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Facile synthesis and biological activities of novel fluorine-containing pyrido[4,3-*d*]pyrimidines

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ABSTRACT

Fourteen novel 3-substituted-5-methyl-4-methylene-7-alkylsulfanyl-3,4-dihydro-pyrido[4,3-*d*]pyrimidine-8-carbonitriles, compounds **4a**–**n**, were designed and synthesized *via* a facile regioselective cyclization process. The intermediate **2** reacted with triethyl orthoformate (molar ratio is 1:4) in the presence of acetic anhydride to give formamidate **3**, which was cycled to **4** regioselectively upon addition of different amines at mild condition. The structures of compounds **4a**–**n** were confirmed by IR, ^{1}H NMR, MS and elemental analysis. The preliminary bioassay indicated that some compounds possess significant herbicidal activity against the roots of rape and barnyard grass, especially when the fluorine atom was introduced to the *para* position of the substitutents on pyrimidine ring; some compounds showed fungicidal activities against *Rhizoctonia solani*, *Botrytis cinereapers*, *Gibberella zeae*, *Dothiorella gregaria*, *Colletotrichum gossypii* as well, and the introduction of fluorine has negative effect on the antifungal activities.

1. Introduction

Pyrido[4,3-*d*]pyrimidines possess various biological activities, especially pharmaceutical activities in terms of EGFR-TK (epidermal growth factor receptor-tyrosine kinase) and DHFR (dihydrofolate reductase) inhibitors. These compounds display antitumor, antiviral, anti-inflammatory and antimicrobial properties [1]. And some of pyrido[4,3-*d*]pyrimidines have been reported as agricultural lead compounds, possessing herbicidal and fungicidal activities [2], which attracted our interest intensely.

The methods described so far on preparation of pyrido[*d*]pyrimidines mainly involve the formation of the pyridine or pyrimidine ring by cyclization of suitable substitutents of another ring [3]. It has been reported that formamidate cyclized with cyano group in the neighboring position to afford pyrimidine ring [4].

As a part of our extensive research program to rapidly synthesize novel bioactive heterocycles, we developed herein a facile regioselective annulation process for a highly efficient assembly of pyrido[4,3-*d*]pyrimidines at mild condition. The formamidate' attacking neighboring carbonyl instead of cyano group regioselectively afford pyrido[4,3-*d*]pyrimidines. The preliminary results *in vitro* bioassay indicated that some title compounds displayed favorable herbicidal activity and moderate fungicidal activity.

2. Results and discussion

2.1. Synthesis

The intermediates of **1** were prepared by the reaction of malononitrile and carbon bisulfide in the presence of KOH as base in acetonitrile, and then treated with ammonium hydroxide in methanol [5]. And the 4-aminopyridines **2**, which were easily obtained from compounds **1** in the presence of zinc nitrate, were converted to the formamidates **3** in good yield *via* reaction with triethyl orthofromate, using acetic anhydride as catalyst. Treated with amines, the formamidates **3** were cyclized easily and regioselectively to afford **4** in good yields (Table 1). This process can be rationalized in Scheme 1.

In previous report, compound **2** was synthesized with moderate yield (58%), using anhydrous stannic chloride as the catalyst [6]. Herein, we use Lewis acid $Zn(NO_3)_2{\cdot}6H_2O$ as catalyst instead of anhydrous stannic chloride to obtain higher yield (83%). Furthermore, we found that formamidates **3** can react with primary amines to afford pyrido[4,3-*d*]pyrimidines, the cyclization proceeded very smoothly and regioselectively by attacking neighboring carbonyl instead of cyano group.

