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Synthesis, Fungicidal Activity, and Sterol 14α-Demethylase Binding Interaction of 2-Azolyl-3,4-dihydroquinazolines on *Penicillium digitatum*

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Supporting Information

ABSTRACT: A series of new 2-azolyl-3,4-dihydroquinazolines **6** was synthesized by direct cyclization of imidazole or 1,2,4-triazole with carbodiimides **4**, which were obtained from aza-Wittig reaction of iminophosphorane **3** with isocyanate. The preliminary bioassay results demonstrated that most of the 2-imidazolyl-3,4-dihydroquinazolines **6a−6i** exhibited good to significant fungicidal activity against *Penicillium digitatum*, whereas 2-triazolyl-3,4-dihydroquinazolines **6j−6t** exhibited low fungicidal activity. Some of the 2-imidazolyl-3,4-dihydroquinazolines **6a−6i** also exhibited strong binding interaction with the cytochrome P450-dependent sterol 14α-demethylase (CYP51). For example, compound **6e** showed the best fungicidal activity against *P. digitatum* with IC_{50} = 4.14 μg/mL and the best CYP51 binding activity with K_d = 0.34 μg/mL, both superior to those of the agricultural fungicide triadimefon.

KEYWORDS: *imidazole, triazole, quinazoline, aza-Wittig reaction, fungicidal activity, 14α-demethylase, binding constant*

INTRODUCTION

Green mold of citrus, caused by *Penicillium digitatum*, is one of the most serious diseases affecting postharvest citrus. Serious postharvest losses are caused every year in most regions of the world due to this pathogen infection.[1,2] Synthetic fungicides are widely utilized to control postharvest diseases in fruits and vegetables. Among various kinds of fungicides, azoles are the most widely used and studied class of antifungal agents in agricultural usage because of their lower application doses, high selectivity, and reduced undesired environmental impact.[3,4] The biochemical site of action of the fungicidal azoles is the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51), encoded by the ERG11 gene in fungal cells.[5] This heme protein binds tetracyclic steroid molecules, inserting one oxygen atom into a CH bond of the C-14 methyl group.[6] Azole antifungal agents inhibit CYP51 by a mechanism in which the heterocyclic nitrogen atom (N3 of imidazole and N4 of triazole) binds to the heme iron atom in the binding site of the enzyme. Unfortunately, azoles are fungistatic against yeasts, and the broad usage of these compounds led to the development of resistance, showing the urgent need for new and effective antifungal agents.

Heterocyclic compounds play an increasingly important role in pesticide discovery because many of them have shown good insecticidal or fungicidal activities.[7−10] Among them, 1,2,4-triazole derivatives such as diniconazole, hexaconazole, triadimefon, and triadimenol represent the most important category of fungicides to date. Imidazole compounds exhibited also siginificant fungicidal activities, and some of these compounds have been utilized as agricultural fungicides. For example, triadimefon, prochloraz, triflumizole, and pefurazoate are widely used to control various fungal pathogens (Figure 1). These compounds have excellent protective, curative, and eradicant power toward a wide spectrum of crop diseases. Although various structure types of azoles have been synthesized and evaluated as fungicides previously,[11] the azole compounds substituted by another heterocycle through N1 of imidazole and the triazole ring were less investigated. Questions concerning synthesis and fungicidal activities of new structures of azole derivatives substituted by another heterocycle and the binding ability of the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51) for these azole fungicides are therefore of particular interest in this study.

In previous studies,[12−15] we have carried out cloning, expression, and inhibition of CYP51 from *P. digitatum*. The structural characteristics of the interaction between heterologous CYP51 and commercial azoles were also analyzed by binding assay. Here we report an efficient synthesis of new structures of 2-azolyl-3,4-dihydroquinazolines by direct cyclization of imidazole or 1,2,4-triazole with carbodiimides, which were obtained from the aza-Wittig reaction of iminophosphorane with isocyanate. Their fungicidal activity against *P. digitatum* and binding interactions with the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51) are also investigated.

MATERIALS AND METHODS

Unless otherwise noted, all materials were commercially available and were used directly without further purification. All solvents were redistilled before use. *P. digitatum* was provided through the courtesy of the Center for Bioassay, Central China Normal University. ^{1}H NMR spectra were recorded on a Mercury-Plus 400 or 600

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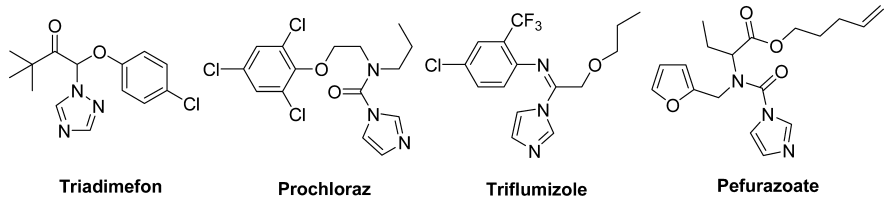
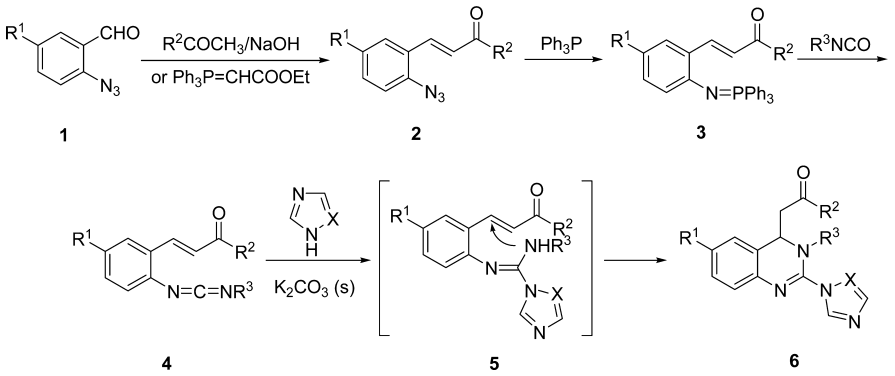


Figure 1. Some representative structures of agrocultural azole fungicides.

Scheme 1. General Synthetic Route for Compounds 6



spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal reference. MS spectra were determined using a Trace MS 2000 organic mass spectrometer. IR were recorded on a PE-983 infrared spectrometer as KBr pellets with absorption in cm^{-1}. Elemental analyses were performed on a Vario EL III elemental analysis instrument. Melting points were taken on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and are uncorrected. The azides **2** were prepared according to the reported method.[16,17] The general synthesis of target compounds is shown in Scheme 1, and the results are listed in Table 1.

General Procedure for Preparation of Iminophosphorane 3. To a stirred solution of azide **2** (3 mmol) in anhydrous methylene chloride (10 mL) was added dropwise triphenylphosphine (0.79 g, 3 mmol) in methylene chloride (10 mL) at room temperature. After the reaction mixture was stirred for 4 h at ambient temperature, the solvent was removed under reduced pressure, and the residual was recrystallized from methylene dichloride/petroleum ether to give iminophosphorane **3**.

