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A facile synthesis and fungicidal activities of novel fluorine-containing pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones

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Abstract

Sixteen novel 2-substituted-5,8,9-trimethyl-3-(4-fluoro-substituted)phenyl-thieno[3′,2′-5,6] pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones **5a–5p** were designed and have been successfully synthesized *via* tandem aza-Wittig and annulation reactions of the corresponding iminophosphoranes **1**, *para*-fluoro phenyl isocyanate, and substituted phenols or amines in 73–90% isolated yields. Their structures were clearly verified by IR, ^{1}H NMR, EI-MS spectroscopy and elemental analysis, and in the case of compound **5a**, analyzed by single-crystal X-ray diffraction further. The results of preliminary bioassay indicated that some compounds possess inhibition activities against *Rhizoctonia solani* and *Botrytis cinereapers* at a dosage of 50 mg/L.

Keywords: Pyrido[4,3-*d*]pyrimidin-4(3*H*)-one; aza-Wittig reaction; Synthesis; Fungicidal activity

1. Introduction

The derivatives of pyridopyrimidines have been the focus of great interest over many years. This is due to the wide range of biological activities associated with this heterocyclic scaffold. For example, some related 4-(phenylamino) pyrido [4,3-*d*]pyrimidines have been reported as selective inhibitors of tyrosine phosphorylation by the epidermal growth-factor receptor (EGFR), and have become an important class of potential anticancer drugs [1,2]. However, few reports so far are available on the pesticidal activities of pyrido[4,3-*d*]pyrimidine derivatives.

It is well known that fluorine can affect the biological activity of compounds in a number of important ways because of the highest electronegativity, high thermal stability and lipophilicity. Therefore, fluorinated compounds in general and fluorinated heterocycles are the focus of many researches [3]. In the area of modern crop protection, fluoro agrochemicals are widely employed as herbicides, insecticides and fungicides [4]. Some examples also demonstrate that the incorporation of fluorine atoms or fluorinated substituents into certain compounds influences the herbicidal activity [5,6], fungicidal activity [7,8] and insecticidal activity [9–11].

The aza-Wittig reactions of iminophosphoranes have received increasing attention in view of their utility in the synthesis of *N*-heterocyclic compounds [12]. Recently, we have become interested in the synthesis of new bioactive heterocycles such as pyrazolopyrimidinones [13], thienopyrimidinones [14], and triazolopyrimidinones [15] from various iminophosphoranes, with the aim of evaluating their biological activities. Herein, we would like to describe a facile synthesis of 2-substituted-5,8,9-trimethyl-3-(4-fluoro-substituted)phenyl-thieno[3′,2′-5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones (**5**) *via* the tandem aza-Wittig and cyclization reaction. The preliminary bioassay showed that some of them have fungicidal activities.

2. Result and discussion

2.1. Synthesis

The 2-amino-3-cyano-4, 5-dimethylthieno [16] (**3**) was converted to thienopyridine derivative **2** *via* reaction with acetoacetic ester and tin tetrachloride under heating. The

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Table 1
Yields of compounds **5**

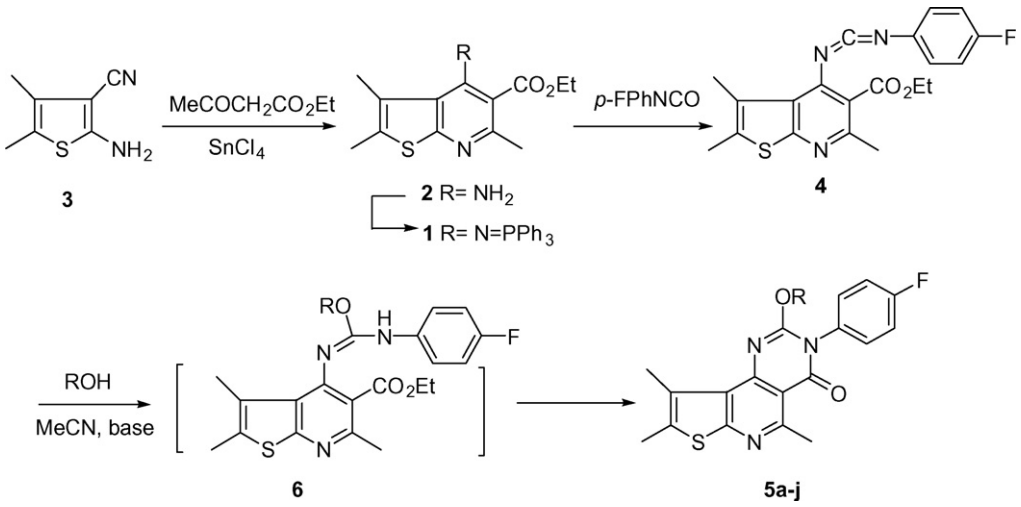
Compounds	RO or R^1R^2N	Yield (%)[a]
5a	4-MePhO	88
5b	1,2,3,4,5-Cl_5PhO	90
5c	2-NO_2PhO	82
5d	2-ClPhO	90
5e	3-MePhO	84
5f	2-MePhO	82
5g	PhO	84
5h	4-ClPhO	86
5i	2,4-Cl_2PhO	85
5j	2-Cl-4-FPhO	82
5k	NCH_2CH_3	83
5l	$NH(CH_2)_2CH_3$	83
5m	$NHCH(CH_3)_2$	83
5n	$NH(CH_2)_3CH_3$	73
5o	$NHC(CH_3)_3$	74
5p	$N(C_4H_9)_2$	79