All products **4** were obtained as white crystal after filtration and recrystallization from acetone/petroleum ether. The structures were fully confirmed by IR, ^{1}H NMR, MS and elemental analysis and all structures were supported spectroscopically. For example, the ^{1}H NMR spectrum of **4d** revealed that the two hydrogen of

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Table 1
Yields of compounds **4**

Compounds	R^1	R^2	Yields
4a	Me	$PhCH_2$–	67
4b	Me	$PhCH_2CH_2$–	60
4c	Me	$PhCH_2CH_2CH_2$–	71
4d	Me	2-$FPhCH_2$–	80
4e	Me	3-$FPhCH_2$–	78
4f	Me	4-$FPhCH_2$–	54
4g	Me	4-$FPhCH_2CH_2$–	79
4h	Et	$PhCH_2$–	70
4i	Et	$PhCH_2CH_2$–	63
4j	Et	$PhCH_2CH_2CH_2$–	59
4k	Et	2-$FPhCH_2$–	61
4l	Et	3-$FPhCH_2$–	49
4m	Et	4-$FPhCH_2$–	61
4n	Et	4-$FPhCH_2CH_2$–	84

4-methylene group in pyrimidine was nuclear isoequivalent, appears at $\delta(H)$ 4.58–4.59 (d, J = 4.0 Hz, =CH[a]) and $\delta(H)$ 4.63–4.64 (d, J = 4.0 Hz, =CH[b]), and 7-SMe group at $\delta(H)$ 2.65(s), 5-Me group at $\delta(H)$ 2.61(s). In IR spectra, the relatively strong absorption of phenyl ring appeared at 1601–1523 cm^{-1}. The stretching resonance of C≡N showed strong absorption at about 2219 cm^{-1}. The mass spectra showed strong molecular ion peaks.

2.2. Biological activities

The herbicidal activity of all compounds **4** against *Brassica napus* (rape) and *Echinochloa crus-galli* (barnyard grass) has been investigated at the dosage of 100 mg/L and 10 mg/L using known procedure [7] compared with distilled water. The results of bioassay showed that some of them exhibited pronounced herbicidal activity against the roots of rape and barnyard grass when fluorine atom is introduced to the *para* position of the substitutent R^2. See Table 2.

Comparing activities among compounds (R^1 = Me), the position of fluorine on the substituent R^2 greatly affected herbicidal activity. For example, compounds **4f** (R^2 = 4-$FPhCH_2$–), **4g** (R^2 = 4-$FPhCH_2CH_2$–) **s**howed higher inhibition rate against the growth of rape and barnyard grass than compounds **4a** (R^2 = $PhCH_2$–), **4b** (R^2 = $PhCH_2CH_2$–), respectively. It showed that fluorine introduced to the *para* position of the substitutent R^2 was useful for the improvement of herbicidal activity. However, the introduction of fluorine to other position (*o*-, *m*-) of the substitutent R^2 decreased the inhibition activity. For example, compounds **4d** (R^2 = 2-$FPhCH_2$–), **4e** (R^2 = 3-$FPhCH_2$–) showed lower activity against the roots of rape and barnyard grass than compound **4a** (R^2 = $PhCH_2$–) at dosage of 100 mg/L. Among compounds

Table 2
The herbicidal activity of compounds **4**

Compounds	Relative inhibition (root/stalk, %[a])			
	Rape		Barnyard grass	
	100 mg/L	10 mg/L	100 mg/L	10 mg/L
4a	90/67	37/na	91/40	51/13
4b	88/80	74/40	96/59	62/na
4c	82/60	30/16	91/na	62/16
4d	56/21	30/na	64/61	59/53
4e	71/5	49/11	54/61	41/22
4f	99/79	68/40	98/70	84/41
4g	88/57	80/46	96/64	67/18
4h	40/35	36/35	78/62	14/21
4i	34/29	26/24	75/62	25/na
4j	33/41	31/29	72/55	14/7
4k	36/35	29/29	67/62	50/45
4l	51/35	36/24	61/55	17/17
4m	70/41	56/35	89/72	69/52
4n	41/29	34/12	83/66	47/31

