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Quinazolinone Compounds Have Potent Antiviral Activity against Zika and Dengue Virus

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Abstract

Dengue (DENV) and Zika (ZIKV) virus are important human pathogens, causing ~100 million symptomatic infections each year. These infections carry 20-fold increased incidence of serious neurological diseases, such as microcephaly in newborns (for ZIKV) and Guillain-Barré syndrome. Moreover, DENV can develop serious and possibly life-threatening dengue hemorrhagic fever in certain patients. Patients recovered from one of the 4 serotypes of DENV are still susceptible to other serotypes with a higher likelihood of serious disease because of antibody-dependent enhancement. Except for mosquito control, there have been no antiviral drugs to prevent and treat ZIKV/DENV infections. Phenotypic screening found 2,3,6-trisubstituted quinazolinone compounds are novel inhibitors of ZIKV replication. Fifty-four analogs were synthesized and their structure-activity relationships are discussed. Additional testing shows that compounds **22**, **27** and **47** exhibited broad and potent activities against ZIKV and DENV with EC_{50} values as low as 86 nM with no significant cytotoxicity to mammalian cells.

Graphical Abstract

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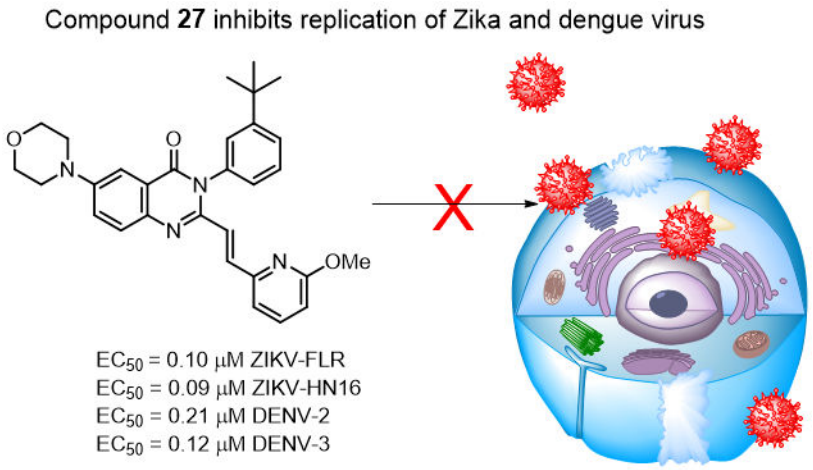
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supporting Information

Figure S1, HPLC tracers of selected most active compounds, and Molecular Formula Strings for all compounds are available free of charge via the Internet at http://pubs.acs.org.



Keywords

Zika virus; Dengue virus; Structure-activity relationship; Antiviral agents

Introduction

Zika virus (ZIKV) and closely related dengue virus (DENV) belong to the genus *Flavivirus* of the virus family *Flaviviridae*, which also includes other major human pathogenic viruses such as yellow fever, Japanese encephalitis and West Nile viruses. ZIKV and DENV are transmitted by *Aedes* mosquitoes in the tropical and subtropical regions, where approximately 3 billion people or 40% of world population live and are at risk of these infections [1, 2].

ZIKV, discovered and isolated in 1947 [3], has caused three major outbreaks in the Yap Island (~7,000 cases in 2007), the French Polynesia (~28,000 cases in 2013) in the Pacific ocean, and Brazil and other American countries (2015–2016) with significantly broader impact and damages [4, 5]. Several millions of people in 48 Pan-American countries and territories have been infected, showing symptoms including fever, rashes and conjunctivitis. Although most people recovered in several days, ZIKV infection has been found to cause 20-fold increased incidence of serious neurological diseases, such as Guillain-Barré syndrome [6–8] and >4,000 cases of microcephaly (small brain/head) and other neurological defects in newborns [9–11]. The prognosis of these infants to have normal brain functions is low and their life expectancy is shorter. WHO announced ZIKV is a “Public Health Emergency”. Moreover, ZIKV can be transmitted through sex or body fluids even when infected people have no symptoms [12, 13]. This secondary transmission route renders it more difficult to contain ZIKV infection.

DENV has been a major human pathogen for the past several decades. It is estimated that DENV infects ~400 million people per year with 100 million developing symptoms including fever, headache, rash, conjunctivitis and pain in muscle and joints, which are usually self-healing in 3–10 days [1]. However, ~500,000 cases/year develop serious and possibly life-threatening dengue hemorrhagic fever and shock syndrome. Approximately

22,000 people (mostly children) die of the disease per year [2]. More problematic is that there are 4 serotypes of DENV (DENV-1–4) with significantly different genomes [14, 15]. Patients who have recovered from one DENV serotype are still susceptible to other DENV serotypes and there is an increased likelihood to develop a more serious disease [16], because of existing antibodies against the previous DENV serotype [14, 17].

Except for mosquito control, there have been no antiviral drugs to prevent and treat ZIKV and DENV infections. The only licensed DENV vaccine, Dengvaxia, has been found to have safety and efficacy concerns for DENV-naïve individuals in clinical trials [18]. A limited number of compounds have been reported to have potent antiviral activities against ZIKV or DENV [19–30]. Most of these compounds are natural products with complex chemical structures that are not amenable for medicinal chemistry optimization. Only a few compounds showed broad activities against both ZIKV and DENV [19–23]. There is therefore a pressing need to find effective antiviral agents against ZIKV and DENV. Here, we report discovery, synthesis and structure-activity relationships (SAR) of a series of tri-substituted quinazolinone compounds that exhibit potent antiviral activities against both ZIKV and DENV. Interestingly, a quinazolinone compound was reported to exhibit modest activities (16.7 μM) against Venezuelan equine encephalitis virus [31], which is a mosquito-borne Alphavirus causing flu-like symptoms in humans.

RESULTS and DISCUSSION

Discovery of novel anti-Zika compounds

A cell-based, phenotypic anti-ZIKV activity assay was developed. Rapid replication of ZIKV (FLR strain, Colombia, 2015 [32]) in Vero (monkey kidney cells that lack interferon-mediated antiviral defense [33]) cells causes significant cytopathic effects (CPE) and eventually cell lysis, which can be clearly observed under the microscope. However, a compound that inhibits ZIKV replication can protect cells from CPE and death. If needed, an MTT [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay may further quantitate the number of viable cells. 0.1 multiplicity of infection (MOI, the number of infectious viral particles per cell) of ZIKV was added to a monolayer of cells in 96-well plates with culturing media containing a compound at 10 μM in duplicate for 72h. Observing CPE followed by MTT assay enabled us to quickly screen compounds to identify anti-ZIKV compounds. Moreover, cytotoxic compounds (at 10 μM), showing similar phenotypes (CPE and/or cell death) in the assay, were also eliminated.

Next, activities of these hits to reduce the viral titers were further determined, using an end-point dilution assay as described in our previous publications [34–36]. Half-log (0.32×) serial dilutions of the supernatant containing newly generated ZIKV particles were added to Vero cells in quadruplicate. Upon incubation for ~5 days, ZIKV infection in each sample was determined with CPE. $TCID_{50}$ (tissue culture infective dose) was calculated based on the highest dilution in which ≥50% of the quadruplicate samples were infected with ZIKV. As compared to $TCID_{50}$ of the untreated samples, ability of a compound to reduce ZIKV replication can be determined.

Through screening of ~1,000 compounds in our in-house compound library, which were synthesized for medicinal chemistry studies targeting H3K4 demethylase LSD1, histone acetyltransferase p300 and transcription cofactor AF9/ENL [37–39], 2,3,6-trisubstituted quinazolinone compound **1** (Chart 1) was found to be a potent anti-ZIKV agent, which can inhibit the viral replication by 99.9% (3-log reduction) at 10 μM. Interestingly, di-substituted quinazolinone compounds **2-4** can also inhibit ZIKV replication by 68%–90% (0.5–1-log reduction). Known anti-ZIKV compounds chloroquine and SYC-1307 (developed by us) [34, 35] were used as positive controls and exhibited expected antiviral activities (90% inhibition by chloroquine at 10 μM and 68% by SYC-1307 at 1 μM). These results show certain quinazolinone compounds are novel, potent antiviral agents against ZIKV and medicinal chemistry optimization based on these compounds are needed.

Chemical synthesis

Synthesis of quinazolinone compounds **1-54** for structure activity relationship studies is shown in Scheme 1. For compounds **2–21** and **43**, isatoic anhydride compound **55** was condensed with an aniline derivative to give a benzamide compound **56**, which was subjected to a cyclization reaction at 110 °C to produce 2-methyl-3-phenylquinazolinone **57**. The methyl group of compound **57** was reacted with an arylaldehyde to yield target compounds **2–21** and **43**.

For synthesis of compounds **1**, **22-28, 35–42** and **44–52**, 6-chloro-quinazolinone **20** was subjected to a Buchwald-Hartwig amination reaction with an amine to give the target compounds. Compound **25** was prepared from compound **24** by deprotection of its *tert*-butoxycarbonyl (BOC) group. Saturated compounds **53** and **54** were synthesized from compounds **22** and **27**, respectively, by Pd/C catalyzed hydrogenation. A Suzuki coupling reaction with 6-bromo-quinazolinone **58** gave compounds **29-34**.

Structure-activity relationships for inhibiting ZIKV replication

Disubstituted quinazolinone compounds **5-11** (Table 1) with a variety of R^3 groups were found to be inactive at 10 μM in the compound screen. Lack of anti-ZIKV activities of these compounds, together with the high activity of compound **1**, show the 3-*tert*-butyl R^3 group in **1-3** seems to be favored. Thus, compounds **12-19** bearing a 3-*tert*-butyl R^3 group and various R^2 groups were synthesized and tested for their activities to inhibit ZIKV replication. As shown in Table 1, none of these compounds exhibited significantly improved activity. SAR analysis suggested an aromatic R^2 group seems not to significantly affect the anti-ZIKV activity. For example, while an electron-deficient pyridin-4-yl or pyrimidine-containing R^2 group in compounds **13** and **14** (68% inhibition) appears to be more favorable than a phenyl in **15** (inactive), compound **12** with a pyridin-3-yl R^2 group was inactive. With both compounds having 90% ZIKV inhibitory activity at 10 μM, the pyridinyl R^2 group in compound **3** does not exhibit a superior activity to the phenyl in **18**. Interestingly, the *para*-CF_3 group in compounds **3** and **18** appears to provide a moderate activity improvement, compared to unsubstituted or methoxy-substituted compounds **2**, **11-17**. A bicyclic naphthyl R^2 group in compound **19** (68% inhibition) does not significantly enhance anti-ZIKV activity.

Next, we focused on optimization of the R^6 group, since compared to unsubstituted compound **11**, the diethylamino group in compound **1** (99.9% inhibition at 10 μM) renders ≥1,000-fold activity enhancement to inhibit ZIKV replication. Further testing showed compound **1** can inhibit ZIKV replication in Vero cells by 68% (0.5-log reduction) at 1 μM (Table 2). With the same 3-*tert*-butyl R^3 and 6-methoxy-pyridin-2-yl R^2 group, compounds **20-34** having various R^6 groups were synthesized and their anti-ZIKV activities at 10 and 1 μM shown in Table 2. While compounds **20** and **21** with a -Cl and -OMe R^6 group were inactive, compound **22** with a piperidin-1-yl group inhibited ZIKV replication by ≥99.9% at 10 μM and 90% at 1 μM, showing superior activities to compound **1** (68% inhibition at 1 μM). However, compound **23** with a 5-membered pyrolidin-1-yl R^6 group is only a modest anti-ZIKV agent (68% inhibition at 10 μM). A larger, 4-BOC protected piperazin-1-yl R^6 group in compound **24** (99% inhibition at 10 μM) is tolerable, although it is considerably less active than **1** and **22**. Deprotection of BOC yielded compound **25** with a piperazin-1-yl R^6 group, which was found to exhibit significant cytotoxicity to Vero cells, which is undesirable for this study. Similarly, compound **26** with a 4-methyl-piperazin-1-yl group is also cytotoxic, suggesting a basic *N* atom at the 4-position of the piperazine ring (in **25** and **26**) is attributed to the toxicity, as compared to **22** and **24**. Indeed, compound **27** having a morpholin-4-yl R^6 substituent, as well as compound **28** with a larger 4-(morpholin-4-yl)piperazin-1-yl R^6 group, is one of our most potent anti-ZIKV agents without cytotoxicity. Both compounds can inhibit ZIKV replication by 99.9% at 10 μM and 90% at 1 μM.

Several compounds with an aromatic R^6 group were investigated. While compounds **29** and **30** with a pyridine-4-yl and 3-methoxyphenyl are inactive, compounds **31-33** with a *para*-F, -dimethylamino and -pyrolidine substituted phenyl R^6 group were moderately active with 99–96.8% ZIKV inhibition at 10 μM (Table 2). Compound **34** with a partially unsaturated tetrahydropyran-4-yl substituent exhibited moderate anti-ZIKV activity (96.8% inhibition at 10 μM). Collectively, SAR studies for the R^6 show that 1) a *N*-atom directly attaching to the 6-position of the quinazolinone core provides a significant activity enhancement (e.g., **27** vs. **34**); 2) 6-membered piperidin-1-yl or morpholin-4-yl (in **22**, **27** and **28**) is the most favored, while smaller 5-membered pyrrolidine ring loses most activity; and 3) a second, basic *N*-atom at the 4-position of the 6-membered ring is cytotoxic.

With the same 3-*tert*-butyl R^3 and a piperidine or related R^6 group, compounds **35-43** (Table 2) were synthesized to optimize the R^2 substituent. Compounds **35** and **36** (99% and 90% inhibition at 10 μM), which contain a *para*-CF_3-substituted pyridine and phenyl R^2 group showing good activities in Table 1, are less active than compound **22**, indicating the 6-methoxy-pyridin-2-yl R^2 group in **22** is more favored. Compound **37** with a pyridine-4-yl R^2 substituent is a modest compound (68% inhibition at 10 μM). Compound **38** having a 2-naphthyl R^2 substituent exhibited a strong anti-ZIKV activity of 99.7% at 10 μM, while compound **39** bearing a 3,5-dimethoxyphenyl R^2 moiety is less potent (96.8% ZIKV inhibition). Its analog **40** with a 4-methyl-piperazin-1-yl R^6 group is also cytotoxic, consistent with the results of compounds **26** and **25**. With a 3-methoxyphenyl R^2 substituent, compound **41** is also a potent anti-ZIKV agent with 99.9% and 68% inhibition at 10 and 1 μM, but it is slightly weaker than compound **22** (90% inhibition at 1 μM), showing a pyridine-containing R^2 substituent is more favorable than a phenyl. Similarly, compounds

42 and **43** with a 3-methoxyphenyl R^2 moiety exhibited comparable anti-ZIKV activities to their pyridine-containing analogs **24** and **21**. These results show the 6-methoxy-pyridin-2-yl R^2 group (in **22**) is the most favored for this series of compounds.

