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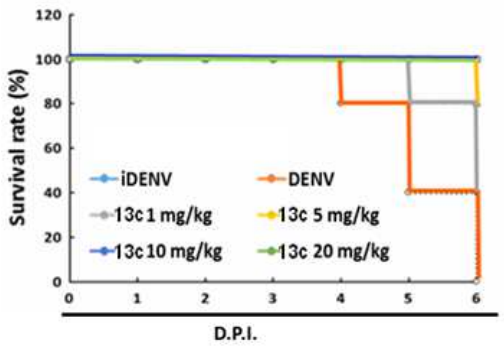
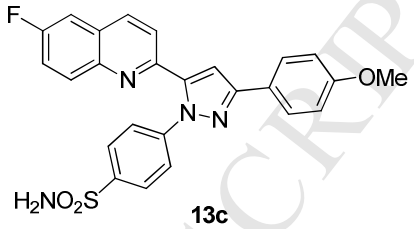
Graphical abstract

New diarylpyrazolylquinolines with anti-DENV activity were designed and synthesized. Among them, compound **13c** was potent inhibited all four sero-types of DENV. It can effectively protect mice from DENV infection by reducing disease symptoms and mortality of DENV-infected mice.





13c

Discovery of novel diarylpyrazolylquinoline derivatives as potent anti-dengue virus agents

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Abstract

A number of diarylpyrazolylquinoline derivatives were synthesized and evaluated for their anti-dengue virus (DENV) activity. Among them, 6-fluoro-2-(1-(4-fluorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-5-yl)quinoline (**11c**), 2-[1,3-bis(4-methoxyphenyl)-1H-pyrazol-5-yl]-6-fluoroquinoline (**12c**), and 4-[5-(6-fluoroquinolin-2-yl)-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (**13c**) exhibited approximately 10-folds more active anti-DENV-2 activity (IC_{50} of 1.36, 1.09 and 0.81 μM, respectively) than that of ribavirin (IC_{50} = 12.61 μM). Compound **13c** was also potent inhibited other sero-types of DENV. It reduced DENV replication in both viral protein and mRNA levels, and no significant cell cytotoxicity was detected, with greater than 50% viability of Huh-7-DV-Fluc cells at a concentration of 200 μM. Furthermore, compound **13c** can effectively protect mice from DENV infection by reducing disease symptoms and mortality of DENV-infected mice. It represents a potential antiviral agent to block DENV replication *in vitro* and *in vivo*. Structural optimization of the initial lead compound, **13c**, and the detailed molecular mechanism of action are ongoing.

Keywords: Diarylpyrazolylquinoline, Ribavirin, Dengue virus, Anti-dengue virus activity

1. Introduction

Dengue is a mosquito-borne viral disease that has become a major public health concern worldwide in recent years. Annually, 100 million cases of dengue fever and 500,000 cases of dengue hemorrhagic fever occur, particularly in tropical Asia, Latin America, and the Caribbean [1]. At present, dengue is endemic in 112 countries around the world [2]. However, there is no vaccine or treatment other than vector control and supportive medical care. The development of safe and effective therapeutics is therefore urgently needed [3-6].

The dengue virus (DENV) belongs to the family *Flaviviridae* and is closely related to the West Nile virus (WNV), the Yellow Fever virus (YFV) and the Hepatitis C virus (HCV) [7]. DENV are transmitted by mosquitos of the species *Aedes aegypti* and cause clinical symptoms ranging from mild fever to Dengue hemorrhagic fever (DHS) and Dengue shock syndrome (DSS) [8, 9]. However, there are no specific approved drugs or vaccines for the treatment or prevention of DHS and DSS.

Quinoline and pyrazole are isosteric with purine and pyrimidine nuclei respectively, which are present in a number of fundamental cellular components and bioactive compounds [10-16]. This heterocycle may represent a kind of privileged substructure, which may interact with different proteins and enzymes. Indeed, a number of important drugs used in different therapeutic areas contain the quinoline ring, as well as several other kinds of still investigational therapeutic agents, including antitumorals [17-20] and antivirals [21-25]. Among these known antiviral quinoline and pyrazole derivatives, compound **1** (*Figure* 1) exhibited inhibitory activities against a DENV serotype 2 (DENV-2) sub-genomic replicon cell line with an IC_{50} value of 5 μM and against a WNV sub-genomic replicon cell line with an IC_{50} of 1.9 μM [26]. Compound **2** inhibited the growth of Hepatitis C virus with an IC_{50} value of 7.0 μM [27]. Recently, we have synthesized a number of 2-aroyl-3-arylquinoline derivatives and evaluated for their anti-DENV activity [28]. Compounds **3** and **4** were found to significantly inhibit the DENV-2 RNA expression in

Huh-7-DV-Fluc cells with potencies approximately equal to that of ribavirin. Both compounds were capable of reducing DENV-2 replication in both viral protein and mRNA levels. Compound **5** is a triarylpyrazoline derivative which has been discovered to inhibit flavivirus infection in cell culture [29]. In continuation of our studies to identify potential antiviral agents with a novel type of structures, we describe herein the preparation of hybrid pyrazolylquinoline derivatives (target compounds, *Figure* 1) and evaluation of their anti-DENV activity *in vitro* and *in vivo*.

< **Insert Figure 1 here** >

2. Chemistry

Oxidation of 6-fluoro-2-methylquinoline (**6**) with selenium dioxide gave 6-fluoro-2-formylquinoline (**7**) which was then condensed with acetophenone to afford exclusively trans conjugated carbonyl product, (*E*)-3-(6-fluoroquinolin-2-yl)-1-phenylprop-2-en-1-one (**8a**) as depicted in *Scheme* 1. Accordingly, compounds **8b** and **8c** were obtained from 4-fluoroactophenone and 4-methoxyacetophenone respectively with compound **7** under the same reaction conditions. Treatment of **8a** with phenylhydrazine followed by DDQ oxidation gave a mixture of 2-(2,3-diphenyl-2*H*-pyrazol-5-yl)-6-fluoroquinoline (**9a**) and 2-(2,5-diphenyl-2*H*-pyrazol-3-yl)-6-fluoroquinoline (**10a**) in 21% and 36% yield respectively as outlined in *Scheme* 2. The mixture of **9a** and **10a** was separated by silica gel column chromatography eluting with solvent mixtures of CH_2Cl_2-MeOH (100:1, 50:1, and 20:1).

Accordingly, a mixture of compounds **9b** and **10b** was obtained from compound **8b** with phenylhydrazine and the mixture of compounds **9c** and **10c** was obtained from compound **8c** under the same reaction conditions. However, reflux of **8a** with 4-fluorophenylhydrazine hydrochloride gave exclusively 6-fluoro-2-[1-(4-fluorophenyl)-3-phenyl-1*H*-pyrazol-5-yl]quinoline (**11a**) as a sole product. Compound **11b** and **11c** was obtained from 4-fluorophenylhydrazine hydrochloride and compound **8b** and **8c** respectively. Reflux of **8a** with 4-methoxyphenylhydrazine hydrochloride gave exclusively 6-fluoro-2-[1-(4-methoxyphenyl)-3-phenyl-1*H*-pyrazol-5-yl]quinoline (**12a**) as a sole product. Compound **12b** and **12c** was obtained from 4-methoxyphenylhydrazine hydrochloride

and compound **8b** and **8c** respectively under the same reaction conditions. Accordingly, reflux of **8a** with 4-hydrazinobenzenesulfonamide hydrochloride gave exclusively 4-[5-(6-fluoroquinolin-2-yl)-3-phenyl-1*H*-pyrazol-1-yl]benzenesulfonamide (**13a**) as a sole product. Compound **13b** and **13c** was obtained from 4-hydrazinobenzenesulfonamide hydrochloride and compound **8b** and **8c** respectively under the same reaction conditions. It is of interest to note that when **14** was reacted with 4-hydrazinobenzenesulfonamide hydrochloride the product was identified as the **13c** and not the expected regioisomer **15** as depicted in *Scheme* 3.

< **Insert Scheme 1-3 here** >

Structure of **9c** and **10c** were unambiguously determined by X-ray crystallographical analysis. The structure was solved and refined by direct methods Shelx 97 suite of programs [30]. Compounds **9c** (R_f = 0.52, (CH_2Cl_2 ∶ hexane = 4/1)) and **10c** (R_f = 0.51, (CH_2Cl_2 ∶ hexane = 4/1)) were obtained by slow evaporation from MeOH/CH_2Cl_2 (30/70) solution. The crystal structure and crystal packing diagram of compounds **9c** and **10c** were shown in *Figure* 2, respectively, while the crystallographic data and structure refinement details were given in *Table* 1. Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC 1054761 for compound **9c**, and CCDC 1054760 for compound **10c**. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via www.ccdc.cam.ac.uk).

The X-ray analysis proved unambiguously the regio-chemistry of the synthesized compounds and was in good agreement with the results by the ^{1}H NMR analysis. As shown in *Figure* 2, compounds **9c** and **10c** with the same formula were clearly assigned as the *N*-2-phenyl and *N*-3-phenyl isomers, respectively. Compounds **9c** and **10c** had a singlet signal appearing at δ = 7.30, 7.18 ppm in $CDCl_3$ and δ = 7.29, 7.49 ppm in DMSO-d_6 were assigned as pyrazole 5-H, respectively (*Figure* 3). The structures of the *N*-2 (**9a-c**) and *N*-3 (**10a-b**, **11a-c**, and **12a-c)** substituted isomers in Table 2 were assigned according to the methods of X-ray and ^{1}H NMR for

9c and **10c**. Consistent with previous observations, the *N*-2 substituted isomers (e.g., **9a-c**) had chemical shifts at the range of 7.30-7.38 ppm (lower fields) for the pyrazole 5-H as a singlet signal in their individual ^{1}H-NMR spectra in $CDCl_3$, while the *N*-3 substituted isomers (e.g., **10a-c, 11a-c, and 12a-c**) had chemical shifts at the range of of 7.16-7.25 ppm (higher fields) for the pyrazole 5-H as a singlet signal in $CDCl_3$. The compounds **13a-c** had chemical shifts at the range of of 7.58-7.68 ppm (lower fields) for the pyrazole 5-H as a singlet signal in their individual ^{1}H-NMR spectra in DMSO-d_6, which was similar to the *N*-3 substituted isomers **10c** with a singlet signal appearing at δ = 7.49 ppm (lower fields) in DMSO-d_6. In conclusion, all the structures of the regioisomers in this series could be definitely assigned by the ^{1}H NMR combined with single-crystal X-ray diffraction.