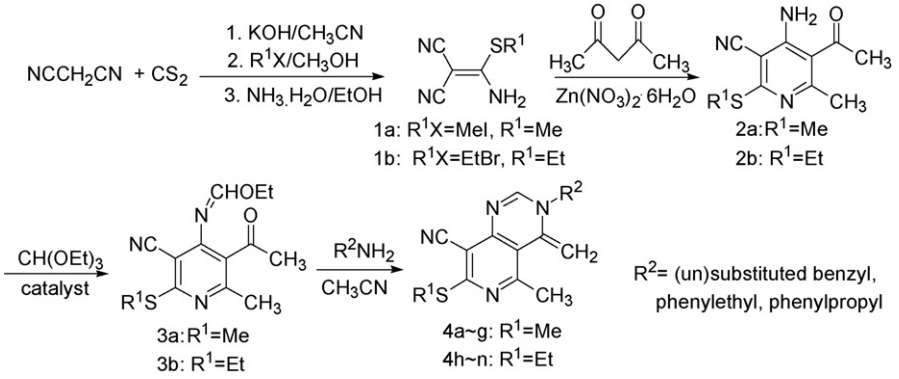
[a] na-inactive.

(R^1 = Et), the fluorine introduced to the *para* position of the substitutent R^2 was also favorable. For example, compounds **4m** (R^2 = 4-$FPhCH_2$–), **4n** (R^2 = 4-$FPhCH_2CH_2$–) showed higher inhibition rate against the roots of rape and barnyard grass than compounds **4h** (R^2 = $PhCH_2$–), **4i** (R^2 = $PhCH_2CH_2$–), respectively. Comparatively, introduction of fluorine to other position (*o*-, *m*-) of the substitutent R^2 has no obvious effect on the inhibition activity.

When the substituents R^2 remain the same, the increment of methylene in the substituents R^1, respectively decreased the herbicidal activity obviously. For example, compounds **4a** (R^1 = Me, R^2 = $PhCH_2$–), **4b** (R^1 = Me, R^2 = $PhCH_2CH_2$–), **4c** (R^1 = Me, R^2 = $PhCH_2CH_2CH_2$–) showed higher inhibition rates than **4h** (R^1 = Et, R^2 = $PhCH_2$–), **4i** (R^1 = Et, R^2 = $PhCH_2CH_2$–), **4j** (R^1 = Et, R^2 = $PhCH_2CH_2CH_2$–), respectively. However, when the substituents R^1 remain the same, the increment of methylene in the substituent R^2 has no obvious influence on the activity.

The fungicidal activity of compounds **4** was screened against six fungi, *Fusarium oxysporium*, *Rhizoctonia solani*, *Botrytis cinereapers*, *Gibberella zeae*, *Dothiorella gregaria* and *Colletotrichum gossypii* according to the reported method [8] at a dosage of 50 mg/L.

As listed in Table 3, among the compounds **4**, the most active is **4a**, where R^1 was methyl and R^2 was an unsubstituted benzyl. Compound **4a** exhibited significant broad spectrum fungicidal activity, with nearly complete inhibition against *F. oxysporium*, *B. cinereapers* and *G. zeae* at dosage of 50 μg/mL. Among compounds (R^1 = Me), most of them showed significant activities. When fluorine was introduced to the compounds, the inhibition rates decreased obviously. It showed that the introduction of fluorine



Scheme 1. Synthesis of the title compounds **4**.