Table 1. Preparation of Compounds 6

compd	X	R^1	R^2	R^3	yield[a] (%)
6a	CH	Cl	Me	i-Pr	85
6b	CH	H	Me	i-Pr	86
6c	CH	H	Me	n-Bu	80
6d	CH	H	Me	4-ClC_6H_4	72
6e	CH	H	Me	4-FC_6H_4	77
6f	CH	H	Me	Ph	73
6g	CH	H	OEt	Ph	77
6h	CH	H	OEt	4-FC_6H_4	83
6i	CH	Cl	Me	4-FC_6H_4	74
6j	N	Cl	Me	Ph	68
6k	N	Cl	OEt	4-ClC_6H_4	62
6l	N	Cl	OEt	4-FC_6H_4	75
6m	N	Cl	Me	i-Pr	87
6n	N	H	Me	Ph	86
6o	N	H	Me	3-MeC_6H_4	91
6p	N	H	Me	4-ClC_6H_4	69
6q	N	H	Me	4-FC_6H_4	62
6r	N	H	Me	i-Pr	84
6s	N	H	t-Bu	Ph	84
6t	N	H	t-Bu	3-MeC_6H_4	81

[a]Isolated yields based on iminophosphorane **3**.

*4-[2-(Triphenylphosphoranylideneamino)phenyl]but-3-en-2-one (**3a**):* yield, 94%; light yellow solid; mp, 119−121 °C; ^1H NMR (400 MHz, $CDCl_3$), δ 8.71 (d, J = 16.4 Hz, 1H, =CH), 7.78−7.45 (m, 16H, Ar—H), 6.90 (t, J = 8.0 Hz, 1H, Ar—H), 6.72−6.63 (m, 2H, Ar—H and =CH), 6.47 (d, J = 8.4 Hz, 1H, Ar—H), 2.43 (s, 3H, CH_3); MS (70 eV), m/z (%) 421 (M^+, 3), 378 (100), 277 (22), 143 (33), 108 (35). Anal. Calcd for $C_{28}H_{24}NOP$ (421.5): C, 79.79; H, 5.74; N, 3.32. Found: C, 79.58; H, 5.83; N, 3.51.

*4-[5-Chloro-2-(triphenylphosphoranylideneamino)phenyl]but-3-en-2-one (**3b**):* yield, 90%; light yellow solid; mp, 155−156 °C; ^1H NMR (600 MHz, $CDCl_3$), δ 8.56 (d, J = 16.8 Hz, 1H, =CH), 7.74−7.45 (m, 16H, Ar—H), 6.83 (d, J = 6.0 Hz, 1H, Ar—H), 6.66 (d, J = 16.8 Hz, 1H, =CH), 6.36 (d, J = 9.0 Hz, 1H, Ar—H), 2.41 (s, 3H, CH_3); MS (70 eV), m/z (%) 457 (M^+, 8), 455 (23), 412 (94), 207 (97), 183 (100), 177 (70), 115 (30), 73 (37). Anal. Calcd for $C_{28}H_{23}ClNOP$ (455.9): C, 73.76; H, 5.08; N, 3.07. Found: C, 73.51; H, 5.13; N, 3.21.

*1-[2-(Triphenylphosphoranylideneamino)phenyl]-4,4-dimethylpent-1-en-3-one (**3c**):* yield, 90%; light yellow solid; mp, 181−183 °C; ^1H NMR (400 MHz, $CDCl_3$), δ 8.69 (d, J = 16.0 Hz, 1H, =CH), 7.78−7.43 (m, 16H, Ar—H), 7.28 (d, J = 15.6 Hz, 1H, =CH), 6.85 (t, J = 8.4 Hz, 1H, Ar—H), 6.63 (t, J = 7.6 Hz, 1H, Ar—H), 6.45 (d, J = 8.4 Hz, 1H, Ar—H), 1.19 (s, 9H, $3CH_3$); MS (70 eV), m/z (%) 463 (M^+, 4), 378 (80), 277 (55), 210 (49), 183 (95), 170 (100), 77 (49), 40 (54). Anal. Calcd for $C_{31}H_{30}NOP$ (463.6): C, 80.32; H, 6.52; N, 3.02. Found: C, 80.58; H, 6.67; N, 3.08.

*Ethyl 3-[5-chloro-2-(triphenylphosphoranylideneamino)phenyl]-acrylate (**3d**):* yield, 85%; light yellow solid; mp, 171−172 °C; ^1H NMR (600 MHz, $CDCl_3$), δ 8.64 (d, J = 16.2 Hz, 1H, =CH), 7.75−7.43 (m, 16H, Ar—H), 6.79 (d, J = 8.4 Hz, 1H, Ar—H), 6.44 (d, J = 16.8 Hz, 1H, =CH), 6.35 (d, J = 8.4 Hz, 1H, Ar—H), 4.26 (q, J = 7.2 Hz, 2H, OCH_2), 1.36 (t, J = 7.2 Hz, 3H, CH_3); MS (70 eV), m/z (%) 485 (M^+, 3), 412 (66), 262 (25), 183 (100), 108 (57), 44 (53). Anal. Calcd for $C_{29}H_{25}ClNO_2P$ (485.9): C, 71.68; H, 5.19; N, 2.88. Found: C, 71.94; H, 5.01; N, 2.89.

General Procedure for Preparation of 2-Azolyl-3,4-dihydroquinazolines 6. To a solution of iminophosphorane **3** (2 mmol) in anhydrous methylene chloride (10 mL) was added isocyanate R^3NCO (2 mmol). After the reaction mixture was stirred at room temperature

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for 2−4 h (R^3 is an aryl group) or refluxed for 8−12 h (R^3 is an alkyl group), the solvent was removed under reduced pressure and ether/petroleum ether (1:2, 20 mL) was added to precipitate triphenylphosphine oxide. Filtered, the solvent was removed to give carbodiimide **4**, which was used directly without further purification. To the solution of **4** prepared above in CH_3CN (10 mL) was added imidazole (0.14 g, 2 mmol) and catalytic solid K_2CO_3 (0.03 g, 0.2 mmol). The mixture was stirred for 1−2 h at room temperature and filtered, the filtrate was condensed, and the residual was recrystallized from methylene dichloride/petroleum ether to give 2-azolyl-3,4-dihydroquinazolines **6**.

*1-(6-Chloro-3,4-dihydro-2-(1H-imidazol-1-yl)-3-isopropylquinazolin-4-yl)propan-2-one (**6a**):* white solid; mp, 97−99 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 8.04 (s, 1H, imidazolyl-2-H), 7.37 (s, 1H, Ar−H), 7.28−7.17 (m, 4H, Ar−H), 5.07 (dd, J_1 = 5.4 Hz, J_2 = 7.8 Hz, 1H, CH), 3.78−3.74 (m, 1H, NCH), 2.82 (dd, J_1 = 8.4 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.64 (dd, J_1 = 5.4 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.08 (s, 3H, CH_3), 1.34 (d, J = 6.0 Hz, 3H, CH_3), 0.97 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 204.9, 146.7, 139.9, 136.6, 131.0, 130.4, 128.9, 128.6, 125.3, 124.9, 117.7, 52.1, 49.5, 48.0, 31.2, 22.1, 20.9; MS (70 eV), m/z (%) 330 (M^+, 6), 273 (46), 231 (100), 177 (23), 163 (16), 68 (34), 43 (50). Anal. Calcd for $C_{17}H_{19}ClN_4O$ (330.8): C, 61.72; H, 5.79; N, 16.94. Found: C, 61.58; H, 5.68; N, 16.96.

