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RESEARCH ARTICLE

The synthesis of 6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinoline carbonate derivatives and their antifungal activity against *Pyricularia oryzae*

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Abstract A series of novel 6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinoline carbonate derivatives were designed and synthesized. Bioassay results showed that some of them exhibited good activity against *Pyricularia oryzae* (*P. oryzae*). It was found that the compound **5q** (benzyl (6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinolin-4-yl) carbonate) possessed good activity against *P. oryzae* whatever protective activity (10 $mg \cdot L^{-1}$) or curative activity (25 $mg \cdot L^{-1}$), which was better than that of control tebufloquin. In addition, the frontier molecular orbit results revealed that the compound held higher activity against *P. oryzae* when the total energy was low and the ClogP was high, which may provide useful information for further design novel fungicides.

Keywords quinoline, synthesis, antifungal activity, rice blast, SAR

1 Introduction

Fungicide resistance had been series problems in modern agriculture. So development of effective broad-spectra fungicides is an important role in crop protecting [1–3]. Natural product quinoline and their derivatives are widely used in pharmaceutical and pesticidal fields because of their high biological activity. To explore new quinoline pesticides, many quinoline structures were discovered by agrochemical companies. For example, the quinoxyfen [4,5], a quinoline fungicide, was discovered by Dow Agrochemicals, mainly through the inhibition of the activity of serine esterase of powdery mildew fungi, and interfere with the protein kinase mediated pathway. Thus it inhibits the fungal germination and spore attachment. The other important quinoline fungicide, tebufloquin [6], discovered by Meiji Seika Kaisha in 2005, an excellent fungicide with effective disease control caused by rice blast (RB). It possesses good disease control efficacy against the resistance fungi and does not inhibit melanin biosynthesis, maybe it held a novel mode of action. From 2014, more than 100 patents [7–11] reported the mixture inclusion of tebufloquin with other mode of action fungicides used to control phytopathogens in agriculture. On the other hand, quinoline derivatives exhibit a variety of biological activities, such as anti-tuberculosis [12], anticancer [13], antioxidant [14], aromatase inhibitors [15], multi-trypanosomatid activity [16], leishmanicidal [17], antibacterial [18]. The famous anti-malaria drug quinine cinchona alkaloid was isolated from the bark of cinchona and related plants with quinolone based substructure.

Previously, our group synthesized a series of new heterocyclic compounds [19–22] as fungicides, nematocides, insecticides and herbicides based on quinoline scafold. In this paper, the fungicide tebufloquin was selected as a lead compound. The main substructure (6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinoline) of tebufloquin was maintained. Then, the ethyl ester group was replaced by ester carbonate. Our strategy is depicted in Scheme 1. Surprisingly, the designed compounds possess higher fungicidal efficacy than tebufloquin against rice blast at 10 $mg \cdot L^{-1}$.

2 Materials and methods

2.1 General information

All chemical reagents were analytical grade or prepared in our laboratory. Melting points were measured using an X-4 apparatus (Taike, Beijing, China) and were uncorrected. ^1H

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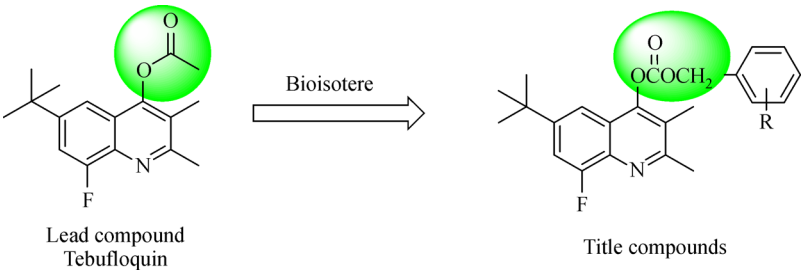


Scheme 1 Design strategy of the title compounds

NMR and ^{13}C NMR spectra were recorded on a BRUKER Avance 400 MHz or 600 MHz spectrometer using $CDCl_3$ as solvent. Mass spectra were determined on a LCQ Advantage LC/mass detector instrument. Elemental analyses were performed on a Vario EL elemental analyzer. The full geometry optimization was carried out using 6-31G(d, p) basis set, and total energy, orbital energy, and CLogP of compound **5q**, **5d** and tebufloquin were calculated using DFT-B3LYP/6-31G (d, p) method in the Gaussian 03 package [23].