Table 3
The fungicidal activity of compounds **4**

Compounds	Inhibition rate (%[a], 50 mg/L)					
	Fusarium oxysporium	*Rhizoctonia solani*	*Botrytis cinereapers*	*Gibberella zeae*	*Dothiorella gregaria*	*Colletotrichum gossypii*
4a	98	87	100	99	89	86
4b	50	67	65	63	75	80
4c	41	74	57	41	56	67
4d	4	15	na	na	6	11
4e	na	21	12	6	26	18
4f	59	84	91	74	81	67
4g	41	80	61	37	56	67
4h	23	0	9	9	24	12
4i	12	na	na	9	3	23
4j	19	0	23	9	21	12
4k	12	2	na	6	na	8
4l	27	na	20	6	3	19
4m	23	4	17	22	21	27
4n	19	5	0	13	7	27

[a] na-inactive.

has negative effect on the fungicidal activity, especially the introduction to the *o*-, *m*-position of substituent R^2. For example, compounds **4d**, **4e**, **4f** showed lower inhibition rates than compound **4a** against all six fungi; and **4g** was lower than **4b** with *R. solani* as exception. When the substituents R^1 remain the same, the increment of methylene in the substituents R^2 decreased the fungicidal activity. Comparatively, all compounds where R^1 is Et exhibited very low fungicidal activities. It indicated that the increment of substituent R^1 induced obvious decrease of fungicidal activity.

3. Conclusion

The title compounds **4** were designed, synthesized by a novel regioselective cyclization process. Some of these compounds had significant herbicidal activity against the roots of rape and barnyard grass, the introduction of fluorine to the *para* position of the substitutent R^2 was useful for the improvement of herbicidal activity. Compound **4a** showed potent broad-spectrum fungicidal activity, and it can be developed as lead compound for further study.

4. Experimental

Melting points were measured on an electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Avatar360 FTIR spectrometer in KBr pellets. ^{1}H NMR spectra was performed on Mercury 400 (Varian, 400 MHz) spectrometer with $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were determined on a Finnigan Trace MS 2000 spectrometer. Elemental analysis was taken on Elementar Vario EL III elementary analyzer. All the solvent and materials are reagent grades and purified before use.

4.1. Synthesis of 2-(amino-alkylsulfanyl-methylene)-malononitrile (1a–b)

Intermediates **1** were synthesized according to the literature [5].

2-(*amino-methylsulfanyl-methylene*)-*malononitrile* (**1a**): Yellowish crystal, mp 218.0–220.0 °C, yield 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (s, 3H, SCH_3), 8.75 (s, 2H, NH_2).

2-(*amino-methylsulfanyl-methylene*)-*malononitrile* (**1b**): Colorless crystal, mp 187.1–187.4 °C, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21–1.25 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 3.12–3.18 (q, 2H, J = 7.4 Hz, SCH_2CH_3), 8.72 (s, 2H, NH_2).

4.2. Synthesis of 5-acetyl-4-amino-6-methyl-2-alkylsulfanyl-nicotinonitrile (2a–b)

Acetylacetone (1.0 g, 10 mmol) and **1** (10 mmol) were added to a stirred solution of zinc nitrate in 30 mL alcohol. The mixture was stirred and refluxed for 12 h. The precipitate was filtered and washed with water (10 mL × 3) and purified from alcohol to afford **2**.

5-*acetyl*-4-*amino*-6-*methyl*-2-*methylsulfanyl-nicotinonitrile* (**2a**): Colorless crystal, mp 163.5–164.5 °C, yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.58 (s, 3H, CH_3), 2.61 (s, 3H, SCH_3), 2.68 (s, 3H, $COCH_3$), 6.60 (s, 2H, NH_2).

5-*acetyl*-4-*amino*-6-*methyl*-2-*ethylsulfanyl-nicotinonitrile* (**2b**): Yellowish crystal, mp 139.0–140.0 °C, yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36–1.40 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.59 (s, 3H, CH_3), 2.70 (s, 3H, $COCH_3$), 3.12–3.18 (q, 2H, J = 7.4 Hz, SCH_2CH_3), 6.68(s, 2H, NH_2).

4.3. Synthesis of N-(3-acetyl-5-cyano-2-methyl-6-alkylsulfanyl-pyridin-4-yl)-formimidic acid ethyl ester (3a–b)

To a solution of **2** (10 mmol) in triethyl orthoformate (5.92 g, 40 mmol) was added catalyzed amount acetic anhydride. The mixture was heated to 130–140 °C for 8–0 h. The extensive triethyl orthoformate was removed at reduced pressure, and the residue was purified by chromatography (ether:petroleum ether = 1:10) to afford formamidate **3**.

