantly reduced viral titer (>400,000-fold), and several analogs were shown to block the activity of the RNA-dependent RNA-polymerase complex of RSV.

INTRODUCTION

Acute bronchiolitis, a common lower respiratory tract infection most seriously affecting infants and the elderly, is predominately caused by the highly infectious human respiratory syncytial virus (hRSV). The virus belongs to the paramyxovirus family, which also includes mumps and measles viruses; however, unlike these related pathogens for which vaccines have been developed, a safe and effective vaccine remains elusive to prevent the contraction and transmission of RSV. In fact, since the discovery of the virus over 50 years ago, the only FDA approved small molecule inhibitor for treatment of the infection is ribavirin, a nucleoside antimetabolite, that is limited to use in critical cases due to its toxicological side effects. In the United States, the prevalence of RSV infection in adults over the age of 65 results in approximately 170,000 hospitalizations and 10,000 deaths annually while the global incidence of RSV infection was estimated in 2005 to result in the hospitalization of 3.4 million children under the age of 5. Furthermore, exposure does not impart full immunity from future infection and, in fact, promotes an inflammatory response that can contribute to chronic lung complications such as asthma. These burdens, coupled with the absence of suitable therapeutic agents for susceptible populations, underscore the importance of identifying effective and safe pharmacological countermeasures for RSV.

The scientific literature is replete with examples from translational development programs aimed at addressing this important need. Replication inhibitors have been investigated, along with several compounds that target RSV’s entry-enabling F protein, though in most cases the compounds were not pursued or clinical development was discontinued. Despite these efforts, the search continues for...

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RSV inhibitors that offer a superior pharmacological and safety profile compared to that of ribavirin. 

As part of the National Institutes of Health Molecular Libraries Initiative, we pursued a subset of RSV-inhibiting hit scaffolds identified through a high-throughput screen of the national compound repository. Optimization of a screening hit led to compound 1, probe ML232, a sulfonamide-based RSV inhibitor with single-digit micromolar in vitro activity, and a proposed entry-based mechanism of inhibition based on time-of-addition studies (Figure 1A). A sulfonamide-based RSV inhibitor with single-digit micromolar in vitro activity, and a proposed entry-based mechanism of inhibition based on time-of-addition studies (Figure 1A). In a titer reduction assay, hit 1 was found to reduce viral plaques by ∼4.2 log (~14,000-fold as compared to control) at a concentration of 10 μM. The team undertook an optimization effort that focused on the five colored regions of the scaffold with the primary aims of broadening the selectivity index by enhancing potency and attenuating cellular toxicity, amplifying the plaque reducing effect, and improving solubility (Figure 1B).

### CHEMISTRY

Analogs of hit 2 were generally prepared using standard peptide coupling conditions of 2-amino benzoic acid with methyl 4-(aminomethyl)benzoate to afford an aminobenzamide intermediate (Scheme 1). Subsequent cyclization with CDI generated quinazolinedione core intermediate 4. Most analogs were made by ester hydrolysis of 4, followed by incorporation of the pendent amido alkyl ether (R2) with routine amide coupling, followed by installation of the N-benzyl appendage (R3) to afford products 5. In some cases, it was advantageous to affix the N-benzyl portion prior to revealing the benzoic acid functionality for coupling to the preferred amine component (i.e., shuffling the sequence of R1 vs R2 integration). In either case, the synthetic route was flexible and offered selective, orthogonal, late-stage diversification to prepare the desired analog sets. Quinazolinones 7 were also prepared from anthranilic acid 3. Amide coupling between 3 and methyl 4-(aminomethyl)benzoate afforded a 2-aminobenzamide intermediate that was treated with an isopropylphenylacetic acid chloride. The resulting bis-amide 6 was treated with hydroxide base to reveal a benzoic acid that could be further manipulated; however, these conditions fortuitously induced the intended hydrolysis and necessary cyclization to generate the quinazolinone core in one step. Subsequent coupling of the unmasked benzoic acid with 3-methoxypropyramine afforded the desired analogs 7.

### RESULTS AND DISCUSSION

#### Medicinal Chemistry Optimization

For this stage of our program, 73 quinazolinedione-derived analogs were prepared and analyzed. All compounds were evaluated for inhibition of an RSV-induced cytopathic effect and assessment of mammalian cell cytotoxicity. Both assays were performed in a 10-point dose response format using HEP-2 cells (hRSV strain Long), and the data from these screens was employed to drive iterative

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Scheme 1. Chemical Synthesis of Quinazolinedione and Quinazolinone Analogs

**Reagents:** (a) DIPEA, HATU, methyl 4-(aminomethyl)benzoate hydrochloride, DMF, rt, 2 h, 68%; (b) DIPEA, 1,1-carbonyldimidazole, CH3Cl, reflux, 16 h, 95%; (c) LiOH, H2O, THF, 40 °C, 1 h, 88%; (d) DIPEA, HATU, 3-methoxypropylamine or other amine for R2, DMF, rt, 2 h, 44—76%; (e) K2CO3, 4-isopropylbenzyl bromide other aryl halide for R3, DMF, 40 °C, 16 h, 11—71%; (f) DIPEA, EDCI, HOBt, methyl 4-(aminomethyl)benzoate hydrochloride, CH3Cl, rt, 74%; (g) 3- or 4-isopropylphenylacetic acid, (COCl)2, cat. DMF, CH3Cl, then pyridine, CH3Cl, 1.5 h, rt, 93—100%; (h) LiOH, THF, H2O, 40 °C, 20 h; (i) 3-methoxypropylamine, EDCI, HOBt, DIPEA, CH3Cl, rt, 10—22% over 2 steps.

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Alterations of the secondary amide (R²) were independently carried out in a parallel effort. Exchange of the methyl ether for the ethyl or isopropyl ether did not appreciably alter potency in the CPE assay; however, the cytotoxic effects associated with those analogs increased relative to the parent hit 2 (Table 2, entries 1–3). Replacement of the methyl ether with a tertiary amine for the purpose of enhancing solubility was inferior in terms of both potency and toxicity (entry 4). Elongation of the alkyl chain by one methylene unit only marginally enhanced potency and reduced the therapeutic window (entry 5); however, truncating the linker afforded analog 28 with comparable potency to hit 2 (entry 6). Introducing an oxetane as a cyclized version of the linear ether chain resulted in a promising potency, cytotoxicity, and viral plaque reduction profile (entry 7). Other modifications were explored to improve solubility or potency without inducing cytotoxicity, but none were found to be more advantageous when considering multiparameter optimization.