< **Insert Figure 2,3 and Table 1,2 here** >

3. Biological Results and Discussion

3.1. *Anti-DENV-2 and Antiproliferative Activities*

The anti-DENV-2 activities and cytotoxicities of diarylpyrazolylquinoline derivatives are summarized in *Table* 3. Huh-7-DV-Fluc cells were treated with compounds **9** – **13** or the positive ribavirin respectively at a concentration of 1 or 10 μM for three days. Cells were then analyzed through the firefly luciferase activity. Compounds which exhibited >32% inhibition of DENV-2 at a concentration of 10 μM were considered as active. Results from *Table 3* indicated that 2-(1,3-diphenyl-1*H*-pyrazol-5-yl)-6-fluoroquinoline (**9a**), which exhibited 15% inhibition of DENV-2 at a concentration of 10 μM, was more active than its fluoro-substituted counterpart, **9b**, and methoxy-substituted counterpart **9c**. Compound **9a** was also more active than its positional isomer, **10a**. However, 6-fluoro-2-[5-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-3-yl]quinoline (**10b**), which exhibited 25% inhibition of DENV-2 at a concentration of 10 μM, was more active than its positional isomer, **9b**. Introduction of 4-methoxy or 4-aminosulfonyl at *N*-3 phenyl ring improved anti-DENV2 in which compounds **12a** and **13a** were more active than **10a** while compounds **12c** and **13c** were more active than **10c**. However, compound **10b** was exceptionally active against

DENV-2 and more active than that of **12a** and **13a**. Among these pyrazolylquinoline derivatives, 6-fluoro-2-(1-(4-fluorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-5-yl)quinoline (**11c**), 2-[1,3-bis (4-methoxyphenyl)-1*H*-pyrazol-5-yl]-6-fluoroquinoline (**12c**), and 4-[5-(6-fluoroquinolin-2-yl)-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (**13c**) are three of the most active which exhibited 55%, 66% and 85% inhibition of DENV-2 replication in the Huh-7-DV-Fluc cells at a concentration of 10 μM compared to 33% inhibition exhibited by the positive ribavirin. The anti-DENV2 activity decreased in an order of **13c** (R_1 = OMe, R_2 = SO_2NH_2; 85% inhibition) > **12c** (R_1 = OMe, R_2 = OMe; 66% inhibition) > **11c** (R_1 = OMe, R_2 = F; 55% inhibition) indicated that R_2-substitution is the most prefer to be a 4-aminosulfonyl group. With the same 4-aminosulfonyl group substituted at R_2-position, the methoxy group substituted at R_1-position is the most favorable in which the anti-DENV2 activity decreased in an order of **13c** (R_1 = OMe; 85% inhibition) > **13b** (R_1 = F; 13% inhibition), **13a** (R_1 = H; 12% inhibition).

The cell cytotoxicity of was determined by XTT assay in the Huh-7 cells after 3 days treatment with 20 and 200 μM of diarylpyrazolylquinoline derivatives. Compound **11c, 12c** and **13c** exhibited low cytotoxicity, with greater than 50% viability at a concentration of 200 μM. The 50% inhibitory concentration of DENV-2 replication (IC_{50}), the 50% cytotoxic concentration for cell growth (CC_{50}), and the selective index (SI : CC_{50}/IC_{50}) of compounds **11c, 12c** and **13c** were determined as shown in *Table* 4. Results indicated that these compounds exhibited approximately 10-folds more active anti-DENV-2 activity (IC_{50} of 1.36, 1.09 and 0.81 μM, respectively) than that of ribavirin (IC_{50} = 12.61 μM). These compounds have also demonstrated a good selectivity with SI value of greater than 147, 183 and 247 respectively which was higher than that of ribavirin (SI = 4.47). Compound **13c** was also tested for other sero-types of DENV, and results shown in *Table 5* indicated that it exhibited potent inhibition of all sero-types of DENV.

< **Insert Table 3-5 here** >

3.2. *Compound **13c** reduced DENV-2 replication in DENV-2-infected Huh-7 cells*

To confirm the results of dose-dependent decrease luciferase activity representing the

DENV-2 replication in the Huh-7-DV-Fluc cells, we performed western blotting and RT-qPCR using a specific antibody against DENV-2 NS2B protein and specific primers targeting DENV-2 NS5 gene, respectively, in which cellular GAPDH was served as a loading control. Both results of western blotting and RT-qPCR revealed that **13c** consistently reduced DENV-2 replication in the Huh-7-DV-Fluc cells at the indication concentrations after 3 days treatment. Treatment of 0.1% DMSO were served as a mock control on inhibition of DENV-2 replication (*Figures* 4 and 5).

< **Insert Figure 4,5 here** >

3.3. *Compoun*d ***13c*** *decreases the mortality of DENV-infected ICR suckling mice.*

To investigate whether **13c** exerted protective effects against DENV infection *in vivo*, 6-day-old ICR suckling mice were injected with DENV-2 intracerebrally and with **13c** (1, 5, 10 and 20 mg/kg) intraperitoneally at 1, 3, and 5 days post-infection (dpi). Mice injected with heat-inactive DENV-2 (iDENV) were used as mock controls. Survival rates and clinical scores of DENV-infected mice treated with or without **13c** were measured daily for 6 days. As shown in *Figure* 6A, DENV-infected mice that were not treated with **13c** developed severe sickness leading to death within 4–6 dpi compared with iDENV-infected control mice. In contrast, **13c** at various concentrations shielded the mice from the life-threatening effects of the DENV-2 infection when compared to the non-**13c**-treated mice. DENV-infected mice that did not receive **13c** developed severe paralysis, anorexia, and asthenia and lost of their body weight compared with iDENV-treated mice at 6 dpi (*Figure* 6B and 6C). In contrast, DENV-infected mice treated with **13c** showed slight paralysis, anorexia, and asthenia compared with iDENV-infected mice at 6 dpi (F*igure* 6B and 6C).

< **Insert Figure 6 here** >

4. Conclusion

We have identified 4-[5-(6-fluoroquinolin-2-yl)-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl]-benzenesulfonamide (**13c**) as a potential anti-DENV agent which exhibited a potent inhibitory activity against DENV-2 (IC_{50} = 0.81 μM, SI > 246.91) and was more active than the positive

ribavirin (IC_{50} = 12.61 μM, SI = 4.47). It was also potent inhibited other sero-types of DENV. Furthermore, it can effectively protect mice from DENV infection by reducing disease symptoms and mortality of DENV-infected mice. It represents a potential antiviral agent to block DENV replication *in vitro* and *in vivo*. Further structural optimization of compound **13c** is ongoing.

5. Experimental

5.1. General

Melting points were determined on an Electrothermal IA9100 melting point apparatus and are uncorrected. IR spectra were measured using a Perkin Elmer System-2000 spectrometer. UV spectra (λmax in nm) were recorded in spectroscopic grade MeOH on a Shimadzu UV-160A UV-vis spectrophptometer. Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co.. Mass spectra were recorded on Bruker APEX II (ESI) mass spectrometer. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Taiwan University using Heraeus CHN-O Rapid EA, and all values are within ± 0.4% of the theoretical compositions.

5.2. General procedure for the preparation of quinolinyl chalcones 8a-c

A mixture **6** (0.48 g, 3.0 mmol) and selenium dioxide (0.66 g, 6.0 mmol) in 1,4-dioxane (50 mL) was heated at 100 ^{o}C for 2 h (TLC monitoring). After cooling, the mixture was treated with 5% $NaHCO_3$ aqueous (80 mL), extracted with CH_2Cl_2 (50 mL X 3), the organic layer was collected, dried over $MgSO_4$, and evaporated. The crude product was crystallized with EtOH to give **7** (0.43 g, 81%) as a white solid. Compound **7** (0.36 g, 2.0 mmol) and appropriate acetophenone (2.0 mmol) were stirred at 0 ^{o}C for 15 min. Aqueous solution of KOH (6 equiv) was added and the mixture was stirred at room temperature for 12 h (TLC monitoring). After the reaction reached completion, the resulting mixture was added 1M HCl until pH 3 resulted and extracted with ethyl acetate (50 mL X

3). The organic layer was collected, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified and crystallized with EtOH to give quinolinyl chalcones **8a-c**.

5.2.1. (*E*)-3-(6-Fluoroquinolin-2-yl)-1-phenylprop-2-en-1-one (8a). Yield: 50% as a yellow solid. Mp. 156.7-157.7°C. UV λ_{max} nm (logε): 327 (4.23), 264 (4.67), 218 (4.69) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.45 (dd, 1H, *J* = 8.4, 2.8 Hz, Ar-H), 7.51-7.56 (m, 3H, Ar-H), 7.60-7.64 (m, 1H, Ar-H), 7.68 (d, 1H, *J* = 8.4 Hz, 3-H), 7.93 (d, 1H, *J* = 15.6 Hz, *trans*-CH=), 8.10-8.17 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.61 (d, J_{CF} = 22.0 Hz), 120.50 (d, J_{CF} = 25.8 Hz), 122.20, 127.02, 128.71 (2C), 128.77 (2C), 128.83 (d, J_{CF} = 9.7 Hz), 132.43 (d, J_{CF} = 9.1 Hz), 133.12, 136.14 (d, J_{CF} = 5.3 Hz), 137.78, 143.18, 145.50, 152.87 (d, J_{CF} = 3.0 Hz), 160.95 (d, J_{CF} = 248.7 Hz), 190.58 (C=O). ESIMS $[M+H]^+$: 278. Anal. calcd for $C_{18}H_{12}FNO \cdot 0.5\ H_2O$: C 75.51, H 4.58, N 4.89; found C 75.50, H 4.38, N 4.65.