Compounds **44-52** (Table 3) with the most favored R^2 and R^6 groups were synthesized to further optimize the R^3 substituent. 3-methyl group in compounds **44-46** (no inhibition at 1 μM) were found to be considerably less active than their 3-*tert*-butyl analogs **22**, **27** and **28**. 3-Methoxy R^3 substituent in compounds **47-49** gives potent anti-ZIKV activities, but it is slightly less favored than 3-*tert*-butyl R^3 group, when combined with a morpholine-containing R^6 (e.g., compounds **48**/**49** vs. **27**/**28**). Compounds **50-52** with an electron-withdrawing 3-CF_3 R^3 substituent are considerably less active, as compared to **22**, **27** and **28**. These results show 3-*tert*-butyl or 3-OMe R^3 group is more favorable.

Finally, the *trans*-C=C double bond in the most potent compounds **22** and **27** was hydrogenated to generate the corresponding saturated compounds **53** and **54** (Chart 2). Both compounds were found to be inactive (at 10 μM) against ZIKV replication in Vero cells. To test whether the double bond is chemically reactive in cells, which could cause covalent binding to a protein, compound **27** was tested in culture media with added glutathione (GSH), a nucleophilic thiol commonly present in cells. As shown in Supporting Information Figure S1, anti-ZIKV activity of compound **27** (as well as ZIKV replication) in Vero cells was not affected in the presence of 1 mM of GSH. These results show that the rigid, *trans*-C=C bond at the 2-position of the quinazolinone core is critical to the anti-ZIKV activity of this series of compounds.

Antiviral activity evaluation

The most potent compounds **22**, **27** and **47** in the anti-ZIKV screen were selected for dose-dependent testing against several ZIKV and DENV strains. The representative results are shown in Figure 1 and the antiviral EC_{50} values summarized in Table 4. Compound **22** inhibited replication of the ZIKV FLR strain in Vero cells by 0%, 90% and >99.9% at 0.3, 1, 3 and 10 μM (Figure 1A) with a calculated EC_{50} value of 900 nM (Table 4). Compound **27** exhibited ~4× more potent antiviral activity with an EC_{50} of 180 nM, suppressing the ZIKV-FLR replication in Vero cells by 68%, 90% and >99.9% at 0.3, 1 and ≥3 μM. Compound **47** (EC_{50} = 210 nM) was found to have similar activities to **27**. Moreover, as shown in Figure 2A, treatment of ZIKV-infected Vero cells with compounds **22** and **27** can dose-dependently reduce the ZIKV RNA copies in the supernatant. Although ~$1/10^4$ of these RNAs represent infectious viruses [32], the quantitative PCR results confirm the antiviral activities of these compounds. Treatment with compound **27** can also significantly reduce the cellular levels of viral NS5, NS3 and capsid proteins (Figure 2B). In addition, these three compounds inhibited the ZIKV-FLR replication in human glioblastoma U87 cells with comparable potencies, with compound **27** showing the most potent antiviral activity. It inhibited the viral replication by 90%, 97%, 99% and >99.9% at 0.3, 1, 3 and 10 μM (Figure 1B) with an EC_{50} of 100 nM. Besides the two mammalian cells, ZIKV-FLR replication in mosquito C6/36 cells was also significantly blocked by these three compounds with EC_{50}s of 230–770 nM (Table 4 and Figure 1C).

Next, the most potent compound **27** was tested against replication of the HN16 strain of ZIKV (Honduras, 2016 [16]) in Vero cells. The compound can potently inhibit ZIKV-HN16 replication in a dose-dependent manner with an EC_{50} of 86 nM (Table 4 and Figure 1D). Moreover, these three compounds did not significantly inhibit proliferation of human U87 cells at 20 μM, showing they are non-cytotoxic with a very high selectivity against ZIKV replication.

We next tested antiviral activity of these compounds against replication of DENV, using a similar end-point dilution assay [34]. We were pleased to find that compounds **22**, **27** and **47** strongly inhibited replication of DENV serotype 2 virus (DENV-2, strain K0049, Thailand, 1995) in Vero cells with EC_{50} values of 160, 210 and 560 nM, respectively (Table 4 and Figure 1E). In addition, replication of DENV serotype 3 virus (DENV-3, strain 7431, Sri Lanka, 1989) was also potently suppressed by compound **27** with an EC_{50} of 120 nM (Table 4 and Figure 1F).

We also performed experiments to see whether delayed addition of compound **27** affects its anti-ZIKV activity in Vero cells. As shown in Figure 3A, adding compound **27** (1 μM) together with the initial virus into the cell culture media and incubating for 2 hours (2h-pretreatment), which is the regular compound treatment procedure for all of the above experiments, provided significantly higher anti-ZIKV activity, consistently reducing the viral titers by ≥90%. However, adding compound **27** (1 μM) immediately (0h-posttreatment) or 2 hours (2h-posttreatment) after removal of the inoculated virus showed reduced antiviral activity with 68% of viral titer reduction. Chloroquine, a known entry inhibitor of ZIKV, showed similar anti-ZIKV activities for these experiments (Figure 3B). These results suggest that compound **27** inhibits the attachment and/or entry of ZIKV to the cells.

Conclusion

DENV and ZIKV are important human pathogens, causing ~100 million symptomatic infections each year. More significantly, these viral infections can further cause serious and life-threatening diseases, such as microcephaly (in newborns), Guillain-Barré syndrome, and dengue hemorrhagic fever and shock syndrome. In addition, patients recovered from one of 4 serotypes of DENV infection are still susceptible to other serotypes with an increased likelihood of serious disease because of antibody-dependent enhancement. Except for mosquito control, there have been no antiviral drugs to prevent and treat ZIKV/DENV infections. There is therefore a pressing need for such antiviral agents. Phenotype-based compound screening found several 2,3,6-trisubstituted quinazolinone compounds are novel inhibitors of ZIKV replication. Fifty-four analogs were synthesized and tested with their structure-activity relationships discussed above. Additional characterization shows that the most potent compounds **22**, **27** and **47** are non-cytotoxic and exhibited potent activities against ZIKV and DENV with EC_{50} values as low as 86 nM in a variety of mammalian and mosquito cells. Their structures are amenable for further medicinal chemistry optimization. Although the protein or nucleic acid target(s) that these compounds inhibit in mammalian and mosquito cells is unknown and is the subject of future investigation, our results suggest they inhibit ZIKV attachment or entry to the cell. Nevertheless, given the scarcity of potent

and broad anti-Flavivirus agents, these compounds are novel pharmacological leads for drug development against ZIKV and DENV infections.

Experimental Section

All the chemicals used for synthesis were purchased from Aldrich (Milwaukee, WI) or Alfa Aesar (Ward Hill, MA). Unless otherwise stated, all solvents and reagents were used as received. All reactions were conducted with the use of a Teflon-coated magnetic stir bar at the indicated temperature and were performed under an inert atmosphere when stated. The identity of the synthesized compounds was characterized by ^{1}H and ^{13}C NMR on a Varian (Palo Alto, CA) 400-MR spectrometer and mass spectrometer (Shimadzu LCMS-2020). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). The identity of the potent inhibitors was confirmed with high resolution mass spectra (HRMS) using an Agilent 6550 iFunnel quadrupole-time-of-flight (Q-TOF) mass spectrometer with electrospray ionization (ESI). The purities of the final compounds were determined to be >95% with a Shimadzu Prominence HPLC using a Zorbax C18 (or C8) column (4.6 × 250 mm) at 0.7 mL/min flow rate (water:acetonitrile with 0.1% formic acid, 90:10 – 5:95 in 3 min) monitored by UV at 254 nm.

Chemical synthesis.

General synthetic methods for compounds 2–21 and 43: A mixture of isatoic anhydride **55** (3.07 mmol, 1 eq) and an aniline (3.37 mmol, 1.1 eq) was heated in 1,4-dioxane (10 mL) at 90 °C for 12 h. The solvent was evaporated, and the crude product purified with column chromatography (silica gel, hexane/ethyl acetate, 6/1) to give compound **56** as a white or pale-yellow powder in 45–89% yield.

A mixture of 2-amino-*N*-phenyl-benzamide **56** (0.565 mmol, 1 eq) and 1,1,1-triethoxy-ethane (0.28 g, 1.696 mmol, 3 eq) were stirred at 110 °C for overnight. The reaction mixture was quenched with water (10 mL) and the product extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. Upon removal of the solvent, the residue was purified with column chromatography (silica gel, hexane/ethyl acetate, 10/1) to provide compound **57** as a white or pale-yellow powder in 32–94% yield.

A mixture of 2-methyl-3-phenyl-4(3*H*)-quinazolinone **57** (0.14 mmol, 1 eq), an aldehyde (0.18 mmol, 1.3 eq) and NaOAc (6.8 mg, 0.08 mmol, 0.6 eq) in AcOH (1 ml) was heated at 110 °C for overnight. The reaction mixture was cooled down and the product extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. Upon removal of the solvent, the residue was purified with column chromatography (silica gel, hexane/ethyl acetate, 5/1) to provide compounds **2–21** and **43** as a white or pale-yellow powder in 35–91% yield.

3-(3-*tert*-Butylphenyl)-2-[2-(2-methoxyl-3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (2)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (d, *J* = 8.3 Hz, 1H), 8.07 (dd, *J* = 4.9, 1.7 Hz, 1H), 7.96 (d, *J* = 15.6 Hz, 1H), 7.82 – 7.73 (m, 2H), 7.60 – 7.40 (m, 4H), 7.29 (d, *J* = 1.7 Hz, 1H), 7.18 – 7.11 (m, 1H), 6.85 (dd, *J* = 7.3, 4.9 Hz, 1H), 6.74 (d, *J* = 15.6 Hz, 1H), 3.79 (s, 3H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 161.9, 153.4, 152.4, 147.9, 147.2, 138.7, 137.1, 134.6, 134.3, 129.5, 127.5, 127.3, 126.7, 126.2, 125.8, 123.4, 121.1, 119.1, 117.1, 53.3, 35.0, 31.4. MS (ESI) calculated for $(C_{26}H_{26}N_3O_2)^+$ $[M+H]^+$ 412.2, found 412.2.

3-(3-*tert*-Butylphenyl)-2-[2-(4-trifluoromethyl-3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (3)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.64 (s, 1H), 8.33 (d, *J* = 7.7 Hz, 1H), 7.95 (d, *J* = 15.7 Hz, 1H), 7.84 – 7.76 (m, 2H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.56 (ddd, *J* = 15.5, 13.8, 8.0 Hz, 4H), 7.29 (s, 1H), 7.15 (dd, *J* = 7.6, 0.9 Hz, 1H), 6.52 (d, *J* = 15.7 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 153.8, 150.7, 149.0, 147.6, 136.4, 135.5, 134.9, 134.0, 129.8, 127.7, 127.4, 126.7, 125.8, 125.7, 124.7, 121.4, 120.6, 110.1, 35.1, 31.4. MS (ESI) calculated for $(C_{26}H_{23}F_3N_3O)^+$ $[M+H]^+$ 450.2, found 450.2.

3-(3-Methoxylphenyl)-2-[2-(3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (4)

1H NMR ($CDCl_3$, 400 MHz) δ 8.58 (s, 1H), 8.49 (d, *J* = 4.1 Hz, 1H), 8.27 (d, *J* = 7.9 Hz, 1H), 7.94 (d, *J* = 15.6 Hz, 1H), 7.77 (d, *J* = 3.6 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.46 (td, *J* = 8.2, 5.0 Hz, 2H), 7.21 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.07 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.91 – 6.80 (m, 2H), 6.48 (d, *J* = 15.6 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 160.8, 151.0, 150.3, 149.4, 147.6, 137.9, 136.1, 134.7, 134.1, 131.1, 130.8, 127.5, 127.2, 127.0, 123.7, 121.9, 121.1, 120.8, 115.4, 114.4, 55.6. MS (ESI) calculated for $(C_{22}H_{18}N_3O_2)^+$ $[M+H]^+$ 356.1, found 356.2.

3-Phenyl-2-[2-(3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (5)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (s, 1H), 8.50 (s, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 15.6 Hz, 1H), 7.80 (s, 2H), 7.57 (t, *J* = 8.3 Hz, 4H), 7.49 (s, 1H), 7.32 (d, *J* = 7.0 Hz, 2H), 7.24 (dd, *J* = 11.8, 6.8 Hz, 1H), 6.45 (d, *J* = 15.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 151.1, 150.4, 149.5, 147.7, 136.9, 136.2, 134.8, 134.1, 131.2, 130.1, 129.7, 128.8, 127.6, 127.3, 127.1, 123.7, 122.0, 121.2. MS (ESI) calculated for $(C_{21}H_{16}N_3O)^+$ $[M+H]^+$ 326.1, found 326.1.

3-(2-Methylphenyl)-2-[2-(3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (6)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (s, 1H), 8.50 (d, *J* = 4.3 Hz, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 7.97 (d, *J* = 15.6 Hz, 1H), 7.81 (d, *J* = 4.2 Hz, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.51 – 7.34 (m, 4H), 7.25 – 7.18 (m, 2H), 6.38 (d, *J* = 15.6 Hz, 1H), 2.12 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.7, 151.1, 150.4, 149.5, 147.9, 136.6, 136.2, 136.0, 134.8, 134.1, 131.7, 131.2, 130.0, 128.7, 127.8, 127.6, 127.4, 127.0, 123.8, 121.3, 121.2, 17.7. MS (ESI) calculated for $(C_{22}H_{18}N_3O)^+$ $[M+H]^+$ 340.1, found 340.1

3-(3-Methylphenyl)-2-[2-(3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (7)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.59 (s, 1H), 8.51 (d, *J* = 3.9 Hz, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 15.6 Hz, 1H), 7.79 (d, *J* = 3.2 Hz, 2H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.51 –

7.41 (m, 2H), 7.36 (d, J = 7.3 Hz, 1H), 7.24 (dd, J = 7.4, 4.7 Hz, 1H), 7.15 – 7.04 (m, 2H), 6.48 (d, J = 15.6 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 151.1, 150.2, 149.3, 147.7, 140.4, 136.7, 136.0, 134.8, 134.3, 131.3, 130.5, 129.9, 129.2, 127.5, 127.3, 127.0, 125.6, 123.8, 122.2, 121.2, 21.5.

134.1, 131.1, 130.8, 127.5, 127.2, 127.0, 123.7, 121.9, 121.1, 120.8, 115.4, 114.4, 55.6. MS (ESI) calculated for $(C_{22}H_{18}N_3O)^+$ $[M+H]^+$ 340.1, found 340.1.