Hybrid analogs derived from the most advantageous individual R¹ or R² modifications were then prepared to assess synergistic effects, and additional SAR data was pursued using these compounds as templates. At this stage, several compounds were also evaluated for improvement in solubility. The initial analog sets (Tables 1 and 2) revealed that analogs with structural variations in the (R¹) region of hit compound 2 were the most promising. Consequently, the N⁴-nitrobenzyl derivative 15 and the N⁴-isopropylbenzyl analog 19 showed the best potency, viral titer assay efficacy, and cytotoxicity profile in the collection. Heterocyclic variants in this region led to suboptimal potency, cytotoxicity, and reduction in viral plaques (entries 14–17).
solubility and at least a 2 log increase in capacity to reduce viral titer. Combining the 4-isopropylbenzyl group (R¹) with the NHCH₂-3-oxetane (R²) component resulted in potency comparable to the best analogs in the series (44, entry 3, Table 3); however, the selectivity index was diminished due to unwanted cytotoxicity. Alternatives to the 4-nitrobenzyl element were examined, such as a 4-benzoic acid derivative which imparted a desirable solubility effect but also abrogated activity, which may be the result of reduced cellular permeability. The N-4-dimethylaminobenzyl analog was also found to have improved solubility but did not robustly provide cytoprotection to the extent observed with other architectural combinations (entry 5). Furfuryl and pyridyl variants in place of the R² appendage were also determined to be suboptimal (entry 5). Furyl and pyridyl variants in place of the benzamide moiety or for compounds with the para-positioned amide functionality as opposed to the para-arrangement present in the hit (data not shown).

Given the idiosyncratic cytotoxicity profile for several of the compounds in the quinazolinedione series and our experience with a related, antiviral quinazolinone scaffold that lacked any detectable mammalian cytotoxicity, two quinazolinone analogs were prepared to explore if this modified core was beneficial against RSV (51–52, Figure 2). Quinazolinone analogs 51 and 52 were generated bearing peripheral components that had been shown to possess anti-RSV activity when integrated with the quinazolinedione core. While the compounds were not protective against RSV (50 μM), the effort underscored the importance of the carbonyl group situated between the core nitrogens to retaining anti-RSV potency. Additionally, the impact of introducing a nitrogen atom into the fused phenyl ring of the quinazolinedione core was studied (53–54, Figure 2). Only analog 54 was weakly active, but the compound also demonstrated some appreciable toxicity. Though limited somewhat in scope, the core alterations represented by these four analogs and those previously discussed in which functional groups were introduced at the C6 or C7 position of the scaffold showed that no core modification made to date was well tolerated.

### Profiling Assays

The purpose of this program was to identify and develop new compounds with compelling in vitro anti-RSV activity that could be used as a platform for deriving suitable probes for future in vivo efficacy studies. Toward this goal, several analogs emerged from the SAR effort as interesting probe candidates worthy of further characterization based on improvements in CPE potency, solubility, and viral titer. Nonetheless, limitations in aqueous solubility or the presence of functionality with suspected metabolic liability prompted the team to assess passive permeability and hepatocyte toxicity for...
select analogs. Compounds 15, 19, and 46 were evaluated accordingly (Table 4).

Aqueous solubility for isopropylbenzyl derivative 19 was the most limited of these three compounds, resulting in a skewed result in aqueous stability (27% parent remaining, entry 2, Table 4). The addition of acetonitrile to the stability experiment to account for compound precipitation in PBS buffer reflected that compound 19 was stable to degradation. Solubility for each compound in CPE assay media was improved compared to PBS buffer, likely due to protein binding. Passive permeability was negligible for 19 in PBS, as expected from the solubility data. Moderate to good permeability was observed for the nitrobenzyl and oxetane-containing derivatives 15 and 46, respectively, at each of 3 pH levels. Toxicity in hepatocytes, most concerning for the nitrobenzyl analog 15, was not observed (>50 μM), nor was it significant with the other two analogs tested. While each compound possessed desirable attributes, compound 19 was selected as our lead, ML275, due to the overall profile which included the most improved potency, selectivity window and reduction in viral titer.

Probe candidates were routinely assessed in a Eurofins PanLabs (formerly Ricerca) Hit LeadProfiling screen against 67 discrete GPCRs, ion channels and transporters. All assays were performed in duplicate with probe ML275 (19) at a concentration of 10 μM, and >50% inhibition was noted for five targets (Table 5). Inhibition of the human adenosine A3 receptor and the human platelet activating factor was determined to be 44% and 43%, respectively; however, inhibition of all remaining targets did not exceed 34%. A full list of the targets and percent inhibition by compound 19

Table 3. hRSV CPE Assay Potency, Cytotoxicity, Selectivity Index, Logarithmic Reduction in Viral Plaques, and Solubility Assessments for Analogs with Tandem Structural Variations in the (R1) and (R2) Regions of Hit Compound 2

| entry | cmpd | R1 | R2 | RSV CPE potency ± standard deviation (EC50 (μM)) | HEP-2 cellular toxicity ± standard deviation (CC50 (μM)) | selectivity index (CC50/EC50) | viral titer reduction at 10 μM (log) | solubility (μM) |
|-------|------|----|----|---------------------------------------------|----------------|----------------|-------------------------------|----------------|
| 1     | 2    | CH2-4-methylphenyl | NH(CH2)3OCH3 | 2.1 ± 0.5 | >50.0 | >23.8 | 4.2 | 0.1 | 2.5 |
| 2     | 43   | CH2-4-nitrophenyl  | NHCH2-3-oxetane | 1.3 ± 0.5 | >50.0 | >38.5 | >6.2 | 1.4 | 7.2 |
| 3     | 44   | CH2-4-iso-propylphenyl | NHCH2-3-oxetane | 0.4 ± 0.1 | 3.6 ± 0.8 | 9.0 | 5.8 | NT | NT |
| 4     | 45   | CH2-4-CO2H-phenyl  | NHCH2-3-oxetane | >50.0 | >50.0 | NA | NT | 98.1 | NT |
| 5     | 46   | CH2-4-N(CH3)2-phenyl | NHCH2-3-oxetane | 1.1 ± 0.2 | 44.7 ± 7.2 | 41.8 | >6.2 | 11.2 | 18.5 |
| 6     | 47   | CH2-4-tert-butylphenyl | NHCH2-3-oxetane | 1.6 ± 0.8 | 3.7 ± 0.3 | 2.3 | 3.4 | NT | NT |
| 7     | 48   | CH2-4-iso-propylphenyl | NHCH2-2-furyl | 1.2 ± 0.1 | >50.0 | >41.7 | 2.8 | 0.3 | NT |
| 8     | 49   | CH2-4-chlorophenyl  | NHCH2-3-oxetane | 0.8 ± 0.1 | >50.0 | >62.5 | NT | NT | 10.2 |
| 9     | 50   | CH2-4-iso-propylphenyl | NH-2-CH2-O-pyridyl | 0.5 ± 0.6 | <1.6 | <3.3 | NT | NT | NT |

a Data were an average of ≥3 experiments. b Data are an average of ≥2 experiments. c Kinetic solubility in 1×PBS, pH 7.4. d Kinetic solubility in CPE assay media: (DMEM/F12(r) (Sigma, Cat # D6434)/1×Pen/Strep/Glutamine (Gibco, Cat # 10378)/2% Heat Inactivated FBS (Gibco Cat # 10082)). NT = not tested; NA = not applicable. Data were analyzed using Microsoft Excel 2010. e Data were an average of outcomes from two separate lots of compound 2.