2.2 Synthesis

Synthesis of *N*-(4-(tert-butyl)phenyl)acetamide (**1**). To a solution of 4-(*tert*-butyl)aniline (40.00 g, 0.268 mol) in MTBE (800 mL) and CH_2Cl_2 (400 mL), was added acetic anhydride (28 mL, 0.30 mol) dropwisely at 0 °C for 30 min. The mixture was stirred at reflux condition for another 2 h, then the mixture was poured into water, *N*-(4-(*tert*-butyl)phenyl)acetamide was given by filter and wash by hexane, yield 97.0%, m.p.168–170 °C。^1H NMR (400 MHz, DMSO-d_6, δ ppm): 1.25 (s, 9H, $C(CH_3)_3$), 2.01 (s, 3H, $COCH_3$), 7.29 (d, 2H, J = 8.0 Hz, Ar-H), 7.47 (d, 2H, J = 8.0 Hz, Ar-H), 9.83 (s, 1H, NH).

Synthesis of *N*-(4-(tert-butyl)-2-fluorophenyl)acetamide (**2**). To a solution of selectfluor (35.5 g, 0.1 mol) in CH_3CN (400 mL), was added compound **1** (19.1 g, 0.1 mol) dropwisely at 60 °C. Then the mixture was stirred at 100 °C for 1 h. Subsequently the reaction mixture was poured with water. The organic layer was washed by saturated salt water and dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on a silica gel using petroleum ether and ethyl acetate as the eluent to afford the intermediate **2**. Yield 76.2%, m.p.162–164 °C。^1H NMR (400 MHz, DMSO-d_6, δ ppm): 1.26 (s, 9H, $C(CH_3)_3$), 2.06 (s, 3H, $COCH_3$), 7.15 (d, 1H, J = 8.0 Hz, Ar-H), 7.21 (d, 1H, J = 8.0 Hz, Ar-H), 7.69–7.72 (m, 1H, Ar-H), 9.63(s, 1H, NH).

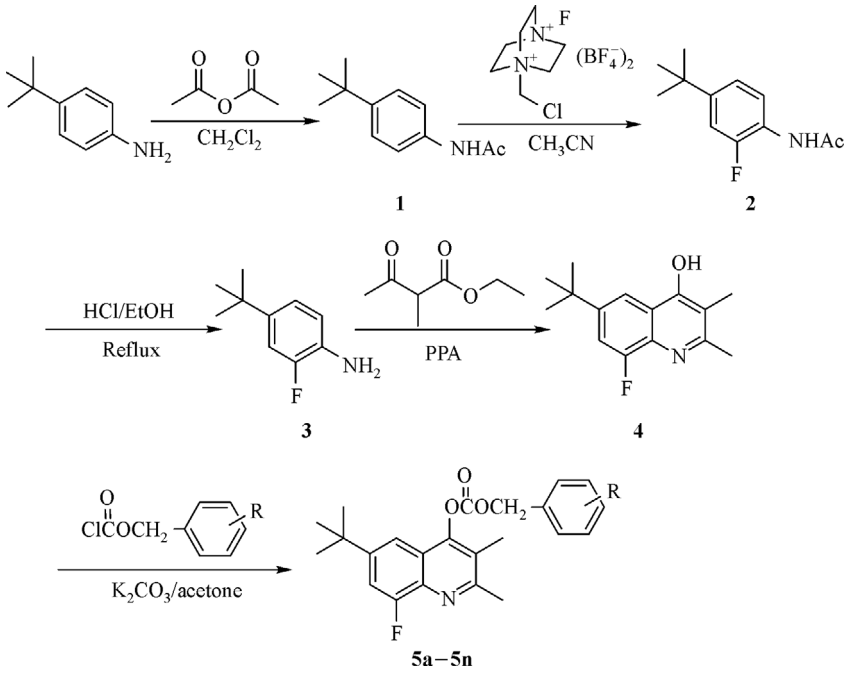
Synthesis of 4-(tert-butyl)-2-fluoroaniline (**3**). Compound **2** (10.45 g, 0.05 mol) was dissolved in the solution of EtOH (100 mL) and 37% HCl (50 mL), then the mixture was refluxed for 8 h. The solvent was removed, subsequently the reaction mixture was poured with water. The organic layer was exacted by CH_2Cl_2 and dried over Na_2SO_4 and evaporated. The intermediate **3** was given as red oil, yield 82.5%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.26 (s, 9H, $C(CH_3)_3$), 3.51 (s, 2H, NH_2), 6.68–6.71 (m, 1H, Ar-H), 6.93–7.01 (m, 2H, Ar-H).