3-(4-*tert*-Butylphenyl)-2-[2-(3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (8)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.59 (s, 1H), 8.51 (s, 1H), 8.30 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 15.5 Hz, 1H), 7.79 (s, 2H), 7.59 (d, J = 7.3 Hz, 3H), 7.49 (s, 1H), 7.23 (d, J = 7.3 Hz, 3H), 6.49 (d, J = 15.6 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4, 152.8, 151.4, 150.3, 149.4, 147.7, 136.1, 134.8, 134.3, 134.1, 131.3, 128.2, 127.6, 127.4, 127.1, 127.0, 123.8, 122.3, 121.2, 35.1, 31.5. MS (ESI) calculated for $(C_{25}H_{24}N_3O)^+$ $[M+H]^+$ 382.2, found 382.2.

3-(4-*tert*-Butylphenyl)-2-[2-(5-bromo-3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (9)

1H NMR ($CDCl_3$, 400 MHz) δ 8.56 (d, J = 2.1 Hz, 1H), 8.46 (d, J = 1.7 Hz, 1H), 8.35 – 8.27 (m, 1H), 7.84 (d, J = 15.6 Hz, 1H), 7.80 (dd, J = 5.8, 1.2 Hz, 2H), 7.66 (t, J = 1.8 Hz, 1H), 7.54 (dddd, J = 11.9, 8.2, 4.3, 1.8 Hz, 3H), 7.28 (t, J = 1.8 Hz, 1H), 7.19 – 7.13 (m, 1H), 6.43 (d, J = 15.6 Hz, 1H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 153.6, 151.1, 150.8, 147.6, 147.1, 136.5, 136.4, 134.8, 134.1, 132.9, 129.8, 127.6, 127.3, 127.2, 126.7, 125.7, 123.7, 121.3, 121.1, 35.1, 31.4. MS (ESI) calculated for $(C_{25}H_{23}BrN_3O)^+$ $[M+H]^+$ 460.1, found 460.1

3-(4-*tert*-Butylphenyl)-2-[2-(5-pyrimidyl)ethenyl]-4(3*H*)-quinazolinone (10)

1H NMR ($CDCl_3$, 400 MHz) δ 9.10 (s, 1H), 8.67 (s, 2H), 8.31 (d, J = 7.9 Hz, 1H), 7.88 (d, J = 15.7 Hz, 1H), 7.79 (s, 2H), 7.59 (d, J = 8.1 Hz, 2H), 7.51 (t, J = 5.7 Hz, 1H), 7.24 (t, J = 7.8 Hz, 2H), 6.57 (d, J = 15.7 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2, 158.6, 155.4, 153.0, 150.7, 147.5, 134.9, 133.8, 132.1, 129.4, 128.1, 127.6, 127.4, 127.2, 124.1, 121.3, 35.1, 31.5. MS (ESI) calculated for $(C_{24}H_{23}N_4O)^+$ $[M+H]^+$ 383.2, found 383.2

3-(3-*tert*-Butylphenyl)-2-[2-(3-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (11)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 8.6 Hz, 3H), 7.60 – 7.43 (m, 4H), 7.32 (s, 1H), 7.14 (d, J = 7.2 Hz, 1H), 7.04 (d, J = 14.8 Hz, 1H), 6.89 (d, J = 7.0 Hz, 1H), 6.62 (d, J = 8.2 Hz, 1H), 3.59 (s, 3H), 1.33 (s, 9H).^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.3, 162.4, 153.5, 152.0, 150.8, 148.0, 139.1, 137.7, 137.1, 134.6, 129.5, 127.5, 127.3, 126.8, 126.2, 125.8, 125.6, 124.1, 121.3, 118.2, 112.2, 52.7, 35.0, 31.4. MS (ESI) calculated for $(C_{26}H_{26}N_3O_2)^+$ $[M+H]^+$ 412.2, found 412.2

3-(3-*tert*-Butylphenyl)-2-[2-(3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (12)

1H NMR ($CDCl_3$, 400 MHz) δ 8.57 (s, 1H), 8.51 (d, J = 4.5 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 15.6 Hz, 1H), 7.80 (d, J = 3.6 Hz, 2H), 7.59 – 7.45 (m, 4H), 7.29 (s, 1H), 7.26 – 7.19 (m, 1H), 7.15 (d, J = 7.5 Hz, 1H), 6.44 (d, J = 15.6 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 153.6, 151.3, 150.3, 149.3, 147.7, 136.6, 135.9,

134.8, 134.1, 131.3, 129.7, 127.6, 127.3, 127.1, 126.6, 125.8, 125.7, 123.8, 122.3, 121.3, 35.1, 31.4. MS (ESI) calculated for $(C_{25}H_{26}N_3O)^+$ $[M+H]^+$ 382.2, found 382.2

3-(3-*tert*-Butylphenyl)-2-[2-(4-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (13)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.55 (d, *J* = 4.5 Hz, 2H), 8.33 (d, *J* = 7.6 Hz, 1H), 7.86–7.80 (m, 3H), 7.62 – 7.47 (m, 3H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.14 (dd, *J* = 19.1, 5.6 Hz, 3H), 6.54 (d, *J* = 15.6 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2, 153.6, 151.0, 150.5, 147.6, 142.6, 136.6, 136.5, 134.8, 129.7, 127.7, 127.4, 127.3, 126.6, 125.8, 125.7, 124.7, 121.6, 121.4, 35.1, 31.4. MS (ESI) calculated for $(C_{25}H_{26}N_3O)^+$ $[M+H]^+$ 382.2, found 382.2

3-(3-*tert*-Butylphenyl)-2-[2-(5-pyrimidyl)ethenyl]-4(3*H*)-quinazolinone (14)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.09 (s, 1H), 8.64 (s, 2H), 8.32 (d, *J* = 7.7 Hz, 1H), 7.92 – 7.75 (m, 3H), 7.54 (dt, *J* = 11.4, 7.8 Hz, 3H), 7.28 (s, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 6.50 (d, *J* = 15.7 Hz, 1H), 1.34 (s, 9H).^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 158.6, 155.3, 153.8, 150.6, 147.5, 136.4, 134.9, 131.9, 129.8, 129.4, 127.7, 127.4, 126.7, 125.7, 125.7, 124.1, 121.4, 35.1, 31.4. MS (ESI) calculated for $(C_{24}H_{23}N_4O)^+$ $[M+H]^+$ 383.2, found 383.2.

3-(3-*tert*-Butylphenyl)-2-(2-phenylethenyl)-4(3*H*)-quinazolinone (15)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 15.5 Hz, 1H), 7.82 – 7.78 (m, 2H), 7.60 – 7.44 (m, 3H), 7.30 (s, 6H), 7.16 (dd, *J* = 7.8, 1.6 Hz, 1H), 6.38 (d, *J* = 15.5 Hz, 1H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4, 153.5, 152.1, 147.9, 139.8, 136.8, 135.5, 134.7, 129.7, 129.6, 128.9, 127.8, 127.5, 127.3, 126.7, 126.4, 125.9, 125.8, 121.2, 120.3, 35.1, 31.4. MS (ESI) calculated for $(C_{26}H_{25}N_2O)^+$ $[M+H]^+$ 381.2, found 381.2.

3-(3-*tert*-Butylphenyl)-2-[2-(2-methoxylphenyl)ethenyl]-4(3*H*)-quinazolinone (16)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 16.0 Hz, 1H), 7.87 – 7.73 (m, 2H), 7.60 – 7.42 (m, 4H), 7.29 (dd, *J* = 6.3, 1.6 Hz, 1H), 7.24 (s, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 6.91 – 6.79 (m, 2H), 6.63 (d, *J* = 15.6 Hz, 1H), 3.71 (s, 3H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 158.5, 153.4, 137.1, 134.6, 130.8, 130.0, 129.5, 127.3, 126.5, 126.2, 125.9, 124.5, 120.8, 111.1, 55.2, 35.1, 31.4. MS (ESI) calculated for $(C_{27}H_{27}N_2O_2)^+$ $[M+H]^+$ 411.2, found 411.2

3-(3-*tert*-Butylphenyl)-2-[2-(3-methoxylphenyl)ethenyl]-4(3*H*)-quinazolinone (17)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 15.5 Hz, 1H), 7.79 (dd, *J* = 3.6, 1.7 Hz, 2H), 7.60 – 7.43 (m, 3H), 7.30 (s, 1H), 7.24 – 7.13 (m, 2H), 6.93 – 6.79 (m, 3H), 6.36 (d, *J* = 15.5 Hz, 1H), 3.76 (s, 3H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4, 159.9, 153.5, 152.0, 147.9, 139.7, 136.9, 136.8, 134.7, 129.9, 129.6, 127.4, 127.3, 126.7, 126.4, 125.8, 121.1, 120.6, 120.3, 115.4, 113.0, 55.3, 35.0, 31.4. MS (ESI) calculated for $(C_{27}H_{27}N_2O_2)^+$ $[M+H]^+$ 411.2, found 411.2.

3-(3-*tert*-Butylphenyl)-2-[2-(4-trifluoromethylphenyl)ethenyl]-4(3*H*)-quinazolinone (18)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 15.6 Hz, 1H), 7.81 (d, *J* = 3.7 Hz, 2H), 7.61 – 7.46 (m, 5H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.30 (s, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 6.45 (d, *J* = 15.6 Hz, 1H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2, 153.6, 151.5, 147.6, 138.9, 137.9, 136.6, 134.8, 129.7, 127.9, 127.5, 127.4, 127.1, 126.6, 125.9, 125.9, 125.8, 125.8, 122.6, 121.3, 35.1, 31.4. MS (ESI) calculated for $(C_{27}H_{24}F_3N_2O)^+$ $[M+H]^+$ 449.2, found 449.2.

3-(3-*tert*-Butylphenyl)-2-[2-(1-naphthyl)ethenyl]-4(3*H*)-quinazolinone (19)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.77 (d, *J* = 15.3 Hz, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 7.93 – 7.74 (m, 4H), 7.59 – 7.46 (m, 5H), 7.39 – 7.30 (m, 3H), 7.19 (d, *J* = 7.5 Hz, 1H), 6.48 (d, *J* = 15.3 Hz, 1H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.6, 136.9, 134.8, 133.8, 133.1, 131.6, 130.1, 129.7, 128.9, 127.5, 127.3, 126.9, 126.5, 126.3, 125.9, 125.8, 125.5, 124.8, 123.9, 122.9, 121.2, 35.1, 31.4. MS (ESI) calculated for $(C_{30}H_{27}N_2O)^+$ $[M+H]^+$ 431.2, found 431.2.

6-Chloro-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (20)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25 (d, *J* = 1.5 Hz, 1H), 7.79 (d, *J* = 14.8 Hz, 1H), 7.75 – 7.66 (m, 2H), 7.51 (ddd, *J* = 13.0, 12.3, 6.7 Hz, 3H), 7.31 (s, 1H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 14.8 Hz, 1H), 6.88 (d, *J* = 7.1 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 1H), 3.58 (s, 3H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.3, 161.4, 153.6, 152.2, 150.6, 146.4, 139.0, 138.1, 136.8, 135.0, 132.4, 129.6, 129.2, 126.6, 126.3, 125.7, 125.5, 123.7, 122.2, 118.2, 112.3, 52.7, 35.0, 31.4. MS (ESI) calculated for $(C_{26}H_{25}ClN_3O_2)^+$ $[M+H]^+$ 446.2, found 446.1.

6-Methoxy-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (21)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 – 7.71 (m, 2H), 7.69 (d, *J* = 3.0 Hz, 1H), 7.56 – 7.44 (m, 3H), 7.39 (dd, *J* = 8.9, 3.0 Hz, 1H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.12 (ddd, *J* = 7.5, 2.2, 1.3 Hz, 1H), 7.01 (d, *J* = 14.8 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 1H), 6.60 (dd, *J* = 8.2, 0.9 Hz, 1H), 3.92 (s, 3H), 3.58 (s, 3H), 1.32 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 158.5, 153.3, 150.8, 149.8, 138.9, 137.0, 129.3, 128.9, 125.9, 125.6, 125.5, 124.8, 117.8, 111.8, 106.5, 55.8, 52.5, 34.8, 31.2. MS (ESI) calculated for $(C_{28}H_{28}N_3O_3)^+$ $[M+H]^+$ 442.2, found 442.2.

6-Methoxy-3-(3-*tert*-butylphenyl)-2-(3-methoxystyryl)-4(3*H*)-quinazolinone (43)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (d, *J* = 15.5 Hz, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.67 (d, *J* = 2.9 Hz, 1H), 7.55 (ddd, *J* = 8.0, 1.9, 1.2 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.38 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.28 (t, *J* = 1.9 Hz, 1H), 7.19 (t, *J* = 7.9 Hz, 1H), 7.13 (ddd, *J* = 7.6, 2.1, 1.2 Hz, 1H), 6.86 (ddd, *J* = 7.6, 1.7, 0.9 Hz, 1H), 6.84 – 6.76 (m, 2H), 6.33 (d, *J* = 15.5 Hz, 1H), 3.91 (s, 3H), 3.74 (s, 3H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.0, 159.7, 158.4, 153.2, 149.8, 136.9, 136.7, 129.6, 129.4, 128.9, 126.1, 125.6, 125.6, 124.9, 121.7, 120.0, 115.0, 112.8, 106.5, 55.8, 55.1, 34.8, 31.2. MS (ESI) calculated for $(C_{28}H_{29}N_3O_2)^+$ $[M+H]^+$ 441.2, found 441.2.

General synthetic methods for compounds 1, 22–28, 35–42, 44–52: A mixture of a compound **20** (35 mg, 0.07 mmol), an amine (0.08 mmol), tris(dibenzylideneacetone)dipalladium(0) (4 mg, 0.004 mol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (3 mg, 0.008 mmol), sodium *tert*-butoxide (10 mg, 0.10 mmol) in toluene (5 mL) was heated at 110 °C for 24 h. The reaction mixture was cooled down and quenched with brine (20 mL). The product was extracted with ethyl acetate (3 × 20 mL), and the combined organic phase was washed with brine and then dried over Na_2SO_4. Upon removal of solvent, the residue was purified with column chromatography (silica gel, hexanes : ethyl acetate, 8: 1) to give the target compounds **1, 22–24, 26–28, 35–42, 44–52** as a pale yellow solid in 30–81% yield.

To the solution of compound **24** (0.1 mmol) in dichloromethane (2 mL) was added dropwise HCl (0.2 mL, 4N in *p*-dioxane) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 12 hours. The solid powder thus obtained was filtered and washed with in dichloromethane to give compound **25** as a hydrochloride salt.

