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Design, Synthesis, and Pesticidal Activities of Pyrimidin-4-amine Derivatives Bearing a 5-(Trifluoromethyl)-1,2,4-oxadiazole Moiety

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ABSTRACT: It is important to discover new pesticides with new modes of action because of the increasing evolution of pesticide resistance. In this study, a series of novel pyrimidin-4-amine derivatives containing a 5-(trifluoromethyl)-1,2,4-oxadiazole moiety were designed and synthesized. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS. Bioassays indicated that the 29 compounds synthesized possessed excellent insecticidal activity against *Mythimna separata*, *Aphis medicagini*, and *Tetranychus cinnabarinus* and fungicidal activity against *Pseudoperonospora cubensis*. Among these pyrimidin-4-amine compounds, 5-chloro-*N*-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U7**) and 5-bromo-*N*-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl) pyrimidin-4-amine(**U8**) had broad-spectrum insecticidal and fungicidal activity. The LC_{50} values were 3.57 ± 0.42, 4.22 ± 0.47, and 3.14 ± 0.73 mg/L for **U7**, **U8**, and flufenerim against *M. separata*, respectively. The EC_{50} values were 24.94 ± 2.13, 30.79 ± 2.21, and 3.18 ± 0.21 mg/L for **U7**, **U8**, and azoxystrobin against *P. cubensis*, respectively. The AChE enzymatic activity testing revealed that the enzyme activities of compounds **U7**, **U8**, and flufenerim are 0.215, 0.184, and 0.184 U/mg prot, respectively. The molecular docking results of compounds **U7**, **U8**, and flufenerim with the AChE model demonstrated the opposite docking mode between compound **U7** or **U8** and positive control flufenerim in the active site of AChE. The structure−activity relationships are also discussed. This work provided excellent pesticide for further optimization. Density functional theory analysis can potentially be used to design more active compounds.

KEYWORDS: *pyrimidin-4-amine derivatives, synthesis, fungicidal, insecticidal, SAR*

INTRODUCTION

Synthetic pesticides have been relied on in agriculture for pest management for 75 years, and resistance to them is evolving rapidly. Therefore, it is urgent to develop new pesticides to which both nontarget site and target-site resistance have not evolved as well as pesticides with new mechanisms of action.

Pyrimidines are important members of nitrogen-containing heterocycles in natural products, such as uracil, thymine, cytosine, caffeine, theophylline, variolin B, vitamin B1, and heteromine. Among them, cytosine, variolin B, vitamin B1, tingitamine, and heteromine contain a pyrimidinamine moiety (Figure 1). Currently, synthetic pyrimidinamine derivatives possess efficient, broad-spectrum, and diverse activities, such as antitumor,[1] fungicidal,[2] insecticidal,[3] acaricidal,[4] antioxidant,[5] anti-HIV,[6] and other biological activities. Due to the high biological activity of 4-substituted pyrimidinamine derivatives, they have been a focus of pesticide discovery. For example, Cuccia et al.[7] reported a series of 4-position pyrimidinamine compounds that possessed good insecticidal activity against spider mites at 100 mg/L. Liu's group[8] reported some new 4-position pyrimidinamine compounds with activity against cucumber downy mildew using an intermediate derivatization method. Another example, the pyrimidinamine-type insecticides pyrimedifen and flufenerim were discovered by Ube Industries (Figure 1). It is reported that flufenerim targets AChE.[9] These properties indicate that flufenerim could be a promising template for the discovery of new insecticide candidates.

1,2,4-Oxadiazole is a five-membered heterocycle containing O and N atoms, which has various biological activities, such as insecticidal,[10,11] anticancer,[12,13] fungicidal,[14] anti-inflammatory,[15] antibactericidal,[16] antiproliferative,[17] and antimalarial.[18] In 2015, BASF[19,20] reported 5-(trifluoromethyl)-1,2,4-oxadiazole compounds that possessed excellent fungicidal activity. Subsequently, some patents[21−26] about fungicidal, herbicidal, or insecticidal 5-(trifluoromethyl)-1,2,4-oxadiazole compounds were filed by BASF, Syngenta, Sumitomo, Bayer, and others.