N-(3-*acetyl*-5-*cyano*-2-*methyl*-6-*methylsulfanyl-pyridin*-4-*yl*)-*formimidic acid ethyl ester* (**3a**): Colorless crystal, mp 78.5–79.9 °C, yield 95%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38–1.42 (t, 3H, J = 8.0 Hz, CH_2CH_3), 2.46 (s, 3H, CH_3), 2.56 (s, 3H, SCH_3), 2.62 (s, 3H, $COCH_3$), 4.36–4.42 (q, 2H, J = 8.0 Hz, CH_2CH_3), 8.06 (s, 1H, N = CH). EI-MS (70 eV, m/z): 277 (M^+, 63), 199 (31), 183 (22), 152 (28), 77 (100).

N-(3-*acetyl*-5-*cyano*-2-*methyl*-6-*ethylsulfanyl-pyridin*-4-*yl*)-*formimidic acid ethyl ester* (**3b**): Yellowish oil, yield 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37–1.39 (t, 3H, J = 3.8 Hz, SCH_2CH_3), 1.39–1.41 (t, 3H, J = 3.8 Hz, CH_2CH_3), 2.45 (s, 3H, CH_3), 2.46 (s, 3H, $COCH_3$), 3.24–3.29 (q, 2H, J = 3.6 Hz, SCH_2CH_3), 4.24–4.29 (q, 2H, J = 3.6 Hz, CH_2CH_3), 7.65 (s, 1H, N = CH).

4.4. General procedure for the preparation of 3,4-dihydro-3-substituted-5-methyl-4-methylene-7-alkylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (4a–m)

To a solution of formamidate **3** (10 mmol) in acetonitrile (10 mL) was added the appropriate amine (15 mmol), and the

mixture was stirred at room temperature for 0.5–1 h. The precipitate was isolated by filtration, recrystallized from acetone/petroleum ether to give pure products **4**.

*4.4.1. 3,4-Dihydro-3-benzyl-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4a**)*

Colorless crystal, yield 67%, mp 173.0–174.0 °C; IR (KBr): υ 3034, 2970, 2929, 2219, 1610, 1544, 1522, 1402, 1277, 916 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.57 (s, 3H, CH_3), 2.68 (s, 3H, SCH_3), 4.65 (s, 2H, CH_2Ph), 4.99 (d, 2H, $=CH_2$), 7.29–7.41 (m, 5H, Ph–H), 8.06 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 320 (M^+). Elemental anal. calcd. for $C_{18}H_{16}N_4S$: C 67.47, H 5.03, N 17.49; found C 66.99, H 5.17, N 17.52.

*4.4.2. 3,4-Dihydro -3-phenethyl-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4b**)*

Colorless crystal, yield 60%, mp 131.7–131.9 °C; IR (KBr): υ 3132, 3023, 2925, 2219, 1629, 1600, 1579, 1396, 1265, 852 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.54 (s, 3H, CH_3), 2.74 (s, 3H, SCH_3), 2.54–2.74 (m, 2H, CH_2CH_2Ph), 3.16 (t, 2H, J = 13.6 Hz, CH_2CH_2Ph), 4.30 (d, 2H, $=CH_2$), 6.89–7.33 (m, 6H, Ph–H and pyrimidine–H). Elemental anal. calcd. for $C_{19}H_{18}N_4S$: C 68.23, H 5.42, N 16.75; found C 67.90, H 5.47, N 16.63.