*1-(3,4-Dihydro-2-(1H-imidazol-1-yl)-3-isopropylquinazolin-4-yl)-propan-2-one (**6b**):* white solid; mp, 61−63 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.07 (s, 1H, imidazolyl-2-H), 7.40−7.16 (m, 6H, Ar−H), 5.11 (dd, J_1 = 5.2 Hz, J_2 = 7.6 Hz, 1H, CH), 3.78−3.72 (m, 1H, NCH), 2.82 (dd, J_1 = 8.0 Hz, J_2 = 16.4 Hz, 1H, CHCO), 2.65 (dd, J_1 = 5.6 Hz, J_2 = 16.4 Hz, 1H, CHCO), 2.03 (s, 3H, CH_3), 1.36 (d, J = 6.8 Hz, 3H, CH_3), 0.97 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.3, 146.3, 141.0, 136.5, 130.0, 128.4, 127.3, 126.0, 124.7, 123.9, 117.7, 51.7, 49.4, 48.4, 31.2, 21.9, 20.7; MS (70 eV), m/z (%) 296 (M^+, 3), 239 (49), 197 (100), 143 (22), 102 (15), 68 (24), 40 (35). Anal. Calcd for $C_{17}H_{20}N_4O$ (296.4): C, 68.89; H, 6.80; N, 18.90. Found: C, 68.54; H, 6.92; N, 18.78.

*1-(3-Butyl-3,4-dihydro-2-(1H-imidazol-1-yl)quinazolin-4-yl)-propan-2-one (**6c**):* light yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$), δ 7.98 (s, 1H, imidazolyl-2-H), 7.31−7.10 (m, 6H, Ar−H), 5.03−4.99 (m, 1H, CH), 3.41−3.37 (m, 1H, NCH), 2.96 (dd, J_1 = 8.4 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.65 (dd, J_1 = 4.2 Hz, J_2 = 12.6 Hz, 1H, CHCO), 2.11 (s, 3H, CH_3), 1.42−1.01 (m, 4H, CH_2CH_2), 0.69 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.7, 145.7, 141.3, 136.6, 129.7, 128.2, 125.9, 125.7, 124.1, 123.7, 117.8, 54.2, 51.5, 48.8, 31.4, 30.7, 19.1, 13.1; MS (70 eV), m/z (%) 310 (M^+, 3), 253 (78), 197 (100), 170 (17), 143 (62), 129 (31), 68 (54). Anal. Calcd for $C_{18}H_{22}N_4O$ (310.4): C, 69.65; H, 7.14; N, 18.05. Found: C, 69.37; H, 7.38; N, 18.21.

*1-(3-(4-Chlorophenyl)-3,4-dihydro-2-(1H-imidazol-1-yl)-quinazolin-4-yl)propan-2-one (**6d**):* white solid; mp, 111−113 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 7.94 (s, 1H, imidazolyl-2-H), 7.42−6.99 (m, 10H, Ar−H), 5.34 (t, J = 7.2 Hz, 1H, CH), 3.10 (dd, J_1 = 7.8 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.76 (dd, J_1 = 5.4 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.15 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 205.6, 143.0, 142.0, 140.2, 136.8, 131.2, 130.2, 129.7, 128.9, 126.7, 126.3, 125.1, 124.5, 123.8, 117.6, 59.1, 48.3, 31.4; MS (70 eV), m/z (%) 364 (M^+, 3), 307 (100), 210 (68), 153 (30), 143 (59), 128 (24), 111 (37), 68 (43). Anal. Calcd for $C_{20}H_{17}ClN_4O$ (364.8): C, 65.84; H, 4.70; N, 15.36. Found: C, 65.89; H, 4.92; N, 15.15.

*1-(3-(4-Fluorophenyl)-3,4-dihydro-2-(1H-imidazol-1-yl)-quinazolin-4-yl)propan-2-one (**6e**):* white solid; mp, 139−141 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 7.94 (s, 1H, imidazolyl-2-H), 7.42−6.91 (m, 10H, Ar−H), 5.31 (t, J = 4.8 Hz, 1H, CH), 3.09 (dd, J_1 = 9.0 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.76 (dd, J_1 = 5.4 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.15 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 205.6, 161.0, 159.4, 143.4, 140.3, 139.5, 136.7, 131.9, 130.0, 128.8, 128.3, 126.6, 126.1, 125.1, 124.5, 117.6, 116.5, 59.4, 48.4, 31.3; MS (70 eV), m/z (%) 348 (M^+, 5), 291 (100), 210 (41), 143 (31), 109 (16), 95 (29), 68 (27). Anal. Calcd for $C_{20}H_{17}FN_4O$ (348.4): C, 68.95; H, 4.92; N, 16.08. Found: C, 68.71; H, 4.90; N, 16.31.

*1-(3-Phenyl-3,4-dihydro-2-(1H-imidazol-1-yl)quinazolin-4-yl)-propan-2-one (**6f**):* white solid; mp, 158−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95 (s, 1H, imidazolyl-2-H), 7.42−6.97 (m, 11H, Ar−H), 5.41 (t, J = 7.2 Hz, 1H, CH), 3.10 (dd, J_1 = 7.2 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.84 (dd, J_1 = 6.4 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.15 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 205.5, 143.4, 140.4, 136.9, 129.9, 129.6, 128.7, 126.5, 126.4, 125.7, 125.2, 124.4, 122.5, 117.7, 59.1, 48.6, 31.3; MS (70 eV), m/z (%) 330 (M^+, 3), 273 (100), 210 (36), 143 (21), 91 (16), 77 (59), 68 (23). Anal. Calcd for $C_{20}H_{18}N_4O$ (330.4): C, 72.71; H, 5.49; N, 16.96. Found: C, 72.79; H, 5.21; N, 16.83.

*Ethyl 2-(3,4-dihydro-2-(1H-imidazol-1-yl)-3-phenylquinazolin-4-yl)acetate (**6g**):* white solid; mp, 44−46 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 7.96 (s, 1H, imidazolyl-2-H), 7.43−6.97 (m, 11H, Ar−H), 5.32 (dd, J_1 = 5.4 Hz, J_2 = 9.0 Hz, 1H, CH), 4.23 (q, J = 7.2 Hz, 2H, OCH_2), 2.88 (dd, J_1 = 9.6 Hz, J_2 = 15.0 Hz, 1H, CHCO), 2.65 (dd, J_1 = 5.4 Hz, J_2 = 15.0 Hz, 1H, CHCO), 1.26 (t, J = 7.2 Hz, 3H, CH_3).; ^{13}C NMR (100 MHz, $CDCl_3$), δ 170.3, 143.3, 143.0, 140.3, 136.9, 129.7, 129.6, 128.8, 126.4, 125.9, 125.6, 125.0, 124.5, 122.3, 117.7, 61.1, 60.6, 40.6, 14.0; MS (70 eV), m/z (%) 360 (M^+, 35), 272 (100), 218 (25), 128 (14), 77 (66), 51 (14). Anal. Calcd for $C_{21}H_{20}N_4O_2$ (360.4): C, 69.98; H, 5.59; N, 15.55. Found: C, 69.81; H, 5.47; N, 15.72.