5.2.2. (*E*)-1-(4-Fluorophenyl)-3-(6-fluoroquinolin-2-yl)prop-2-en-1-one (8b). Yield: 69% as a yellow solid. Mp. 194.7-195.4°C. UV λ_{max} nm (logε): 327 (4.07), 263 (4.65), 218 (4.70) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.18-7.24 (m, 2H, Ar-H), 7.45 (dd, 1H, *J* = 8.4, 2.8 Hz, Ar-H), 7.54 (ddd, 1H, *J* = 9.2, 8.4, 2.8 Hz, Ar-H), 7.67 (d, 1H, *J* = 8.4 Hz, 3-H), 7.93 (d, 1H, *J* = 15.6 Hz, *trans*-CH=) , 8.12-8.18 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.66 (d, J_{CF} = 21.9 Hz), 115.86 (2C, d, J_{CF} = 21.3 Hz), 120.58 (d, J_{CF} = 25.7 Hz), 122.37, 126.49, 128.91 (d, J_{CF} = 9.8 Hz), 131.41 (2C, d, J_{CF} = 9.8 Hz), 132.42 (d, J_{CF} = 9.1 Hz), 134.12 (d, J_{CF} = 2.8 Hz), 136.20 (d, J_{CF} = 6.1 Hz), 143.28, 145.49, 152.66 (d, J_{CF} = 3.1 Hz), 160.97 (d, J_{CF} = 248.6 Hz), 165.82 (d, J_{CF} = 253.1 Hz), 188.84 (C=O). ESIMS $[M+H]^+$: 296. Anal. calcd for $C_{18}H_{11}F_2NO \cdot 0.4\ H_2O$: C 71.47, H 3.93, N 4.63; found C 71.57, H 3.96, N 4.65.

5.2.3. (*E*)-3-(6-Fluoroquinolin-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (8c). Yield: 79% as a yellow solid. Mp. 157.7-158.6°C. UV λ_{max} nm (logε): 333 (4.43), 264 (4.71), 218 (4.74) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.91 (s, 3H, $OC\underline{H}_3$), 7.01 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.44 (dd, 1H, *J* = 8.8, 2.8 Hz, Ar-H), 7.52 (ddd, 1H, *J* = 9.2, 8.4, 2.8 Hz, Ar-H) , 7.67 (d, 1H, *J* = 8.4 Hz, 3-H), 7.92 (d, 1H, *J* = 15.6 Hz, *trans*-CH=) , 8.12-8.19 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.51,

110.62 (d, J_{CF} = 21.9 Hz), 113.92 (2C), 120.43 (d, J_{CF} = 25.7 Hz), 122.29, 126.92, 128.80 (d, J_{CF} = 9.8 Hz), 130.76, 131.14 (2C), 132.37 (d, J_{CF} = 9.1 Hz), 136.09 (d, J_{CF} = 5.3 Hz), 142.30, 145.48, 153.05 (d, J_{CF} = 3.0 Hz), 160.87 (d, J_{CF} = 248.7 Hz), 163.70, 188.66 (C=O). ESIMS $[M+H]^+$: 308. Anal. calcd for $C_{19}H_{14}FNO_2 \cdot 0.5\ H_2O$: C 72.14, H 4.78, N 4.43; found C 72.15, H 4.51, N 4.18.

5.3. General procedure for the preparation of 1,3,5-trisubstituted pyrazole derivatives 9a-10c via cyclocondensation of quinolinyl chalcones 8a-c and phenylhydrazines.

To a solution of quinolinyl chalcones **8a, 8b** or **8c** (2 mmol) in EtOH (10 mL) was added phenylhydrazine (2.1 mmol). The resulting solution was refluxed until the reaction was completed as monitored by TLC (ca. 18 h). The solvent was evaporated in vacuo, and then added 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in 1,4-dioxane (10 mL). The reaction mixture was refluxed for 12 h (TLC monitoring) and then concetntrated under vacuum. The residue was extracted with CH_2Cl_2 (50 mL X 3). The organic layer was collected, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by silica gel column chromatography eluting with solvent mixtures of CH_2Cl_2-MeOH (100:1, 50:1, and 20:1) to afford the pyrazole products **9a-10c**.

5.3.1. 2-(2,3-Diphenyl-1*H*-pyrazol-5-yl)-6-fluoroquinoline (9a) and 2-(2,5-diphenyl-1*H*-pyrazol-3-yl)-6-fluoroquinoline (10a). Compound **9a** was obtained in 21% yield (0.16 g) as a yellow solid. Mp. 153.4-154.4^{o}C. R_f = 0.52, (CH_2Cl_2 : hexane = 3/1). UV λ_{max} nm (logε): 331 (4.10), 260 (4.78), 218 (4.78) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.33-7.51 (m, 13H, Ar-H), 8.14-8.17 (m, 2H, Ar-H), 8.30 (d, 1H, J = 8.8 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 106.83, 110.68 (d, J_{CF} = 21.2 Hz), 119.55 (d, J_{CF} = 25.0 Hz), 119.56, 125.41 (2C), 127.75, 128.34 (d, J_{CF} = 10.6 Hz), 128.39, 128.48 (2C), 128.77 (2C), 128.98 (2C), 130.33, 131.88 (d, J_{CF} = 9.1 Hz), 135.77 (d, J_{CF} = 5.3 Hz), 140.07, 144.87, 145.20, 151.62, 152.30, 160.30 (d, J_{CF} = 246.3 Hz). ESIMS $[M+H]^+$: 366. Anal. calcd for $C_{24}H_{16}FN_3 \cdot 0.1\ H_2O$: C 78.50, H 4.45, N 11.44; found C 78.37, H 4.52, N 11.45.

Compound **10a** was obtained in 36% yield (0.15 g) as a pink solid. Mp. 114.5-115.2^{o}C. R_f = 0.42,

(CH_2Cl_2 : hexane = 3/1). UV λ_{max} nm (logε): 258 (4.78), 219 (4.78) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.25-7.30 (m, 2H, Ar-H), 7.34-7.51 (m, 10H, Ar-H), 7.96-8.01 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 106.62, 110.57 (d, J_{CF} = 22.0 Hz), 120.26 (d, J_{CF} = 25.8 Hz), 121.87, 125.54 (2C), 125.88 (2C), 127.68 (d, J_{CF} = 9.8 Hz), 127.80, 128.11, 128.65 (2C), 128.93 (2C), 133.14 (d, J_{CF} = 9.1 Hz), 132.82, 135.55 (d, J_{CF} = 5.3 Hz), 140.48, 143.56, 144.99, 148.81 (d, J_{CF} = 3.0 Hz), 152.23, 160.77 (d, J_{CF} = 247.8 Hz). ESIMS $[M+H]^+$: 366. Anal. calcd for $C_{24}H_{16}FN_3 \cdot 0.1\ H_2O$: C 78.50, H 4.45, N 11.44; found C 78.22, H 4.54, N 11.37.

5.3.2. 6-Fluoro-2-[5-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-3-yl]quinoline (9b) and 6-fluoro-2-[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-5-yl]quinoline (10b) Compound **9b** was obtained in 22% yield (0.17 g) as a yellow solid. Mp. 178.0-178.9°C. R_f = 0.63, (CH_2Cl_2 : hexane = 4/1). UV λ_{max} nm (logε): 330 (4.12), 259 (4.80), 219 (4.81) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.01-7.06 (m, 2H, Ar-H), 7.27-7.32 (m, 2H, Ar-H), 7.34 (s, 1H, pyrazolyl-H), 7.35-7.51 (m, 7H, Ar-H), 8.13-8.17 (m, 2H, Ar-H), 8.29 (d, 1H, *J* = 9.2 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 106.81, 110.69 (d, J_{CF} = 22.0 Hz), 115.65 (2C, d, J_{CF} = 21.2 Hz), 119.50, 119.60 (d, J_{CF} = 25.0 Hz), 125.41 (2C), 126.45 (d, J_{CF} = 2.8 Hz), 127.89, 128.36 (d, J_{CF} = 9.8 Hz), 129.07 (2C), 130.59 (2C, d, J_{CF} = 7.6 Hz), 131.86 (d, J_{CF} = 9.1 Hz), 135.80 (d, J_{CF} = 5.3 Hz), 139.87, 143.84, 145.18, 151.47 (d, J_{CF} = 3.0 Hz), 152.34, 160.26 (d, J_{CF} = 234.0 Hz), 162.33 (d, J_{CF} = 234.1 Hz). ESIMS $[M+H]^+$: 384. Anal. calcd for Anal. calcd for $C_{24}H_{15}F_2N_3 \cdot 0.1\ H_2O$: C 74.83, H 3.98, N 10.91; found C 74.68, H 4.16, N 10.87.

Compound **10b** was obtained in 35% yield (0.19 g) as a yellow solid. Mp. 68.8-69.8°C. R_f = 0.58, (CH_2Cl_2 : hexane = 4/1). UV λ_{max} nm (logε): 323 (4.07), 260 (4.81), 219 (4.81) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.11-7.15 (m, 2H, Ar-H), 7.20 (s, 1H, pyrazolyl-H), 7.34-7.52 (m, 8H, Ar-H), 7.92-8.01 (m, 4H, Ar-H) , 8.00 (d, 2H, *J* = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 106.38, 110.59 (d, J_{CF} = 22.0 Hz), 115.59 (2C, d, J_{CF} = 21.2 Hz), 120.33 (d, J_{CF} = 25.0 Hz), 121.82, 125.51 (2C), 127.57 (d, J_{CF} = 9.8 Hz), 127.70 (2C, d, J_{CF} = 9.9 Hz), 127.89, 128.98 (2C), 132.13 (d, J_{CF} = 9.1 Hz), 135.59 (d, J_{CF} = 6.0 Hz), 135.82 (d, J_{CF} = 2.8 Hz), 140.36, 143.69, 144.99, 148.69 (d, J_{CF} =

2.8 Hz), 151.36, 160.78 (d, J_{CF} = 247.8 Hz), 162.82 (d, J_{CF} = 244.7 Hz). ESIMS $[M+H]^+$: 384. Anal. calcd for $C_{24}H_{15}F_2N_3 \cdot 0.1\ H_2O$: C 74.83, H 3.98, N 10.91; found C 74.99, H 4.33, N 10.61.