Synthesis of 6-(tert-butyl)-8-fluoro-2,3-dimethylquinolin-4-ol (**4**). In a 500 mL three neck bottom flask, compound **3** (7.71 g, 0.04 mol), ethyl 2-methyl-3-oxobutanoate (5.76 g, 0.04 mol) and polyphosphoric acid (PPA, 8.3 g) was stirred at 150 °C. TLC was used to monitor the reaction. After the reaction was completed, the mixture was poured into water, which gave substantial amount of solids. After being dried, 9.51 g of solid resulted, indicating a yield of 96.2%, m.p. 225–228 °C. ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 1.32 (s, 9H, $C(CH_3)_3$), 1.97 (s, 3H, quinolyl-3-CH_3), 2.42 (s, 3H, quinolyl-2-CH_3), 7.60 (d, 1H, J = 8.0 Hz, Ar-H), 7.84 (s, 1H, Ar-H), 11.29 (s, 1H, OH); ESI: 246 $[M - H^+]^-$.

General procedure for the synthesis of target compounds **5a**–**5q**. To a solution of **4** (0.35 g, 1.6 mmol) and K_2CO_3 (0.084 g, 1.4 mmol) in acetone (50 mL), the substituted benzyl carbonochloridate (1.4 mmol) was added dropwise. The reaction was monitored by TLC. After the reaction was completed, the mixture was filtered and evaporated. The target compound was purified by chromatography on a silica gel using petroleum ether and ethyl acetate (V_{EA}:V_{PE} = 1:8) as the eluent to afford compounds **5a**–**5q**. The synthetic route is shown in Scheme 2.

6-(tert-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (4-methylbenzyl) carbonate **5a**. White solid, m.p. 125–128 °C, yield 70.3%, ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.30 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, quinolyl-3-CH_3), 2.38 (s, 3H, Ar-CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.30 (s, 2H, OCH_2), 7.21 (d, 2H, J = 8.0 Hz, Ar-H), 7.34 (d, 3H, J = 8.0 Hz, Ar-H), 7.40–7.43 (m, 1H, Ar- H); ^{13}C NMR (150 MHz, $CDCl_3$, δ ppm): 12.50, 21.72, 24.19, 30.95, 35.18, 71.04, 111.08, 112.15, 112.28, 122.53, 122.65, 128.65, 129.48, 131.61, 135.97, 136.06, 149.91, 149.95, 151.26, 151.29, 152.33, 156.54, 159.93; ESI-MS: 396 $[M + H]^+$; Elemental anal. calculated for $C_{24}H_{26}FNO_3$ (%): C, 72.89; H, 6.63; N, 3.54; found C, 72.98; H, 6.56; N, 3.31.

4-Bromobenzyl (6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinolin-4-yl) carbonate **5b.** White solid, m.p. 161–163 °C, yield 56.6%. ^1H NMR (400 MHz, $CDCl_3$,