In our previous work, some 4-substituted pyrimidinamines[3,27−29] with good insecticidal activity were designed and synthesized. In this paper, in order to discover new pyrimidinamine compounds, the insecticide flufenerim was selected as a template molecule. The amino group of the pyrimidine ring was linked by a 4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl group, the 5-position of pyrimidine was replaced by another halogen, and 1-fluoroethyl was replaced with an isopropyl group. Some of the synthetic pyrimidinamine

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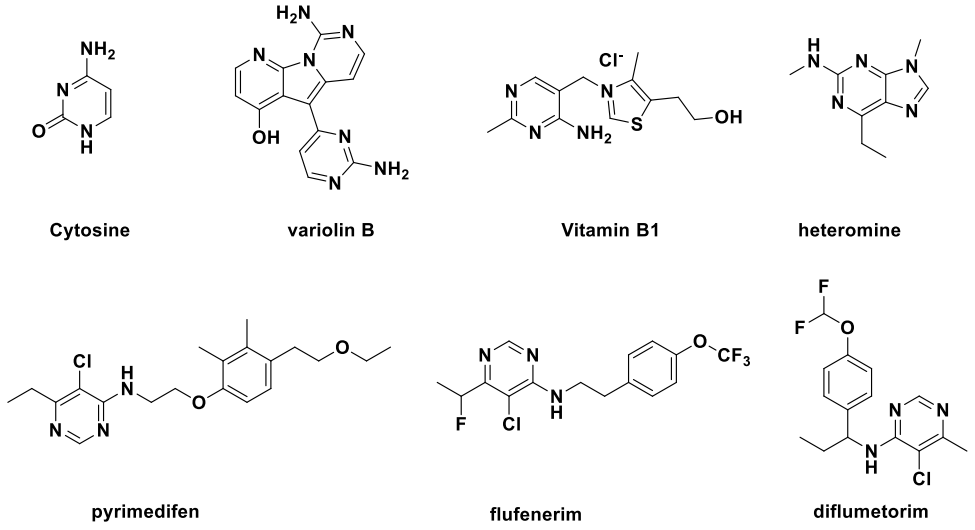


Figure 1. Some pyrimidinamine natural products and commercial pesticides.

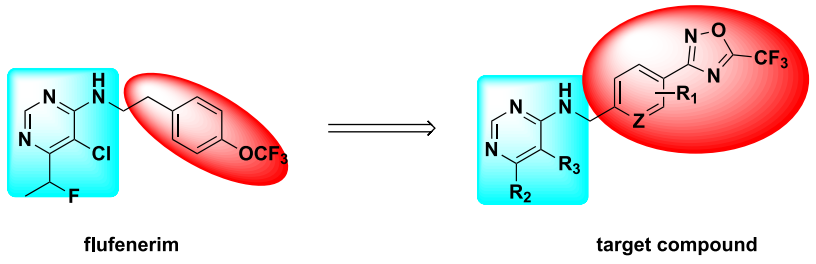
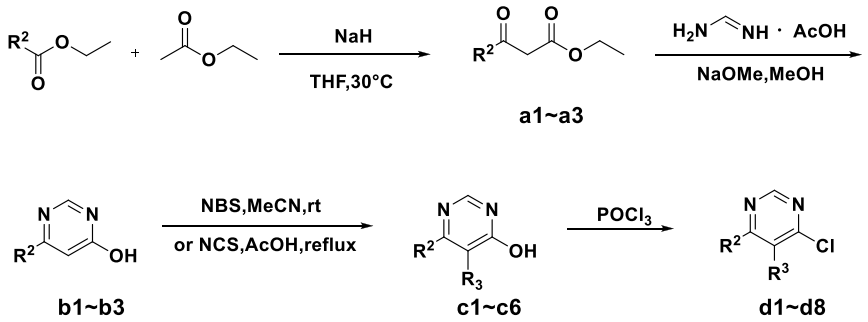


Figure 2. Design strategy for new pyrimidinamine compounds.