*4.4.3. 3,4-Dihydro-3-(3-phenylpropyl)-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4c**)*

Colorless crystal, yield 71%, mp 152.1–152.9 °C; IR (KBr): υ 3187, 3029, 2943, 2221, 1609, 1550, 1524, 1395, 1280, 918 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.60 (s, 3H, CH_3), 2.71 (s, 3H, SCH_3), 2.09–2.13 (m, 4 H, $CH_2CH_2CH_2Ph$), 3.62 (t, 2H, J = 14.0 Hz, $CH_2CH_2CH_2Ph$), 4.53 (d, 1H, J = 3.6 Hz, $=CH^a$), 4.61 (d, 1 H^b, J = 3.6Hz, $=CH^b$), 7.17–7.30 (m, 5H, Ph–H), 7.43 (s, 1 H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 348 (M^+). Elemental anal. calcd. for $C_{20}H_{20}N_4S$: C 68.93, H 5.79, N 16.08; found C 68.65, H 5.95, N 16.17.

*4.4.4. 3,4-Dihydro-3-(2-fluorobenzyl)-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4d**)*

Colorless crystal, yield 80%, mp 151.9–152.3 °C; IR (KBr): υ 3149, 3036, 2929, 2219, 1395, 1278, 915 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.61 (s, 3H, CH_3), 2.65 (s, 3H, SCH_3), 4.58–4.59 (d, 1H, J = 4.0 Hz, $=CH^a$), 4.63–4.64 (d, 1H, J = 4.0 Hz, $=CH^b$), 4.83 (s, 2H, CH_2Ph), 7.11–7.36 (m, 4H, Ph–H), 7.60 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 340 (M^++2), 338 (M^+). Elemental anal. calcd. for $C_{18}H_{15}FN_4S$: C 63.89, H 4.47, N 16.56; found C 64.02, H 4.82, N 16.35.

*4.4.5. 3,4-Dihydro-3-(3-fluorobenzyl)-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4e**)*

Colorless crystal, yield 78%, mp 164.1–165.7 °C; IR (KBr): υ 3178, 2928, 2218, 1607, 1554, 1522, 1396, 1277, 915 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.62 (s, 3H, CH_3), 2.65 (s, 3H, SCH_3), 4.47–4.48 (d, 1H, J = 4.0 Hz, $=CH^a$), 4.63–4.64 (d, 1H, J = 4.0 Hz, $=CH^b$) 4.79 (s, 2H, CH_2Ph), 6.96–7.38 (m, 4H, Ph–H), 7.59 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 340 (M^+ + 2), 338 (M^+). Elemental anal. calcd. for $C_{18}H_{15}FN_4S$: C 63.89, H 4.47, N 16.56; found C 63.62, H 4.72, N 16.45.

*4.4.6. 3,4-Dihydro-3-(4-fluorobenzyl)-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4f**)*

Colorless crystal, yield 54%, mp 136.6–136.9 °C; IR (KBr): υ 3148, 2932, 2222, 1627, 1563, 1510, 1397, 1285, 900 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.47 (s, 3H, CH_3), 2.61 (s, 3H, SCH_3), 2.69 (s, 2H, CH_2Ph), 4.64 (d, 2H, $=CH_2$), 7.02–7.37 (m, 4H, Ph–H), 7.82 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 365 (M^++1). Elemental anal. calcd. for $C_{18}H_{15}FN_4S$: C 63.91, H 4.44, N 16.57; found C 64.02, H 4.82, N 16.35.

*4.4.7. 3,4-Dihydro-3-(4-fluorophenethyl)-5-methyl-4-methylene-7-methylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4g**)*

Colorless crystal, yield 79%, mp 205.9–206.2 °C; IR (KBr): υ 3170, 2919, 2850, 2217, 1607, 1547, 1524, 1389, 1280, 916 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.61 (s, 3H, CH_3), 2.73 (s, 3H, SCH_3), 3.00–3.04 (t, 2H, J = 8.0 Hz, CH_2CH_2Ph), 3.82–3.86 (t, 2H, J = 8.0 Hz, CH_2CH_2Ph), 4.73 (d, 2H, $=CH_2$), 6.98–7.14 (m, 4H, Ph–H), 7.18 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 352 (M^+). Elemental anal. calcd. for $C_{19}H_{17}FN_4S$: C 64.77, H 4.83, N 15.91; found C 64.63, H 4.76, N 15.23.