*Ethyl 2-(3-(4-fluorophenyl)-3,4-dihydro-2-(1H-imidazol-1-yl)-quinazolin-4-yl)acetate (**6h**):* white solid; mp, 39−41 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 7.94 (s, 1H, imidazolyl-2-H), 7.43−6.90 (m, 10H, Ar−H), 5.24 (dd, J_1 = 4.2 Hz, J_2 = 9.6 Hz, 1H, CH), 4.24 (q, J = 7.2 Hz, 2H, OCH_2), 2.86 (dd, J_1 = 9.6 Hz, J_2 = 15.0 Hz, 1H, CHCO), 2.62 (dd, J_1 = 4.8 Hz, J_2 = 15.6 Hz, 1H, CHCO), 1.28 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 170.5, 161.0, 159.3, 143.0, 140.1, 139.4, 136.9, 129.8, 128.9, 126.6, 125.6, 124.9, 124.5, 124.3, 117.7, 116.5, 116.4, 61.2, 60.9, 40.4, 14.0; MS (70 eV), m/z (%) 378 (M^+, 5), 236 (13), 217 (12), 134 (26), 108 (21), 94 (100), 75 (80). Anal. Calcd for $C_{21}H_{19}FN_4O_2$ (378.4): C, 66.66; H, 5.06; N, 14.81. Found: C, 66.69; H, 5.21; N, 14.73.

*1-(6-Chloro-3-(4-fluorophenyl)-3,4-dihydro-2-(1H-imidazol-1-yl)-quinazolin-4-yl)propan-2-one (**6i**).* white solid; mp, 146−147 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 7.93 (s, 1H, imidazolyl-2-H), 7.35−6.92 (m, 9H, Ar−H), 5.29−5.26 (m, 1H, CH), 3.12−3.08 (m, 1H, CHCO), 2.78−2.74 (m, 1H, CHCO), 2.18 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 205.1, 161.1, 159.4, 143.5, 139.3, 138.9, 136.8, 131.5, 130.1, 128.8, 127.5, 125.6, 125.1, 124.5, 117.5, 116.6, 116.4, 58.8, 48.1, 31.1; MS (70 eV), m/z (%) 382 (M^+, 5), 325 (100), 244 (33), 122 (13), 95 (27), 75 (9). Anal. Calcd for $C_{20}H_{16}ClFN_4O$ (382.8): C, 62.75; H, 4.21; N, 14.64. Found: C, 62.74; H, 4.13; N, 14.83.

*1-(6-Chloro-3,4-dihydro-3-phenyl-2-(1H-1,2,4-triazol-1-yl)-quinazolin-4-yl)propan-2-one (**6j**):* white solid; mp, 152−154 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 8.79 (s, 1H, triazolyl-5-H), 7.86 (s, 1H, triazolyl-3-H), 7.38−7.13 (m, 6H, Ar−H), 6.94 (d, J = 7.2 Hz, 2H, Ar−H), 5.45 (t, J = 6.6 Hz, 1H, CH), 3.17 (dd, J_1 = 6.0 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.95 (dd, J_1 = 7.2 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.19 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.2, 152.8, 144.6, 143.1, 142.8, 138.7, 132.0, 129.5, 128.9, 128.1, 126.0, 125.8, 125.6, 122.6, 58.6, 48.9, 31.4; MS (70 eV), m/z (%) 365 (M^+, 6), 322 (45), 308 (100), 281 (23), 245 (21), 218 (18), 163 (21), 77 (70). Anal. Calcd for $C_{19}H_{16}ClN_5O$ (365.8): C, 62.38; H, 4.41; N, 19.14. Found: C, 62.40; H, 4.63; N, 19.02.

*Ethyl 2-(6-chloro-3-(4-chlorophenyl)-3,4-dihydro-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)acetate (**6k**):* white solid; mp, 152−153 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 8.76 (s, 1H, triazolyl-5-H), 7.89 (s, 1H, triazolyl-3-H), 7.41−7.14 (m, 5H, Ar−H), 6.95 (d, J = 8.4 Hz, 2H, Ar−H), 5.29 (t, J = 7.2 Hz, 1H, CH), 4.24−4.21 (m, 2H, OCH_2), 2.93 (dd, J_1 = 7.8 Hz, J_2 = 15.6 Hz, 1H, CHCO), 2.74 (dd, J_1 = 7.2 Hz, J_2 = 15.6 Hz, 1H, CHCO), 1.21 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 169.8, 152.9, 144.5, 142.4, 141.3, 138.4, 132.1, 131.6, 129.5, 129.1, 127.2, 126.0, 125.2, 123.9, 61.3, 60.0, 40.4, 14.0; MS (70 eV), m/z (%) 429 (M^+, 9), 342 (100), 315 (17), 253 (20), 163 (20), 111 (37), 74 (34). Anal. Calcd for $C_{20}H_{17}Cl_2N_5O_2$ (430.3): C, 55.83; H, 3.98; N, 16.28. Found: C, 55.95; H, 3.87; N, 16.56.

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Table 2. Fungicidal Activities and IC_{50} of Compounds 6 on *Penicillium digitatum*

					inhibition under different concentrations (%, μg/mL)						
compd	X	R^1	R^2	R^3	50	25	10	5	1	toxicological eq	IC_{50} (μg/mL)
6a	CH	Cl	Me	i-Pr	30		12	8	0	$y = 0.0924x - 0.1066$	128
6b	CH	H	Me	i-Pr	28		15	4	0	$y = 0.0954x - 0.1198$	126
6c	CH	H	Me	n-Bu	100	57	29	14	5	$y = 0.2325x - 0.2881$	15.58
6d	CH	H	Me	4-ClC_6H_4	100	100	35	14	0	$y = 0.3211x - 0.4289$	10.40
6e	CH	H	Me	4-FC_6H_4	100	100	86	52	9	$y = 0.3071x - 0.1498$	4.14
6f	CH	H	Me	Ph	100		61	29	8	$y = 0.2664x - 0.1024$	5.06
6g	CH	H	OEt	Ph	100		35	20	6	$y = 0.2964x - 0.3376$	10.94
6h	CH	H	OEt	4-FC_6H_4	61	48	23	14	0	$y = 0.156x - 0.178$	29.79
6i	CH	Cl	Me	4-FC_6H_4	100	100	64	21	9	$y = 0.3155x - 0.305$	7.12
6j	N	Cl	Me	Ph	8						>50
6k	N	Cl	OEt	4-ClC_6H_4	6						>50
6l	N	Cl	OEt	4-FC_6H_4	4						>50
6m	N	Cl	Me	i-Pr	17						>50
6n	N	H	Me	Ph	24		11	0	0	$y = 0.0844x - 0.1219$	129
6o	N	H	Me	3-MeC_6H_4	44		19	12	0	$y = 0.137x - 0.1562$	60.06
6p	N	H	Me	4-ClC_6H_4	10						>50
6q	N	H	Me	4-FC_6H_4	14						>50
6r	N	H	Me	i-Pr	10						>50
6s	N	H	t-Bu	Ph	35		15	8	0	$y = 0.1124x - 0.1351$	89.95
6t	N	H	t-Bu	3-MeC_6H_4	40		22	8	0	$y = 0.1337x - 0.1597$	67.08
triadimefon					100	100	60	27	0	$y = 0.3328x - 0.3642$	7.72