Scheme 1. Synthesis of Key Pyrimidine Intermediates d1−d8



derivatives exhibited good insecticidal and fungicidal activity. Their mode of action was also studied. The design strategy in this paper is shown in Figure 2.

MATERIALS AND METHODS

Instruments. Melting points were determined using an X-4 apparatus, and the temperature was uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 600 MHz instrument using TMS as an internal standard and $CDCl_3$ as the solvent. High-resolution mass spectra (HRMS) were recorded on a JEOL Accu TOF 4G instrument. All the reagents were of analytical grade or freshly prepared before use in Schemes 1 and 2.

Synthesis of Key Pyrimidine Intermediates. *Synthesis of Intermediates **a1−a3**.* To a solution of 60% NaH (12.0 g, 0.300 mol) in THF (100 mL), a mix solution of ethyl 2-fluoropropanoate (20.0 g, 0.167 mol) and ethyl acetate (22.0 g, 0.250 mol) was added dropwise for 30 min. The mixture was stirred for 5 h at 30 °C and monitored with TLC. The reaction was quenched by 1 mol/L HCl. Ethyl acetate (EA) was extracted and dried by $MgSO_4$, giving colorless oil **a1**[30] (21.1 g, yield 78.3%). The other intermediates **a2** (R_2 = $(CH_3)_2CH$, colorless oil, yield 76.9%) and **a3** (R_2 = CHF_2, colorless oil, yield 65.7%) were synthesized using the same method.

*Synthesis of Intermediates **b1−b3**.* To a solution of 30% $NaOCH_3$ (23.3 g, 0.129 mol) in CH_3OH (100 mL), formamidine acetate (6.42 g, 0.0617 mol) was added in batches for 30 min. Then, ethyl 4-fluoro-3-oxopentanoate (10.0 g, 0.0617 mol) was added dropwise. The mixture was stirred for further 24 h. The reaction was quenched by concentrated HCl to pH = 3−4. EA was extracted and dried by $MgSO_4$, giving white solid **b1**[31] (8.60 g, m.p. 155−156 °C, yield 99.0%). The other intermediates **b2** ((R_2 = $CH_3)_2CH$, white solid, m.p. 161−162 °C, yield 85.5%) and **b3** (R_2 = CHF_2, white solid, m.p. 150−151 °C, yield 80.3%) were synthesized using the same method.

*Synthesis of Intermediates **c1−c3**.* To a 100 mL round flask, intermediate **b1** (4.30 g, 30.3 mmol) and NBS (6.40 g, 36.0 mmol) were dissolved in CH_3CN (50 mL). The mixture was stirred at room temperature for 8 h. Pure **c1**[32] was produced by rapid column chromatography (white solid, m.p. 93−94 °C, yield 98.1%). The other intermediates **c2** ((R_2 = $CH_3)_2CH$, yellow solid, m.p. 95−96