Figure 2. Activity of quinazolinones and azaquinazolinediones.

Table 5. hRSV CPE Assay Potency, Cytotoxicity, Selectivity Index, Logarithmic Reduction in Viral Plaques, and Solubility Assessments for Analogs with Tandem Structural Variations in the (R1) and (R2) Regions of Hit Compound 2

| entry | cmpd | R1 | R2 | RSV CPE potency ± standard deviation (EC50 (μM)) | HEP-2 cellular toxicity ± standard deviation (CC50 (μM)) | selectivity index (CC50/EC50) | viral titer reduction at 10 μM (log) | solubility (μM) |
|-------|------|----|----|---------------------------------------------|----------------|----------------|-------------------------------|----------------|
| 1     | 2    | CH2-4-methylphenyl | NH(CH2)3OCH3 | 2.1 ± 0.5 | >50.0 | >23.8 | 4.2 | 0.1 | 2.5 |
| 2     | 43   | CH2-4-nitrophenyl  | NHCH2-3-oxetane | 1.3 ± 0.5 | >50.0 | >38.5 | >6.2 | 1.4 | 7.2 |
| 3     | 44   | CH2-4-iso-propylphenyl | NHCH2-3-oxetane | 0.4 ± 0.1 | 3.6 ± 0.8 | 9.0 | 5.8 | NT | NT |
| 4     | 45   | CH2-4-CO2H-phenyl  | NHCH2-3-oxetane | >50.0 | >50.0 | NA | NT | 98.1 | NT |
| 5     | 46   | CH2-4-N(CH3)2-phenyl | NHCH2-3-oxetane | 1.1 ± 0.2 | 44.7 ± 7.2 | 41.8 | >6.2 | 11.2 | 18.5 |
| 6     | 47   | CH2-4-tert-butylphenyl | NHCH2-3-oxetane | 1.6 ± 0.8 | 3.7 ± 0.3 | 2.3 | 3.4 | NT | NT |
| 7     | 48   | CH2-4-iso-propylphenyl | NHCH2-2-furyl | 1.2 ± 0.1 | >50.0 | >41.7 | 2.8 | 0.3 | NT |
| 8     | 49   | CH2-4-chlorophenyl  | NHCH2-3-oxetane | 0.8 ± 0.1 | >50.0 | >62.5 | NT | NT | 10.2 |
| 9     | 50   | CH2-4-iso-propylphenyl | NH-2-CH2-O-pyridyl | 0.5 ± 0.6 | <1.6 | <3.3 | NT | NT | NT |

a Data were an average of ≥3 experiments. b Data are an average of ≥2 experiments. c Kinetic solubility in 1×PBS, pH 7.4. d Kinetic solubility in CPE assay media: (DMEM/F12(r) (Sigma, Cat # D6434)/1×Pen/Strep/Glutamine (Gibco, Cat # 10378)/2% Heat Inactivated FBS (Gibco Cat # 10082)). NT = not tested; NA = not applicable. Data were analyzed using Microsoft Excel 2010. e Data were an average of outcomes from two separate lots of compound 2.
Table 4. Comparative SAR, Physicochemical, and In Vitro ADME Data for Select Analogs

| entry | analog | RSV CPE potency (μM) | HEp2 cellular toxicity (μM) | selectivity (CC50/EC50) | viral titer reduction (μM) | solubility (μM) | aqueous stability (%) | 1:1 PBS/ACN permeability (×10⁻⁶ cm/s) | hepatocyte toxicity LC50 (μM) |
|-------|--------|----------------------|---------------------------|-------------------------|---------------------------|-----------------|----------------------|---------------------------------|--------------------------|
| 1 | 2 | 2.1 ± 0.5 | >50.0 | 23.8 | 4.2 | 0.1 NT | NT | NT | NT |
| 2 | 15 | 0.5 ± 0.05 | >50.0 | >100 | 5.6 | 1.3 | 2.8 | 83.4 | 94.9 | 364/349/356 >50 |
| 3 | 19 | 0.3 ± 0.03 | >50.0 | >166.7 | 6.7 | 0.4 | 10.2 | 27.0 | 100 | 718/631/703 >30 |
| 4 | 46 | 1.1 ± 0.2 | 44.7 ± 7.2 | 41.8 | >6.2 | 11.2 | 18.5 | 90.2 | 100 | 780/686/614 >50 |

aData were an average of ≥3 experiments. bData were an average of outcomes from three separate lots of compound 15. cData were an average of outcomes from two separate lots of compound 19. dData were obtained from a 3-day exposure experiment versus 5-day duration due to precipitation of compound after 3 days. eData were an average of outcomes from two separate lots of compound 15. fData collected by Ms. Arianna Mangravita-Novos at the Conrad Prebys Sanford Burnham Medical Research Institute. gKinetic solubility in CPE assay media: (DMEM/F12(3) (Sigma, Cat # D6434)/1X Pen/Strep/Glutamine (Gibco, Cat # 10378)/2% Heat Inactivated FBS (Gibco Cat # 10082)). hData collected by Mr. Patrick Porubsky at the University of Kansas Analytical Chemistry Core, Specialized Chemistry Center. iResults are represented as percent remaining after 48 h; Stability assessment was done independently in PBS or with 1:1 PBS and acetonitrile; the latter was used to account for limitations in solubility affecting PBS results. jPAMPA donor pH: 5.0/6.2/7.4, acceptor pH: 7.4; controls: verapamil (222/197/1936—highly permeable), metoprolol (46/472—moderately permeable), ranitidine (<10/<10/<10—poorly permeable). kPAMPA done in PBS, no additives. lPAMPA done with 20% acetonitrile added to compensate for PBS solubility. mWithout acetonitrile, Pe was insignificant at each pH. nFa2N-4 immortalized human hepatocytes. Data were analyzed using Microsoft Excel 2010; NT = not tested.

Table 5. Off Target Profiling Results for Compound 19 at 10 μM (>50% Inhibition)

| entry | biological target | Eurofins Panlabs assay code | species | percent inhibition (%) |
|-------|-------------------|-----------------------------|---------|------------------------|
| 1 | calcium channel, L-type, benzothiazepine | 214510 | rat | 52 |
| 2 | calcium channel, L-type, dihydropyridine | 214600 | rat | 70 |
| 3 | cannabinoid CB1 | 217030 | human | 86 |
| 4 | serotonin | 217100 | human | 54 |
| 5 | (5-hydoxytryptamine) 5-HT2b | 204410 | human | 54 |

aDetailed assay descriptions can be found at https://www.eurofinspanlabs.com/catalog. bA full list of targets and percent inhibition by compound 19 is provided in the Supporting Information.

is provided in the Supporting Information. The liability posed by significant inhibition of any given host target depends on a multitude of factors that includes but is not limited to potency, metabolism, and physiological compartmental exposure (e.g., CNS). These results, while not negligible, were considered informational at this stage of development, as pursuit of individual IC50 values and bioavailability data was cost-prohibitive and revision of the scaffold architecture was expected prior to finding a suitable tool for in vivo assessment. Nonetheless, the profiling outcome served as a useful alert to potential adverse effects associated with the series that will need to be surveyed as development continues toward an advanced lead candidate.