*Ethyl 2-(6-chloro-3-(4-fluorophenyl)-3,4-dihydro-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)acetate (**6l**):* white solid; mp, 128−130 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 8.74 (s, 1H, triazolyl-5-H), 7.88 (s, 1H, triazolyl-3-H), 7.41−6.91 (m, 7H, Ar−H), 5.26 (t, *J* = 7.2 Hz, 1H, CH), 4.24−4.21 (m, 2H, OCH_2), 2.93 (dd, J_1 = 7.8 Hz, J_2 = 15.0 Hz, 1H, CHCO), 2.74 (dd, J_1 = 6.6 Hz, J_2 = 15.6 Hz, 1H, CHCO), 1.26 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$), δ 169.8, 161.2, 159.6, 152.8, 144.5, 142.8, 138.9, 138.5, 132.0, 129.1, 127.0, 126.0, 125.2, 124.9, 116.4, 116.2, 61.3, 60.2, 40.5, 14.0; MS (70 eV), *m*/*z* (%) 413 (M^+, 11), 326 (100), 299 (18), 163 (15), 95 (43), 75 (17). Anal. Calcd for $C_{20}H_{17}ClFN_5O_2$ (413.8): C, 58.05; H, 4.14; N, 16.92. Found: C, 58.04; H, 4.33; N, 16.70.

*1-(6-Chloro-3,4-dihydro-3-isopropyl-2-(1H-1,2,4-triazol-1-yl)-quinazolin-4-yl)propan-2-one (**6m**):* white solid; mp, 128−130 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 8.87 (s, 1H, triazolyl-5-H), 8.10 (s, 1H, triazolyl-3-H), 7.28−7.18 (m, 3H, Ar−H), 5.17 (dd, J_1 = 4.2 Hz, J_2 = 9.0 Hz, 1H, CH), 3.95−3.90 (m, 1H, NCH), 2.93 (dd, J_1 = 9.6 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.69 (dd, J_1 = 4.2 Hz, J_2 = 17.4 Hz, 1H, CHCO), 2.07 (s, 3H, CH_3), 1.39 (d, *J* = 6.6 Hz, 3H, CH_3), 1.08 (d, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.3, 153.0, 145.8, 145.0, 140.0, 130.8, 128.8, 128.6, 125.1, 125.0, 51.6, 49.5, 48.0, 31.2, 21.9, 20.9; MS (70 eV), *m*/*z* (%) 331 (M^+, 4), 274 (28), 232 (100), 205 (23), 163 (16), 43 (25). Anal. Calcd for $C_{16}H_{18}ClN_5O$ (331.8): C, 57.92; H, 5.47; N, 21.11. Found: C, 57.93; H, 5.25; N, 21.38.

*1-(3,4-Dihydro-3-phenyl-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)-propan-2-one (**6n**):* white solid; mp, 165−166 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.81 (s, 1H, triazolyl-5-H), 7.87 (s, 1H, triazolyl-3-H), 7.47−7.09 (m, 7H, Ar−H), 6.96 (d, *J* = 8.0 Hz, 2H, Ar−H), 5.49 (t, *J* = 7.2 Hz, 1H, CH), 3.17 (dd, J_1 = 6.0 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.95 (dd, J_1 = 7.6 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.16 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.8, 152.7, 144.6 143.0, 142.9, 140.0, 129.4, 128.8, 126.9, 126.8, 125.8, 125.5, 124.6, 122.5, 59.1, 49.0, 31.6; MS (70 eV), *m*/*z* (%) 331 (M^+, 6), 288 (26), 274 (100), 253 (31), 247 (25), 219 (25), 197 (36), 143 (43), 129 (38), 77 (67). Anal. Calcd for $C_{19}H_{17}N_5O$ (331.4): C, 68.87; H, 5.17; N, 21.13. Found: C, 68.96; H, 5.02; N, 21.15.

*1-(3,4-Dihydro-3-m-tolyl-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)propan-2-one (**6o**):* white solid; mp, 163−164 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.79 (s, 1H, triazolyl-5-H), 7.88 (s, 1H, triazolyl-3-H), 7.46−7.06 (m, 5H, Ar−H), 6.91 (d, *J* = 8.0 Hz, 1H, Ar−H), 6.78 (s, 1H, Ar−H), 6.73 (d, *J* = 8.0 Hz, 1H, Ar−H), 5.46 (t, *J* = 7.2 Hz, 1H, CH), 3.15 (dd, J_1 = 6.0 Hz, J_2 = 15.2 Hz, 1H, CHCO), 2.96 (dd, J_1 = 7.6 Hz, J_2 = 15.6 Hz, 1H, CHCO), 2.24 (s, 3H, CH_3), 2.15 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.7, 152.7, 144.6, 143.0, 142.9, 140.0, 139.5, 129.2, 128.8, 126.8, 126.7, 125.6, 124.6, 123.0, 119.7, 59.1, 49.0, 31.6, 21.3; MS (70 eV), *m*/*z* (%) 345 (M^+, 6), 302 (28), 288 (100), 261 (18), 233 (13), 211 (27), 129 (18), 91 (31). Anal. Calcd for $C_{20}H_{19}N_5O$ (345.4): C, 69.55; H, 5.54; N, 20.28. Found: C, 69.78; H, 5.37; N, 20.26.

*1-(3,4-Dihydro-3-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-yl)-quinazolin-4-yl)propan-2-one (**6p**):* white solid; mp, 134−135 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.84 (s, 1H, triazolyl-5-H), 7.88 (s, 1H, triazolyl-3-H), 7.46−7.13 (m, 6H, Ar−H), 6.91 (d, *J* = 9.2 Hz, 2H, Ar−H), 5.42 (t, *J* = 6.8 Hz, 1H, CH), 3.16 (dd, J_1 = 6.4 Hz, J_2 = 17.2 Hz, 1H, CHCO), 2.89 (dd, J_1 = 7.2 Hz, J_2 = 17.2 Hz, 1H, CHCO), 2.17 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.6, 152.8, 144.4, 142.5, 141.5, 139.7, 131.2, 129.4, 128.8, 127.0, 126.6, 125.3, 124.6, 123.8, 59.1, 48.6, 31.5; MS (70 eV), *m*/*z* (%) 365 (M^+, 5), 322 (28), 308 (100), 281 (33), 253 (51), 197 (62), 143 (98), 129 (59), 90 (48), 68 (52). Anal. Calcd for $C_{19}H_{16}ClN_5O$ (365.8): C, 62.38; H, 4.41; N, 19.14. Found: C, 62.21; H, 4.20; N, 19.37.