[a] Yields of isolated products based on iminophosphorane **1**.

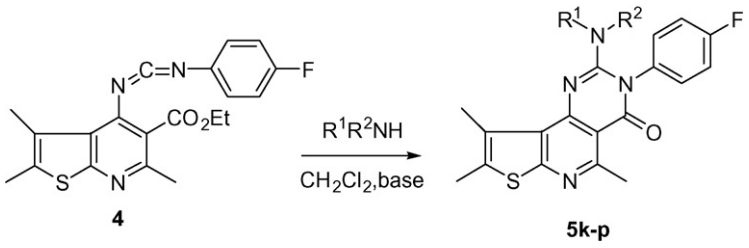
iminophosphorane **1** was subsequently obtained in a satisfactory yield when **2** was treated with triphenylphosphine, hexachloroethane and Et_3N. Iminophosphorane **1** reacted with *para*-fluorophenyl isocyanate to give carbodiimide **4**. The direct reaction of carbodiimide **4** with phenols did not produce 2-aryloxyl-thieno[3′,2′:5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**5**). However, the reaction took place to give **5a–j** in good yields under the condition of heating for 1–2 h in the presence of catalytic amount of K_2CO_3 (Table 1). The formation of **5** can be rationalized in terms of an initial nucleophilic addition of phenoxides to the carbodiimides **4** to give the intermediate **6** which cyclize to give **5a–j** (Scheme 1). Irrespective of the fact whether the substituents on the phenols were electronwithdrawing or electronreleasing groups, the cyclization was completed smoothly.

In refluxing toluene, **4** did not react with alkylamines to the target compounds. However, in CH_2Cl_2 and in the presence of a catalytic amount of EtONa, compounds **4** were converted smoothly into the 2-(alkylamino)-thieno[3′,2′:5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones **5k–p** in satisfactory yields at room temperature for 0.5–1 h whether primary or secondary amines were used (Scheme 2). Worth noting the reaction between carbodiimides **4** and primary amines gives mainly 2-(alkylamino)-thieno [3′,2′:5,6]pyrido[4,3-*d*] pyrimidin-4(3*H*)-ones (**5**) and another kind of cyclization compounds 2-(arylamino)-thieno[3′,2′:5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones (**7**) were not founded (Scheme 3), this might be due to the geometry of the guanidine intermediate and conjugative effect of compounds **5**. As the amines were reacted with **4**, intermediates **6a** were formed since the amines would attack **4** mainly from the opposite direction of CO_2Et group due to the steric hindrance of CO_2Et group. At the same time, the compounds **5** are more stable than compounds **7** because of the conjugative effect between the pyridopyrimidine ring and phenyl ring. Therefore, only compounds **5** were obtained regioselectively.