Mechanism of Action Studies. To gain an understanding of how this quinazolinedione class of compounds was inhibiting RSV, a cell-based, time-of-addition study was initially performed to determine the ability of these compounds to inhibit different stages of a single round of viral replication. The assay was performed by infecting cells with RSV and adding compound at a 10 μM concentration at each of several time points up to 24 h postinfection and then tracking cell viability over the course of the experiment. Compounds 15, 19, and 46 were evaluated as a panel alongside ribavirin. While ribavirin treatment protected cells from RSV-induced CPE (approximately 100%) for up to 7 h postinfection, indicating that it targets the period of infection during which viral replication is in progress, none of the three quinazolinediones showed dramatic changes in cell viability between 7 and 24 h postinfection, suggesting that these compounds acted at a later stage of the viral life cycle (data not shown). This time-of-addition profile suggested possible inhibition of the viral RNA-dependent RNA-polymerase (RdRp) activity rather than interference with receptor binding and/or membrane fusion. To test this hypothesis experimentally, compounds 15, 19, and 46 were subjected, along with a known literature-described RdRp inhibitor 55,18 to a plasmid-based RSV minigenome reporter assay that specifically monitored bioactivity of the viral polymerase machinery (Figure 3). 38 Luciferase reporter interference was tested by comparing the activity of 19 in assays employing recRSV-luciferase or the equivalent measles virus recombinant (recMeV-luciferase), both of which rely on luciferase activity as the assay readout. While recRSV-luciferase was efficiently inhibited, compound 19 was inactive against recMeV-luciferase, excluding direct reporter interference. All four analogs showed a dose-dependent inhibition of reporter expression with active concentrations similar to those observed against live virus, indicating that the compounds blocked RSV RdRp activity.

At this point the exact target with which these compounds interact leading to the observed block of RdRp activity is unknown, but sequencing of RSV-resistant mutant viruses resulting from compound treated cells is currently underway. These studies are expected to pinpoint the location of mutation within the genome that may be responsible for resistance and identify a potential target to investigate further. Attempts to unravel the nature of inhibition are being actively pursued.

CONCLUSION

In summary, we have identified a quinazolinedione class of compounds that show promising cellular activity against RSV. While the SAR for this set of quinazolinediones was relatively limited in texture, we have discovered distinct scaffold regions that tolerate structural change and permit tuning of physicochemical properties and whose modification has led to
a 7-fold improvement in selectivity over the hit compound 2. Several compounds in the series inhibited a virus-induced cytopathic effect in the submicromolar range without exhibiting significant cellular toxicity, and importantly, these same compounds also significantly reduced viral plaque formation at a concentration of 10 μM. Furthermore, the mechanism of action for a subset of quinazolinedione analogs was explored and demonstrated to block the activity of the viral RNA-dependent RNA–polymerase complex which is responsible for RSV genome replication and transcription. These results, combined with the insights from structural modifications of the quinazolinedione scaffold and in vitro ADME data of select analogs, suggest that a favorable pharmacological profile can be tuned to produce a lead antiviral compound suitable for in vivo efficacy assessment against RSV. As such, the current set of compounds will be useful tools in establishing baseline pharmacokinetic parameters and further investigating how these agents interact with the RdRp complex to inhibit viral replication.

### EXPERIMENTAL SECTION

**Chemistry.** The purity of all final compounds was >95% and was confirmed by HPLC/MS analysis employing an Agilent 1200 RRL chromatograph with photodiode array UV detection and an Agilent 6224 TOF mass spectrometer. The chromatographic method utilized a Waters Acquity BEH C-18 2.1 × 50 mm, 1.7 μm column; UV detection wavelength = 214 nm; flow rate = 0.4 mL/min; gradient = 5−100% acetonitrile over 3 min with a hold of 0.8 min at 100% acetonitrile; the aqueous mobile phase contained 0.15% ammonium hydroxide (v/v). The mass spectrometer utilized the following parameters: an Agilent multimode source which simultaneously acquires ESI+/APCI+; a reference mass solution consisting of purine and hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazene; and a make-up solvent of 90:10:0.1 MeOH:water:formic acid which was introduced to the LC flow prior to the source to assist ionization. [1H and 13C NMR spectra were recorded on a Bruker AM 400 spectrometer (operating at 400 and 101 MHz, respectively) or a Bruker AVIII spectrometer (operating at 500 and 126 MHz, respectively) in CDCl3 with 0.03% TMS as an internal standard or DMSO-d6. The chemical shifts (δ) reported are given in parts per million (ppm) and the coupling constants (J) are in Hertz (Hz). The spin multiplicities are reported as s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, hept = heptet, and m = multiplet. Melting points were determined on a Stanford Research Systems OptiMelt apparatus. Compounds were generally prepared according to Scheme 1 and the protocols detailed for compound 19, below, unless otherwise specified.

**Synthesis of 4-((1-(4-isopropylbenzyl)-2,4-dioxo-1,2-dihydroquinoxalin-3(4H)-yl)(methyl)-N-(3-methoxypropyl)benzamide (19).**

**Step 1.** To a solution of 2-aminobenzoic acid 3 (5.00 g, 36.50 mmol) in DMF (45 mL) was added methyl 4-(aminomethyl)benzoate hydrochloride (7.35 g, 36.55 mmol), HATU (15.25 g, 40.10 mmol) and N,N-diisopropylethylamine (18.08 mL, 109 mmol). The reaction mixture was stirred for 16 h at room temperature, then diluted with CH2Cl2 (200 mL) and washed sequentially with 1 M HCl (150 mL), sat. aqueous NaHCO3 (150 mL) and water (2 × 800 mL). The separated organic extract was dried (MgSO4), filtered, and concentrated under reduced pressure to afford a crude product which was purified by silica gel flash column chromatography (0–60% v/v EtOAc/Hexane), yielding the product, methyl 4-((2-aminobenzamido)(methyl)benzoate, as a white solid (5.00 g, 17.59 mmol, 48% yield). [1H NMR (400 MHz, CDCl3): δ 8.01 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.35 (dd, J = 7.9, 1.4 Hz, 1H), 7.25–7.19 (m, 1H), 6.70 (dd, J = 8.3, 0.9 Hz, 1H), 6.67–6.61 (m, 1H), 6.43 (broad s, 1H), 5.56 (broad s, 2H), 4.66 (d, J = 5.9 Hz, 2H), 3.91 (s, 3H).**