Scheme 2 The synthetic route of title compounds

δ ppm): 1.31 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, quinolyl-3-CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.29 (s, 2H, OCH_2), 7.34 (d, 3H, J = 8.0 Hz, Ar-H), 7.41–7.44 (m, 1H, Ar-H), 7.54 (d, 2H, J = 8.0 Hz, Ar-H); ESI-MS: 461 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{23}BrFNO_3$ (%): C, 60.01; H, 5.04; N, 3.04; found C, 59.95; H, 5.12; N, 3.15.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (3,4-dichlorobenzyl) carbonate **5c**. White solid, m.p. 152–155 °C, yield 67.5%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.31 (s, 9H, $C(CH_3)_3$), 2.29 (s, 3H, quinolyl-3-CH_3), 2.75 (s, 3H, quinolyl-2-CH_3), 5.28 (s, 2H, OCH_2), 7.28–7.32 (m, 2H, Ar-H), 7.42–7.47 (m, 2H, Ar-H), 7.56 (s, 1H, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$, δ ppm): 12.52, 24.20, 30.93, 35.19, 69.27, 110.80, 112.28, 112.40, 122.30, 122.55, 127.78, 130.48, 130.86, 133.09, 133.38, 134.70, 136.08, 150.14, 150.18, 151.11, 152.16, 156.57, 158.25, 159.96; ESI-MS: 451 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{22}Cl_2FNO_3$ (%): C, 61.34; H, 4.92; N, 3.11; found C, 61.54; H, 5.01; N, 3.16.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (4-methoxybenzyl) carbonate **5d.** White solid, m.p. 134–137 °C, yield 56.4%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.35 (s, 9H, $C(CH_3)_3$), 2.12 (s, 3H, quinolyl-3-CH_3), 2.45 (s, 3H, quinolyl-2-CH_3), 3.81 (s, 3H, OCH_3), 4.63 (s, 2H, OCH_2), 6.80–6.90 (m, 2H, Ar-H), 7.28–7.37 (m, 3H, Ar-H), 8.10 (s, 1H, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$, δ ppm): 12.63, 24.27, 31.11, 35.13, 55.33, 76.17, 111.54, 112.60, 114.10, 122.21, 124.21, 128.67, 130.17, 148.60, 156.74, 158.42, 159.42, 159.93, 160.44; ESI-MS: 412 $[M + H]^+$; Elemental anal. calculated for $C_{24}H_{26}FNO_4$ (%): C, 70.06; H, 6.37; N, 3.40; found C, 69.89; H, 6.45; N, 3.44.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (4-fluorobenzyl) carbonate **5e.** White solid, m.p. 114–116 °C, yield 48.8%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.34 (s, 9H, $C(CH_3)_3$), 2.35 (s, 3H, quinolyl-3-CH_3), 2.72 (s, 3H, quinolyl-2-CH_3), 5.01 (s, 2H, OCH_2), 7.10 (t, 2H, J = 8.0 Hz, Ar-H), 7.38–7.45 (m, 3H, Ar-H), 7.61 (s, 1H, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$, δ ppm): 12.48, 24.19, 30.95, 35.19, 70.25, 110.91, 112.20, 112.33, 115.75, 115.89, 122.41, 122.59, 130.49, 130.78, 130.84, 135.98, 151.17, 151.20, 152.26, 156.55, 159.95, 162.31, 163.96; ESI-MS: 400 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{23}F_2NO_3$ (%): C, 69.16; H, 5.80; N, 3.51; found C, 69.25; H, 5.87; N, 3.54.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (4-chlorobenzyl) carbonate **5f.** White solid, m.p. 125–128 °C, yield 62.9%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.30 (s, 9H, $C(CH_3)_3$), 2.27 (s, 3H, quinolyl-3-CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.30 (s, 2H, OCH_2), 7.32 (s, 1H, Ar-H), 7.37–7.39 (m, 3H, Ar-H), 7.44–7.46 (m, 2H, Ar-H); ESI-MS: 417 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{23}ClFNO_3$ (%): C, 66.42; H, 5.57; N, 3.37; found C, 66.57; H, 5.46; N, 3.54.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (2,4-dichlorobenzyl) carbonate **5g.** White solid, m.p. 143–145 °C, yield 43.7%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.33 (s, 9H, $C(CH_3)_3$), 2.38 (s, 3H, quinolyl-3-CH_3), 2.73 (s, 3H, quinolyl-2-CH_3), 5.10 (s, 2H, OCH_2), 7.33 (d, 1H, J = 8.0Hz, Ar-H), 7.41 (d, 1H, J = 8.0 Hz, Ar-H), 7.48 (s, 1H, Ar-H), 7.57 (d, 1H, J = 8.0Hz, Ar-H), 7.62 (s, 1H, Ar-H); ESI-MS: 451 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{22}Cl_2FNO_3$ (%): C, 61.34; H, 4.92; N, 3.11; found C, 61.38; H, 5.06; N, 3.01.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (2-chlorobenzyl) carbonate **5h.** White solid, m.p. 137–139 °C, yield 59.1%, ^{1}H NMR(400 MHz, $CDCl_3$)δ: 1.34 (s, 9H, $C(CH_3)_3$), 2.31 (s, 3H, quinolyl-3-CH_3), 2.75 (s, 3H, quinolyl-2-CH_3), 5.46 (s, 2H, OCH_2), 7.31–7.37 (m, 3H, Ar-H), 7.42-7.47 (m, 2H, Ar-H), 7.51–7.53 (m, 1H, Ar-H); ESI-MS: 417 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{23}ClFNO_3$ (%): C, 66.42; H, 5.57; N, 3.37; found C, 66.51; H, 5.49; N, 3.41.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (2-fluorobenzyl) carbonate **5i.** White solid, m.p. 108–111 °C, yield 62.2%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.33 (s, 9H, $C(CH_3)_3$), 2.29 (s, 3H, quinolyl-3-CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.43 (s, 2H, OCH_2), 7.12–7.18 (m, 2H, Ar-H), 7.38–7.44 (m, 2H, Ar-H), 7.47–7.50 (m, 2H, Ar-H); ESI-MS: 400 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{23}F_2NO_3$ (%): C, 69.16; H, 5.80; N, 3.51; found C, 69.23; H, 5.79; N, 3.56.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (2-methylbenzyl) carbonate **5j.** White solid, m.p. 118–121 °C, yield 69.4%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.32 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, quinolyl-3-CH_3), 2.42 (s, 3H, CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.37 (s, 2H, OCH_2), 7.23 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.28–7.32 (m, 1H, Ar-H), 7.38–7.44 (m, 3H, Ar-H); ESI-MS: 396 $[M + H]^+$; Elemental anal. calculated for $C_{24}H_{26}FNO_3$ (%): C, 72.89; H, 6.63; N, 3.54; found C, 72.66; H, 6.58; N, 3.64.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (3-methylbenzyl) carbonate **5k.** White solid, m.p. 101–103 °C, yield 58.6%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.31 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, quinolyl-3-CH_3), 2.37 (s, 3H, CH_3), 2.73 (s, 3H, quinolyl-2-CH_3), 5.30 (s, 2H, OCH_2), 7.18–7.25 (m, 3H, Ar-H), 7.27–7.31 (m, 1H, Ar-H), 7.37–7.43 (m, 2H, Ar-H); ESI-MS: 396 $[M + H]^+$; Elemental anal. calculated for $C_{24}H_{26}FNO_3$ (%): C, 72.89; H, 6.63; N, 3.54; found C, 72.94; H, 6.76; N, 3.45.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (3-fluorobenzyl) carbonate **5l.** White solid, m.p. 95–98 °C, yield 42.8%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.32 (s, 9H, $C(CH_3)_3$), 2.29 (s, 3H, quinolyl-3-CH_3), 2.75 (s, 3H, quinolyl-2-CH_3), 5.32 (s, 2H, OCH_2), 7.07–7.11 (m, 1H, Ar-H), 7.17 (d, 1H, J = 8.0 Hz, Ar-H), 7.23 (d, 1H, J = 8.0 Hz, Ar-H), 7.38–7.44 (m, 3H, Ar-H); ESI-MS: 400 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{23}F_2NO_3$ (%): C, 69.16; H, 5.80; N, 3.51; found C, 68.99; H, 5.91; N, 3.54.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (2,6-dichlorobenzyl) carbonate **5m.** White solid, m.p. 162–164 °C, yield 44.3%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.37 (s, 9H, $C(CH_3)_3$), 2.34 (s, 3H, quinolyl-3-CH_3), 2.76 (s, 3H, quinolyl-2-CH_3), 5.66 (s, 2H, OCH_2), 7.32 (s, 1H, Ar-H), 7.34 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.48 (s, 1H, Ar-H); ESI-MS: 451 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{22}Cl_2FNO_3$ (%): C, 61.34; H, 4.92; N, 3.11; found C, 61.44; H, 5.12; N, 3.05.