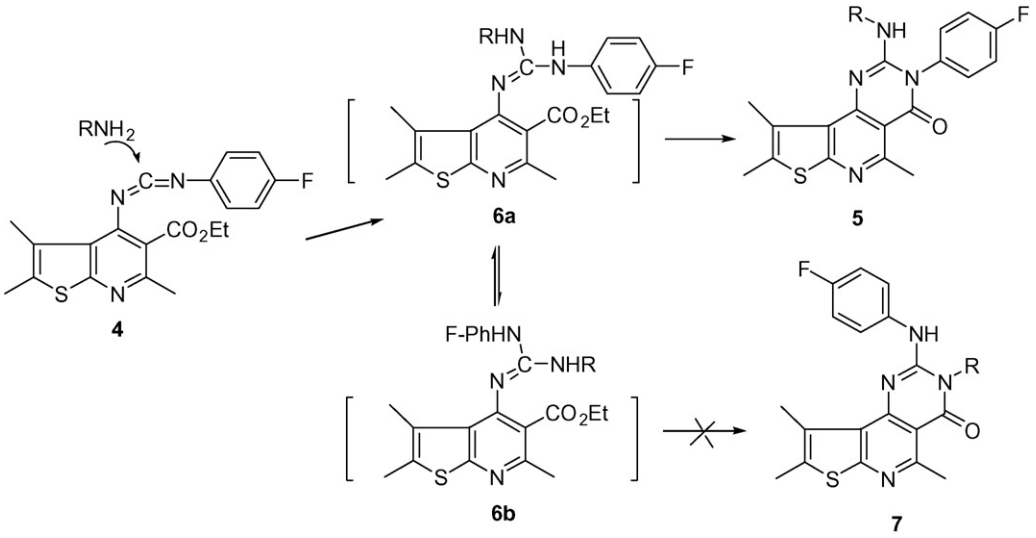
All products of type **5** were obtained as white solid after recrystallization from CH_2Cl_2/petroleum ether, and were



Scheme 1. Synthesis of the title compounds **5a–j**.



Scheme 2. Synthesis of the title compounds **5k–p**.



Scheme 3. Formation of compounds **5**.

characterized by IR, ^{1}H NMR and elemental analysis, and some of them were confirmed by EI-MS. For example, the ^{1}H NMR spectrum of 5 m showed the signal of NH at δ 4.37 as singlet, which was not the same as the proton of PhNH, of which the chemical shift is greater than δ 7.0 [17]. In IR spectrum, the relatively strong absorption of C=O appeared at 1674–1704 cm^{-1}. The MS spectrum displayed strong molecule ion peaks. All the fragmentation ions are consistent with their structures and can be clearly assigned. In the case of **5a**, the structure was additionally solved by single-crystal X-ray diffraction (Fig. 1). In the crystal structure, the C–S bond lengths are 1.730(2) Å and 1.744(2) Å, respectively, which are greater than those observed in free thiophene [1.714(2) Å] [18]. The C5–S1–C6 angle of 91.29(10)° in (I) is slightly less than

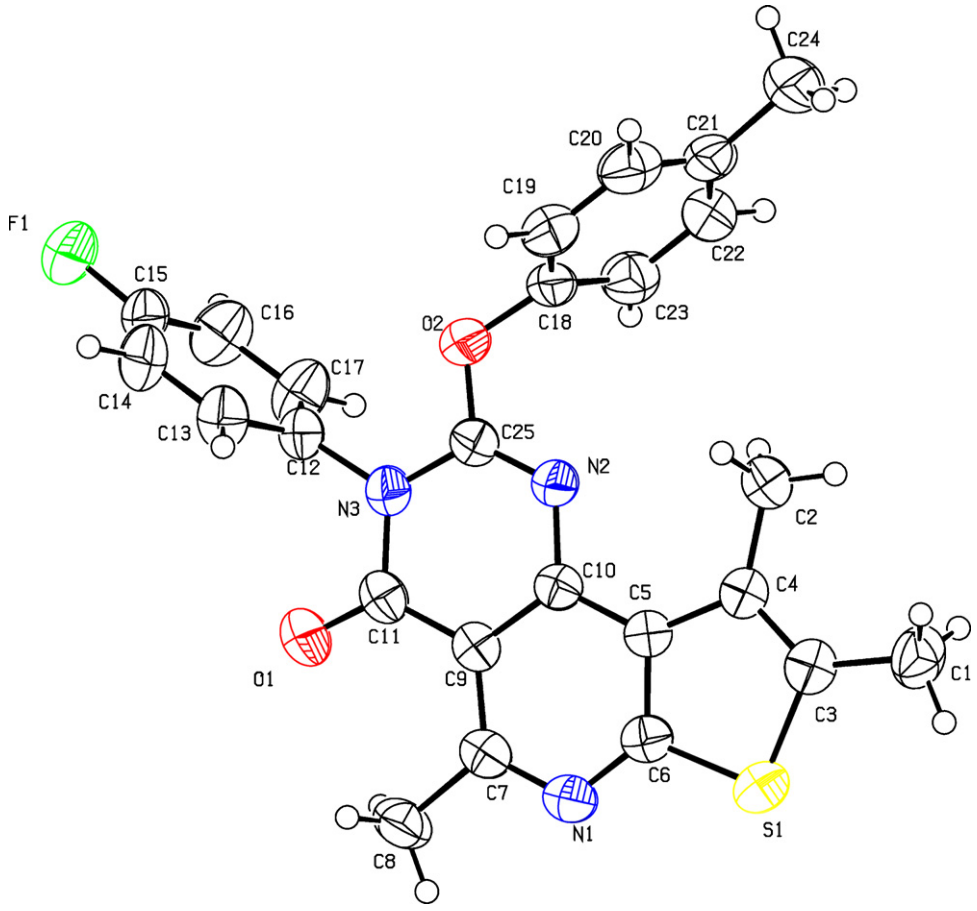
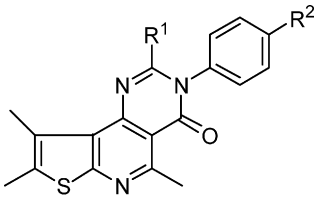