*4.4.8. 3,4-Dihydro-3-benzyl-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4h**)*

Colorless crystal, yield 70%, mp 146.6–147.6 °C; IR (KBr): υ 3034, 2961, 2869, 2218, 1607, 1546, 1520, 1400, 916 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.36–1.40 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.63 (s, 3H, CH_3), 3.22–3.27 (q, 2H, J = 7.3 Hz, SCH_2CH_3), 4.52–4.53 (d, 1H, J = 3.6 Hz, $=CH^a$), 4.61–4.62 (d, 1H, J = 3.2 Hz, $=CH^b$), 4.79 (s, 2H, CH_2Ph), 7.25–7.40 (m, 5H, Ph–H), 7.59 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 334 (M^+). Elemental anal. calcd. for $C_{19}H_{18}N_4S$: C 68.23, H 5.42, N 16.75; found C 68.39, H 5.65, N 16.69.

*4.4.9. 3,4-Dihydro-3-phenethyl-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4i**)*

Colorless crystal, yield 63%, mp 131.3–132.3 °C; IR (KBr): υ 3029, 2956, 2931, 2216, 1605, 1548, 1521, 1389, 954 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.37–1.40 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.72 (s, 3H, CH_3), 3.01–3.05 (t, 2H, J = 7.0 Hz, CH_2CH_2Ph), 3.22–3.28 (q, 2H, J = 7.3 Hz, SCH_2CH_3), 3.84–3.87 (t, 2H, J = 7.2 Hz, CH_2CH_2Ph), 4.72–4.73 (d, 1H, J = 3.6 Hz, $=CH^a$), 4.74–4.75 (d, 1H, J = 3.2 Hz, $=CH^b$), 7.15–7.31 (m, 6H, Ph–H and pyrimidine–H); EI-MS (70 eV): *m*/*z* 348 (M^+). Elemental anal. calcd. for $C_{20}H_{20}N_4S$: C 68.93, H 5.79, N 16.08; found C 68.56, H 5.85, N 16.27.

*4.4.10. 3,4-Dihydro-3-(3-phenylpropyl)-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4j**)*

Colorless crystal, yield 59%, mp 152.0–152.9 °C; IR (KBr): υ 3024, 2927, 2222, 1608, 1554, 1524, 1392, 921 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.36–1.40 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.09–2.41 (m, 2H, $CH_2CH_2CH_2Ph$), 2.64–2.77 (m, 5H, $CH_2CH_2CH_2Ph$ and CH_3), 3.22–3.27 (q, 2H, J = 7.3 Hz, SCH_2CH_3), 3.60–3.64 (t, 2H, J = 7.2 Hz, $CH_2CH_2CH_2Ph$), 4.52–4.53 (d, 1H, J = 3.2 Hz, $=CH^a$), 4.60–4.61 (d, 1H, J = 3.2 Hz, $=CH^b$), 7.16–7.31 (m, 5H, Ph–H), 7.43 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 360 (M^+). Elemental anal. calcd. for $C_{21}H_{22}N_4S$: C 69.58, H 6.12, N 15.46; found C 69.49, H 6.35, N 15.65.