6-(tert-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl (2,4-difluorobenzyl) carbonate **5n**. White solid, m.p. 116–119 °C, yield 44.3%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.33 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, quinolyl-3-CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.38 (s, 2H, OCH_2), 6.87–6.92 (m, 2H, Ar-H), 7.37–7.48 (m, 3H, Ar-H); ESI-MS: 418 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{22}F_3NO_3$ (%): C, 66.18; H, 5.31; N, 3.36; found C, 66.25; H, 5.55; N, 3.44.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl(2-chloro-6-fluorobenzyl) carbonate **5o**. White solid, m.p. 157–160 °C, yield 35.1%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.36 (s, 9H, $C(CH_3)_3$), 2.32 (s, 3H, quinolyl-3-CH_3), 2.75 (s, 3H, quinolyl-2-CH_3), 5.55 (s, 2H, OCH_2), 7.07–7.11 (m, 1H, Ar-H), 7.21–7.25 (m, 1H, Ar- H), 6.87–6.92 (m, 2H, Ar-H), 7.28–7.31 (m, 1H, Ar-H), 7.34–7.38 (m, 1H, Ar-H), 7.46 (s, 1H, Ar-H); ESI-MS: 435 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{22}ClF_2NO_3$ (%): C, 63.67; H, 5.11; N, 3.23; found C, 63.78; H, 5.23; N, 3.45.

6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl(2-chloro-5-(trifluoromethyl)benzyl) **5p**. White solid, m.p. 69–72 °C, yield 30.6%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.35 (s, 9H, $C(CH_3)_3$), 2.32 (s, 3H, quinolyl-3-CH_3), 2.76 (s, 3H, quinolyl-2-CH_3), 5.50 (s, 2H, OCH_2), 7.43 (s, 1H, Ar-H), 7.47–7.48 (m, 1H, Ar-H), 7.58–7.61 (m, 2H, Ar-H), 7.80 (s, 1H, Ar-H); ESI-MS: 485 $[M + H]^+$; Elemental anal. calculated for $C_{24}H_{22}ClF_4NO_3$ (%): C, 59.57; H, 4.58; N, 2.89; found C, 59.68; H, 4.75; N, 3.01.