*1-(3,4-Dihydro-3-(4-fluorophenyl)-2-(1H-1,2,4-triazol-1-yl)-quinazolin-4-yl)propan-2-one (**6q**):* white solid; mp, 127−129 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.81 (s, 1H, triazolyl-5-H), 7.87 (s, 1H, triazolyl-3-H), 7.46−6.89 (m, 6H, Ar−H), 6.91 (d, *J* = 9.2 Hz, 2H, Ar−H), 5.42 (t, *J* = 6.8 Hz, 1H, CH), 3.16 (dd, J_1 = 6.4 Hz, J_2 = 17.2 Hz, 1H, CHCO), 2.89 (dd, J_1 = 7.2 Hz, J_2 = 17.2 Hz, 1H, CHCO), 2.17 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 205.8, 161.3, 159.6, 152.9, 144.5, 143.0, 140.0, 139.2, 128.9, 127.0, 126.6, 125.4, 124.9, 124.7, 116.3, 116.2, 59.5, 49.0, 31.6; MS (70 eV), *m*/*z* (%) 349 (M^+, 7), 306 (36), 293 (22), 292 (100), 265 (33), 211 (28), 129 (22), 95 (37). Anal. Calcd for $C_{19}H_{16}FN_5O$ (349.4): C, 65.32; H, 4.62; N, 20.05. Found: C, 65.48; H, 4.84; N, 19.81.

*1-(3,4-Dihydro-3-isopropyl-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)propan-2-one (**6r**).* white solid; mp, 87−89 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ 8.89 (s, 1H, triazolyl-5-H), 8.10 (s, 1H, triazolyl-3-H), 7.31−7.15 (m, 4H, Ar−H), 5.20 (t, *J* = 4.8 Hz, 1H, CH), 3.91−3.88 (m, 1H, NCH), 2.94 (dd, J_1 = 9.0 Hz, J_2 = 16.2 Hz, 1H, CHCO), 2.68 (dd, J_1 = 3.6 Hz, J_2 = 16.8 Hz, 1H, CHCO), 2.03 (s, 3H, CH_3), 1.41 (d, *J* = 6.6 Hz, 3H, CH_3), 1.07 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C

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NMR (150 MHz, $CDCl_3$), δ 205.7, 152.8, 145.4, 144.8, 140.1, 128.3, 127.2, 125.8, 125.0, 123.6, 51.2, 49.3, 48.3, 31.4, 21.7, 20.8; MS (70 eV), m/z (%) 297 (M^+, 3), 240 (35), 198 (100), 171 (26), 129 (18). Anal. Calcd for $C_{16}H_{19}N_5O$ (297.4): C, 64.63; H, 6.44; N, 23.55. Found: C, 64.37; H, 6.68; N, 23.45.

*1-(3,4-Dihydro-3-phenyl-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)-3,3-dimethylbutan-2-one (**6s**).* white solid; mp, 146−147 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.79 (s, 1H, triazolyl-5-H), 7.86 (s, 1H, triazolyl-3-H), 7.46−7.01 (m, 7H, Ar−H), 6.99 (d, J = 7.6 Hz, 1H, Ar−H), 5.56 (t, J = 7.6 Hz, 1H, Ar−H), 3.08 (d, J = 7.6 Hz, 2H, CH_2CO), 0.99 (s, 9H, $3CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$), δ 212.1, 152.6, 144.4, 143.0, 142.8, 139.8, 129.3, 128.6, 126.7, 126.6, 125.6, 125.4, 124.4, 122.4, 59.3, 44.4, 42.2, 25.3.; MS (70 eV), m/z (%) 373 (M^+, 3), 288 (70), 274 (100), 247 (19), 219 (15), 129 (14), 77 (26). Anal. Calcd for $C_{22}H_{23}N_5O$ (373.5): C, 70.76; H, 6.21; N, 18.75. Found: C, 70.94; H, 6.14; N, 18.84.

*1-(3,4-Dihydro-3-m-tolyl-2-(1H-1,2,4-triazol-1-yl)quinazolin-4-yl)-3,3-dimethylbutan-2-one (**6t**):* white solid; mp, 185−187 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.77 (s, 1H, triazolyl-5-H), 7.88 (s, 1H, triazolyl-3-H), 7.46−6.76 (m, 8H, Ar−H), 5.54 (t, J = 7.0 Hz, 1H, CH), 3.08 (dd, J_1 = 8.4 Hz, J_2 = 13.6 Hz, 1H, CHCO), 2.24 (s, 3H, CH_3), 0.96 (s, 9H, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$), δ 212.2, 152.6, 144.5, 143.1, 140.0, 139.5, 129.1, 128.7, 126.8, 126.6, 125.6, 124.5, 123.0, 119.7, 59.5, 44.5, 42.3, 25.4, 21.3; MS (70 eV), m/z (%) 387 (M^+, 3), 302 (63), 288 (100), 261 (17), 171 (13), 129 (14), 91 (19). Anal. Calcd for $C_{23}H_{25}N_5O$ (387.5): C, 71.29; H, 6.50; N, 18.07. Found: C, 71.17; H, 6.51; N, 18.31.

Fungicidal Activity Determination (Growth Inhibition Test). The in vitro fungicidal activities against *P. digitatum* were tested according to the reported method.[18,19] The medium was amended with aliquots of each tested compound solution to provide a concentration of 50 mg/L. The tested compounds were dissolved in 0.3 mL of dimethyl sulfoxide (DMSO) and added aseptically to molten agar after autoclaving, when the agar had cooled to approximately 45−50 °C. The concentration of solvent never exceeded 0.1 mg/L. The mixed medium without sample was used as the blank control. The inocula, 5 mm in diameter, were removed from the margins of actively growing colonies of mycelium, placed in the centers of the above plates. Three replicates were done for each concentration, and the control plates were sealed with parafilm and incubated at 26 °C in darkness. The diameter of the mycelium was measured after 48 h. The inhibition percent was used to describe the control efficiency of the compounds. Inhibition percent (%) = (hyphal diameter in the control − hyphal diameter in the treatment)/hyphal diameter in the control. The results are summarized in Table 2.