Fig. 1. View and atom labeling of **5a**.

Table 2
Fungicidal comparison with unfluorinated compounds



5

Compounds	R^1	R^2	Relative inhibition (%), 50 mg/L				
			F. oxyporium	*R. solani*	*B. cinereapers*	*G. zeae*	*D. gregaria*
5q	PhO	H	40.00	75.00	83.33	55.56	66.67
5r	PhO	Cl	72.00	98.33	100.00	88.89	85.71
5g	PhO	F	86.36	99.43	98.91	93.55	96.15
5s	$NH(CH_2)_3CH_3$	H	37.50	77.76	72.41	62.55	58.82
5t	$NH(CH_2)_3CH_3$	Cl	72.73	96.47	98.15	96.30	97.06
5n	$NH(CH_2)_3CH_3$	F	59.09	91.95	97.83	67.74	84.62

that observed in free thiophene [92.2(2)°]. As expected for a nonprotonated ring system, the C1–N1–C9 angle of 117.17(16)° is smaller than 120°[19]. The torsion angles C4–C3–C9–N1 and C9–C8–C5–S1 are 179.41(17)° and 179.89(13)°, respectively, showing the essential planarity of the tricyclic system. There is no intermolecular non-bonded interaction with fluorine or sulfur atom from the data. And the crystal packing is stabilized by Π–Π stacking interactions and van der Waals forces.

2.2. Fungicidal activity

The preliminary fungicidal activities of compounds **5** were measured in a concentration of 50 mg/L using a reported procedure [20] and the inhibition rates are listed in Tables 2 and 3. It was found that most of the compounds induce a good inhibition effect against *Rhizoctonia solani* and *Botrytis cinereapers* when fluorine atom is introduced but has not obvious influence on *Gibberella zeae*, *Fusarium oxyporium* and *Dothiorella gregaria.* For example, the inhibitory rate of compounds **5b**, **5i** and **5l** to *B. cinereapers* were 100, 100 and 100%, compounds **5g** and **5l** to *R. solani* were 99 and 98%. As the results listed in Table 2, fluorine-containing compounds **5g** and **5n** displayed much better fungicidal activities than non-substituted phenyl compounds 5**q** and 5**s** but showed the same effect with *para*-chlorosubstituted phenyl compounds **5r** and **5t**, respectively [21].

In summary, we have developed a novel approach of synthesizing 2-substituted-5,8,9-trimethyl-3-(4-fluoro-substituted)phenyl-thieno[3′,2′-5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones (**5**) *via* tandem aza-Wittig and annulation reactions. The biological evaluation showed that these compounds have fungicidal activities and could be further developed as lead compounds.

3. Experimental

Melting points were measured on an electrothermal melting point apparatus and are uncorrected. Mass spectra were measured on a Finnigan Trace MS 2000 spectrometer. IR spectra were recorded on a PE-983 infrared spectrometer as KBr pellets with absorption in cm^{-1}. ^1H NMR were recorded in $CDCl_3$ as solvent on a Varian Mercury 400 spectrometer and resonances are given in ppm (δ) relative to TMS. Elementary analyses were taken on a Vario EL III elementary analysis instrument. All of the solvents and materials were reagent grade and purified as required.