6-Diethylamino-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (1)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 – 7.59 (m, 2H), 7.54 – 7.37 (m, 4H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.21 (d, *J* = 9.2 Hz, 1H), 7.11 (ddd, *J* = 7.4, 2.1, 1.2 Hz, 1H), 7.00 (d, *J* = 14.9 Hz, 1H), 6.84 (d, *J* = 7.2 Hz, 1H), 6.57 (d, *J* = 8.3 Hz, 1H), 3.57 (s, 3H), 3.46 (q, *J* = 7.1 Hz, 4H), 1.31 (s, 9H), 1.21 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.0, 162.2, 153.1, 151.1, 147.3, 146.8, 138.8, 137.4, 135.1, 129.2, 128.5, 125.7, 125.5, 124.3, 122.2, 120.0, 117.5, 111.3, 105.6, 52.5, 44.6, 34.8, 31.2, 12.4. MS (ESI) calculated for $(C_{30}H_{35}N_4O_2)^+$ $[M+H]^+$ 483.3, found 483.2.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (22)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, *J* = 4.2 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.48 (ddd, *J* = 11.3, 10.7, 5.7 Hz, 4H), 7.31 (s, 1H), 7.13 (d, *J* = 7.3 Hz, 1H), 7.02 (d, *J* = 14.9 Hz, 1H), 6.85 (d, *J* = 7.1 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 1H), 3.58 (s, 3H), 3.36 – 3.26 (m, 4H), 1.72 (d, *J* = 4.4 Hz, 4H), 1.62 (d, *J* = 4.7 Hz, 2H), 1.32 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.2, 162.4, 153.3, 151.2, 150.7, 148.9, 140.7, 139.0, 137.5, 135.9, 129.4, 128.4, 125.9, 125.9, 125.7, 124.8, 124.4, 122.0, 117.8, 111.7, 110.1, 52.7, 50.3, 35.0, 31.4, 25.7, 24.3. HRMS (ESI) calculated for $(C_{31}H_{35}N_4O_2)^+$ $[M+H]^+$ 495.2760, found 495.2754.

6-(Pyrrolidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (23)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 – 7.66 (m, 2H), 7.55 – 7.44 (m, 3H), 7.34 – 7.28 (m, 2H), 7.15 – 7.09 (m, 2H), 7.01 (d, *J* = 14.9 Hz, 1H), 6.86 (d, *J* = 7.2 Hz, 1H), 6.58 (d, *J* = 8.3 Hz, 1H), 3.58 (s, 3H), 3.41 (d, *J* = 6.4 Hz, 4H), 2.07 (t, *J* = 6.4 Hz, 4H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.3, 153.3, 151.3, 147.0, 139.0, 137.6, 135.4, 129.4, 128.5, 125.9, 125.8, 122.3, 120.3, 117.8, 111.6, 110.2, 105.8, 52.8, 48.0, 35.0, 31.4, 25.7. MS (ESI) calculated for $(C_{30}H_{33}N_4O_2)^+$ $[M+H]^+$ 481.3, found 481.2

tert-Butyl-4-(3-(3-(*tert*-butyl)phenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4-oxo-3,4-dihydroquinazolin-6-yl)piperazine-1-carboxylate (24)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (s, 2H), 7.64 (d, *J* = 2.8 Hz, 1H), 7.57 – 7.44 (m, 4H), 7.29 (d, *J* = 1.9 Hz, 1H), 7.11 (dt, *J* = 7.6, 1.6 Hz, 1H), 6.99 (dd, *J* = 15.0, 1.4 Hz, 1H), 6.95 (d, *J* = 7.2 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 1H), 3.62 (t, *J* = 5.2 Hz, 4H), 3.56 (d, *J* = 1.3 Hz, 3H), 3.30 (t, *J* = 5.3 Hz, 4H), 1.46 (s, 9H), 1.32 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 161.4, 154.6, 153.5, 150.3, 149.9, 139.0, 136.5, 129.5, 129.4, 126.2, 125.5, 125.4, 124.7, 121.3, 118.7, 112.3, 110.4, 80.1, 52.5, 48.7, 43.3, 34.9, 31.2, 28.4, 28.4. MS (ESI) calculated for $(C_{35}H_{42}N_5O_4)^+$ $[M+H]^+$ 596.3, found 596.3.

6-(Piperazin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone hydrochloride (25)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.54 (s, 2H), 7.93 – 7.83 (m, 2H), 7.72 – 7.62 (m, 2H), 7.55 (dt, *J* = 8.0, 1.5 Hz, 1H), 7.53 – 7.44 (m, 3H), 7.23 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.08 (d, *J* = 7.2 Hz, 1H), 6.84 (d, *J* = 15.0 Hz, 1H), 6.72 (d, *J* = 8.3 Hz, 1H), 3.53 (t, *J* = 5.1 Hz, 4H), 3.46 (d, *J* = 1.1 Hz, 3H), 3.21 (s, 4H), 1.24 (d, *J* = 1.0 Hz, 9H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.0, 160.9, 153.1, 150.3, 150.2, 149.3, 140.5, 138.7, 138.0, 137.2, 129.7, 127.0, 126.3, 126.2, 126.0, 124.9, 122.5, 121.5, 119.3, 112.7, 109.9, 52.5, 45.4, 42.7, 35.0, 31.4. MS (ESI) calculated for $(C_{30}H_{34}N_5O_2)^+$ $[M+H]^+$ 496.3, found 496.3

6-(4-Methylpiperazin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (26)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 – 7.62 (m, 3H), 7.54 – 7.41 (m, 4H), 7.29 (q, *J* = 3.4, 2.5 Hz, 1H), 7.14 – 7.06 (m, 1H), 7.04 – 6.95 (m, 1H), 6.84 (d, *J* = 7.2 Hz, 1H), 6.58 (d, *J* = 8.6 Hz, 1H), 3.56 (d, *J* = 1.4 Hz, 3H), 3.43 – 3.31 (m, 4H), 2.63 (t, *J* = 4.9 Hz, 4H), 2.38 (d, *J* = 2.6 Hz, 3H), 1.31 (d, *J* = 1.3 Hz, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.2, 153.2, 150.9, 149.7, 149.1, 138.8, 137.2, 136.0, 129.3, 128.4, 125.8, 125.7, 125.5, 124.2, 124.0, 121.8, 117.7, 111.6, 110.1, 54.7, 52.5, 48.5, 45.9, 34.8, 31.2. MS (ESI) calculated for $(C_{31}H_{36}N_5O_2)^+$ $[M+H]^+$ 510.3, found 510.3

6-Morpholino-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (27)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 – 7.68 (m, 2H), 7.65 (d, *J* = 2.9 Hz, 1H), 7.55 – 7.38 (m, 4H), 7.29 (d, *J* = 2.0 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 7.00 (d, *J* = 14.9 Hz, 1H), 6.85 (d, *J* = 7.1 Hz, 1H), 6.59 (d, *J* = 8.3 Hz, 1H), 4.00 – 3.80 (m, 4H), 3.57 (s, 3H), 3.29 (dd, *J* = 5.9, 3.8 Hz, 4H), 1.31 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.2, 153.2, 150.8, 149.9, 149.3, 138.9, 137.1, 129.3, 128.4, 125.9, 125.6, 125.5, 123.7, 121.8, 117.7, 111.7, 109.9, 66.7, 52.5, 48.9, 34.8, 31.2; HRMS (ESI) calculated for $(C_{30}H_{33}N_4O_3)^+$ $[M+H]^+$ 497.2553, found 497.2536.

6-(4-Morpholinopiperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxyl-2-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (28)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, *J* = 3.0 Hz, 1H), 7.66 (dd, *J* = 5.8, 2.9 Hz, 2H), 7.54 – 7.42 (m, 4H), 7.29 (d, *J* = 2.0 Hz, 1H), 7.10 (dt, *J* = 7.2, 1.5 Hz, 1H), 6.99 (d, *J* = 14.8 Hz,

1H), 6.84 (d, *J* = 7.1 Hz, 1H), 6.58 (d, *J* = 8.3 Hz, 1H), 3.90 (d, *J* = 12.8 Hz, 2H), 3.74 (t, *J* = 4.7 Hz, 4H), 3.56 (s, 3H), 2.85 (t, *J* = 12.2 Hz, 2H), 2.59 (s, 4H), 2.39 (s, 1H), 1.98 (d, *J* = 12.4 Hz, 2H), 1.67 (q, *J* = 11.6 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) 163.1, 153.1, 150.9, 149.7, 138.8, 137.2, 135.9, 129.3, 128.4, 125.8, 125.7, 125.5, 124.5, 124.2, 117.7, 111.6, 110.1, 61.9, 52.5, 49.7, 48.6, 48.5, 34.8, 31.2, 27.8. MS (ESI) calculated for $(C_{35}H_{42}N_5O_3)^+$ $[M+H]^+$ 580.3, found 580.3.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-trifluoromethyl-3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (35)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1H), 7.83 (d, *J* = 15.7 Hz, 1H), 7.68 (dd, *J* = 12.1, 6.1 Hz, 3H), 7.62 – 7.45 (m, 4H), 7.29 (s, 1H), 7.16 – 7.09 (m, 1H), 6.50 (d, *J* = 15.7 Hz, 1H), 3.40 – 3.29 (m, 4H), 1.74 (s, 4H), 1.64 (d, *J* = 4.7 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2, 153.6, 148.8, 147.6, 136.9, 135.2, 134.4, 132.1, 129.7, 128.6, 126.5, 125.9, 125.8, 125.0, 122.2, 120.6, 110.0, 50.1, 35.1, 31.4, 25.6, 24.3. MS (ESI) calculated for $(C_{31}H_{32}F_3N_4O)^+$ $[M+H]^+$ 533.3, found 533.2

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(4-trifluoromethyl-3-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (36)

^{1}H NMR ($CDCl_3$, 100 MHz) δ 7.82 (d, *J* = 15.6 Hz, 1H), 7.72 – 7.61 (m, 2H), 7.58 – 7.44 (m, 5H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.27 (d, *J* = 2.0 Hz, 1H), 7.13 (ddd, *J* = 7.5, 2.1, 1.1 Hz, 1H), 6.40 (d, *J* = 15.6 Hz, 1H), 3.32 (t, *J* = 5.4 Hz, 4H), 1.73 (t, *J* = 5.8 Hz, 4H), 1.62 (p, *J* = 5.9 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 153.2, 148.2, 139.0, 136.8, 135.9, 129.4, 128.3, 127.4, 126.1, 125.7, 125.6, 125.6, 122.8, 121.8, 34.8, 31.2, 29.6, 25.4, 24.1. LC-MS (M + H) = 532.3. MS (ESI) calculated for $(C_{32}H_{33}F_3N_3O)^+$ $[M+H]^+$ 532.3, found 532.3.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(4-pyridinyl)ethenyl]-4(3*H*)-quinazolinone (37)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.51 (d, *J* = 5.2 Hz, 2H), 7.77 – 7.61 (m, 3H), 7.59 – 7.44 (m, 3H), 7.26 (d, *J* = 1.8 Hz, 1H), 7.17 – 7.04 (m, 3H), 6.51 (dd, *J* = 15.6, 1.2 Hz, 1H), 3.32 (t, *J* = 5.4 Hz, 4H), 1.72 (t, *J* = 5.6 Hz, 4H), 1.61 (p, *J* = 5.7 Hz, 2H), 1.32 (d, *J* = 1.3 Hz, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.0, 153.3, 150.8, 149.9, 147.6, 143.2, 140.1, 136.7, 134.4, 129.4, 128.4, 126.2, 125.7, 125.6, 125.1, 124.4, 122.0, 121.3, 109.8, 49.9, 34.8, 31.2, 29.6, 25.4, 24.1. LC-MS (M + H) = 465.3. MS (ESI) calculated for $(C_{30}H_{33}N_4O)^+$ $[M+H]^+$ 465.3, found 465.3.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(2-naphthalenyl)ethenyl]-4(3*H*)-quinazolinone (38)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, *J* = 15.5 Hz, 1H), 7.82 – 7.62 (m, 6H), 7.61 – 7.38 (m, 5H), 7.33 – 7.25 (m, 2H), 7.17 (dt, *J* = 7.5, 1.6 Hz, 1H), 6.45 (d, *J* = 15.5 Hz, 1H), 3.31 (t, *J* = 5.4 Hz, 4H), 1.74 (s, 4H), 1.62 (s, 2H), 1.34 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 153.2, 136.9, 133.7, 133.3, 133.0, 129.4, 129.1, 128.4, 128.3, 127.6, 126.8, 126.5, 126.1, 125.7, 125.7, 123.1, 121.5, 34.9, 31.2, 29.6, 25.3, 23.9. MS (ESI) calculated for $(C_{35}H_{36}N_3O)^+$ $[M+H]^+$ 514.3, found 514.3.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-(2,6-dimethoxystyryl)-4(3*H*)-quinazolinone (39)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (d, *J* = 15.9 Hz, 1H), 7.72 (d, *J* = 9.0 Hz, 1H), 7.65 (d, *J* = 2.6 Hz, 1H), 7.55 – 7.43 (m, 3H), 7.27 (d, *J* = 2.0 Hz, 1H), 7.18 – 7.08 (m, 2H), 6.95 (d, *J* = 15.8 Hz, 1H), 6.45 (d, *J* = 8.4 Hz, 2H), 3.65 (s, 6H), 3.28 (t, *J* = 5.4 Hz, 4H), 1.73 (s, 4H), 1.61 (q, *J* = 5.9 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 159.6, 152.9, 137.7, 130.0, 129.1, 128.3, 125.8, 125.7, 125.5, 123.2, 121.3, 113.3, 103.5, 55.3, 50.5, 34.8, 31.2, 29.6, 25.4, 25.2, 24.0. LC-MS (M + H) = 524.3. MS (ESI) calculated for $(C_{33}H_{38}N_3O_3)^+$ $[M+H]^+$ 524.3, found 524.3.