Benzyl(6-(*tert*-Butyl)-8-fluoro-2,3-dimethylquinolin-4-yl) carbonate **5q**. White solid, m.p. 88–90 °C, yield 65.2%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.31 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, quinolyl-3-CH_3), 2.74 (s, 3H, quinolyl-2-CH_3), 5.35 (s, 2H, -OCH_2), 7.28–7.47 (m, 7H, Ar-H, quinolyl-7-H, quinolyl-5-H); ^{13}C NMR (150 MHz, $CDCl_3$, δ ppm): 12.49, 24.19, 30.98, 35.19, 71.01, 111.00, 112.17, 112.29, 122.62, 128.31, 128.51, 128.75, 129.01, 134.60, 135.97, 149.96, 150.00, 151.21, 152.30, 156.53, 158.22, 159.93; ESI-MS: 382 $[M + H]^+$; Elemental anal. calculated for $C_{23}H_{24}FNO_3$ (%): C, 72.42; H, 6.34; N, 3.67; found C, 72.46; H, 6.25; N, 3.77.

2.3 Antifungal evaluation

Antifungal activity. The fungi *Pyricularia oryzae* (*P. oryzae*) was provided by Zhejiang University, Hangzhou, China, and stored in our laboratory. *P. oryzae* was identified by Rui-Rui Zhang and comparing the morphological, cultural, spore, and mycelia characteristics with those of a standard culture. Pure culture isolates were grown on potato dextrose agar (PDA) at 26 °C for up to 7 d prior to use.

Protective activity against *P*. oryzae. Compounds were sprayed on two-week-old rice-leaf seedlings. A concentra-

tion of 2×10^5 RB spores mL^{-1} in sterile water with 0.2% (w/v) gelatin was sprayed on leaves after 24 h. Inoculated plants were kept in a 25 °C with 100% humidity and in a 14/10 h light/dark cycle. Lesion formation was observed daily and photographed 7 days after inoculation [24]. The positive control was tebufloquin. The protective control efficacy was calculated as follows: Protective efficacy = [(number of disease spots of control − number of disease spots of treated group)/number of disease spots of control] × 100%

Curative activity against *P.* oryzae. A concentration of 2×10^5 RB spores mL^{-1} in sterile water with 0.2% (w/v) gelatin was sprayed on two-week-old rice-leaf seedlings. Inoculated plants were kept in a 25 °C with 100% humidity and in a 14/10 h light/dark cycle. Compounds were sprayed after 12 h. Lesion formation was observed daily and photographed 7 days after inoculation [24]. The positive control was tebufloquin. The protective control efficacy was calculated as follows: Protective efficacy = [(number of disease spots of control − number of disease spots of treated group)/number of disease spots of control] × 100%. All experiments were replicated three times.

EC_{50} Test. The *in vitro* fungicidal activities of the target compounds **5a, 5c, 5d, 5e** and **5q** against *P. oryzae* were evaluated using the mycelium growth rate method. The culture media, with known concentrations (40, 10, 2.5, 0.625 and 0.15625 $mg \cdot L^{-1}$) of the test compounds, were obtained by mixing the 1% tween-20 water suspension (1 mL) of **5a, 5c, 5d, 5e** and **5q** with potato dextrose agar (PDA, 9 mL) at 50 °C. The medium was then poured into a 9-cm Petri dish and cooled to room temperature, which was inoculated with 5-mm mycelium of *P. oryzae*. The Petri dish was then placed in a light incubator at 25±1 °C for 72 h. A commercial fungicide tebufloquin was used as the positive control, and sterile water was used as the blank. Three replications were employed for each treatment. The inhibition rate was expressed as the mean of values obtained in three independent experiments. Effective concentration (EC_{50}) was obtained using log-probit analysis (Table 3). The inhibition rate was calculated according to the formula: Inhibitor rate (%) = (CK − PT)/CK × 100%. Where CK is the expansion diameter of mycelia in the blank test, and PT is the expansion diameter of mycelia in the presence of tested compounds.

3 Results and discussion

3.1 Synthesis and spectra

The synthetic route of title quinoline compounds were outlined in Scheme 2. Many classic synthetic methods about quinoline derivatives were reported, such as Skraup method, Camps method, Combes method, Friedlander method, Niementowski method and Pfitzinger methods. After comparing these methods, the Combes method was selected, in which the starting material were cyclized under acid conditions. In this step, PPA was used as dehydrating agent. Many dehydrating agents can work, such as H_3PO_4, HF, $AlC1_3$, BF_3, P_2O_5 and H_2SO_4, but the reaction is quicker and product is clean when PPA was used. The final 6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinoline carbonates were synthesized using THF as the solvent and Et_3N as the base at first, but the yield was low and they were difficult to purify. To optimize the yield and process, we selected acetone as the solvent and K_2CO_3 as the base, and the yield increased and the product was more easily purified.