3.1. Synthesis of 4-amino-2,5,6-trimethyl-thieno[2,3-b]pyridine-3-carboxylate (*2*) [22]

2-Amino-4,5-dimethyl-thiophene-3-carbonitrile (**3**; 2.4 g, 16 mmol) and $SnCl_4$ (3.7 mL, 32 mmol) were added to a stirred solution of ethyl acetoacetate (2.08 mL, 16 mmol) in anhydrous toluene (20 mL). The mixture was stirred at r.t. for 1 h, and then heated at reflux for 5 h. The mixture was added to sat.aq. Na_2CO_3 solution (60 mL, pH 10), and the resulting suspension was extracted with AcOEt (3× 50 mL). The

Table 3
The fungicidal activity of compound **5**

Compounds	Relative inhibition (%), 50 mg/L				
	F. oxyporium	*R. solani*	*B. cinereapers*	*G. zeae*	*D. gregaria*
5a	59.09	86.21	91.30	67.74	76.92
5b	54.17	90.20	100.00	62.16	55.00
5c	39.13	80.00	90.48	52.94	56.52
5d	65.22	83.81	88.10	50.00	65.22
5e	52.17	90.48	95.24	79.41	82.61
5f	54.55	85.06	82.61	58.06	69.23
5g	86.36	99.43	98.91	93.55	96.15
5h	81.82	95.40	97.83	83.87	84.62
5i	59.09	91.95	100.00	70.97	84.62
5j	50.00	85.06	84.78	70.97	61.54
5k	54.55	83.91	86.96	51.61	61.54
5l	66.67	98.04	100.00	75.68	85.00
5m	45.45	80.46	80.43	51.61	53.85
5n	59.09	91.95	97.83	67.74	84.62
5o	54.17	89.22	97.44	59.46	60.00
5p	45.45	80.46	93.48	51.61	92.31

combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford **2** in 67% yield. Colorless crystals. m.p. 132–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, 3H, J = 7.2 Hz, CH_3), 2.39 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.77 (s, 3H, CH_3), 4.40 (q, 2H, J = 7.2 Hz, OCH_2CH_3), 6.65–6.73 (s, 2H, NH_2); Elemental Anal. Calcd. for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60. Found: C, 59.47; H, 6.03; N, 10.05.

3.2. Synthesis of iminophosphorane *1*

To a solution of **2** (1.56 g, 6 mmol) in MeCN (20 mL) was added Ph_3P (3.2 g, 12 mmol), C_2Cl_6 (2.8 g, 12 mmol) and, in this order, Et_3N (5.0 ml). The mixture was stirred for 6–7 h at r.t. Then, the solution was concentrated, and the residue was recrystallized from EtOH to give **1** in 90.2% yield. m.p. 217–218 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (t, 3H, J = 7.2 Hz, CH_3), 2.11 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.16 (q, 2H, J = 7.2 Hz, CH_2CO), 7.42–7.63 (m, 18H, Ar-H); Elemental Anal. Calcd. for $C_{31}H_{29}N_2O_2PS$: C, 70.97; H, 5.57; N, 5.34. Found: C, 71.43; H, 5.39; N, 5.80.

3.3. General procedure for the preparation of 2-aryloxy-5,8,9-trimethyl-3-(4-fluoro-substituted) phenyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-ones (*5a–j*)

To a solution of iminophosphorane **1** (1.1 g, 2 mmol) in dry methylene chloride (10 mL), *para*-fluorophenyl isocyanate (0.21 g, 2 mmol) was added under nitrogen at room temperature. After the reaction mixture was left unstirred for 6–12 h, the solvent was removed under vacuum and Et_2O/petroleum ether (1:2 20 mL) was added to precipitate triphenylphosphine oxide. Removal of the solvent gave carbodiimides **4**, which were used directly without further purification.

To the solution of **4** prepared above in CH_3CN (15 mL) was added substituted phenol (2 mmol) and cat solid K_2CO_3 (0.024 g, 0.2 mmol). The mixture was stirred for 1–2 h at 75 °C and filtered, the filtrate was condensed and the residue was recrystallized from dichloromethane/petroleum ether to give pure *2-aryloxy*-5,8,9-trimethyl-3-(4-fluoro-substituted)phenyl-thieno[3′,2′-5,6]pyrido[4,3-*d*] pyrimidin-4(3*H*)-ones **5a–j** [23].