5.3.3. 6-Fluoro-2-[5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl]quinoline (9c) and 6-fluoro-2-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-5-yl]quinoline (10c). Compound **9c** was obtained in 23% yield (0.18 g) as a white solid. Mp. 175.3-176.0°C. R_f = 0.52, (CH_2Cl_2 : hexane = 4/1). UV λ_{max} nm (logε) : 331 (4.12), 261 (4.81), 219 (4.81) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.82 (s, 3H, OC<u>H</u>$_3$), 6.86 (d, 2H, *J* = 8.8 Hz, ArH), 7.25 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.30 (s, 1H, pyrazolyl-H), 7.32-7.51 (m, 7H, Ar-H), 8.13-8.17 (m, 2H, Ar-H), 8.29 (d, 1H, *J* = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.25, 106.28, 110.67 (d, J_{CF} = 22.0 Hz), 113.93 (2C), 119.52 (d, J_{CF} = 25.7 Hz), 119.57, 122.73, 125.41 (2C), 127.66, 128.32 (d, J_{CF} = 9.1 Hz), 128.96 (2C), 130.07 (2C), 131.86 (d, J_{CF} = 9.1 Hz), 135.73 (d, J_{CF} = 5.3 Hz), 140.18, 144.73, 145.19, 151.71 (d, J_{CF} = 2.3 Hz), 152.19, 159.63, 160.28 (d, J_{CF} = 246.2 Hz). ESIMS $[M+H]^+$: 396. Anal. calcd for $C_{25}H_{18}FN_3O$: C 75.93, H 4.59, N 10.63; found C 75.63, H 4.61, N 10.96.

Compound **10c** was obtained in 40% yield (0.32 g) as a white solid. Mp. 142.8-143.5°C. R_f = 0.51, (CH_2Cl_2 : hexane = 4/1). UV λ_{max} nm (logε) : 260 (4.81), 219 (4.82) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.86 (s, 3H, OC<u>H</u>$_3$), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.18 (s, 1H, pyrazolyl-H), 7.27 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.33-7.51 (m, 7H, Ar-H), 7.89 (d, 2H, *J* = 8.8 Hz, Ar-H) , 7.98-8.01 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 55.31, 106.22, 110.57 (d, J_{CF} = 21.9 Hz), 114.06 (2C), 120.24 (d, J_{CF} = 25.7 Hz), 121.90, 125.51 (2C), 125.59, 127.16 (2C), 127.61, 127.70, 128.92 (2C), 132.13 (d, J_{CF} = 9.1 Hz), 135.51 (d, J_{CF} = 5.3 Hz), 140.49, 143.46, 144.99, 148.90 (d, J_{CF} = 3.1 Hz), 152.08, 159.67, 160.74 (d, J_{CF} = 247.8 Hz). ESIMS $[M+H]^+$: 396. Anal. calcd for $C_{25}H_{18}FN_3O$: C 75.93, H 4.59, N 10.63; found C 75.95, H 4.61, N 10.83.

5.4. General procedure for the preparation of 1,3,5-trisubstituted pyrazole derivatives 11a-13c via cyclocondensation of quinolinyl chalcones 8a-c and 4-substituted phenylhydrazines.

To a solution of quinolinyl chalcones **8a, 8b** or **8c** (2 mmol) in EtOH (10 mL) was added

4-substituted phenylhydrazine hydrochloride (2.1 mmol). The resulting solution was refluxed until the reaction was completed as monitored by TLC (ca.12 h). The solvent was evaporated in vacuo, and purified by flash chromatography on silica gel, using CH_2Cl_2/ MeOH(20:1) as eluant and recrystallized with EtOH to afford the pyrazole products (**11a-13c**).

5.4.1. 6-Fluoro-2-[1-(4-fluorophenyl)-3-phenyl-1*H*-pyrazol-5-yl]quinoline (11a). Yield 54% as a yellow solid. Mp. 151.2-152.8^{o}C. UV λ_{max} nm (logε)：323 (4.17), 246 (4.59) 222 (4.52) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.05-7.10 (m, 2H, Ar-H), 7.23 (s, 1H, pyrazolyl-H), 7.35-7.51 (m, 8H, Ar-H), 7.92-7.96 (m, 3H, Ar-H), 8.04 (d, 1H, J = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 106.45, 110.60 (d, J_{CF} = 22.0 Hz), 115.73 (2C, d, J_{CF} = 22.7 Hz), 120.39 (d, J_{CF} = 25.8 Hz), 121.66, 125.88 (2C), 127.47 (d, J_{CF} = 8.3 Hz), 127.69 (d, J_{CF} = 9.9 Hz), 128.21, 128.70 (2C), 132.12 (2C, d, J_{CF} = 9.1 Hz), 132.69, 135.76, 136.87, 143.56, 144.92, 148.48, 152.26, 160.83 (d, J_{CF} = 247.9 Hz), 161.92 (d, J_{CF} = 245.7 Hz). ESIMS $[M+H]^+$: 384. Anal. calcd for $C_{24}H_{15}F_2N_3$: C 75.19, H 3.94, N 10.96; found C 75.30, H 3.63, N 10.87.

5.4.2. 2-[1,3-Bis(4-fluorophenyl)-1*H*-pyrazol-5-yl]-6-fluoroquinoline (11b). Yield 57% as a yellow solid. Mp. 188.2-189.8^{o}C. UV λ_{max} nm (logε)：326 (4.08), 262 (4.61) 220 (4.51) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 7.06-7.16 (m, 4H, ArH), 7.18 (s, 1H, pyrazolyl-H), 7.35 (d, 1H, J = 8.8 Hz, ArH), 7.40-7.52 (m, 4H, ArH), 7.90-7.96 (m, 3H, Ar-H), 8.04 (d, 1H, J = 8.8 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 106.23, 110.61 (d, J_{CF} = 21.9 Hz), 115.64 (2C, d, J_{CF} = 21.3 Hz), 115.78 (2C, d, J_{CF} = 22.7 Hz), 120.45 (d, J_{CF} = 25.7 Hz), 121.61, 127.39 (2C, d, J_{CF} = 9.1 Hz), 127.55 (2C, d, J_{CF} = 8.4 Hz), 127.69 (d, J_{CF} = 10.6 Hz), 128.87 (d, J_{CF} = 3.1 Hz), 132.08 (d, J_{CF} = 9.1 Hz), 135.83 (d, J_{CF} = 5.3 Hz), 136.67 (d, J_{CF} = 3.0 Hz), 143.65, 144.88, 148.29 (d, J_{CF} = 3.1 Hz), 151.35, 160.82 (d, J_{CF} = 247.8 Hz), 161.91 (d, J_{CF} = 246.3 Hz), 162.84 (d, J_{CF} = 245.6 Hz). ESIMS $[M+H]^+$: 402. Anal. calcd for $C_{24}H_{14}F_3N_3$: C 71.82, H 3.52, N 10.47; found C 71.98, H 3.21, N 10.40.

5.4.3. 6-Fluoro-2-(1-(4-fluorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-5-yl)quinoline (11c). Yield 59% as a yellow solid. Mp. 180.4-181.6^{o}C. UV λ_{max} nm (logε)：326 (4.17), 262 (4.62), 221 (4.51) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.86 (s, 3H, O$\underline{CH_3}$), 6.96-7.10 (m, 4H, Ar-H), 7.16

(s, 1H, pyrazolyl-H), 7.35 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.39-7.51 (m, 4H, Ar-H), 7.86-7.89 (m, 2H, Ar-H), 7.94 (dd, 1H, *J* = 9.2, 5.2 Hz, Ar-H), 8.03 (d, 1H, *J* = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.31, 106.05, 110.59 (d, J_{CF} = 21.2 Hz), 114.07 (2C), 115.72 (2C, d, J_{CF} = 22.7 Hz), 120.36 (d, J_{CF} = 25.9 Hz), 121.68, 125.39, 127.13 (2C), 127.39 (2C, d, J_{CF} = 9.1 Hz), 127.65 (d, J_{CF} = 9.9 Hz), 132.08 (d, J_{CF} = 9.1 Hz), 135.74 (d, J_{CF} = 5.3 Hz), 136.80 (d, J_{CF} = 3.1 Hz), 143.44, 144.88, 148.51 (d, J_{CF} = 3.0 Hz), 152.07, 159.71, 160.77 (d, J_{CF} = 247.8 Hz), 161.82 (d, J_{CF} = 246.3 Hz). ESIMS $[M+H]^+$: 414. Anal. calcd for $C_{25}H_{17}F_2N_3O$: C 72.63, H 4.14, N 10.16; found C 72.86, H 4.23, N 9.94.