**Step 2.** After stirring a solution of methyl 4-((2-aminobenzamido)(methyl)benzoate (5.00 g, 17.59 mmol) and N,N-diisopropylethylamine (14.53 mL, 88 mmol) in CH2Cl2 (260 mL) for 10 min at room temperature under nitrogen, 1,1-carbonyldimidazole (8.55 g, 52.80 mmol) was added, and the reaction mixture was heated 16 h at reflux. The formed precipitate was filtered, dried under vacuum and the desired product, methyl 4-((2,4-dioxo-1,2-dihydroquinoxalin-3(4H)-yl)(methyl)benzoate 4, was furnished as a white solid without further purification (4.65 g, 14.99 mmol, 85% yield). [1H NMR (400 MHz,
DMso-d$_6$): $\delta$ 11.58 (s, 1H), 7.94 (dd, $J = 8.3, 1.4$ Hz, 1H), 7.90 (d, $J = 8.4$ Hz, 2H), 7.72–7.64 (m, 1H), 7.43 (d, $J = 8.4$ Hz, 2H), 7.26–7.18 (m, 2H), 5.15 (s, 2H), 3.83 (s, 3H).

**Step 3.** To a solution of methyl 4-((2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)ethyl)benzoate (2.60 g, 8.38 mmol) in THF (50 mL) was added 1 M lithium hydroxide (50 mL, 50.3 mmol). The reaction mixture was stirred at 40 °C for 1 h, at which point TLC confirmed reaction completion. Then 1 M HCl was cautiously added until the reaction mixture reached pH 2, at which point the product precipitated out of solution. The precipitate was collected by filtration, washed with water (2 × 70 mL), dried under high vacuum to afford the desired product, 4-((2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)ethyl)-N-(3-methoxypropyl)benzamide (3), as a white solid (2.19 g, 7.39 mmol, 88% yield). $^1$H NMR (400 MHz, DMso-d$_6$): $\delta$ 12.90 (broad s, 1H), 11.58 (s, 1H), 7.95 (d, $J = 7.3$ Hz, 1H), 7.88 (d, $J = 8.3$ Hz, 2H), 7.74–7.65 (m, 1H), 7.40 (d, $J = 8.3$ Hz, 2H), 7.28–7.18 (m, 2H), 5.15 (s, 2H).

**Step 4.** To a solution of 4-((2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)ethyl)benzoic acid (1.00 g, 3.38 mmol) in DMF (15 mL) was added 3-methoxypropylamine (0.35 mL, 3.38 mmol), HATU (1.41 g, 3.71 mmol) and N,N-diisopropylethylamine (1.67 mL, 10.13 mmol). The reaction mixture was stirred for 16 h at room temperature, then diluted with CHCl$_3$ (90 mL) and washed sequentially with 1 M HCl (60 mL), sat. aqueous NaHCO$_3$ (60 mL) and water (2 × 180 mL). The organic extract was separated, dried (MgSO$_4$), filtered, and concentrated under reduced pressure to afford a crude product which was purified by silica gel flash column chromatography (0–5% v/v MeOH/CHCl$_3$) yielding the desired product, 4-((2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)ethyl)-N-(3-methoxypropyl)benzamide (3), as a white solid (0.91 g, 2.48 mmol, 73% yield), mp 244–246 °C. $^1$H NMR (400 MHz, DMso-d$_6$): $\delta$ 11.56 (s, 1H), 8.40 (t, $J = 5.6$ Hz, 1H), 7.95 (d, $J = 7.3$ Hz, 1H), 7.76 (d, $J = 8.4$ Hz, 2H), 7.71–7.63 (m, 1H), 7.36 (d, $J = 8.4$ Hz, 2H), 7.26–7.17 (m, 2H), 5.13 (s, 2H), 3.35 (t, $J = 6.3$ Hz, 2H), 3.31–3.24 (m, 2H), 3.22 (s, 3H), 1.78–1.67 (m, 2H).

**Step 5.** To a solution of 4-((2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)ethyl)-N-(3-methoxypropyl)benzamide (0.050 g, 0.14 mmol) in DMF (2 mL) was added 4-isopropylbenzyl bromide (0.028 mL, 0.16 mmol) and potassium carbonate (0.056 g, 0.41 mmol). The resulting reaction mixture was stirred at 40 °C for 16 h. The formed residue was dissolved in CHCl$_3$ (6 mL) and sequentially washed several times with 1 M HCl (4 mL), water (3 × 20 mL) and brine (8 mL). The organic layer was separated, dried (MgSO$_4$), filtered, and concentrated under reduced pressure to give a crude product which was purified by silica gel flash column chromatography (0–5% v/v MeOH/CHCl$_3$) yielding 4-((1-(4-Chlorobenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)-N-(3-methoxypropyl)benzamide (3), as a white solid (0.030 g, 0.060 mmol, 44% yield), mp 178–180 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.24 (dd, $J = 7.9, 1.6$ Hz, 1H), 7.74–7.68 (m, 2H), 7.61–7.52 (m, 3H), 7.25–7.20 (m, 1H), 7.20–7.12 (m, 1H), 6.89 (t, $J = 5.3$ Hz, 1H), 5.36 (s, 2H), 5.33 (s, 2H), 3.59–3.52 (m, 4H), 3.37 (s, 3H), 2.87 (l, $J = 6.9$ Hz, 1H), 1.91–1.83 (m, 2H), 1.21 (d, $J = 6.9$ Hz, 6H). $^1$C NMR (126 MHz, CDCl$_3$): $\delta$ 167.04, 161.92, 130.16, 135.40, 134.15, 132.89, 129.25, 129.11, 129.17, 127.17, 126.57, 123.29, 115.74, 114.69, 72.59, 59.10, 47.36, 44.94, 39.31, 33.89, 28.93, 24.06. LCMS Retention time: 3.422 min. LCMS purity 99.8%. HRMS (ESI): m/z calcd for C$_{24}$H$_{22}$ClN$_2$O$_2$ [M + H]$^+$ 505.1396, found 505.1396.