3.3.1. 3-(4-Fluorophenyl)-2-[(4-methylphenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (*5a*)

White solid, yield 88%, m.p. 254.7–255.3 °C; IR (KBr): υ 2929, 2852, 1693 (C=O), 1620, 1562 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.05 (s, 6H, 2*CH_3), 7.21–7.41 (m, 8H, Ar-H); EI-MS (70 eV, *m/z*): 445 (M^+, 100), 308 (98), 188 (85); Elemental Anal. Calcd. for $C_{25}H_{20}FN_3O_2S$: C, 67.40; H, 4.52; N, 9.43. Found: C, 67.25; H, 4.59; N, 9.59.

3.3.2. 3-(4-Fluorophenyl)-2-[(1,2,3,4,5-pentachlorophenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyri-do[4,3-d]pyrimidin-4(3H)-one (*5b*)

White solid, yield 90%, m.p. >280 °C; IR (KBr): υ 2919, 1704 (C=O), 1626, 1563 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.98 (s, 3H, CH_3), 7.32–7.50 (m, 4H, Ar-H); EI-MS (70 eV, *m/z*): 605 (M^+ + 2, 78), 603 (M^+, 100), 601 (M^+ − 2, 65), 338 (52), 75 (23); Elemental Anal. Calcd. for $C_{24}H_{13}Cl_5FN_3O_2S$: C, 47.72; H, 2.17; N, 6.96. Found: C, 47.23; H, 2.25; N, 6.90.

3.3.3. 3-(4-Fluorophenyl)-2-[(2-nitrophenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrim-idin-4(3H)-one (*5c*)

White solid, yield 82%, m.p. 242.7–244 °C; IR (KBr): υ 3193, 1610 (C=O), 1540 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 7.28–7.77 (m, 8H, Ar-H); EI-MS (70 eV, *m/z*): 476 (M^+, 100), 460 (41), 338 (38), 277 (59); Elemental Anal. Calcd. for $C_{24}H_{17}FN_4O_4S$: C, 60.50; H, 3.57; N, 11.76; S, 6.73. Found: C, 60.12; H, 3.52; N, 11.45; S, 7.06.

3.3.4. 2-[(2-Chlorophenyl)oxy]-3-(4-fluorophenyl)-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (*5d*)

White solid, yield 90%, m.p. 270.9–271.4 °C; IR (KBr): υ 2936, 1692 (C=O), 1656, 1561 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.98 (s, 3H, CH_3), 7.16–7.51 (m, 8H, Ar-H); EI-MS (70 eV, *m/z*): 467 (M^+ + 2, 32), 465 (M^+, 100), 431 (96), 338 (74); Elemental Anal. Calcd. for $C_{24}H_{17}ClFN_3O_2S$: C, 61.87; H, 3.68; N, 9.02; S, 6.88. Found: C, 61.90; H, 3.67; N, 8.94; S, 7.18.

3.3.5. 3-(4-Fluorophenyl)-2-[(3-methylphenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (*5e*)

White solid, yield 84%, m.p. 259.1–260.1 °C; IR (KBr): υ 2930, 2857, 1688 (C=O), 1656, 1561 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H, CH_3), 2.39 (s, 6H, 2*CH_3), 3.04 (s, 3H, CH_3), 6.95–7.45 (m, 8H, Ar-H); EI-MS (70 eV, *m/z*): 445 (M^+, 100), 338 (43); Elemental Anal. Calcd. for $C_{25}H_{20}FN_3O_2S$: C, 67.40; H, 4.52; N, 9.43; S, 7.19. Found: C, 67.43; H, 4.22; N, 9.33; S, 7.45.

3.3.6. 3-(4-Fluorophenyl)-2-[(2-methylphenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]-pyrimidin-4(3H)-one (*5f*)

White solid, yield 82%, m.p. 280.1–281.2 °C; IR (KBr): υ 2927, 1718, 1688 (C=O), 1654, 1620, 1580, 1543 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 7.07–7.44 (m, 8H, Ar-H); Elemental Anal. Calcd. for $C_{25}H_{20}FN_3O_2S$: C, 67.40; H, 4.52; N, 9.43; S, 7.19. Found: C, 67.81; H, 4.17; N, 9.53; S, 7.29.

3.3.7. 3-(4-Fluorophenyl)-2-phenoxy-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (*5g*)

White solid, yield 84%, m.p. 266.9–267.5 °C; IR (KBr): υ 2933, 2853, 1735, 1680 (C=O), 1616, 1561 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 7.13–7.44 (m, 9H, Ar-H); Elemental Anal. Calcd. for $C_{24}H_{18}FN_3O_2S$: C, 66.81; H, 4.20; N, 9.74; S, 7.43. Found: C, 66.46; H, 4.42; N, 9.39; S, 7.37.