3.2 *In vivo* antifungal activity and SAR

The *in vivo* protective activity and curative activity of the compounds developed in our study against *P. oryzae* were shown in Table 1 and Table 2. Most of the compounds possessed excellent *in vivo* protective activity (100%) against *P. oryzae* at 100 $mg \cdot L^{-1}$, except compound **5o** (95%). On the other hand, the title compounds exhibited moderate to good *in vivo* curative activity with the range of 65%–90% against *P. oryzae* at 100 $mg \cdot L^{-1}$, which is better than that of control tebufloquin (70%). Among them, compound **5c** (90%), **5k** (90%) and **5q** (90%) exhibited good curative activity against *P. oryzae*.

On the basis of the preliminary results, the title compounds were selected for further bioassay at lower dose against *P. oryzae*. The following results showed that the title compounds still possessed good protective activity

Table 1 The *in vivo* protective efficacy against rice blast at different concentration

No.	R	100 $mg \cdot L^{-1}$	50 $mg \cdot L^{-1}$	10 $mg \cdot L^{-1}$
5a	4-Me	100	95	65
5b	4-Br	100	80	55
5c	3,4-Cl_2	100	85	70
5d	4-OMe	100	99	65
5e	4-F	100	90	80
5f	4-Cl	100	85	45
5g	2,4-Cl_2	100	80	30
5h	2-Cl	100	65	35
5i	2-F	100	75	30
5j	2-Me	100	75	40
5k	3-Me	100	75	45
5l	3-F	100	70	34
5m	2,6-Cl_2	100	85	45
5n	2,4-F_2	100	90	75
5o	2-Cl-6-F	95	70	50
5p	2-Cl-5-CF_3	100	75	45
5q	H	100	95	75
Tebufloquin		100	70	0

Table 2 The *in vivo* curative efficacy against rice blast at different concentration

No.	R	100 mg·L^{-1}	50 mg·L^{-1}	25 mg·L^{-1}
5a	4-Me	85	65	25
5b	4-Br	80	45	0
5c	3,4-Cl_2	90	75	45
5d	4-OMe	85	65	35
5e	4-F	85	65	35
5f	4-Cl	65	35	0
5g	2,4-Cl_2	80	45	0
5h	2-Cl	75	35	0
5i	2-F	85	50	0
5j	2-Me	65	55	25
5k	3-Me	90	45	0
5l	3-F	75	35	0
5m	2,6-Cl_2	85	40	0
5n	2,4-F_2	75	70	0
5o	2-Cl-6-F	75	35	0
5p	2-Cl-5-CF_3	75	45	0
5q	H	90	65	45
Tebufloquin		70	45	0

against *P. oryzae* at 50 mg·L^{-1}. Among them, only the compound **5h** (65%) exhibit a little weaker than that of control (70%) at 50 mg·L^{-1}. While for the curative activity at 50 mg·L^{-1}, it had the similar trends with the preliminary results. For example, the compound **5f** (35%), **5h** (35%), **5l** (35%) and **5o** (35%) exhibited low curative activity, compared with the control (45%), while the compound **5a** (65%), **5c** (75%), **5d** (65%), **5n** (70%) and **5q** (65%) exhibited high curative activity, compared with the control (45%). When the test concentration is at 10 mg·L^{-1}, whatever the protective activity or curative activity, the control Tebufloquin had no activity. Surprisingly, some of title compounds exhibited good protective activity efficacy, such as compound **5c** (70%), **5e** (80%), **5n** (75%) and **5q** (75%) respectively. For the curative activity, only compound **5c** (45%) and **5q** (45%) exhibited highest activity at the concentration 25 mg·L^{-1}.

For EC_{50} testing, the fungicide tebufloquin was selected as positive control (Table 3). The *in vitro* EC_{50} value of compound **5a**, **5c**, **5d**, **5e** and **5q** values is 2.79 mg·L^{-1}, 3.59 mg·L^{-1}, 0.94 mg·L^{-1}, 6.77 mg·L^{-1} and 5.18 mg·L^{-1} against *P. oryzae* respectively, which are the comparable as that of the positive control tebufloquin (1.27 mg·L^{-1}).

3.3 DFT calculation and SAR

To study their structure-active relationship, we choose a high active compound **5q, 5d** and lead compound tebufloquin as model compounds, the frontier orbital and

Table 3 The EC_{50} of high active compound against rice blast (*in vitro*)

Compound	EC_{50}/(mg·L^{-1})	$Y = BX + A$	R
5a	2.79(1.72–4.58)	$4.7522 + 0.5567X$	0.98
5c	3.59(2.31–5.82)	$4.6658 + 0.6018X$	0.98
5d	0.94(0.61–1.37)	$5.0203 + 0.7815X$	0.98
5e	6.77(4.93–9.57)	$4.2012 + 0.9621X$	0.99
5q	5.18(3.68–7.66)	$4.4027 + 0.8363X$	0.99
Tebufloquin	1.27(0.91–1.70)	$4.8943 + 1.0124X$	0.99

Table 4 CLogP, total energy, energy gap and frontier orbital energy

DFT	**5q**	**5d**	Tebufloquin
E_{total}/Hartree[a)]	−1270.85620776	−1385.33947946	−1865.96608237
E_{HOMO}/Hartree	−0.22710	−0.22504	−0.22894
E_{LUMO}/Hartree	−0.05235	−0.05014	−0.07096
ΔE[b)]/Hartree	0.17475	0.1749	0.15798
CLogP	6.33	6.95	4.81

a) 1 Hartree = 4.35974417 × 10^{-18} J = 27.2113845 eV; b) $\Delta E = E_{LUMO} - E_{HOMO}$.