**N-(3-Methoxypropyl)-4-((1-(4-methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)ethyl)benzamide (2).** Isolated as a white solid (36 mg, 56% yield), mp 197–199 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.23 (dd, $J = 7.9, 1.6$ Hz, 1H), 7.74–7.68 (m, 2H), 7.60–7.51 (m, 1H), 7.22 (ddd, $J = 8.0, 7.3, 0.9$ Hz, 1H), 7.16–7.09 (m, 9H), 6.89 (t, $J = 5.2$ Hz, 1H), 5.36 (s, 2H), 5.33 (s, 2H), 3.59–3.52 (m, 4H), 3.37 (s, 3H), 2.31 (s, 3H), 1.91–1.83 (m, 2H). $^1$C NMR (126 MHz, CDCl$_3$): $\delta$ 167.01, 161.92, 151.49, 140.41, 140.11, 137.59, 135.38, 134.16, 132.59, 129.80, 129.26, 129.11, 127.17, 126.54, 123.30, 115.76, 114.64, 72.58, 59.09, 47.37, 44.95, 39.31, 28.95, 24.13. LCMS Retention time: 1.98 min. LCMS purity 97.8%. HRMS (ESI): m/z calcd for C$_{21}$H$_{17}$N$_2$O$_2$ [M + H]$^+$ 472.2158, found 472.2241.
2H), 5.35 (s, 2H), 3.62 (m, 1H), 6.98 (d, 7.61 (m, 2H), 7.61 (s, 1H), 5.46 (s, 2H), 3.56 (s, 2H), 3.60–3.53 (m, 4H), 3.38 (s, 3H), 1.91–1.84 (m, 2H).

13C NMR (126 MHz, CDCl3): δ 166.99, 161.61, 140.11, 130.58, 134.14, 132.78, 129.12, 128.60, 127.28, 126.87, 115.17, 114.55, 112.68, 57.12, 28.94, 28.39, 28.15. LCMS Retention time: 2.97 min. LCMS purity 98.8%. HRMS (ESI): m/z calc for C23H18N3O4 [M + H]+ 499.1465, found 499.1459.

2-(4-(Dioxa-1,2-dihydroquinazolin-3(4H)-yl)ethyl)methyl)-N-(3-methoxypropyl)benzamide (15). Isolated as white solid (29 mg, 56% yield), mp 170–173 °C. 1H NMR (500 MHz, CDCl3): δ 8.29 (dd, J = 7.9, 1.6 Hz, 1H), 7.75–7.69 (m, 2H), 7.66–7.53 (m, 5H), 7.34 (d, J = 8.3 Hz, 2H), 7.30–7.24 (m, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.91 (t, J = 5.3 Hz, 1H), 5.46 (s, 2H), 3.56 (s, 2H), 3.60–3.53 (m, 4H), 3.38 (s, 3H), 1.91–1.84 (m, 2H).

13C NMR (126 MHz, CDCl3): δ 166.99, 161.61, 140.11, 130.58, 134.14, 132.78, 129.12, 128.60, 127.17, 126.58, 123.28, 115.74, 114.66, 72.56, 59.08, 47.36, 44.93, 39.29, 28.95, 28.59, 15.61. LCMS Retention time: 3.32 min. LCMS purity 99.1%. HRMS (ESI): m/z calc for C23H18N3O4 [M + H]+ 499.1465, found 498.2180, 498.2180, 488.2180.

2-(4-(Dioxa-1,2-dihydroquinazolin-3(4H)-yl)ethyl)methyl)benzamide (15). Isolated as white solid (29 mg, 56% yield), mp 170–173 °C. 1H NMR (500 MHz, CDCl3): δ 6.42 (s, 1H), 3.96 (m, 2H), 3.56 (s, 2H), 1.92–1.84 (m, 2H).

13C NMR (126 MHz, CDCl3): δ 166.81, 161.61, 140.79, 134.77, 129.74, 129.21, 127.39, 127.23, 124.7, 118.85, 113.97, 72.65, 59.11, 47.12, 45.07, 39.38, 28.94. LCMS Retention time: 2.973 min. LCMS purity 97.8%. HRMS (ESI): m/z calc for C23H18N3O4 [M + H]+ 503.1925, found 503.1951.

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a white solid (12 mg, 22% yield), mp 185–187 °C. 1H NMR (500 MHz, CDCl3): δ 8.44 (t, J = 4.8 Hz, 1H), 8.24 (dd, J = 7.9, 1.6 Hz, 1H), 7.76–7.69 (m, 2H), 7.61–7.51 (m, 3H), 7.22 (dd, J = 8.1, 1.7 Hz, 0.9 Hz), 7.17–7.09 (m, 9H), 5.36 (s, 2H), 5.33 (s, 2H), 5.38–3.51 (m, 2H), 2.52–2.46 (m, 2H), 2.31 (s, 3H), 2.28 (s, 6H), 1.80–1.70 (m, 2H). 13C NMR (126 MHz, CDCl3): δ 166.75, 161.93, 151.49, 140.19, 131.17, 135.57, 134.16, 132.59, 129.79, 129.26, 129.06, 127.18, 126.55, 123.29, 115.77, 114.63, 59.64, 47.36, 45.62, 44.95, 40.87, 25.31, 21.23. LCMS Retention time: 3.18 min. LCMS purity 98.7%. HRMS (ESI): m/z calc for C16H12N2O2 [M + H]+ 248.2547, found 248.2545.

N-(4-Methoxybutyl)-2-(4-(1-(4-methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-ylyl)benzyl)(dimethyl)amine (38). Isolated as a white solid (15 mg, 29% yield), mp 171–173 °C. 1H NMR (500 MHz, CDCl3): δ 8.23 (dd, J = 7.9, 1.6 Hz, 1H), 7.60–7.51 (m, 3H), 3.73–3.72 (m, 2H), 7.21 (dd, J = 8.0, 7.3, 0.9 Hz, 1H), 7.16–7.10 (m, 5H), 5.39–5.30 (m, 4H), 3.69 (apparent broad s, 2H), 3.32 (apparent broad s, 2H), 2.31 (s, 3H), 1.72–1.57 (m, 4H), 4.81 (apparent broad s, 2H). 13C NMR (126 MHz, CDCl3): δ 167.20, 161.89, 141.59, 140.09, 138.39, 137.55, 135.91, 135.31, 132.61, 129.78, 129.21, 129.17, 127.14, 126.55, 125.78, 114.61, 48.88, 43.94, 42.36, 26.59, 25.73, 21.23. LCMS Retention time: 3.41 min. LCMS purity 96.7%. HRMS (ESI): m/z calc for C30H28N2O2 [M + H]+ 468.2282, found 468.2235.

N-Methyl-4-(1-(4-methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)benzyl)malononitrile (39). Isolated as a white solid (15 mg, 29% yield), mp 171–173 °C. 1H NMR (500 MHz, CDCl3): δ 8.23 (dd, J = 7.9, 1.6 Hz, 1H), 7.73–7.68 (m, 2H), 7.60–7.51 (m, 3H), 7.22 (dd, J = 8.0, 7.3, 0.9 Hz, 1H), 7.16–7.10 (m, 5H), 5.39–5.30 (m, 4H), 3.69 (apparent broad s, 2H), 3.32 (apparent broad s, 2H), 2.31 (s, 3H), 1.72–1.57 (m, 4H), 4.81 (apparent broad s, 2H). 13C NMR (126 MHz, CDCl3): δ 167.20, 161.89, 141.59, 140.09, 138.39, 137.55, 135.91, 135.31, 132.61, 129.78, 129.21, 129.17, 127.14, 126.55, 125.78, 114.61, 48.88, 43.94, 42.36, 26.59, 25.73, 21.23. LCMS Retention time: 3.41 min. LCMS purity 96.7%. HRMS (ESI): m/z calc for C30H28N2O2 [M + H]+ 468.2282, found 468.2235.