*3.3.8. 2-[(4-Chlorophenyl)oxy]-3-(4-fluorophenyl)-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (**5h**)*

White solid, yield 86%, m.p. 270.2–270.9 °C; IR (KBr): υ 3076, 2935, 2852, 1733, 1680 (C=O), 1615,1551 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 7.09–7.42 (m, 8H, Ar-H); Elemental Anal. Calcd. for $C_{24}H_{17}ClFN_3O_2S$: C, 61.87; H, 3.68; N, 9.02; S, 6.88. Found: C, 61.53; H, 3.49; N, 8.97; S, 7.04.

*3.3.9. 3-(4-Fluorophenyl)-2-[(2,4-dichlorophenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d] pyrimidin-4(3H)-one (**5i**)*

White solid, yield 85%, m.p. 277.7–279.1 °C; IR (KBr): υ 3040, 2919, 2840, 1688 (C=O), 1621, 1562 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 7.17–7.51 (m, 7H, Ar-H); Elemental Anal. Calcd. for $C_{24}H_{17}Cl_2FN_3O_2S$: C, 57.61; H, 3.22; N, 8.40; S, 6.41. Found: C, 58.04; H, 3.40; N, 8.34; S, 6.72.

*3.3.10. 3-(4-Fluorophenyl)-2-[(2-chloro-4-fluorophenyl)oxy]-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido [4,3-d]pyrimidin-4(3H)-one (**5j**)*

White solid, yield 82%, m.p. 260.1–261.9 °C; IR (KBr): υ 2988, 2935, 2852, 1687 (C=O), 1664, 1621, 1580 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 7.06–7.68 (m, 7H, Ar-H); Elemental Anal. Calcd. for $C_{24}H_{16}ClF_2N_3O_2S$: C, 59.57; H, 3.33; N, 8.68; S, 6.61. Found: C, 59.86; H, 3.76; N, 8.44; S, 6.64.

*3.4. General procedure for the preparation of 2-alkylamino-5,8,9-trimethyl-3-(4-fluoro-substituted) phenyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-ones (**5k–p**)*

Alkylamine (2 mmol) was added into the solution of **4** prepared above in CH_2Cl_2 (10 mL). After the reaction mixture was stirred continuously for an addition 6 h, the solvent was removed and 10 mL of anhydrous ethanol with several drops of sodium ethoxide in ethanol (3 M) were added. After stirring for another 0.5–1 h, the solution was condensed and the residue was recrystallized from dichloromethane/petroleum ether to give pure *2-alkylamino*-5,8,9-trimethyl-3-(4-fluoro-substituted) phenyl-thieno[3′,2′-5,6]pyrido [4,3-*d*]pyrimidin-4(3*H*)-ones **5k–p**.

*3.4.1. 2-Ethylamino-3-(4-fluorophenyl)-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (**5k**)*

White solid, yield 83%, m.p. 275.7–276.1 °C; IR (KBr): υ 3425 (N–H), 2929, 2852, 1693 (C=O), 1620, 1562 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 3H, J = 7.0 Hz, $CH_2\mathbf{CH_3}$), 2.48 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 3.52–3.58 (m, 2H, CH_2), 4.36 (s, 1H, NH), 7.15–7.33 (m, 4H, Ar-H); Elemental Anal. Calcd. for $C_{20}H_{19}FN_4OS$: C, 62.81; H, 5.01; N, 14.65. Found: C, 62.75; H, 5.37; N, 14.66.

*3.4.2. 3-(4-Fluorophenyl)-2-n-propylamino-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4 (3H)-one (**5l**)*

White solid, yield 83%, m.p. >280 °C; IR (KBr): υ 3443 (N–H), 2963, 2930, 2874, 1686 (C=O), 1581, 1561 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 7.4 Hz, $CH_2\mathbf{CH_3}$), 1.62 (q, 2H, J = 7.4 Hz, $\mathbf{CH_2}CH_3$), 2.48 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 2.94 (s, 3H, CH_3), 3.45 (q, 2H, J = 6.8 Hz, $NH\mathbf{CH_2}CH_2$), 4.37 (s, 1H, N–H), 7.26–7.33 (m, 4H, Ar-H); EI-MS (70 eV, *m/z*): 396 (M^+, 100), 354 (86), 338 (20); Elemental Anal. Calcd. for $C_{21}H_{21}FN_4OS$: C, 63.62; H, 5.34; N, 14.13. Found: C, 63.45; H, 5.51; N, 14.11.