6-(4-Methylpiperazin-1-yl)-3-(3-*tert*-butylphenyl)-2-(2,6-dimethoxystyryl)-4(3*H*)-quinazolinone (40)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (d, *J* = 15.8 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.64 (d, *J* = 2.9 Hz, 1H), 7.52 (dt, *J* = 8.1, 1.4 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.46 – 7.39 (m, 1H), 7.27 (d, *J* = 1.9 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.13 – 7.08 (m, 1H), 6.94 (d, *J* = 15.9 Hz, 1H), 6.45 (d, *J* = 8.4 Hz, 2H), 3.65 (s, 6H), 3.39 (t, *J* = 5.0 Hz, 4H), 2.70 (t, *J* = 4.9 Hz, 4H), 2.42 (s, 1H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.5, 152.9, 148.9, 137.7, 130.0, 129.1, 128.8, 128.5, 125.8, 125.7, 125.5, 124.2, 123.3, 121.3, 113.3, 110.3, 103.5, 55.3, 55.3, 54.6, 48.4, 45.6, 34.8, 31.2, 31.1. MS (ESI) calculated for $(C_{33}H_{39}N_4O_3)^+$ $[M+H]^+$ 539.3, found 539.3.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-(3-methoxystyryl)-4(3*H*)-quinazolinone (41)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, *J* = 15.5 Hz, 1H), 7.71 – 7.62 (m, 2H), 7.57 – 7.42 (m, 3H), 7.27 (d, *J* = 4.0 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.12 (ddd, *J* = 7.5, 2.1, 1.2 Hz, 1H), 6.89 – 6.83 (m, 1H), 6.83 – 6.76 (m, 2H), 6.32 (d, *J* = 15.6 Hz, 1H), 3.73 (s, 3H), 3.30 (t, *J* = 5.4 Hz, 4H), 1.73 (m, 4H), 1.61 (t, *J* = 5.3 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.7, 153.1, 148.9, 137.8, 137.0, 137.0, 129.6, 129.3, 128.2, 126.0, 125.7, 125.7, 124.8, 121.6, 120.7, 120.0, 114.8, 112.6, 55.1, 34.8, 31.2, 29.6, 25.4, 24.1. MS (ESI) calculated for $(C_{32}H_{36}N_3O_2)^+$ $[M+H]^+$ 494.3, found 494.3.

tert-Butyl 4-[3-(3-*tert*-butylphenyl)-2-(3-methoxystyryl)-4-oxo-3,4-dihydroquinazolin-6-yl]piperazine-1-carboxylate (42)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (d, *J* = 15.5 Hz, 1H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.64 (d, *J* = 2.8 Hz, 1H), 7.54 (dt, *J* = 8.1, 1.5 Hz, 1H), 7.52 – 7.41 (m, 2H), 7.27 (d, *J* = 3.9 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.12 (ddd, *J* = 7.5, 2.0, 1.2 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.83 – 6.79 (m, 1H), 6.78 (t, *J* = 2.0 Hz, 1H), 6.31 (d, *J* = 15.5 Hz, 1H), 3.73 (s, 3H), 3.60 (t, *J* = 5.1 Hz, 4H), 3.26 (t, *J* = 5.1 Hz, 4H), 1.47 (s, 9H), 1.32 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.0, 159.7, 154.6, 153.2, 149.7, 149.5, 136.9, 136.8, 129.6, 129.4, 128.2, 126.1, 125.6, 125.6, 124.7, 121.5, 120.0, 115.0, 112.7, 110.5, 80.0, 55.1, 48.9, 34.8, 31.2, 28.4. MS (ESI) calculated for $(C_{36}H_{43}N_4O_4)^+$ $[M+H]^+$ 595.3, found 595.3.

6-(Piperidin-1-yl)-3-(*m*-tolyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (44)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 – 7.63 (m, 3H), 7.48 (dd, *J* = 8.3, 7.2 Hz, 2H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.16 – 7.03 (m, 3H), 6.86 (d, *J* = 7.2 Hz, 1H), 6.59 (d, *J* = 8.3 Hz, 1H), 3.61 (d, *J* = 1.2 Hz, 3H), 3.31 (t, *J* = 5.4 Hz, 4H), 2.41 (s, 3H),

1.74 (p, *J* = 5.6 Hz, 4H), 1.66 – 1.60 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.2, 151.0, 139.7, 138.9, 137.4, 129.5, 129.4, 129.2, 128.3, 125.7, 124.1, 121.8, 117.6, 111.5, 52.5, 29.6, 25.5, 24.1, 21.2. MS (ESI) calculated for $(C_{28}H_{29}N_4O_2)^+$ $[M+H]^+$ 453.2, found 453.2.

6-Morpholino-3-(*m*-tolyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (45)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 – 7.69 (m, 2H), 7.64 (d, *J* = 2.9 Hz, 1H), 7.51 – 7.39 (m, 3H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.15 – 7.04 (m, 3H), 6.87 (d, *J* = 7.2 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 1H), 3.89 (t, *J* = 4.8 Hz, 4H), 3.61 (s, 3H), 3.38 – 3.17 (m, 4H), 2.41 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.0, 150.8, 149.9, 149.2, 139.8, 138.9, 137.2, 129.7, 129.5, 129.1, 128.2, 125.6, 123.7, 121.7, 117.9, 111.8, 109.9, 66.6, 52.5, 48.8, 21.2. MS (ESI) calculated for $(C_{27}H_{27}N_4O_3)^+$ $[M+H]^+$ 455.2, found 455.2.

6-(4-Morpholinopiperidin-1-yl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-3-(*m*-tolyl)-4(3*H*)-quinazolinone (46)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 – 7.61 (m, 3H), 7.51 – 7.38 (m, 3H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.15 – 7.03 (m, 3H), 6.85 (d, *J* = 7.1 Hz, 1H), 6.59 (d, *J* = 8.2 Hz, 1H), 3.90 (d, *J* = 12.5 Hz, 2H), 3.75 (bs, 4H), 3.60 (s, 3H), 2.91 – 2.77 (m, 2H), 2.61 (bs, 4H), 2.40 (s, 4H), 2.05 – 1.92 (m, 2H), 1.68 (d, *J* = 12.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 150.9, 149.7, 148.7, 140.8, 139.8, 138.9, 137.4, 136.0, 129.5, 129.5, 129.2, 128.4, 125.7, 124.5, 124.1, 121.8, 117.6, 111.6, 110.2, 62.0, 49.7, 48.5, 21.2. MS (ESI) calculated for $(C_{32}H_{36}N_5O_3)^+$ $[M+H]^+$ 538.3, found 538.2

6-(Piperidin-1-yl)-3-(3-methoxyphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (47)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 – 7.62 (m, 3H), 7.53 – 7.39 (m, 3H), 7.09 (d, *J* = 14.9 Hz, 1H), 7.02 (ddd, *J* = 8.5, 2.6, 1.0 Hz, 1H), 6.90 (ddd, *J* = 7.7, 1.9, 0.9 Hz, 1H), 6.88 – 6.83 (m, 2H), 6.59 (d, *J* = 8.2 Hz, 1H), 3.80 (s, 3H), 3.63 (s, 3H), 3.31 (t, *J* = 5.4 Hz, 4H), 1.73 (t, *J* = 5.7 Hz, 4H), 1.62 (q, *J* = 6.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.1, 160.6, 151.0, 148.5, 138.9, 138.6, 136.0, 130.3, 128.3, 124.6, 124.0, 121.7, 121.0, 117.6, 115.0, 114.1, 111.5, 55.4, 52.6, 25.5, 24.1; HRMS (ESI) calculated for $(C_{28}H_{29}N_4O_3)^+$ $[M+H]^+$ 469.2240, found 469.2227.

6-Morpholino-3-(3-methoxyphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (48)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 – 7.69 (m, 2H), 7.64 (d, *J* = 2.9 Hz, 1H), 7.53 – 7.39 (m, 3H), 7.09 (d, *J* = 14.8 Hz, 1H), 7.03 (dd, *J* = 8.5, 2.7 Hz, 1H), 6.94 – 6.83 (m, 3H), 6.60 (d, *J* = 8.2 Hz, 1H), 3.89 (t, *J* = 4.8 Hz, 4H), 3.81 (d, *J* = 1.7 Hz, 3H), 3.63 (d, *J* = 1.7 Hz, 3H), 3.29 (dd, *J* = 5.8, 3.7 Hz, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 161.9, 160.7, 150.8, 149.9, 149.1, 138.9, 138.3, 130.4, 128.2, 123.7, 121.6, 120.9, 117.8, 115.1, 114.0, 111.8, 109.9, 66.6, 55.5, 52.6, 48.8. MS (ESI) calculated for $(C_{27}H_{27}N_4O_4)^+$ $[M+H]^+$ 471.2, found 471.2

6-(4-Morpholinopiperidin-1-yl)-3-(3-methoxyphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (49)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 – 7.62 (m, 3H), 7.51 – 7.40 (m, 3H), 7.09 (d, *J* = 14.9 Hz, 1H), 7.03 (ddd, *J* = 8.5, 2.6, 1.0 Hz, 1H), 6.90 (ddd, *J* = 7.8, 1.9, 1.0 Hz, 1H), 6.85 (d, *J* = 6.8 Hz, 2H), 6.59 (d, *J* = 8.3 Hz, 1H), 3.91 (d, *J* = 12.5 Hz, 2H), 3.80 (d, *J* = 1.1 Hz, 3H), 3.75 (s, 4H), 3.62 (d, *J* = 1.1 Hz, 3H), 2.85 (td, *J* = 12.5, 2.2 Hz, 2H), 2.62 (d, *J* = 6.1 Hz, 4H), 2.07 – 1.93 (m, 2H), 1.77 – 1.62 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz)) δ 163.1, 162.3, 153.3, 151.6, 150.9, 150.6, 139.4, 138.9, 136.9, 136.7, 133.3, 129.4, 127.7, 126.7, 126.0, 125.9, 125.7, 125.5, 124.6, 121.3, 118.1, 112.0, 52.6, 34.8, 34.6, 31.3, 31.2. MS (ESI) calculated for $(C_{32}H_{36}N_5O_4)^+$ $[M+H]^+$ 554.3, found 554.3.

6-(Piperidin-1-yl)-3-(3-trifluoromethylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (50)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 – 7.59 (m, 6H), 7.58 – 7.42 (m, 3H), 6.97 (dd, *J* = 14.8, 1.7 Hz, 1H), 6.86 (d, *J* = 7.1 Hz, 1H), 6.60 (t, *J* = 6.1 Hz, 1H), 3.56 (s, 3H), 3.42 – 3.23 (m, 4H), 1.81 – 1.68 (m, 4H), 1.63 (q, *J* = 5.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.2, 162.1, 150.6, 138.9, 138.2, 136.5, 132.6, 130.3, 128.5, 126.0, 125.6, 124.6, 123.2, 121.5, 117.9, 111.9, 52.4, 25.4, 24.1. MS (ESI) calculated for $(C_{28}H_{26}F_3N_4O_2)^+$ $[M+H]^+$ 507.2, found 507.2.

6-Morpholino-3-(3-trifluoromethylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (51)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 – 7.67 (m, 4H), 7.65 (s, 1H), 7.62 (d, *J* = 2.9 Hz, 1H), 7.55 – 7.42 (m, 3H), 6.97 (d, *J* = 14.7 Hz, 1H), 6.86 (d, *J* = 7.1 Hz, 1H), 6.61 (d, *J* = 8.2 Hz, 1H), 4.00 – 3.82 (m, 4H), 3.56 (s, 3H), 3.38 – 3.22 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.2, 161.9, 150.4, 150.1, 148.3, 138.9, 138.1, 137.2, 132.5, 132.2, 130.4, 128.5, 126.0, 125.9, 125.8, 125.7, 124.7, 123.8, 122.9, 121.5, 118.0, 112.1, 109.7, 66.6, 52.4, 48.7. MS (ESI) calculated for $(C_{27}H_{24}F_3N_4O_3)^+$ $[M+H]^+$ 509.2, found 509.2.

6-(4-Morpholinopiperidin-1-yl)-3-(3-(trifluoromethyl)phenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (52)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 – 7.56 (m, 6H), 7.56 – 7.38 (m, 3H), 6.97 (dt, *J* = 14.9, 3.0 Hz, 1H), 6.86 (dd, *J* = 7.1, 1.9 Hz, 1H), 6.60 (dd, *J* = 8.3, 1.9 Hz, 1H), 3.91 (d, *J* = 12.4 Hz, 2H), 3.74 (s, 4H), 3.56 (d, *J* = 1.9 Hz, 3H), 2.87 (t, *J* = 12.5 Hz, 2H), 2.60 (s, 4H), 2.48 – 2.35 (m, 1H), 1.99 (d, *J* = 12.4 Hz, 2H), 1.76 – 1.57 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.2, 162.0, 150.5, 149.9, 147.9, 140.5, 138.9, 138.2, 136.7, 132.5, 130.3, 128.6, 125.9, 125.7, 124.6, 123.2, 121.5, 117.9, 112.0, 110.0, 61.9, 52.4, 49.7, 48.4, 27.7. LC-MS (M + H) = 592.3. MS (ESI) calculated for $(C_{32}H_{33}F_3N_5O_3)^+$ $[M+H]^+$ 592.3, found 592.2

General synthetic methods for compounds 29–34: A mixture of compound **58** (0.25 mmol, 1 eq), an aryl or vinyl boronic acid (0.30 mmol, 1.2 eq), tetrakis(triphenylphosphine)palladium(0) (0.029 g, 0.0125 mmol, 0.05 eq), and Na_2CO_3 (0.053 g, 0.5 mmol, 2 eq) in 1,4-dioxane/H_2O (8/2 mL) was heated to 110 °C for 24 h. The reaction mixture was cooled down and quenched with brine (20 mL). The product was

extracted with ethyl acetate (3 × 20 mL), and the combined organic phase was washed with brine and then dried over Na_2SO_4. Upon removal of solvent, the residue was purified with column chromatography (silica gel, hexanes : ethyl acetate, 9:1 – 3:1) to give the target compounds as an off-white or pale yellow solid in 45–80% yield.

6-(Pyridin-4-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone dihydrochloride (29)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.71 (d, *J* = 5.4 Hz, 2H), 8.62 (d, *J* = 2.2 Hz, 1H), 8.08 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.91 (d, *J* = 8.5 Hz, 1H), 7.85 (d, *J* = 14.8 Hz, 1H), 7.77 – 7.70 (m, 2H), 7.65 (ddd, *J* = 12.0, 8.2, 1.4 Hz, 1H), 7.58 – 7.43 (m, 4H), 7.32 (t, *J* = 1.9 Hz, 1H), 7.14 (ddd, *J* = 7.4, 2.2, 1.2 Hz, 1H), 7.04 (d, *J* = 14.8 Hz, 1H), 6.91 (d, *J* = 7.1 Hz, 1H), 6.63 (d, *J* = 8.3 Hz, 1H), 3.58 (s, 3H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.2, 162.0, 153.5, 152.9, 150.4, 148.7, 148.6, 138.9, 138.5, 136.6, 135.2, 132.8, 132.1, 132.0, 132.0, 131.9, 131.9, 129.5, 128.6, 128.5, 128.4, 126.2, 125.9, 125.5, 125.4, 123.6, 121.8, 121.6, 118.3, 112.3, 52.6, 34.9, 31.2. MS (ESI) calculated for $(C_{31}H_{29}N_4O_2)^+$ $[M+H]^+$ 489.2, found 489.2

6-(3-Methoxyphenyl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (30)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.56 – 8.50 (m, 1H), 8.03 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.90 – 7.78 (m, 2H), 7.57 – 7.44 (m, 3H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.35 – 7.26 (m, 2H), 7.23 (dd, *J* = 2.5, 1.6 Hz, 1H), 7.14 (ddd, *J* = 7.4, 2.1, 1.3 Hz, 1H), 7.04 (d, *J* = 14.8 Hz, 1H), 6.93 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 6.90 (d, *J* = 7.1 Hz, 1H), 6.62 (dd, *J* = 8.3, 0.7 Hz, 1H), 3.87 (s, 3H), 3.58 (s, 3H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.3, 160.1, 153.3, 151.8, 150.6, 141.1, 139.4, 138.9, 137.7, 136.9, 133.5, 129.9, 129.4, 127.8, 126.0, 125.6, 125.5, 125.0, 123.8, 121.3, 119.6, 118.1, 113.4, 112.6, 112.0, 55.3, 52.6, 34.8, 31.2. MS (ESI) calculated for $(C_{33}H_{32}N_3O_3)^+$ $[M+H]^+$ 518.2, found 518.2.