*4.4.11. 3,4-Dihydro-3-(2-fluorobenzyl)-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4k**)*

Colorless crystal, yield 61%, mp 145.1–146.1 °C; IR (KBr): υ 3041, 2967, 2928, 2217, 1599, 1547, 1524, 1393, 918 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.37–1.38 (t, 3H, J = 2.2 Hz, SCH_2CH_3), 2.64 (s, 3H, $-CH_3$), 3.24–3.26 (q, 2H, J = 2.4 Hz, SCH_2CH_3), 4.57–4.58 (d, 1H, J = 4.0 Hz, $=CH^a$), 4.63–4.64 (d, 1H, J = 3.6 Hz, $=CH^b$), 4.83 (s, 2H, CH_2Ph), 7.11–7.36 (m, 4H, Ph–H), 7.61 (s, 1H, pyrimidine–H); EI-MS (70 eV): *m*/*z* 352 (M^+). Elemental anal. calcd. for $C_{19}H_{17}FN_4S$: C 64.75, H 4.86, N 15.90; found C 64.50, H 5.05, N 16.17.

*4.4.12. 3,4-Dihydro-3-(3-fluorobenzyl)-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4l**)*

Colorless crystal, yield 49%, mp 145.3–145.8 °C; IR (KBr): υ 3041, 2965, 2872, 2218, 1608, 1551, 1521, 1401, 917 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.36–1.40 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.64 (s, 3H, CH_3), 3.22–3.28 (q, 2H, J = 7.2 Hz, SCH_2CH_3), 4.47 (d, 1H, J = 3.6 Hz, $=CH^a$), 4.62–4.63 (d, 1H, J = 3.2 Hz, $=CH^b$), 4.80 (s, 2H, CH_2Ph), 6.96–7.38 (m, 4H, Ph–H), 7.60 (s, 1H, pyrimidine–H); EI-

MS (70 eV): m/z 352 (M^+). Elemental anal. calcd. for $C_{19}H_{17}FN_4S$: C 64.75, H 4.86, N 15.90; found C 64.54, H 5.10, N 16.07.

*4.4.13. 3,4-Dihydro-3-(4-fluorobenzyl)-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4m**)*

Colorless crystal, yield 61%, mp 128.6–129.2 °C; IR (KBr): υ 2965, 2928, 2220, 1611, 1553, 1524, 1403 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.36–1.40 (t, 3H, J = 7.2 Hz, $-SCH_2CH_3$), 2.63 (s, 3H, $-CH_3$), 3.22–3.28 (q, 2H, J = 7.4 Hz, $-SCH_2CH_3$), 4.48–4.49 (d, 1H, J = 3.2 Hz, $=CH^a$), 4.62 (d, 1H, J = 3.2 Hz, $=CH^b$), 4.77 (s, 2H, $-CH_2Ph$), 7.07–7.10 (t, 2H, J = 7.0 Hz, Ph–H), 7.23–7.26 (t, 2H, J = 7.0 Hz, Ph–H), 7.59 (s, 1H, pyrimidine–H); EI-MS (70 eV): m/z 352 (M^+). Elemental anal. calcd. for $C_{19}H_{17}FN_4S$ (352.1): C 64.75, H 4.86, N 15.90; found C 64.49, H 4.95, N 15.67.

*4.4.14. 3,4-Dihydro-3-(4-fluorophenethyl)-5-methyl-4-methylene-7-ethylsulfanyl-pyrido[4,3-d]pyrimidine-8-carbonitrile (**4n**)*

Colorless crystal, yield 84%, mp 168.3–169.3 °C; IR (KBr): υ 3032, 2979, 2933, 2218,1607, 1545, 1518, 1390, 915 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.37–1.41(t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.72 (s, 3H, CH_3), 3.00–3.03 (t, 2H, J = 6.8 Hz, CH_2CH_2Ph), 3.22–3.28 (q, 2H, J = 7.4 Hz, SCH_2CH_3), 2.82–3.86 (t, 2H, J = 7.2 Hz, CH_2CH_2Ph), 4.72 (d, 2H, $=CH_2$), 6.98–7.14 (m, 4H, Ph–H), 7.19 (s, 1H, pyrimidine–H); EI-MS (70 eV): m/z 366 (M^+). Elemental anal. calcd. for $C_{20}H_{19}FN_4S$: C 65.55, H 5.23, N 15.29; found C 65.40, H 5.35, N 14.97.

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