*3.4.3. 3-(4-Fluorophenyl)-2-iso-propylamino-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4(3H)-one (**5m**)*

White solid, yield 83%, m.p. >280 °C; IR (KBr): υ 3437 (N–H), 2966, 2924, 2868, 1674 (C=O), 1581, 1561 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (d, 6H, J = 6.8, 2*CH_3), 2.48 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 2.94 (s, 3H, CH_3), 4.04 (d, 1H, J = 6.8 Hz, CH), 4.33 (d, 1H, J = 6.8 Hz, N–H), 7.26–7.33 (m, 4H, Ar-H); Elemental Anal. Calcd. for $C_{21}H_{21}FN_4OS$: C, 63.62; H, 5.34; N, 14.13. Found: C, 63.40; H, 5.44; N, 13.71.

*3.4.4. 2-n-Butylamino-3-(4-fluorophenyl)-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4 (3H)-one (**5n**)*

White solid, yield 73%, m.p. >280 °C; IR (KBr): υ 3443 (N–H), 2930, 2863, 1682 (C=O), 1580 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 7.2 Hz, CH_3), 1.25–1.34 (m, 2H, CH_2), 1.54–1.61 (m, 2H, CH_2), 2.48 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 3.49 (q, 2H, J = 4.2 Hz, CH_2), 4.35 (s, 1H, N–H), 7.26–7.33 (m, 4H, Ar-H); Elemental Anal. Calcd. for $C_{22}H_{23}FN_4OS$: C, 64.37; H, 5.65; N, 13.65. Found: C, 64.67; H, 5.45; N, 13.47.

*3.4.5. 2-tert-Butylamino-3-(4-fluorophenyl)-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4 (3H)-one (**5o**)*

White solid, yield 74%, m.p. >280 °C; IR (KBr): υ 3430 (N–H), 3054 (Ph-H), 2964, 2924, 1677 (C=O), 1579, 1559 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, 3*CH_3), 2.49 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 2.96 (s, 3H, CH_3), 4.21 (s, 1H, N–H), 7.26–7.33 (m, 4H, Ar-H); Elemental Anal. Calcd. for $C_{22}H_{23}FN_4OS$: C, 66.92; H, 6.70; N, 12.01. Found: C, 66.90; H, 6.99; N, 11.68.

*3.4.6. 2-Di-butylamino-3-(4-fluorophenyl)-5,8,9-trimethyl-thieno[3′,2′-5,6]pyrido[4,3-d]pyrimidin-4 (3H)-one (**5p**)*

White solid, yield 78%, m.p. >280 °C; IR (KBr): υ 2956, 2928, 2868, 1680 (C=O), 1604, 1556 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 (q, 6H, J = 7.6 Hz, 2*CH_3), 1.14–1.45 (m, 8H, 4*CH_2), 2.49 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 2.96(s, 3H, CH_3), 3.12 (q, 4H, J = 2.4 Hz, 2*CH_2), 7.19–7.40 (m, 4H, Ar-H); Elemental Anal. Calcd. for $C_{26}H_{31}FN_4OS$: C, 66.92; H, 6.70; N, 12.01. Found: C, 66.78; H, 6.79; N, 11.92.

3.5. Fungicidal testing

The newly synthesized compounds were tested for in vitro antifungal activity against six plant diseases which are *R. solani*, *B. cinereapers*, *G. zeae*, *Bipilaris maydis*, *F. oxyporium* and *Botryosphaeria berengerinan*. The tested compounds were dissolved in DMF and added to a sterile agarized Czapek-Dox medium at 45 °C. In preliminary screenings compounds were used in a concentration of 50 mg/L. The media were poured onto 8-cm Petri dishes (10 mL for each dish) and were inoculated with 5-mm PDA discs of overgrown mycelium. After the tested dishes being incubated at 25 °C in the dark for 48 h, the diameters of the mycelium were measured. The percentage inhibition of fungal growth was determined by comparison between the development of fungi colonies on media containing compounds and that on the control. Three replicates of each test were carried out.

3.6. Crystal structure determination

Single crystal X-ray diffraction data for **5a** at 292 K on a Bruker Smart Apex Area CCD equipped with Mo Ka radiation (=0.71073Å). Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 282310. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk).

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