6-(4-fluorophenyl)-3-(3-tert-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (31)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (d, *J* = 2.2 Hz, 1H), 7.99 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.90 – 7.78 (m, 2H), 7.70 – 7.61 (m, 2H), 7.57 – 7.45 (m, 3H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.20 – 7.11 (m, 3H), 7.04 (d, *J* = 14.8 Hz, 1H), 6.89 (d, *J* = 7.1 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 1H), 3.58 (s, 3H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 153.4, 151.8, 150.6, 138.9, 138.5, 136.8, 133.2, 129.4, 128.8, 128.7, 127.9, 126.1, 125.6, 125.5, 124.8, 121.3, 118.1, 116.0, 115.8, 112.1, 52.6, 34.8, 31.2. MS (ESI) calculated for $(C_{32}H_{29}N_3O_2)^+$ $[M+H]^+$ 506.2, found 506.2

6-(4-dimethylaminophenyl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (32)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.49 (d, *J* = 2.3 Hz, 1H), 8.02 (ddd, *J* = 8.5, 2.3, 0.9 Hz, 1H), 7.85 – 7.74 (m, 2H), 7.68 – 7.59 (m, 2H), 7.56 – 7.44 (m, 3H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.18 – 7.11 (m, 1H), 7.04 (d, *J* = 14.8 Hz, 1H), 6.88 (d, *J* = 7.1 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 2H), 6.60 (d, *J* = 8.2 Hz, 1H), 3.58 (d, *J* = 1.0 Hz, 3H), 3.01 (s, 6H), 1.32 (d, *J* = 1.0 Hz, 9H). ^{13}C

NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.4, 153.2, 151.0, 150.8, 146.0, 139.6, 138.9, 137.1, 137.0, 132.6, 129.3, 127.7, 127.7, 125.9, 125.7, 125.5, 124.1, 123.3, 121.3, 117.9, 112.8, 111.8, 52.6, 40.5, 34.8, 31.2, 29.6. MS (ESI) calculated for $(C_{34}H_{35}N_4O_2)^+$ $[M+H]^+$ 531.3, found 531.3.

6-[4-(Pyrrolidin-1-yl)phenyl]-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (33)

^1H NMR ($CDCl_3$, 400 MHz) δ 8.48 (d, *J* = 2.2 Hz, 1H), 8.02 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.85 – 7.74 (m, 2H), 7.63 (d, *J* = 8.6 Hz, 2H), 7.56 – 7.44 (m, 3H), 7.32 (d, *J* = 1.9 Hz, 1H), 7.14 (dt, *J* = 7.4, 1.7 Hz, 1H), 7.03 (d, *J* = 14.8 Hz, 1H), 6.88 (d, *J* = 7.1 Hz, 1H), 6.66 (d, *J* = 8.3 Hz, 2H), 6.60 (d, *J* = 8.3 Hz, 1H), 3.58 (s, 3H), 3.34 (t, *J* = 8.0, 4H), 2.13 – 1.91 (m, 4H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.4, 153.2, 150.8, 150.8, 147.6, 139.9, 138.9, 137.1, 132.5, 129.3, 127.8, 127.6, 125.9, 125.7, 125.5, 124.1, 123.1, 121.3, 117.9, 111.8, 52.6, 47.6, 34.8, 31.2, 25.4. MS (ESI) calculated for $(C_{36}H_{37}N_4O_2)^+$ $[M+H]^+$ 557.3, found 557.3.

6-(3,6-dihydro-*2H*-pyran-4-yl)-3-(3-tert-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)vinyl]-4(3*H*)-quinazolinone (34)

^1H NMR ($CDCl_3$, 400 MHz) δ 8.27 (d, *J* = 2.1 Hz, 1H), 7.88 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.84 – 7.72 (m, 2H), 7.56 – 7.44 (m, 3H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.12 (ddd, *J* = 7.4, 2.2, 1.3 Hz, 1H), 7.02 (d, *J* = 14.8 Hz, 1H), 6.88 (d, *J* = 7.2 Hz, 1H), 6.61 (d, *J* = 8.3 Hz, 1H), 6.32 (tt, *J* = 3.2, 1.5 Hz, 1H), 4.37 (q, *J* = 2.8 Hz, 2H), 3.96 (t, *J* = 5.4 Hz, 2H), 3.57 (s, 3H), 2.69 – 2.51 (m, 2H), 1.32 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.2, 153.3, 151.6, 150.6, 138.9, 138.4, 136.8, 133.0, 131.0, 129.4, 127.4, 126.0, 125.6, 125.4, 123.8, 122.4, 120.9, 118.1, 112.0, 65.8, 64.3, 52.6, 34.8, 31.2, 26.9. MS (ESI) calculated for $(C_{31}H_{32}N_3O_3)^+$ $[M+H]^+$ 494.2, found 494.3.

General synthetic methods for compounds 53–54: A mixture of a compound (**22, 27)** (0.25 mmol, 1 eq) and palladium on activated charcoal (10% w/w) in methanol was stirred under hydrogen gas. Upon completion, the reaction mixture was filtered using Celite bed, and solvent was removed under reduced pressure. Obtained residue was purified with column chromatography (silica gel, hexanes : ethyl acetate, 9:1 – 3:1) to give the target compounds as an off-white or orange solid in 80–88% yield.

6-(Piperidin-1-yl)-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)ethyl]-4(3*H*)-quinazolinone (53)

^1H NMR ($CDCl_3$, 400 MHz) δ 7.68 – 7.52 (m, 2H), 7.52 – 7.28 (m, 4H), 7.28 – 7.17 (m, 1H), 7.06 – 6.95 (m, 1H), 6.61 (d, *J* = 7.2 Hz, 1H), 6.46 (d, *J* = 8.2 Hz, 1H), 3.70 (s, 3H), 3.26 (t, *J* = 5.5 Hz, 4H), 3.08 (t, *J* = 7.7 Hz, 2H), 2.88 – 2.68 (m, 2H), 1.71 (q, *J* = 5.7 Hz, 4H), 1.59 (t, *J* = 6.2 Hz, 2H), 1.31 (s, 9H).^{13}C NMR (100 MHz, $cdcl_3$) δ 162.5, 157.8, 153.1, 138.6, 137.2, 129.3, 125.9, 125.3, 125.2, 121.3, 115.3, 107.9, 52.9, 34.8, 34.6, 34.6, 31.2, 25.4, 24.0. MS (ESI) calculated for $(C_{31}H_{37}N_4O_2)^+$ $[M+H]^+$ 497.3, found 497.3.

6-Morpholino-3-(3-*tert*-butylphenyl)-2-[2-(6-methoxypyridin-2-yl)ethyl]-4(3*H*)-quinazolinone (54)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 (d, *J* = 8.9 Hz, 1H), 7.61 (d, *J* = 2.8 Hz, 1H), 7.52 – 7.34 (m, 4H), 7.24 (d, *J* = 2.8 Hz, 1H), 7.01 (dt, *J* = 7.6, 1.6 Hz, 1H), 6.62 (d, *J* = 7.2 Hz, 1H), 6.47 (d, *J* = 8.2 Hz, 1H), 3.87 (t, *J* = 4.8 Hz, 4H), 3.69 (s, 3H), 3.25 (t, *J* = 4.8 Hz, 4H), 3.09 (t, *J* = 7.7 Hz, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 163.4, 153.2, 149.8, 138.6, 129.3, 126.1, 125.2, 125.2, 123.8, 115.3, 109.9, 108.0, 66.7, 52.9, 49.0, 34.8, 34.6, 31.2, 29.6. MS (ESI) calculated for $(C_{30}H_{35}N_4O_3)^+$ $[M+H]^+$ 499.3, found 499.3.

Cellular antiviral assays.

The cellular antiviral activities of these compounds were determined in Vero, U87 glioma or mosquito C6/36 cells, following our previous methods [34]. Briefly, 2×10^4 cells/well were seeded in 96-well plates and cultured in DMEM media with 2% FBS to form a monolayer of cells. For regular compound treatment (2h-pretreatment), 0.1 MOI (multiplicity of infection) of ZIKV or DENV, together with various concentrations of a compound, was added to each well. After incubation for 2h, the supernatant was removed and cells were washed with PBS to remove unattached viral particles. Fresh medium (150 μL/well) containing various concentrations of a compound in duplicate were added. For 0h-post-treatment, a compound was added into cell culture media right after removal of the unattached viruses. For 2h-post-treatment, a compound was added into cell culture media 2 hours after removal of the unattached viruses. Upon incubation at 37 °C for 48h, aliquots of the supernatant from each well were used to determine viral $TCID_{50}$ (tissue culture infectious dose) using end-point dilution assay. Half-log serial dilution of the viral supernatant (50 μL) was added to a monolayer of Vero cells in quadruplicate in 96-well plates and cultured for 5–7 days. CPE or cell lysis was determined with microscope followed by MTT assay. $TCID_{50}$ was calculated based on the highest dilution in which ≥50% (i.e., ≥2 out of the 4 quadruplicate wells) of Vero cells were infected with ZIKV. Compared to controls, the ability for a compound to reduce $TCID_{50}$ can be determined. The results were from at least 2 independent experiments. Inhibition data were imported into Prism (version 5.0) and EC_{50} values with standard deviation were obtained by using a standard dose-response curve fitting.

Quantitative RT-PCR to determine ZIKV RNA copies.

Viral RNA was extracted from the supernatant (50 μL) using TRIzol (ThermoFisher). qPCR is based on amplification of ZIKV envelope gene region 3, using ZIKV-specific primers and probes, following our previous method [34]. PCR was performed using a TaqMan Fast Virus 1-step Master Mix kit on a StepOnePlus RT-PCR system (Applied Biosystems). Concentrations of ZIKV RNA (copies/mL) were calculated by using a standard curve.

Western blot.

8×10^5 Vero cells in petri dishes were infected with ZIKA (MOI = 0.1) for 2h. Upon removal of the virus, the cells were cultured with fresh media contain 2% FBS and compound **27** for 48h. The cells were harvested and lysed using ice-cold

radioimmunoprecipitation assay buffer (Invitrogen). Equal amounts of total proteins were then separated on SDS-PAGE and transferred to PVDF membranes. The blots were probed and visualized with primary antibodies against Zika virus NS3 (GTX133309, GeneTex), Zika virus capsid (GTX133317, GeneTex), Zika virus NS5 (GTX133327, GeneTex), and human β-Actin (4967S, Cell Signaling).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation:

SAR	structure–activity relationships
DENV	Dengue virus
ZIKV	Zika virus
RNA	Ribonucleic acid
EC_{50}	half maximal effective concentration
WHO	World Health Organization
CPE	cytopathic effects
MOI	multiplicity of infection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
$TCID_{50}$	half maximal tissue culture infectious dose
LCMS	liquid chromatography–mass spectrometry
ESI	electrospray ionization
HPCL	high performance liquid chromatography
DMF	N,N-dimethylformamide

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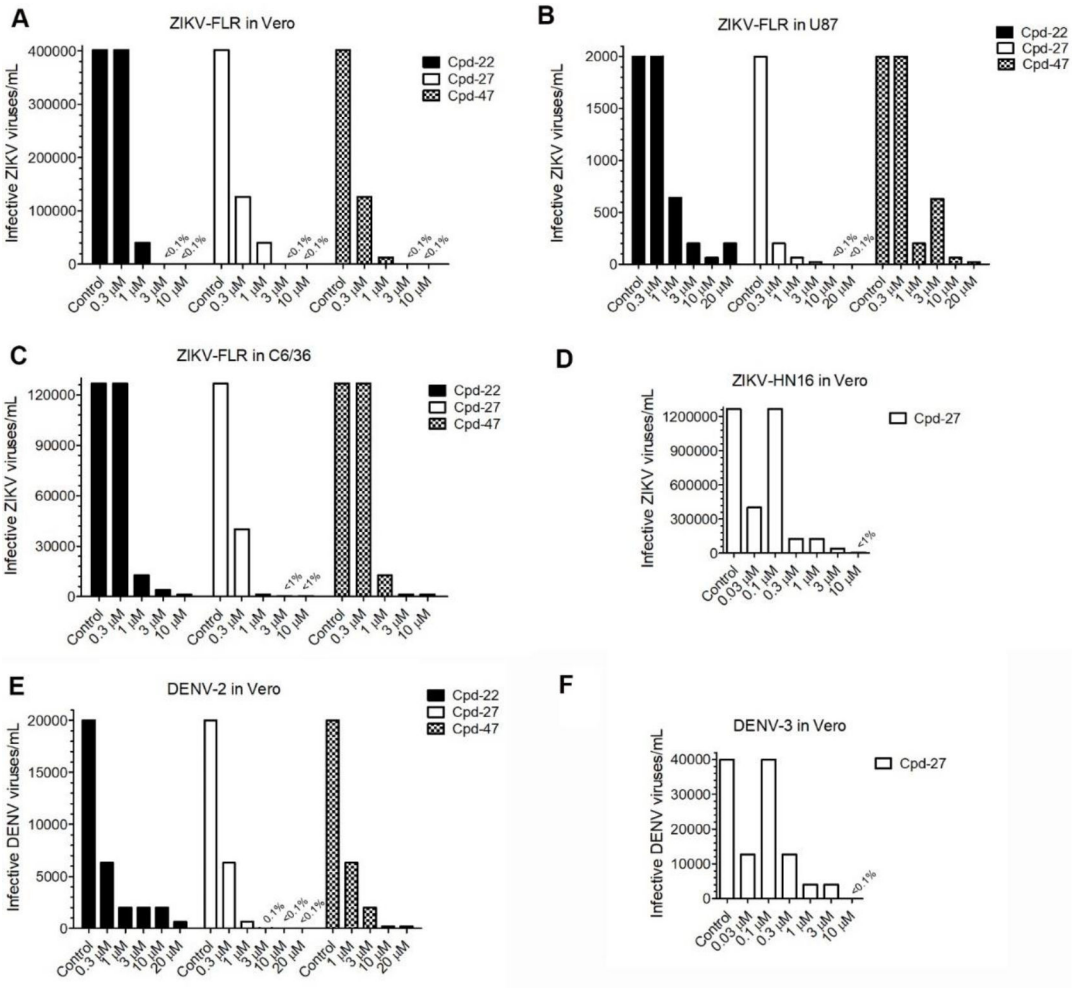


Figure 1.

Representative antiviral activities of compounds **22**, **27**, and **47** in cells. (**A-C**, **E**) Treatment of Vero (**A**, **E**), U87 (**B**) and mosquito C6/36 cells (**C**) with compounds **22**, **27**, and **47** caused dose-dependent reduction of infectious ZIKV-FLR (**A-C**) and DENV-2 (**E**); (**D**, **F**) Treatment of Vero cells with compound **27** inhibited generation of infectious ZIKV-HN16 (**D**) and DENV-3 (**F**).