5.4.4. 6-Fluoro-2-[1-(4-methoxyphenyl)-3-phenyl-1*H*-pyrazol-5-yl]quinoline (12a). Yield: 58% as a brown solid. Mp. 203.6-204.5^{o}C. UV λ_{max} nm (logε) : 258 (4.72), 215 (4.81) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H, OC$\underline{H_3}$), 6.88-6.91 (m, 2H, Ar-H), 7.25 (s, 1H, pyrazolyl-H), 7.26-7.27 (m, 1H, Ar-H), 7.33-7.53 (m, 7H, Ar-H), 7.95-8.05 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.52, 106.17, 110.55 (d, J_{CF} = 21.9 Hz), 114.11 (2C), 120.24 (d, J_{CF} = 25.0 Hz), 121.80, 125.84 (2C), 126.95 (2C), 127.63 (d, J_{CF} = 9.9 Hz), 128.01, 128.63 (2C), 132.12 (d, J_{CF} = 9.1 Hz), 132.89, 133.67, 135.51 (d, J_{CF} = 5.3 Hz), 143.56, 144.98, 148.83 (d, J_{CF} = 3.0 Hz), 151.91, 159.15, 160.73 (d, J_{CF} = 247.8 Hz). ESIMS $[M+H]^+$: 396. Anal. calcd for $C_{25}H_{18}FN_3O \cdot 0.1\ H_2O$: C 75.59, H 4.62, N 10.58; found: C 75.49, H 4.47, N 10.54.

5.4.5. 6-Fluoro-2-[3-(4-fluorophenyl)-1-(4-methoxyphenyl)-1*H*-pyrazol-5-yl]quinoline (12b). Yield: 59% as a brown solid. Mp. 167.5-168.3^{o}C. UV λ_{max} nm (logε) : 259 (4.84), 219 (4.84) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H, OC$\underline{H_3}$), 6.88-6.92 (m, 2H, Ar-H), 7.10-7.15 (m, 2H, Ar-H), 7.19 (s, 1H, pyrazolyl-H), 7.24 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.33-7.36 (m, 2H, Ar-H), 7.40 (dd, 1H, *J* = 8.8, 2.8 Hz, Ar-H), 7.47-7.52 (m, 1H, Ar-H) , 7.90-7.94 (m, 2H, Ar-H) , 7.98 (d, 1H, *J* = 8.8 Hz, 4-H) , 8.03 (dd, 1H, *J* = 9.2, 5.6 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.53, 105.91 (d, J_{CF} = 22.0 Hz), 114.15 (2C), 114.19, 115.55 (d, J_{CF} = 22.0 Hz), 120.28 (d, J_{CF} = 25.7 Hz), 121.74, 126.91 (2C), 127.52 (2C, d, J_{CF} = 8.4 Hz), 127.65 (d, J_{CF} = 9.9 Hz), 129.13 (d, J_{CF} = 3.0 Hz), 132.13 (d, J_{CF} = 9.1 Hz), 133.55, 135.53 (d, J_{CF} = 5.3 Hz), 143.72, 144.99, 148.71 (d, J_{CF} = 3.0

Hz), 151.03, 159.20, 160.75 (d, J_{CF} = 247.8 Hz), 162.76 (d, J_{CF} = 245.5 Hz). ESIMS $[M+H]^+$: 414. Anal. calcd for $C_{25}H_{17}F_2N_3O \cdot 0.2\ H_2O$: C 72.00, H 4.21, N 10.08; found C 71.90, H 4.33, N 9.79.

5.4.6. 2-[1,3-Bis(4-methoxyphenyl)-1*H*-pyrazol-5-yl]-6-fluoroquinoline (12c). Yield: 62% as a brown solid. Mp. 63.5-63.9 °C. UV λ_{max} nm (logε)：261 (4.85), 218 (4.86) in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H, OC$\underline{H_3}$), 3.86 (s, 3H, OC$\underline{H_3}$), 6.88-6.90 (m, 2H, Ar-H), 6.96-6.99 (m, 2H, Ar-H), 7.17 (s, 1H, pyrazolyl-H), 7.25 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.334-7.37 (m, 2H, Ar-H), 7.40 (dd, 1H, *J* = 8.8, 2.8 Hz, Ar-H), 7.47-7.52 (m, 1H, Ar-H), 7.88-7.90 (m, 2H, Ar-H), 7.98 (d, 1H, *J* = 8.8 Hz, 4-H) , 8.03 (dd, 1H, *J* = 8.8, 5.2 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.30, 55.53, 105.71, 110.54 (d, J_{CF} = 22.0 Hz), 114.04 (2C), 114.11 (2C), 120.19 (d, J_{CF} = 25.0 Hz), 121.82, 125.71, 126.94 (2C), 127.12 (2C), 127.58 (d, J_{CF} = 9.8 Hz), 132.15 (d, J_{CF} = 9.1 Hz), 133.75, 135.45 (d, J_{CF} = 5.3 Hz), 143.53, 145.03, 148.96, 151.78, 159.10, 159.60, 160.72 (d, J_{CF} = 247.9 Hz). ESIMS $[M+H]^+$: 426. Anal. calcd for $C_{26}H_{20}FN_3O_2$: C 73.40, H 4.74, N 9.88; found C 73.29, H 4.95, N 9.84.

5.4.7. 4-[5-(6-Fluoroquinolin-2-yl)-3-phenyl-1*H*-pyrazol-1-yl]benzenesulfonamide (13a). Yield: 55% as a brown solid. Mp. 244.5~245.3°C. UV λ_{max} nm (logε)：260 (4.86), 219 (4.86) in MeOH. ^{1}H NMR (400 MHz, DMSO-d_6): 7.39-7.44 (m, 1H, Ar-H), 7.49-7.52 (m, 4H, Ar-H), 7.68 (s, 1H, pyrazolyl-H), 7.61-7.75 (m, 4H, Ar-H), 7.82-7.89 (m, 4H, Ar-H), 7.99-8.01 (m, 2H, Ar-H), 8.46 (d, 1H, *J* = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 107.58, 111.11 (d, J_{CF} = 22.0 Hz), 120.37 (d, J_{CF} = 25.8 Hz), 121.94, 125.53 (2C), 125.70 (2C), 126.27 (2C), 127.67 (d, J_{CF} = 9.8 Hz), 128.45, 128.88 (2C), 131.62 (d, J_{CF} = 9.1 Hz), 132.12, 136.74 (d, J_{CF} = 5.3 Hz), 142.90 , 142.95, 143.29, 144.02, 147.97 (d, J_{CF} = 2.3 Hz), 151.65, 160.24 (d, J_{CF} = 244.8 Hz). ESIMS $[M+H]^+$: 445. Anal. calcd for $C_{24}H_{17}FN_4O_2S$: C 64.85, H 3.86, N 12.60; found C 64.60, H 3.94, N 12.45.

5.4.8. 4-[3-(4-Fluorophenyl)-5-(6-fluoroquinolin-2-yl)-1*H*-pyrazol-1-yl]benzenesulfonamide (13b). Yield: 48% as a green solid. Mp. 236.5-237.7°C. UV λ_{max} nm (logε): 332 (4.10), 260 (4.61), 224 (4.52) in MeOH. ^{1}H NMR (400 MHz, DMSO-d_6): 7.32-7.37 (m, 2H, Ar-H), 7.49 (s, 2H, N$\underline{H_2}$), 7.67 (s, 1H, pyrazolyl-H), 7.60-7.75 (m, 4H, Ar-H), 7.82-7.88 (m, 4H, Ar-H), 8.02-8.05 (m, 2H,

Ar-H), 8.46 (d, 1H, J = 8.8 Hz, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 107.50, 111.10 (d, J_{CF} = 22.0 Hz), 115.79 (2C, d, J_{CF} = 21.3 Hz), 120.38 (d, J_{CF} = 25.8 Hz), 121.90, 125.68 (2C), 126.27 (2C), 127.59 (2C, d, J_{CF} = 8.3 Hz), 127.67 (d, J_{CF} = 9.1 Hz), 128.70 (d, J_{CF} = 3.0 Hz), 131.61 (d, J_{CF} = 9.8 Hz), 136.74 (d, J_{CF} = 5.3 Hz), 142.87, 142.93, 143.37, 144.01, 147.92, 150.76, 160.04 (d, J_{CF} = 244.8 Hz), 162.25 (d, J_{CF} = 244.0 Hz). ESIMS $[M+H]^+$: 463. Anal. calcd for $C_{24}H_{16}F_2N_4O_2S \cdot 0.5\ H_2O$: C 61.14, H 3.63, N 11.88; found C 61.43, H 3.40, N 11.76.

5.4.9. 4-[5-(6-Fluoroquinolin-2-yl)-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (13c). Yield 53% as a yellow solid. Mp. 233.1-234°C. UV λ_{max} nm (logε)：262(4.89), 219 (4.89) in MeOH. ^{1}H NMR (400 MHz, DMSO-d_6): 3.37 (s, 3H, OC$\underline{H_3}$), 7.05-7.07 (m, 2H, Ar-H), 7.49 (s, 2H, N$\underline{H_2}$), 7.58 (s, 1H, pyrazolyl-H), 7.59-7.76 (m, 5H, Ar-H), 7.82-7.93 (m, 6H, Ar-H), 8.46 (d, 1H, J = 8.8Hz, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 55.20, 107.21, 111.11 (d, J_{CF} = 22.0 Hz), 114.27 (2C), 120.36 (d, J_{CF} = 25.7 Hz), 121.95, 124.70, 125.58 (2C), 126.27 (2C), 126.92 (2C), 127.66 (d, J_{CF} = 11.3 Hz), 131.63 (d, J_{CF} = 9.9 Hz), 136.71 (d, J_{CF} = 5.3 Hz), 142.73, 143.00, 143.18, 144.05, 148.09 (d, J_{CF} = 3.0 Hz), 151.59, 159.52, 160.04 (d, J_{CF} = 244.8 Hz). ESIMS $[M+H]^+$: 475. Anal. calcd for $C_{25}H_{19}FN_4O_3S \cdot 0.5\ H_2O$: C 62.10, H 4.17, N 11.59; found C 61.72, H 3.85, N 11.51.

5.5. Cytotoxicity and antiviral activity assays

5.5.1. Compounds

Compounds were dissolved in DMSO at 10 mM and then diluted in culture medium.

5.5.2. Cells and virus

Human hepatoma Huh-7 cells were purchased from Bioresources Collection and Research Center, Taiwan. Huh-7-DV-Fluc cells and C6/36 cells were kindly provided by Dr Huey Nan Wu, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC. The DENV-2 strain 16681 was isolated from a patient with dengue hemorrhage fever (DHF) from Thailand [31], and was amplified in C6/36 mosquito cells.