Preparation of the Heterogeneous Expressed CYP51 Protein of *P. digitatum*. Cloning and expression of PdCYP51 were carried out by using reverse transcription PCR (RT-PCR) with the purified mRNA as a template isolated from *P. digitatum*.[12] The amplified PdCYP51 cDNA fragment was cloned into pMD-T vector for sequencing and then subcloned into pET-28 for expression. The recombinant plasmid pET-PdCYP51 was transformed into *Escherichia coli* BL21 (DE3), and the positive colonies were selected. The expression of recombinant protein in *E. coli* BL21 (DE3) was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 6 h at 25 °C before harvesting at 5000*g* for 10 min. After harvesting, cells were washed twice with 100 mM phosphate-buffered saline (PBS) and suspended in buffer A (50 mM KH_2PO_4, 50 mM K_2HPO_4, 20% glycerol, 1 mM EDTA, 0.5% Triton X-100) with 1 mM dithiothreitol (DTT) and 1 mM PMSF. The cells were lysed by sonication on ice, in a UP200S cell disruptor at an intensity of 60 W for 5 min, with a 30 s rest period between each burst. The lysate was centrifuged at 15000*g* (30 min, 4 °C), and the supernatant containing soluble cellular material was purified using the Ni-NTA affinity resin according to the manufacturer's protocol with certain modifications.[20] All of the buffers were adjusted to pH 7.2. The purified protein was dialyzed into 10 mM PBS (pH 7.2) to desalt, concentrated, and stored at −80 °C until use.[21]

Determination of Protein Content. The protein concentration was determined according to the Bradford method using bovine serum albumin (BSA) as the protein standard.[22]

Determination of CYP51 Activity and Binding Spectrum Analysis. According to the methods described in the literature,[23] the CYP51 content and the activity were determined. The solutions of CYP51 were prepared with 100 mM PBS (pH 7.2), and UV−visible absorption spectra were recorded for the oxidized species on a UV-240 IPC spectrophotometer at 25 °C. CYP51 solutions were reduced using a small amount of sodium dithionite and bubbled briefly with carbon monoxide to form the P450−CO complex and measure the precise P450 concentration at 25 °C.[24] The purified soluble PdCYP51 was diluted to appropriate concentration and filled into a cuvette. After its baseline was scanned at 350−500 nm on an S-3100 spectrophotometer, the drug was added to the cuvette. After staying for 1 min under room temperature, the absorption spectrum of the mixture was recorded (concentration of DMSO in the mixture was maintained at <0.5%). The binding constant K_d was calculated by using the following equation and Hanes−Woolf[25] chart [I] versus [I]/ΔA:

$$A = A_{max}[I]/(K_d + [I])$$

In the above equation, A is the value of the maximum, subtracting the minimum observed shift in absorption at different drug concentrations; A_{max} is the value of the maximum, subtracting the minimum observed shift in absorption at saturation; [I] is the drug concentration; and K_d is the binding constant.

RESULTS AND DISCUSSION

Synthesis of Compounds 6. Many 1-substituted imidazole and 1,2,4-triazole compounds are generally prepared by N-alkylations of imidazole and 1,2,4-triazole with alkylating reagents; however, many of these reactions are limited by the simultaneous formation of the unwanted and biologically inactive 2-isomer of imidazoles or 4-isomer of 1,2,4-triazoles.[26] It was reported that 1,2,4-triazole derivatives can be regiospecifically synthesized by addition reactions of 1,2,4-triazole with aldehydes or carbodiimide, and this method was successfully utilized to prepare many novel triazole derivatives.[27−29] The aza-Wittig reaction of iminophosphorane provides an efficient way to synthesize various heterocycles.[30−33] It has been reported that quinazolines may be prepared efficiently by tandem aza-Wittig reaction of a suitable iminophosphorane, isocyanate, and various nucleophiles.[17] Cyclization was achieved by initial nucleophilic addition of various nucleophiles to the carbodiimide intermediate and further intramolecular Michael addition. We envisioned that 2-azolyl-3,4-dihydroquinazolines would be prepared directly if imidazole and 1,2,4-triazole were used as the nucleophile.

As shown in Scheme 1, iminophosphoranes **3**, obtained from Staudinger reaction of azides **2** with triphenylphosphine, reacted with isocyanates to give the carbodiimides **4** in anhydrous methylene chloride at room temperature or under reflux. Then carbodiimides **4** were allowed to react with imidazole or 1*H*-1,2,4-triazole to provide 2-azolyl-3,4-dihydroquinazolines **6** in the presence of a catalytic amount of solid potassium carbonate in 62−91% yields (Table 1). It is noteworthy that the reaction proceeds under mild conditions to give 2-azolyl-3,4-dihydroquinazolines **6**, and the overall transformation is realized in a simple one-pot procedure from iminophosphoranes **3** in good overall yields. The formation of **6** can be viewed as an initial nucleophilic addition of imidazole or 1*H*-1,2,4-triazole under potassium carbonate to give the intermediate **5**, which directly cyclized to give **6** through intramolecular Michael addition.

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Bioassay Activity of Compounds 6. The fungicidal activities of compounds **6** were evaluated in vitro using agar diffusion and broth dilution assay, the results of which are presented in Table 2. Some of the compounds showed significant activity in the initial screening against *P. digitatum* cultures when tested at 50 μg/mL concentration using agar diffusion assay. For example, compounds **6c**, **6d**, **6e**, **6f**, **6g**, and **6i** all exhibited 100% inhibitive activity. At lower (25 μg/mL) concentration, compounds **6d**, **6e**, and **6i** still showed 100% inhibitive activity against *P. digitatum*. The fungicidal activities of some compounds **6** were further determined at lower concentrations (10, 5, and 1 μg/mL). The toxicological equation and IC_{50} of the compounds were then obtained, and the results are listed in Table 2.

As indicated in Table 2, the structure−activity relationships clearly suggest that 2-triazolyl-substituted 3,4-dihydroquinazolines **6** (compounds **6j−6t**) show low fungicidal activity against *P. digitatum* with 4−44% inhibition at 50 μg/mL and all with IC_{50} > 50 μg/mL, whereas most of the 2-imidazolyl-substituted 3,4-dihydroquinazolines **6** (compounds **6c−6i**) exhibited good fungicidal activity except of compounds **6a** and **6b**.

The fungicidal activity of imidazoles **6a−6i** is obviously related to R^3 substituents. When R^3 is an aromatic substituent, good to excellent activity was reached, and the best three compounds (**6e**, **6f**, and **6i**) have the smallest IC_{50} values (4.14, 5.06, and 7.12 μg/mL). The presence of a group such as 4-F (**6e** and **6i**) substitution on the phenyl ring also plays a significant role in imparting antifungal activity to the compounds. As R^3 is an alkyl substituent, good activity was obtained when R^3 is a butyl group (**6c**), whereas low activities resulted in the case that R^3 is an isopropyl group (**6a** and **6b**). When R^2 is a methyl group, better activity is generally obtained than when R is a methoxy group. For example, compounds **6e** and **6f** (R^2 = Me) displayed better fungicidal activities than compounds **6g** and **6h** (R^2 = OEt) did.

Triadimefon, a broad-spectrum fungicide, was used as a reference to study the activity of the potential compounds **6**. As indicated in Table 2, compound **6i** showed in vitro activity (IC_{50} = 7.12 μg/mL) as compared to triadimefon (IC_{50} = 7.72 μg/mL). Two compoundst **6e** and **6f** had comparably high inhibitory activity (**6e**, IC_{50} = 4.14 μg/mL; **6f**, IC_{50} = 5.06 μg/mL) as compared to triadimefon against *P. digitatum*.