N-(tert-Butyl)-4-((1-(4-methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)benzyl)malononitrile (39). Isolated as a white solid (15 mg, 29% yield), mp 171–173 °C. 1H NMR (500 MHz, CDCl3): δ 8.23 (dd, J = 7.9, 1.6 Hz, 1H), 7.73–7.68 (m, 2H), 7.60–7.51 (m, 3H), 7.22 (dd, J = 8.0, 7.3, 0.9 Hz, 1H), 7.16–7.10 (m, 5H), 5.39–5.30 (m, 4H), 3.69 (apparent broad s, 2H), 3.32 (apparent broad s, 2H), 2.31 (s, 3H), 1.72–1.57 (m, 4H), 4.81 (apparent broad s, 2H). 13C NMR (126 MHz, CDCl3): δ 167.20, 161.89, 141.59, 140.09, 138.39, 137.55, 135.91, 135.31, 132.61, 129.78, 129.21, 129.17, 127.14, 126.55, 125.78, 114.61, 48.88, 43.94, 42.36, 26.59, 25.73, 21.23. LCMS Retention time: 3.41 min. LCMS purity 96.7%. HRMS (ESI): m/z calc for C30H28N2O2 [M + H]+ 468.2282, found 468.2235.
N-Benzyl-4-((1-(4-methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)methyl)-N-(oxetan-3-ylmethyl)benzamide (39). Isolated as a white solid (18 mg, 30% yield) mp 247–249 °C. 1H NMR (500 MHz, CDCl3): δ 8.24 (dd, J = 7.9, 1.7 Hz, 1H), 7.86–7.77 (m, 3H), 7.63 (d, J = 8.4 Hz, 4H), 7.55 (dd, J = 8.7, 1.3, 1.7 Hz, 1H), 7.40–7.32 (m, 2H), 7.22 (apparent t, J = 7.2 Hz, 1H), 7.18–7.09 (m, 6H), 5.38 (s, 2H), 5.34 (s, 2H), 2.31 (s, 3H). 13C NMR (126 MHz, CDCl3): δ 165.57, 161.92, 151.49, 141.12, 140.11, 138.06, 137.62, 135.44, 133.49, 132.54, 129.81, 129.34, 129.26, 129.22, 127.36, 126.53, 124.65, 126.33, 120.23, 115.72, 114.68, 47.39, 44.49, 21.23. LCMS Retention time: 3.456 min. LCMS purity 98.9%. HRMS (ESI): m/z calc for C38H29N3O4 [M + H]+ 529.2125, found 529.2124.

N-Furan-2-ylmethyl-4-((1-(4-methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)methyl)benzamide (40). Isolated as a white solid (29 mg, 54% yield) mp 219–220 °C. 1H NMR (500 MHz, CDCl3): δ 8.22 (dd, J = 7.9, 1.6 Hz, 1H), 7.78–7.72 (m, 2H), 7.61–7.56 (m, 2H), 7.54 (dd, J = 8.7, 1.3, 1.7 Hz, 1H), 7.37–7.31 (m, 4H), 7.31–7.27 (m, 1H), 7.21 (dd, J = 8.0, 3.9, 0.9 Hz, 1H), 7.16–7.09 (m, 6H), 6.99 (t, J = 5.7 Hz, 1H), 5.35 (s, 2H), 5.32 (s, 2H), 4.63 (d, J = 5.7 Hz, 2H), 2.31 (s, 3H). 13C NMR (126 MHz, CDCl3): δ 167.13, 163.19, 151.47, 140.80, 140.09, 137.59, 135.39, 133.74, 132.56, 129.80, 129.25, 129.20, 128.91, 128.01, 127.74, 127.29, 115.33, 113.51, 111.73, 114.64, 47.37, 44.92, 44.23, 21.23. LCMS Retention time: 3.387 min. LCMS purity 100%. HRMS (ESI): m/z calc for C33H29N2O3S [M + H]+ 490.2125, found 490.2124.

4-((1-(4-Methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)-methyl)-(N-thiazol-2-yl)benzamide (41). Isolated as a colorless oil (8 mg, 17% yield). 1H NMR (500 MHz, CDCl3): δ 10.02 (s, 1H), 8.25 (dd, J = 7.9, 1.6 Hz, 1H), 7.95–7.88 (m, 2H), 7.70–7.64 (m, 2H), 7.56 (dd, J = 8.7, 3.3, 1.7 Hz, 1H), 7.39 (d, J = 3.6 Hz, 1H), 7.30–7.20 (m, 1H), 7.19–7.09 (m, 6H), 6.99 (d, J = 3.6 Hz, 1H), 5.41 (s, 2H), 5.35 (s, 2H), 2.31 (s, 3H). 13C NMR (126 MHz, CDCl3): δ 164.29, 161.92, 156.85, 151.49, 142.38, 140.13, 137.74, 137.67, 135.11, 135.50, 132.52, 131.37, 129.84, 129.53, 129.32, 128.72, 126.55, 123.41, 115.71, 114.70, 110.45, 47.41, 44.92, 21.24. LCMS Retention time: 3.278 min. LCMS purity 100%. HRMS (ESI): m/z calc for C28H23N2O3S [M + H]+ 483.1485, found 483.1482.

4-((1-(4-Methylbenzyl)-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)methyl)-(N-pyridin-4-yl)benzamide (42). Isolated as a colorless oil (7 mg, 14% yield). 1H NMR (500 MHz, DMSO-d6): δ 7.15 (s, 1H), 8.74 (d, J = 6.6 Hz, 2H), 8.30 (d, J = 6.9 Hz, 2H), 8.11 (d, J = 7.9, 1.7 Hz, 1H), 8.04–7.98 (m, 2H), 7.70 (dd, J = 8.7, 1.7, 1.7 Hz, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.36–7.27 (m, 2H), 7.21 (d, J = 8.9 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H), 5.36 (s, 2H), 5.31 (s, 2H), 2.62 (s, 3H). 13C NMR (126 MHz, DMSO): δ 167.07, 161.22, 150.88, 142.80, 142.55, 139.74, 136.48, 135.50, 133.13, 131.96, 129.29, 128.62, 128.14, 127.39, 126.48, 123.12, 115.24, 115.07, 115.04, 106.96, 46.23, 44.41, 20.66. LCMS Retention time: 3.159 min. LCMS purity 98%. HRMS (ESI): m/z calc for C32H21N2O4S [M + H]+ 477.1921, found 477.1917.