5.5.3. Cytotoxicity assays

For cytotoxicity tests, run in parallel with antiviral assays, plates at an initial density of (5 x 10^3

cells/well), in maintenance medium, with or without test compounds at two concentrations (20 and 200 μM) or 0.1% DMSO as control. Cell viability was determined after 3 days at 37 ^{o}C in a humidified CO_2 (5%) atmosphere by the (2,3-bis [2-methyloxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide) (XTT) method [32].

5.5.4. Analysis of luciferase activity

Huh-7-DV-Fluc cells were seeded in 24-well plates at a density of 2 × 10^4 cells per well, and treated with the diarylpyrazolylquinoline compounds at two concentrations (1 and 10 μM) or 0.1% DMSO as control. After 3 days of incubation, the luciferase activity assay was performed using the Bright-Glo Luciferase assay system (Promega) according to the manufacturer's instructions.

5.5.5. Immunoblot analysis

The standard procedure was followed to perform western blotting as described previously [33]. The membranes were incubated overnight with rabbit polyclonal antibodies against NS2B (1：3000, Genetex, Irvine, CA, USA) and GAPDH (1：10000, Genetex, Irvine, CA, USA) serving as loading control.

5.5.6. Quantification of DENV RNA

Total cellular RNA was extracted after 3 days compounds treatment through Total RNA Miniprep Purification Kit (GMbiolab, Taiwan) according to the manufacturer's instructions. The expression of DENV NS5 mRNA level was determine through quantification real-time RT-PCR (RT-PCR) with specific primers corresponding to the DENV NS5 gene：Forward primer, 5-AAG GTG AGA AGC AAT GCA GC-3；reverse primer, 5-CCA CTC AGG GAG TTC TCT CT-3. The copy number of NS5 in each sample was normalized to cellular endogenous reference gene glyceraldehydes-3-phosphate dehydrogenase (gapdh)；Forward primer: 5-GTC TTC ACC ACC ATG GAG AA-3 and Reverse primer: 5-ATG GCA TGG ACT GTG GTC AT-3. The CT value of each sample was determined by the ABI Step One Real-Time PCR-System (ABI Warrington, UK).

5.5.7. DENV infection assay

Huh-7 cells were seeded at the density of 4 X 10^4 cells/well in the 24 well culture plate for 16-20

hours and then infected with DENV-2 (16681 strain) at an MOI of 0.1 for 2 hours at 37 ^{o}C. Cells were washed with PBS once and then re-filled with DMEM-2% FBS medium containing various indicating concentration of **13c**.

5.5.8. Anti-DENV activity assay *in vivo*

Six-days-old ICR suckling mice were randomly divided into 3 groups (n = 5/group): Group 1: intracerebrally injected 2.5x10^{5} pfu 60°C heat-inactive DENV (iDENV). Group 2: intracerebrally injected 2.5x10^{5} pfu DENV + intraperitoneally injected saline (DENV). Group 3; intracerebrally injected 2.5x10^{5} pfu DENV virus+ intraperitoneally injected 1, 5, 10 and 20 mg/kg **13c**. Mice were given saline, 1, 5, 10 and 20 mg/kg **13c** by intraperitoneal injection at 1, 3, 5 days post-infection (D.P.I). The survival rate, body weight and clinical score were measured every day after DENV injection. Illness symptom were scored ad follow; 0 for no symptom; 1 for slight losing weight and ruffled hair; 2 for slowing of activity; 3 for asthenia; 4 for paralysis and mortally ill; 5 for death [34].

5.5.9. Statistical analysis

The results were expressed as means±S.D. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) and Student's t-test. For the septic shock assay, we used the log-rank test. Statistical significance was assessed as $p<0.05$ [*$p<0.05$; **$p<0.01$].

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Figure captions:

Figure 1. The structure of quinoline and pyrazole derivatives.

Figure 2. ORTEP diagram of 6-fluoro-2-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-5-yl] quinoline (**9c**) (A) and 6-fluoro-2-[5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl]quinoline (**10c**) (B).

Figure 3. The chemical shift differences for pyrazole 5-H of regioisomers, **9c** (N_2 substituted) and **10c** (N_3 substituted) in both $CDCl_3$ and in DMSO-d_6.

Figure 4. Inhibition of DENV-2 RNA expression in DENV-infected Huh-7 cells by **13c**. DENV-infected Huh-7 cells were treated with 0.5, 1, 2.5 and 5 μM of **13c** for 3 days. Total RNA was extracted and quantified DENV2 RNA levels by RT-qPCR. DENV2 RNA expression was normalized by cellular GAPDH mRNA. Treatment with 0.1% DMSO served as a mock control. The results are expressed as the means ± standard deviations (SD) of triplicate experiments.

Figure 5. Inhibition of DENV-2 protein expression in DENV-infected Huh-7 cells by **13c**. DENV-infected Huh-7 cells were treated with 0.5, 1, 2.5 and 5 μM of **13c** for 3 days. Total cell lysate was collected for performing western blotting to analyze DENV protein synthesis. Levels of GAPDH were used as equal loading control.

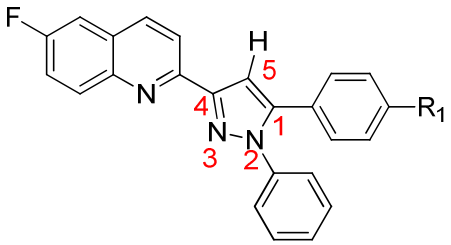
Figure 6. 13c shows therapeutic efficacy in DENV-infected ICR suckling mice. Six-day-old ICR suckling mice were injected with 2.5×10^5 pfu DENV-2 intracerebrally and with 1, 5, 10 and 20 mg/kg of **13c** intraperitoneally at 1, 3 and 5 dpi. (A) Survival rate, (B) clinical score, and (C) body weight of the mice were measured every day after the DENV injection. Illness symptoms were scored as follow: 0, no symptom; 1, slight weight loss and ruffled hair; 2, slow activity; 3, asthenia and anorexia; 4, paralysis and fatal illness; and 5, death.

Table 1. The crystallographic data and structure refinement details of **9c** and **10c.**

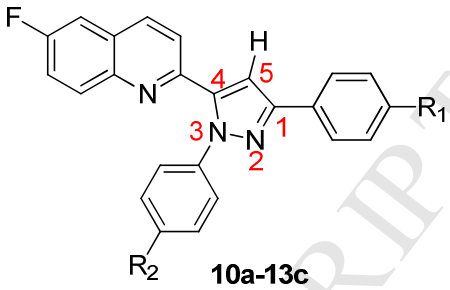
Identification code	**9c**	**10c**
Empirical formula	$C_{25}H_{18}FN_3O$	$C_{25}H_{18}FN_3O$
Formula weight	395.42	395.42
Temperature	100(2) K	100(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system, space group	Triclinic, P -1	Monoclinic, P 1 21/c 1
Unit cell dimensions	a = 6.262(3) Å α= 68.380(11)° b = 11.961(5) Å β= 87.617(13)° c = 13.502(6) Å γ = 83.998(12)°	a = 12.8551(14) Å α= 90° b = 14.1552(15) Å β= 98.879(2)° c = 10.8351(13) Å γ = 90°
Volume	935.0(8) $Å^3$	1948.0(4) $Å^3$
Z, Calculated density	2, 1.405 Mg/m^3	4, 1.348 Mg/m^3
Absorption coefficient	0.094 mm^{-1}	0.091 mm^{-1}
F(000)	412	824
Crystal size	0.20 x 0.05 x 0.05 mm^3	0.30 x 0.30 x 0.08 mm^3
Theta range for data collection	1.62 to 26.51°	1.60 to 26.41°
Limiting indices	-4<=h<=7, -14<=k<=14, -16<=l<=16	-16<=h<=15, -15<=k<=17, -13<=l<=13
Reflections collected/unique	13276/3808 [R(int) = 0.0258]	16028/3984 [R(int) = 0.0338]
Completeness to	θ = 26.51, 98.3 %	θ = 26.41, 99.6 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.9486 and 0.8995	0.9486 and 0.8609
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3808 / 0 / 272	3984 / 0 / 272
Goodness-of-fit on F2	1.006	1.019

Final R indices [I >2σ (I)]	R1 = 0.0370, wR2 = 0.0865	R1 = 0.0358, wR2 = 0.0834
R indices (all data)	R1 = 0.0536, wR2 = 0.0952	R1 = 0.0495, wR2 = 0.0904
Largest diff. peak and hole	0.215 and -0.280 e.Å^{-3}	0.222 and -0.228 e.Å^{-3}

Table 2. Comparative studies on the chemical shifts for ^{1}H NMR signals of the pyrrazoyl part of diarylpyrazolylquinoline derivatives.