Binding Spectrum Analysis of Compounds 6 with PdCYP51. The binding interactions of some 2-imidazolyl-3,4-dihydroquinazolines **6c**, **6d**, **6e**, **6h** and **6i** with PdCYP51 were investigated with the commercial fungicide triadimefon as standard. UV−visible absorption spectroscopy provided a simple and accurate method for the determination of the level of binding of substrates and inhibitors to P450s. Similar to our previous results, compounds **6c**, **6d**, **6e** and **6i** induced type II binding spectra with a maximum absorbance at 415−420 nm and a minimum at 390−400 nm (Figures 2 and 3), indicating a shift in the heme iron of the cytochrome P450 to a low-spin state on inhibitor binding. However, compound **6h** induced type I binding spectra with a maximum absorbance at 400 nm and a minimum at 420 nm (Figure 4), which might be due to the special influence of the ester group of compound **6h**. The magnitude of the spectral response increased steadily with increasing fungicide concentration and attained saturation when an equilibrium concentration of drug was added to the PdCYP51 fractions. These spectra most likely resulted from an interaction of the imidazole N-3 of **6** with the heme of the P450 causing a shift toward the high-spin state. This exhibited the

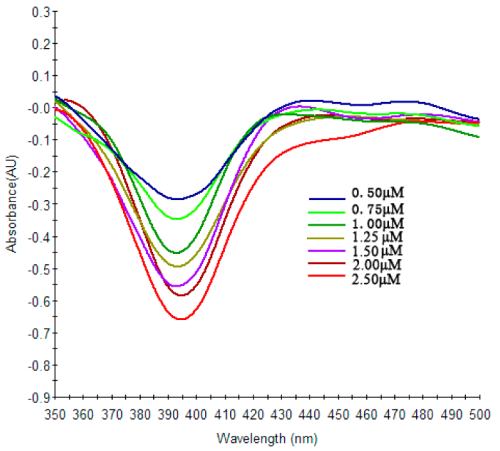

Figure 2. CYP51 type II binding spectrum in the presence of compound **6e**. Type II spectral response to **6e** and the concentrations of the **6e** added to the reaction mixture were 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, and 2.50 μg/mL.

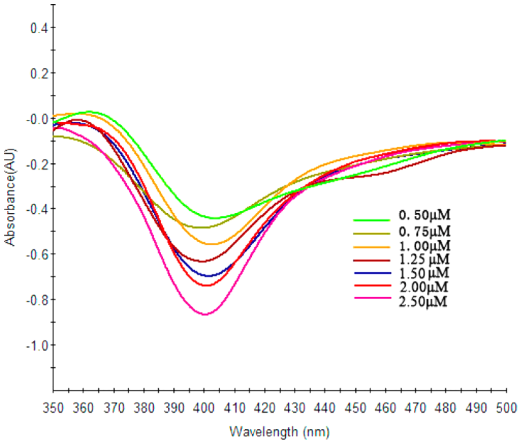

Figure 3. CYP51 type II binding spectrum in the presence of compound **6i**. Type II spectral response to **6i** and the concentrations of the **6i** added to the reaction mixture were 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, and 2.50 μg/mL.

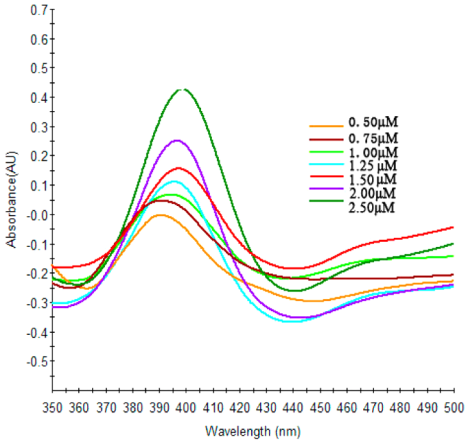

Figure 4. CYP51 type I binding spectrum in the presence of compound **6h**. Type I spectral response to **6h** and the concentrations of **6h** added to the reaction mixture were 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, and 2.50 μg/mL.

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displacement of the native sixth ligand of the heme iron by the nitrogen atom in the imidazole ring of **6**.

The spectral results showed that the affinity of most of the compounds **6** with the purified PdCYP51 was tight. The K_d values of compounds **6c**, **6d**, **6e**, **6h**, and **6i** were calculated to be 4.13, 1.06, 0.34, 1.79, and 0.92 μg/mL, respectively (Table 3). The five imidazoles **6** exhibited a high and gradually

Table 3. In Vitro Binding Constants (K_d) and IC_{50} of Some Compounds 6

	compd					
	6c	**6d**	**6e**	**6h**	**6i**	triadimefon
K_d (μM)	4.13	1.06	0.34	1.79	0.92	0.42
IC_{50} (μg/mL)	15.58	10.40	4.14	29.79	7.12	7.72

weakening affinity for PdCYP51 in the order **6e** > **6i** > **6d** > **6h** > **6c**. Although compounds **6c**, **6d**, **6h**, and **6i** displayed lower binding activities than triadimefon, compound **6e** (K_d = 0.34 μg/mL) did exhibit stronger binding activities than triadimefon (K_d = 0.42 μg/mL). This is consistent with the fact that compound **6e** displays a better fungicidal activity (IC_{50} = 4.14 μg/mL) against *P. digitatum* than triadimefon does (IC_{50} = 7.72 μg/mL).

Docking Model of Compound 6e with PdCYP51. On the basis of our previous homology modeling of PdCYP51,[12] compound **6e** was docked in the active site with the N3 of the imidazole ring forming a coordinate bond with the Fe of heme (Figure 5). In the binding model, the distance between the N

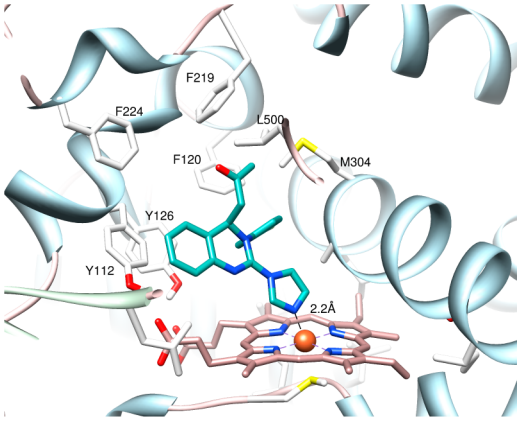

Figure 5. Docking model of compound **6e** with PdCYP51.

atom of compound **6e** and the heme atom was 2.2 Å; there should therefore be interactions between them. The 4-fluorophenyl ring of compound **6e** shows hydrophobic interactions with F120. The 2-oxopropyl group interacts hydrophobically with F219, M304, and L500. The phenyl ring of the quinazoline has only weak hydrophobic interactions with Y112.

In conclusion, a novel series of 2-azolyl-3,4-dihydroquinazolines have been designed and synthesized by tandem aza-Wittig reaction of iminophosphorane, isocyanate, and imidazole or 1,2,4-triazole. The preliminary investigation on the biological activities of compounds **6** shows that some of the 2-imidazolyl-3,4-dihydroquinazolines exhibited significant activity in the initial screening against *P. digitatum*. UV−visible absorption spectroscopy of some compounds **6** on PdCYP51 shows strong binding interactions with the cytochrome P450-dependent lanosterol 14α-demethylase. We are currently looking further into the biological activities of this type of compound on other plant pathogenic fungi.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR for intermediates **3a−3d** and ^{1}H NMR and ^{13}C NMR spectra for compounds **6a−6t**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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