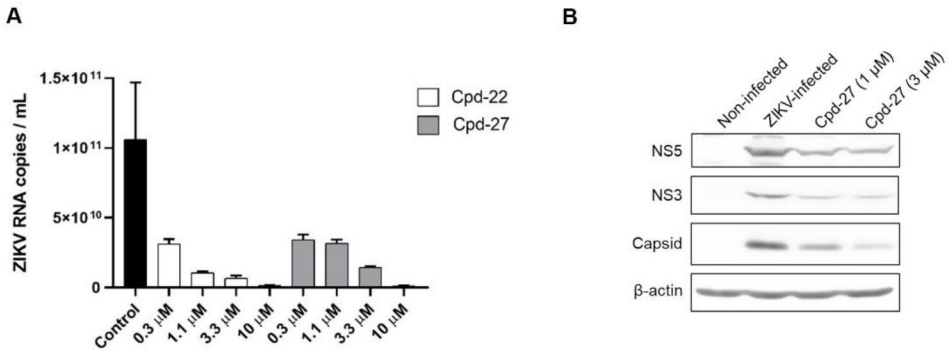


Figure 2.

Treatment of Vero cells with compounds **27** and/or **22** dose-dependently reduced (**A**) the ZIKV RNA copies in the cell supernatant and (**B**) cellular viral NS5, NS3 and capsid proteins.

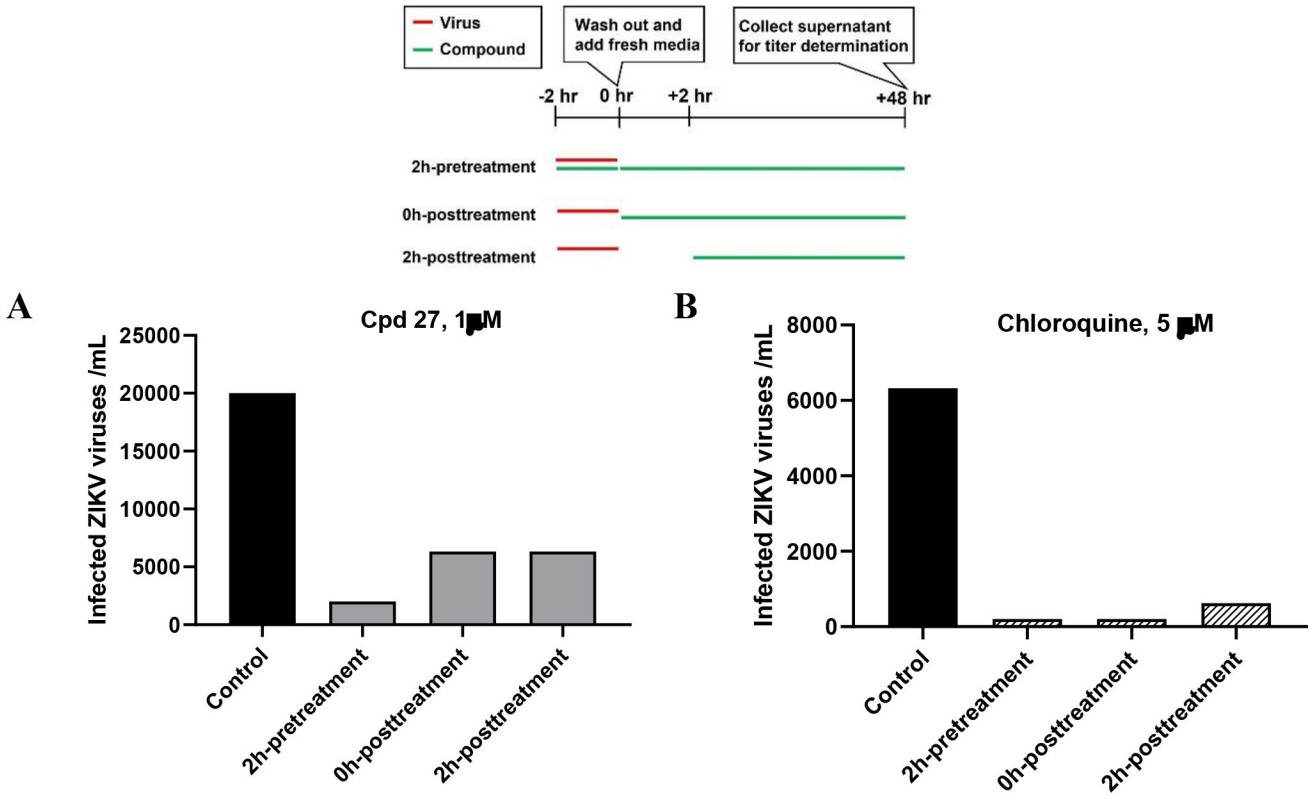


Figure 3.

Anti-ZIKV (FLV strain) activities of (**A**) compound **27** and (**B**) chloroquine when added into the cell culture media at −2, 0 and 2 hours before removal of the inoculated virus (designated as 2h-pretreatment, 0h-, and 2h-posttreatment, respectively).

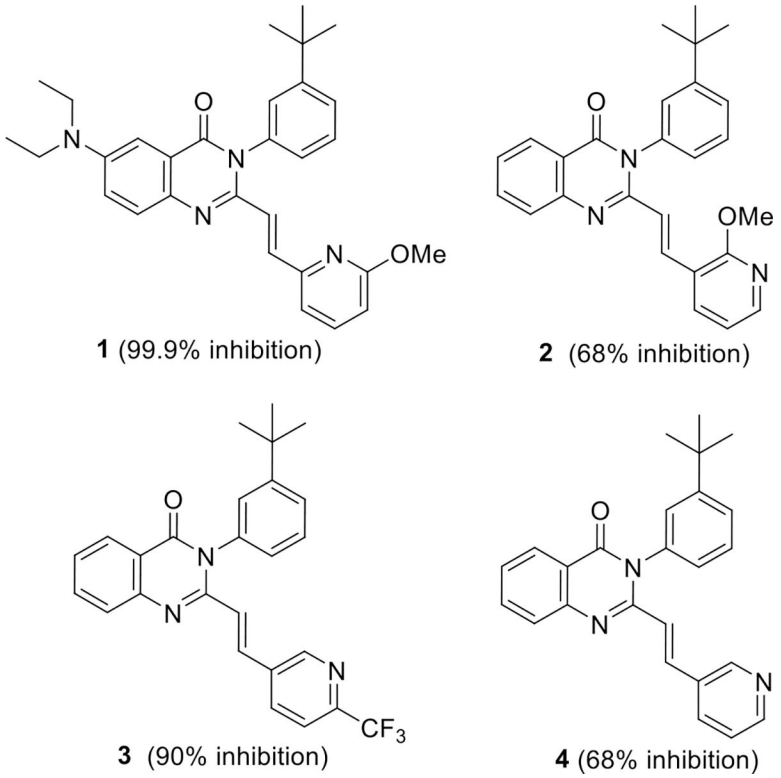


Chart 1.
Structures and anti-ZIKV (FLV strain) activities in Vero cells (at 10 μM) of compounds **1–4**.

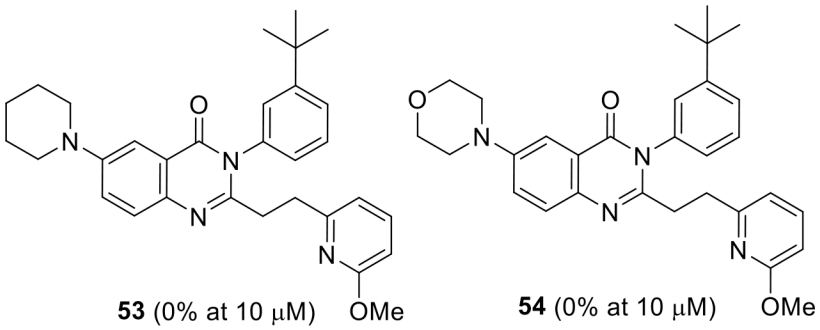
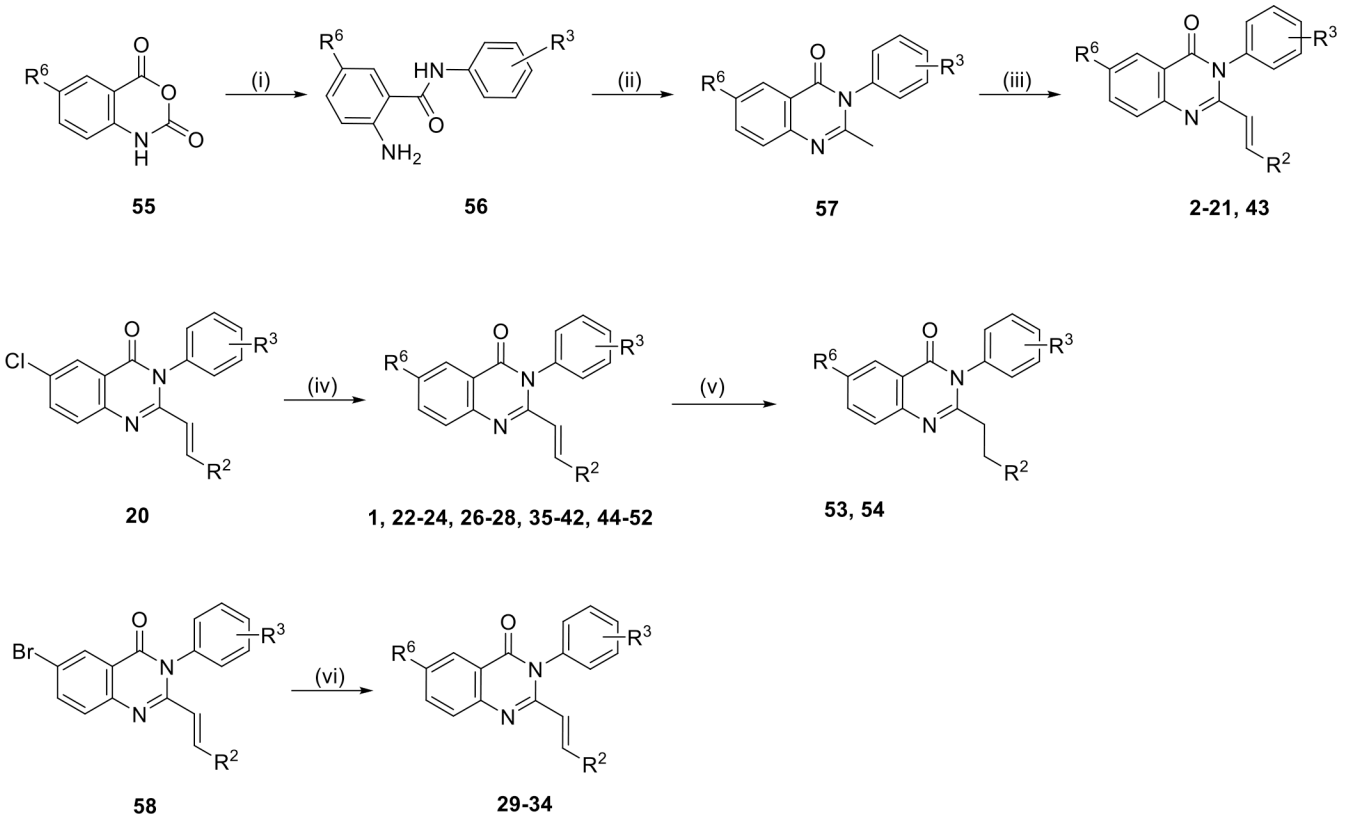


Chart 2.

Structures and anti-ZIKV (FLV strain) activities of compounds **53** and **54** in Vero cells.



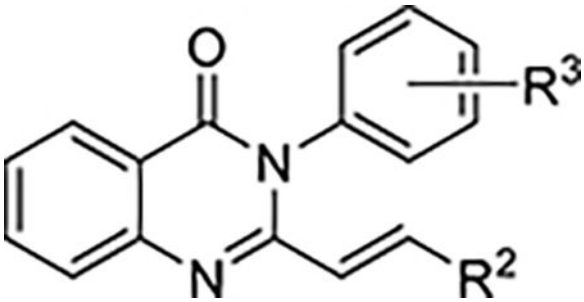
Scheme 1:

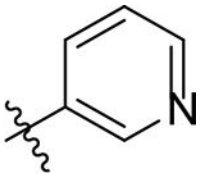
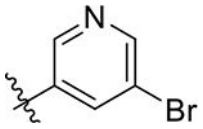
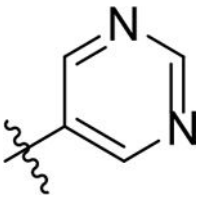
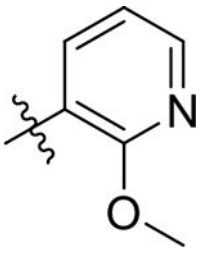
Synthesis of compounds **1–54**.[a]

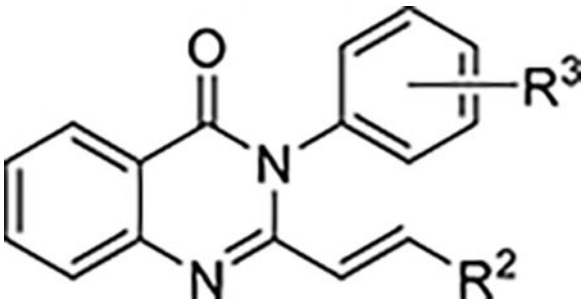
[a]*Reagents and conditions*: (i) an aniline, 1,4-dioxane, 90 °C, 12 h, 45–81%; (ii) 1,1,1-triethoxyethane, 110 °C, overnight, 32–94%; (iii) aryl-CHO, AcONa, AcOH, reflux, overnight, 35–91%; (iv) an amine, tris(dibenzylideneacetone)dipalladium(0), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl, sodium *tert*-butoxide, toluene, 110 °C, 24 h, 30–81%; (v) Pd/C, H_2, MeOH, 25 °C, overnight, 80–88%; (vi) tetrakis(triphenylphosphine)palladium(0), 1,4-dioxane, H_2O, 110 °C, 24 h, 45–80%.

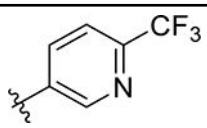
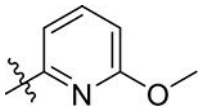
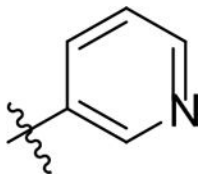
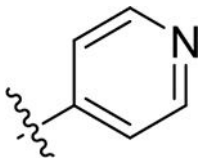
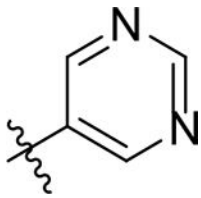
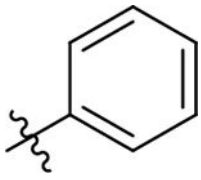
Table 1:

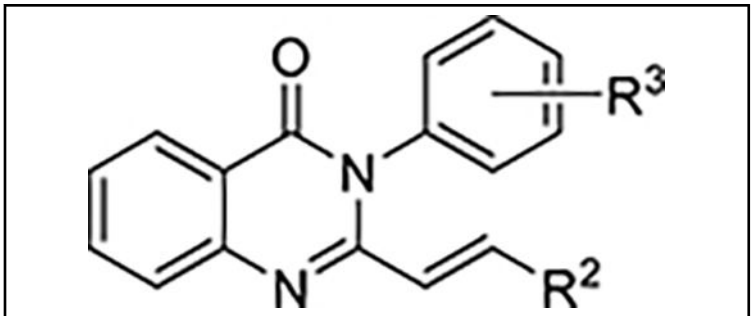
Structures and anti-ZIKV (FLV strain) activities of compounds **2–19** in Vero cells.