9a-c



10a-13c

Compounds	R_1	R_2	Pyrazole-5-*H* chemical shift (ppm)	
			N-2-Aryl	*N*-3-Aryl
9a	H	--	7.38[a]	--
9b	F	--	7.34[a]	--
9c	OMe	--	7.30[a] (7.29)[b]	--
10a	H	H	--	7.25[a]
10b	F	H	--	7.20[a]
10c	OMe	H	--	7.18[a] (7.49)[b]
11a	H	F	--	7.23[a]
11b	F	F	--	7.18[a]
11c	OMe	F	--	7.16[a]
12a	H	OMe	--	7.25[a]
12b	F	OMe	--	7.19[a]
12c	OMe	OMe	--	7.17[a]
13a	H	SO_2NH_2	--	7.68[b]
13b	F	SO_2NH_2	--	7.67[b]
13c	OMe	SO_2NH_2	--	7.58[b]

[a]$CDCl_3$; [b]DMSO-d_6

Table 3. Anti-DENV-2 activities and cytotoxicities of diarylpyrazolylquinoline derivatives.

compounds	DENV-2		Huh-7 cell	
	% Inhibition at 1 μM	% Inhibition at 10 μM	% viability at 20 μM	% viability at 200 μM
9a	5.21 ± 3.1	15.31 ± 2.3	124.10 ± 7.48	115.31 ± 11.43
9b	3.58 ± 1.7	6.14 ± 2.1	88.26 ± 9.46	89.72 ± 1.71
9c	4.14 ± 2.3	7.15 ± 1.9	95.56 ± 5.07	93.68 ± 5.75
10a	3.02 ± 1.8	5.13 ± 1.4	125.54 ± 6.35	98.29 ± 7.59
10b	11.23 ± 2.8	25.39 ± 3.4	88.13 ± 1.84	87.49 ± 7.61
10c	5.31 ± 1.6	8.09 ± 2.3	103.99 ± 3.75	98.90 ± 9.26
11a	4.36 ± 1.7	8.31 ± 1.5	97.82 ± 3.68	91.56 ± 3.85
11b	8.91 ± 1.3	22.35 ± 1.7	95.32 ± 2.61	86.51 ± 4.51
11c	41.42 ± 2.4	55.32 ± 1.8	98.75 ± 2.87	95.37 ± 3.64
12a	7.81 ± 2.5	20.68 ± 3.7	115.73 ± 3.28	97.35 ± 8.21
12b	6.12 ± 2.1	15.83 ± 3.2	89.08 ± 8.16	52.55 ± 1.89
12c	46.87 ± 5.3	65.71 ± 4.1	95.81 ± 4.82	98.40 ± 16.44
13a	4.52 ± 2.7	11.81 ± 1.9	89.22 ± 4.03	83.64 ± 2.68
13b	5.71 ± 1.7	12.75 ± 3.8	91.12 ± 2.04	42.97 ± 2.47
13c	58.96 ± 2.8	85.34 ± 6.7	83.71 ± 2.52	89.53 ± 2.41
ribavirin	10.14 ± 1.98	32.53 ± 2.3	71.37 ± 1.31	26.78 ± 1.59

Table 4. Anti-DENV-2 activities [IC_{50} (μM)][a] of the compounds tested.

Compounds	IC_{50}[a]	CC_{50}[b]	SI[c]
11c	1.36 ± 0.13	> 200	> 147.06
12c	1.09 ± 0.15	> 200	> 183.49
13c	0.81 ± 0.07	> 200	> 246.91
ribavirin	12.61 ± 1.17	56.31 ± 2.32	4.47

[a] The IC_{50} is the concentration of the compound resulting in a 50% inhibition in virus production.

[b] The CC_{50} is the concentration of the compound causing a 50% growth inhibition of uninfected Huh-7 cells.

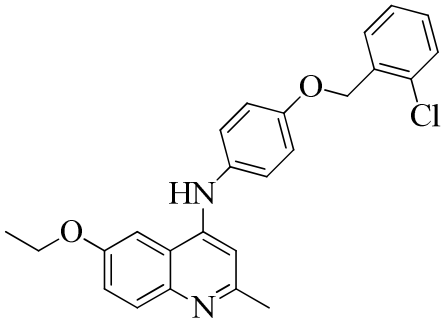
[c]SI: selectivity index. SI = CC_{50}/IC_{50}.

Table 5. Anti-DENV-1-4 activity of **13c.**

IC_{50} (μM)[a]			
DENV-1	DENV-2	DENV-3	DENV-4
1.21 ± 0.16	0.81 ± 0.07	0.73 ± 0.14	1.56 ± 0.09

[a] The IC_{50} is the concentration of the compound resulting in a 50% inhibition in virus production.

Figure 1.



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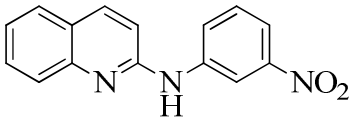
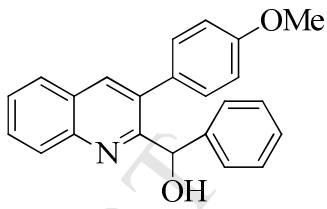


2



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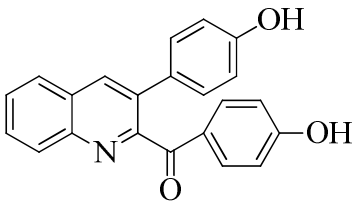

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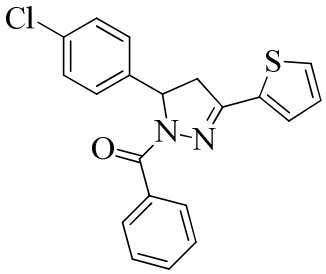

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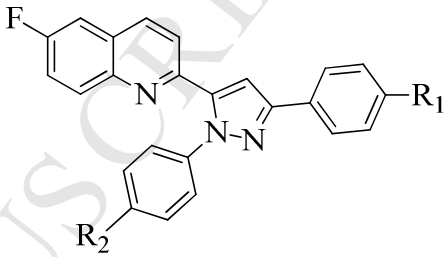
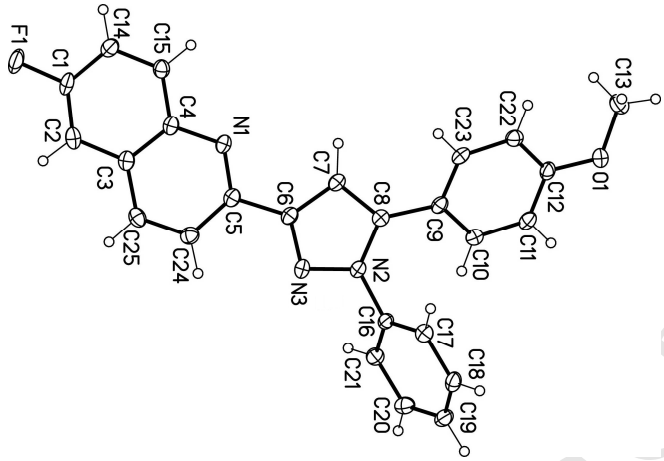


Target compounds

Figure 2.

(A)



(B)

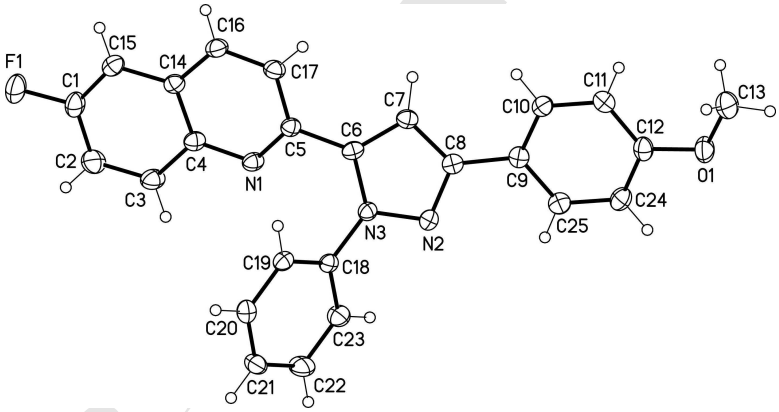
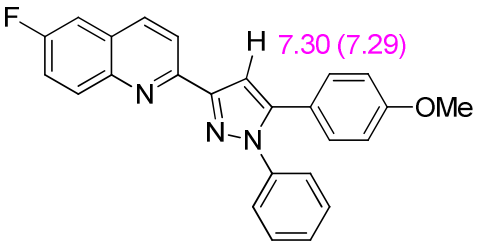
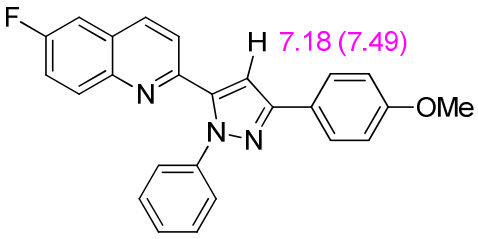


Figure 3.



9c



10c

Figure 4.

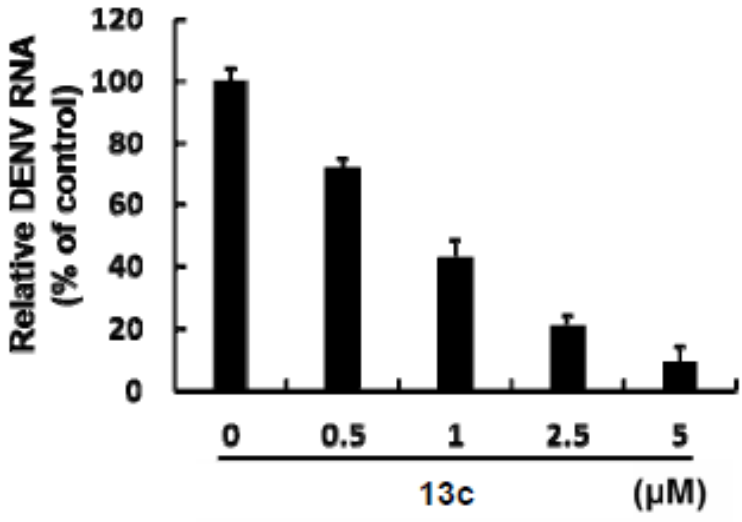


Figure 5.