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NaHCO₃, and water. The organic extract was separated, dried (MgSO₄), filtered, and concentrated under reduced pressure to afford a crude product which was purified by silica gel flash column chromatography (0–5% v/v MeOH/CH₂Cl₂) yielding 46 as a white solid (33 mg, 36% yield). ¹H NMR (500 MHz, CDCl₃): δ 8.21 (d, J = 7.9, 1.6 Hz, 1H), 7.74–7.68 (m, 2H), 7.62–7.52 (m, 3H), 7.25–7.18 (m, 2H), 7.17–7.10 (m, 2H), 6.70–6.63 (m, 2H), 6.35 (s, J = 5.5, 1H), 5.36 (s, 2H), 5.27 (s, 2H), 4.82 (d, J = 7.7, 6.3 Hz, 2H), 4.46 (apparent t, J = 6.0 Hz, 2H), 3.75–3.71 (m, 2H), 3.34–3.21 (m, 3H), 2.91 (s, 3H). ¹C NMR (126 MHz, CDCl₃): δ 167.83, 161.97, 151.49, 150.18, 141.01, 140.23, 135.35, 133.58, 129.18, 129.15, 128.78, 127.22, 123.7, 123.02, 115.71, 114.79, 117.85, 75.20, 47.15, 44.88, 42.58, 40.64, 35.15. LCMS Retention time: 1.82 min. LCMS purity 100%. ¹H NMR (500 MHz, CDCl₃): δ 7.84 (s, 1H), 7.36 (d, J = 1.9, 0.9 Hz, 1H), 7.22 (d, J = 8.0, 7.2, 0.9 Hz, 1H), 7.10–7.02 (m, 3H), 6.43 (t, J = 5.5 Hz, 1H), 6.32 (d, J = 3.2, 1.9 Hz, 1H), 6.28 (d, J = 3.1 Hz, 1H), 5.35 (s, 2H), 5.33 (s, 2H), 4.62 (d, J = 5.5 Hz, 2H), 2.87 (h, J = 6.9 Hz, 1H), 1.21 (d, J = 6.9 Hz, 6H). ¹C NMR (126 MHz, CDCl₃): δ 167.00, 161.89, 151.45, 151.28, 148.54, 142.42, 140.85, 140.14, 135.41, 133.52, 132.87, 129.24, 129.17, 127.33, 127.18, 126.55, 123.30, 115.70, 114.69, 110.63, 107.79, 47.35, 44.91, 37.12, 33.88, 24.65. LCMS Retention time: 3.35 min. LCMS purity 97.8%. HRMS (ESI): m/z calcd for C₂₄H₂₂N₂O₃ [M + H]⁺ 512.2527, found 512.2527.

- **Step 2: Synthesis of ethyl-2-(3-prop-1-en-2-yl)phenylacetate.** A solution of methyl-4-((2-(3-prop-1-en-2-yl)phenyl)acetate (273 mg, 1.34 mmol) in EtOH (9 mL) was added to the mixture at rt for 2 hrs. The reaction mixture was concentrated and the product was purified by flash chromatography (0–15% EtOAc/hexanes) to give the title compound (230 mg, 89%) as a clear, pale-yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 7.35–7.26 (m, 1H), 7.34 (dt, J = 7.7, 1.5 Hz, 1H), 7.25 (s, J = 7.6 Hz, 1H), 7.17 (dt, J = 7.5, 1.4 Hz, 1H), 5.35 (dq, J = 1.5, 1.3 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.58 (s, 2H), 2.12 (dd, J = 1.5, 0.8 Hz, 3H), 1.72 (t, J = 7.1 Hz, 3H)

- **Step 4: Synthesis of 2-(3-isopropylphenyl)acetic acid.** A solution of LiOH (217 mg, 9.1 mmol) in water (5 mL) was added to a solution of ethyl-2-(3-prop-1-en-2-yl)phenylacetate (267 mg, 1.33 mmol) in THF (9 mL). The mixture was stirred at rt for 45 h. The reaction mixture was quenched with 1 M aq. HCl (20 mL). The layers were separated and the aq. layer was extracted with CH₂Cl₂ (2 × 20 mL). The combined organic extracts were dried with Na₂SO₄ to give the title compound (234 mg, 100%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 7.17 (s, 1H), 7.05–7.02 (m, 3H), 7.00–6.97 (m, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.56 (s, 2H), 2.87 (hept, J = 6.9 Hz, 1H), 1.26–1.18 (m, 9H), 0.84–0.73 (m, 3H).
4-(2-(3-isopropylbenzyl)-4-oxoquinazolin-3(4H)-yl)methyl)benzoic acid (113 mg, 0.27 mmol) and 4-(2-(3-isopropylphenyl)-acetamido)benzamido)methyl)benzoic acid was dissolved in dry CH2Cl2 (10 mL) and to this solution 3-methoxypropylamine (0.28 mL, 2.4 mmol) and 4-((2-(4-isopropylphenyl)acetamido)benzamido)methyl)benzoate was added. The mixture was stirred at rt for 30 min and then concentrated. The remaining residue was purified by flash chromatography (0–100% EtOAc/hexanes). Single-spot fractions (by TLC) were combined and concentrated to give the title compound (13 mg, 10%) as a yellow solid.1H NMR (400 MHz, CDCl3) δ 8.34 (dd, J = 8.5, 1.2 Hz, 1H), 7.97 (td, J = 7.7, 1.2 Hz, 2H), 7.31 (d, J = 8.5 Hz, 1H), 7.03–7.07 (m, 1H), 6.88 (t, J = 8.5 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 4.06 (s, 2H), 3.56 (d, J = 6.9 Hz, 2H), 3.44 (s, 3H), 2.87 (hept, J = 6.9 Hz, 6H), 1.84 (m, 2H).13C NMR (126 MHz, CDCl3) δ 166.7, 162.5, 155.9, 150.2, 146.8, 139.3, 135.0, 134.6, 134.4, 129.4, 127.7, 127.9, 127.5, 126.5, 126.4, 125.7, 125.5, 120.4, 77.5, 59.1, 46.3, 24.3, 39.5, 34.2, 28.9, 24.1. LC-MS: R′ = 3.40 min, purity = 100%. HRMS (m/z): calcd for C30H34N3O3 (M + H)+ 484.2595; found 484.2593.

**Notes:** Providing accurate technical details is critical for reproducibility in scientific research. The authors appreciate your commitment to this practice.
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### Abbreviations

ACN, acetonitrile; ADME, absorption, distribution, metabolism, and excretion; CC50, concentration that reduced the cell viability by 50% when compared to untreated controls; CDI, carbonyldimidazole; CPE, cytopathic effect; DIPSA, N,N-disopropylethylamine; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; GPCR, G-protein coupled receptor; hRSV, human respiratory syncytial virus; MOI, multiplicity of infection; PAMPA, parallel artificial membrane permeability assay; PBS, phosphate buffered saline; RdRp, RNA-dependent RNA-polymerase; SAR, structure–activity relationship; SI, selectivity index; SPR, structure–property relationship

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