Cpd #	R^2	R^3	% inhibition of ZIKV at 10 μM
5		H	0
6	same as above	2-CH_3	0
7	same as above	3-CH_3	0
4	same as above	3-OCH_3	68.4%
8	same as above	4-*t*-Bu	0
9		same as above	0
10		same as above	0
2		3-*t*-Bu	68.4%



Cpd #	R^2	R^3	% inhibition of ZIKV at 10 μM
3		same as above	90.0%
11		same as above	0
12		same as above	0
13		same as above	68.4%
14		same as above	68.4%
15		same as above	0



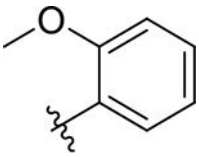
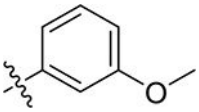
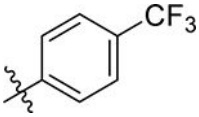
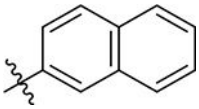
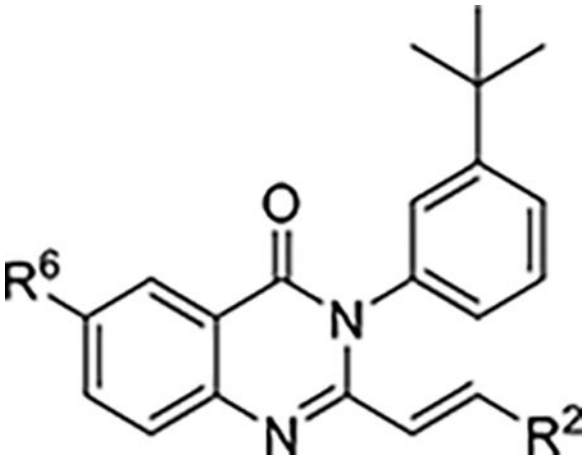
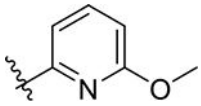
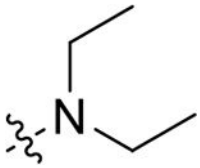
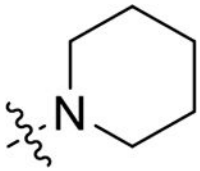
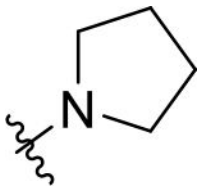
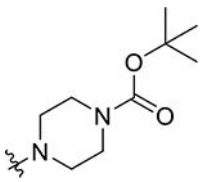
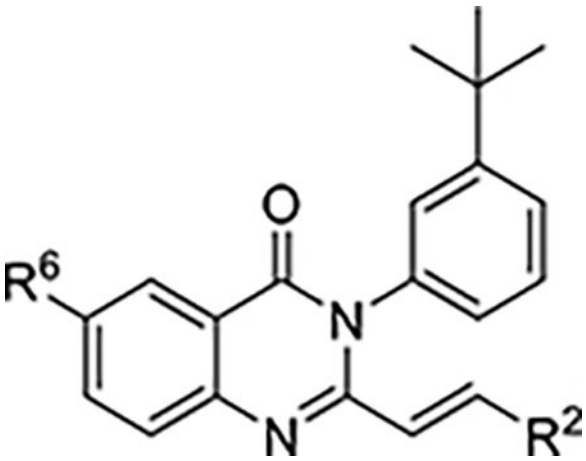
Cpd #	R^2	R^3	% inhibition of ZIKV at 10 μM
16		same as above	0
17		same as above	0
18		same as above	90.0%
19		same as above	68.4%

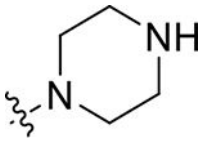
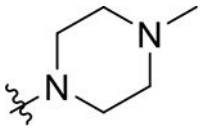
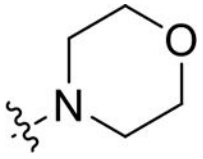
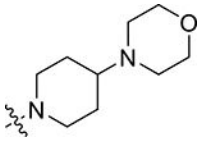
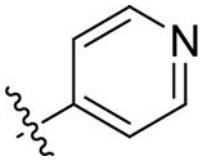
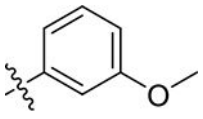
Table 2:

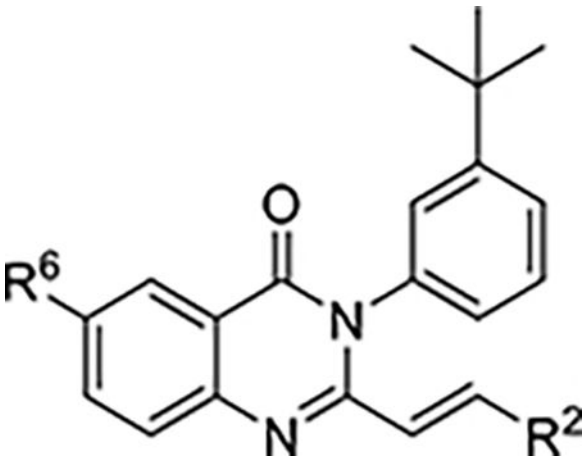
Structures and anti-ZIKV (FLV strain) activities of compounds **1**, **20–43** in Vero cells.

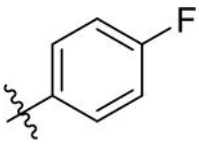
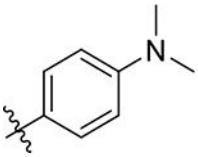
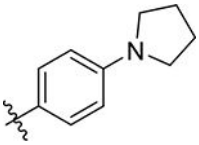
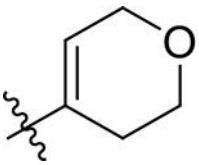
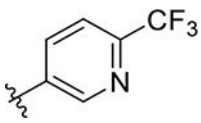
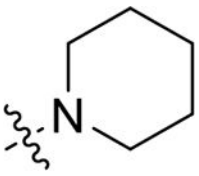
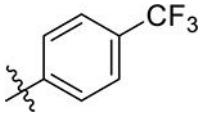


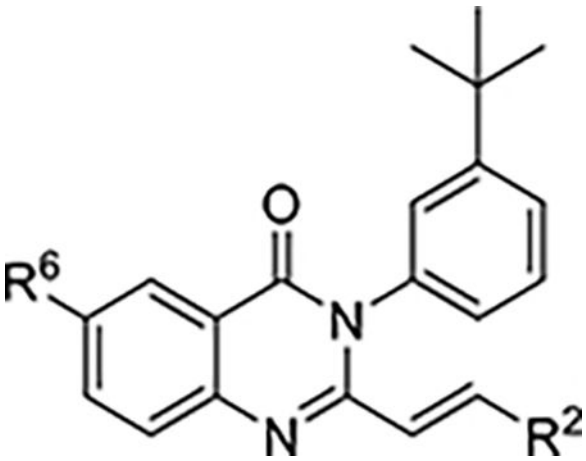
Cpd #	R^2	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
1			99.9%	68.4%
20	same as above	-Cl	0	NT [a]
21	same as above	$-OCH_3$	0	NT [a]
22	same as above		99.9%	90.0%
23	same as above		68.4%	NT [a]
24	same as above		99.0%	NT [a]

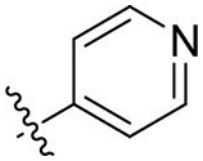
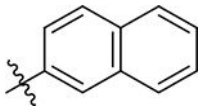
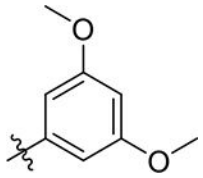
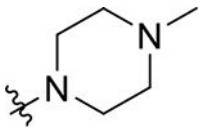
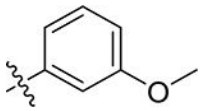
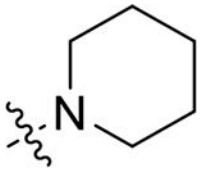


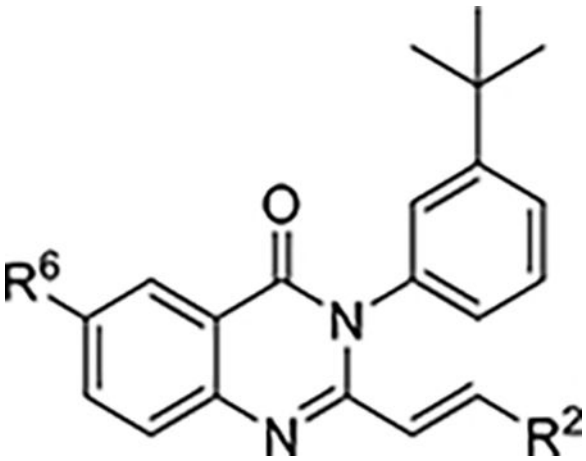
Cpd #	R^2	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
25	same as above		Cytotoxic	NT [a]
26	same as above		Cytotoxic	NT [a]
27	same as above		99.9%	90.0%
28	same as above		99.9%	90.0%
29	same as above		0	NT [a]
30	same as above		0	NT [a]

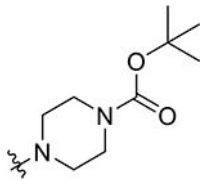


Cpd #	R^2	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
31	same as above		99.0%	NT [a]
32	same as above		96.8%	NT [a]
33	same as above		96.8%	NT [a]
34	same as above		96.8%	NT [a]
35			90.0%	NT [a]
36		same as above	99.0%	NT [a]



Cpd #	R^2	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
37		same as above	68.4%	NT [a]
38		same as above	99.7%	NT [a]
39		same as above	96.8%	NT [a]
40	same as above		Cytotoxic	NT [a]
41			99.9%	68.4%

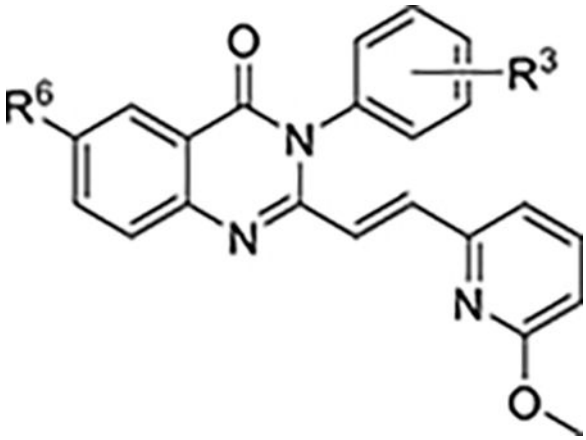


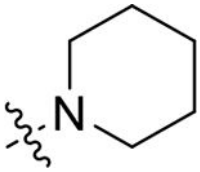
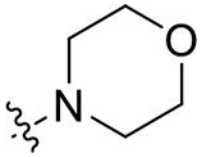
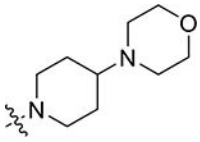
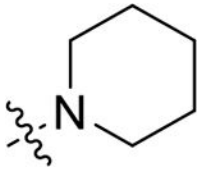
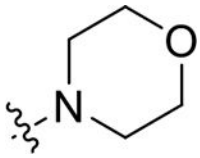
Cpd #	R^2	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
42	same as above		99.0%	NT [a]
43	same as above	-OCH_3	0	NT [a]

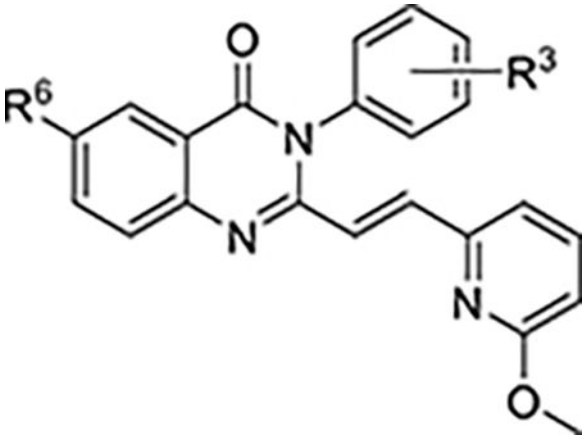
[a]Not tested.

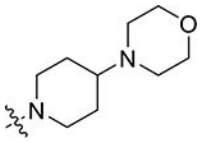
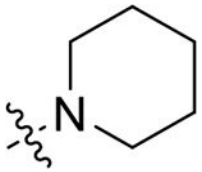
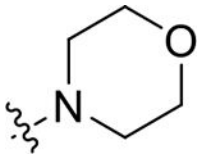
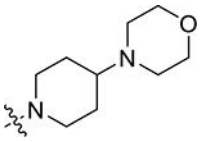
Table 3:

Structures and anti-ZIKV (FLV strain) activities of compounds **44–52** in Vero cells.



Cpd #	R^3	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
44	3-CH_3		99.9%	0%
45	same as above		90.0%	NT [a]
46	same as above		99.9%	0%
47	3-OCH_3		99.9%	90.0%
48	same as above		99.0%	NT [a]



Cpd #	R^3	R^6	% inhibition of ZIKV at 10 μM	% inhibition of ZIKV at 1 μM
49	same as above		99.9%	68.4%
50	3-CF_3		99.0%	NT [a]
51	same as above		0%	NT [a]
52	same as above		68.4%	NT [a]

[a]Not tested.

Table 4:

Antiviral activity (EC_{50} in μM) against ZIKV and DENV.

	ZIKV-FLR in Vero cells	ZIKV-FLR in U87 cells	ZIKV-FLR in C6/36 cells	ZIKV-HN16 in Vero cells	DENV-2 in Vero cells	DENV-3 in Vero cells	CC_{50} (μM) in U87 cells
22	0.90 ± 0.32	0.82 ± 0.09	0.75 ± 0.40	NT [a]	0.16 ± 0.04	NT [a]	>20
27	0.18 ± 0.02	0.10 ± 0.022	0.23 ± 0.01	0.086 ± 0.057	0.21 ± 0.02	0.12 ± 0.07	>20
47	0.21 ± 0.01	0.69 ± 0.47	0.77 ± 0.41	NT [a]	0.56 ± 0.02	NT [a]	>20

[a]Not tested.