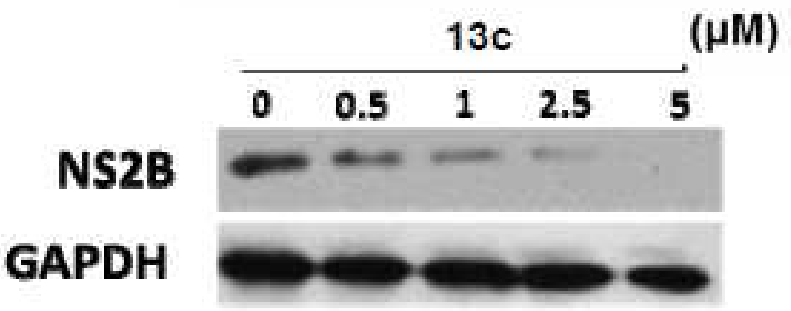
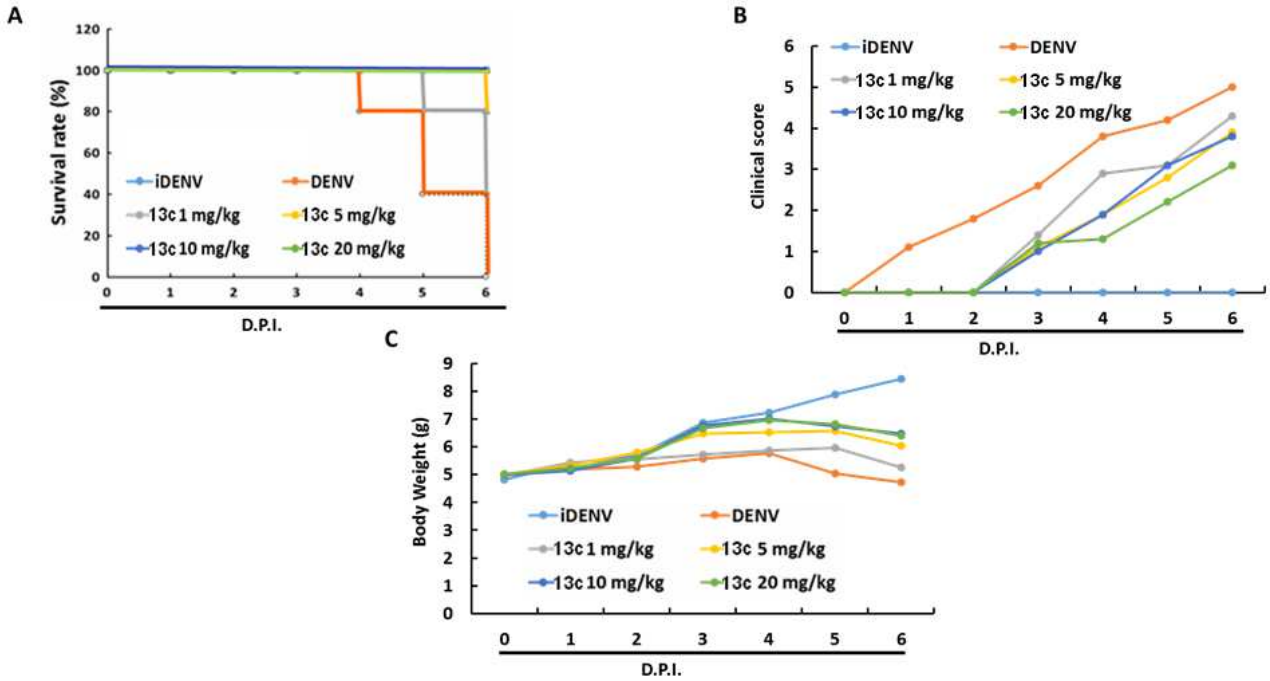
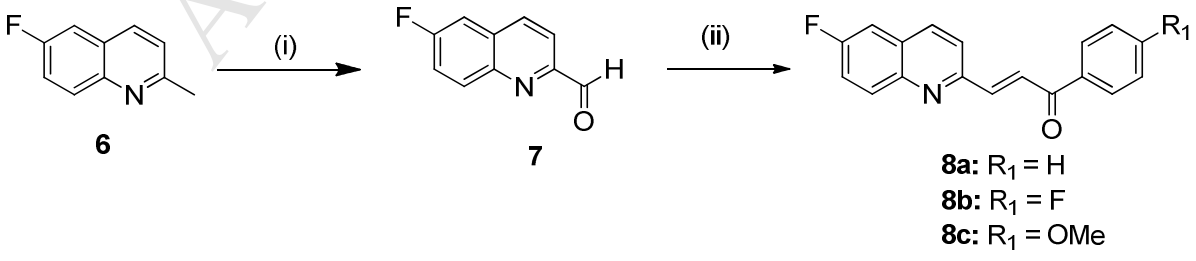
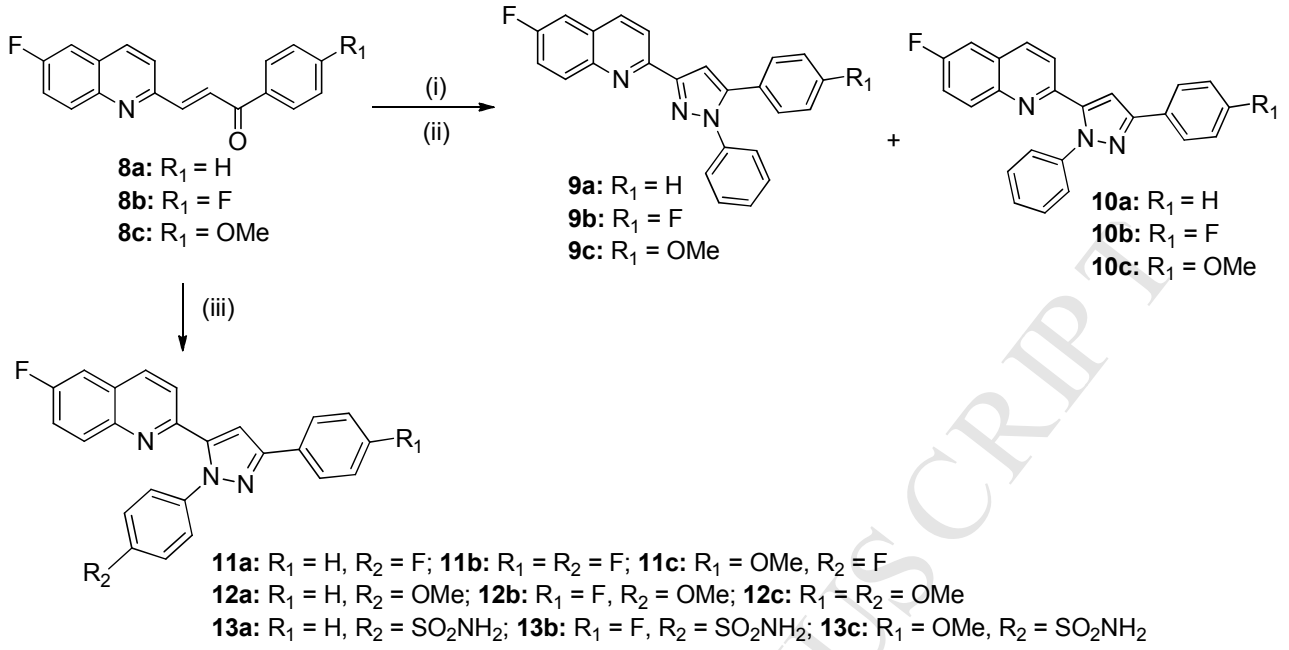


Figure 6.

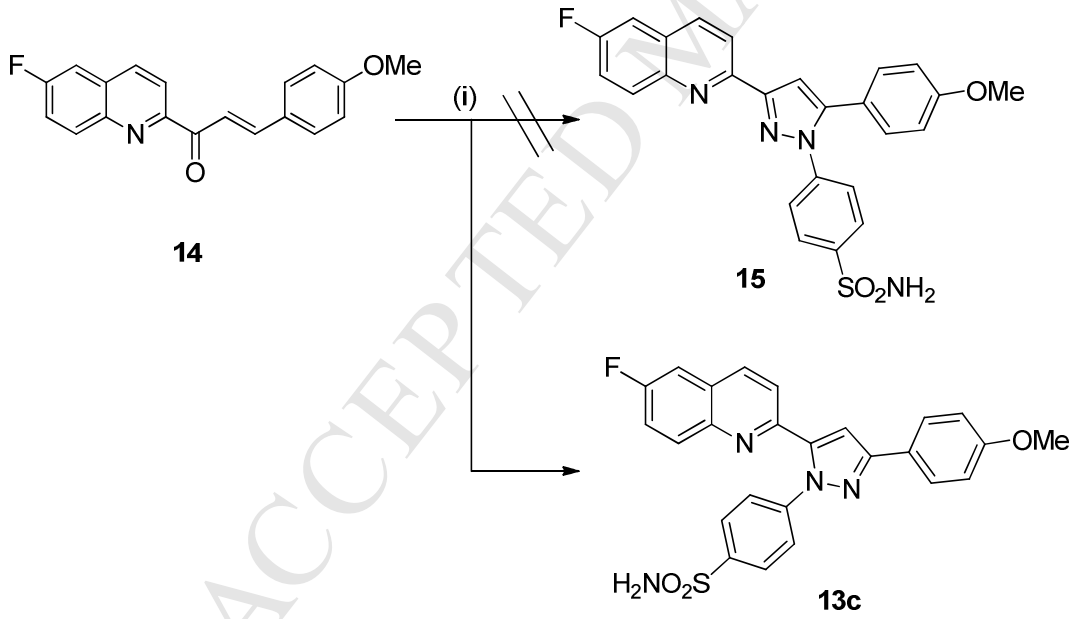




Scheme 1: Reagents and condictions: (i) SeO_2, 90°C, 2h (81%); (ii) 4-substituted acetophenone 50%KOH, 0°C, 20 min.



Scheme 2: Reagents and condictions: (i) phenylhydrazine, reflux, 6hr; (ii) DDQ, reflux, 6h; (iii) 4-substituted phenylhydrazine hydrochloride, reflux, 24h.



Scheme 3: Reagents and condictions: (i) 4-hydrazinobenzenesulfonamide hydrochloride, reflux, 24h (61%).

Highlights

- Diarylpyrazolylquinoline compounds were synthesized.
- **13c** was potent inhibited all four sero-types of DENV.
- **13c** reduced DENV replication in both viral protein and mRNA levels, and no significant cell cytotoxicity was detected.
- **13c** represents a potential antiviral agent to block DENV replication *in vitro* and *in vivo*.