CLogP was calculated. The CLogP, energy of HOMO and LUMO, total energy and energy gap are listed in Table 4.

According to the frontier molecular orbital theory, HOMO has the priority to provide electrons, while LUMO can accept electrons first. As we can see from Fig. 1, the LUMO and HOMO are different between the high active compounds **5q, 5d** and lead compound tebufloquin, especially in the orient of electron transition and energy gap. For the HOMO, the electron of compound **5q, 5d** and control tebufloquin had the same condition: it is mainly concentrated on the quinoline ring, methyl group and a little on the ester carbonate bridge or ester bridge. But for the LUMO, the two compounds are different. The electron of compound **5q** and **5d** is evenly distributed among the quinoline ring, methyl group, carbonate bridge and fluorine group. But the electron of tebufloquin is mainly located on the ester group, methyl group and a little quinolone ring. Perhaps the reason of different fungicidal activity between the compound **5q, 5d** and tebufloquin is electron transition direction and energy gap. From the Fig. 1, we assumed that the compound with higher energy gap and lower total energy exhibited higher fungicidal activity. The other fact is the CLogP. From Table 4, the CLogP is different between the three compounds.

4 Conclusions

Most of the synthesized compounds exhibited excellent *in vivo* antifungal activity against *P. oryzae* at 100 mg·L^{-1}. Among them, compounds **5c**, **5e**, **5n** and **5g** are highly active at 10 mg·L^{-1}. In particular, quinoline derivatives containing strong electron-withdrawing at para position of

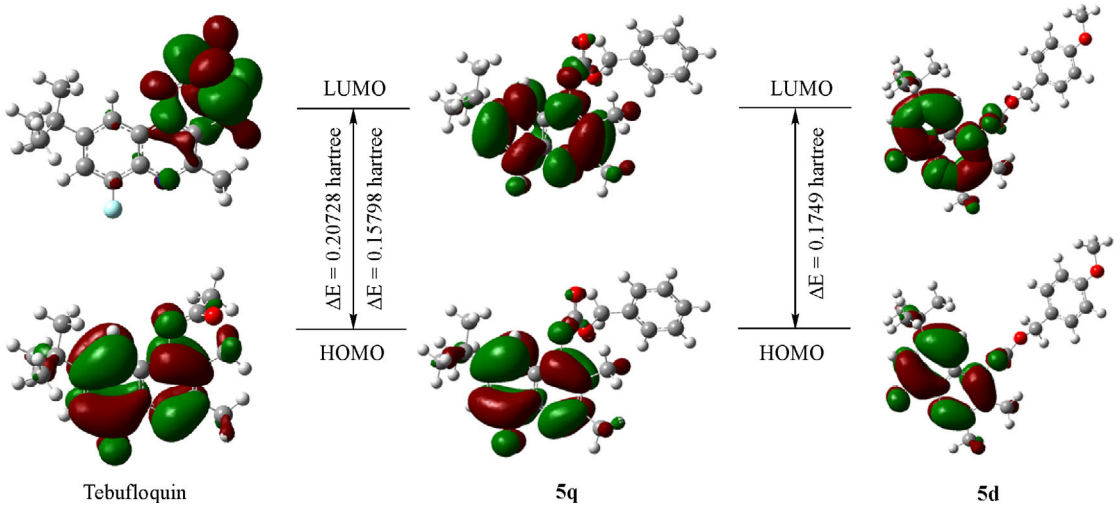


Fig. 1 Frontier molecular orbitals of compound **5q, 5d** and tebufloquin

benzene ring or with no substitution on benzene ring exhibited significantly potent antifungal activity against *P. oryzae* pathogens. Furthermore, a density functional theory study established the structure-activity relationships of the synthesized compounds. It can be found that the electron transit orient is different between the high active compound and lead compound Tebufloquin. Quinoline derivatives, especially benzyl (6-(*tert*-butyl)-8-fluoro-2,3-dimethylquinolin-4-yl) carbonate, which possess good control effective against *P. oryzae*, may become new lead compounds for the development of antifungals with further structure modification.

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