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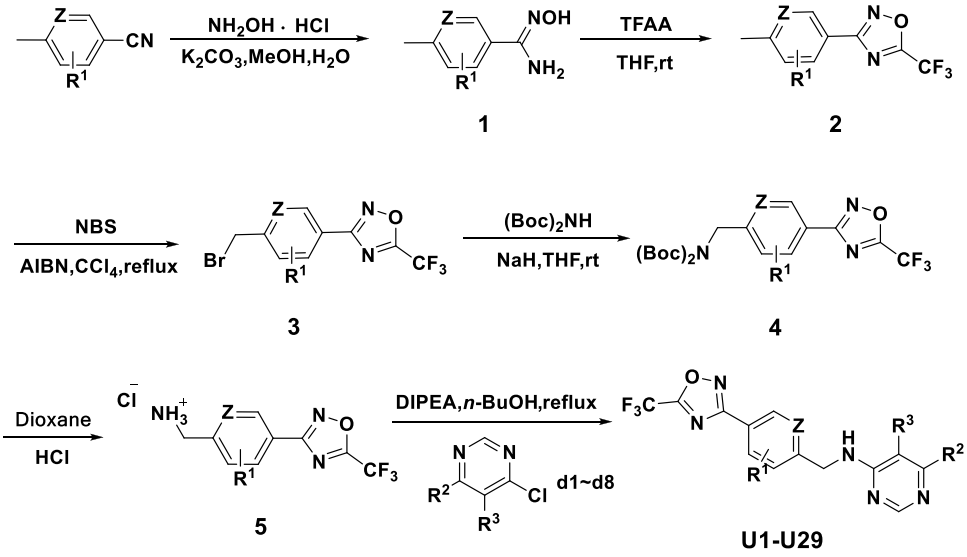
Scheme 2. Synthetic Route of Title Pyrimidinamine Compounds



°C, yield 80.5%) and **c3** (R_2 = CHF_2, yellow solid, m.p. 86−87 °C, yield 95.6%) were synthesized using the same method.

*Synthesis of Intermediates **c4−c6***. To a 100 mL round flask, intermediate **b1** (4.30 g, 30.3 mmol) and NCS (6.80 g, 50.9 mmol) were dissolved in CH_3CN (50 mL). The mixture was stirred at room temperature for 8 h. Pure **c4**[32] was obtained after rapid column chromatography (yellow solid, m.p. 83−84 °C, yield 75.6%). The other intermediates **c5** ((R_2 = $CH_3)_2CH$, yellow solid, m.p. 81−82 °C, yield 65.5%) and **c6** (R_2 = CHF_2, yellow solid, m.p. 75−76 °C, yield 77.7%) were synthesized using the same method.

*Synthesis of Intermediates **d1−d8***. To a 100 mL round flask, the intermediate **c1** (6.50 g, 29.4 mmol) and $POCl_3$ (30 mL) were refluxed at 90 °C for 3 h. The reaction was quenched by H_2O and adjusted to neutral by saturated sodium bicarbonate solution. EA was extracted and dried by $MgSO_4$, giving a white solid **d1**[33] (6.00 g, colorless oil, yield 85.0%). The other intermediates **d2** (R_2 = $(CH_3)_2CH$, R_3 = Br, colorless oil, yield 56.5%), **d3** (R_2 = CHF_2, R_3 = Br, colorless oil, yield 37.6%), **d4** (R_2 = CH_3CHF, R_3 = Cl, colorless oil, yield 55.6%), **d5** (R_2 = $(CH_3)_2CH$, R_3 = Cl, colorless oil, yield 67.5%), **d6** (R_2 = CHF_2, R_3 = Cl, colorless oil, yield 27.7%), **d7** (R_2 = CH_3CHF, R_3 = H, colorless oil, yield 47.5%), and **d8** (R_2 = CHF_2, R_3 = H, colorless oil, yield 65.5%) were synthesized using the same method.

Synthesis of Target Compounds. *Synthesis of Intermediates **1a−1e***. To a solution of *p*-methylbenzonitrile (35.0 g, 299 mmol) and K_2CO_3 (51.8 g, 375 mmol) in anhydrous methanol (300 mL), a solution of hydroxylamine hydrochloride (41.7 g, 600 mmol) in MeOH (90 mL) and H_2O (45 mL) was added dropwise at room temperature for 1 h and then heated to 80 °C for 5.5 h. The mixture was monitored by TLC. Then, methanol was evaporated, water (100 mL) and ethyl acetate (200 mL) were added, and the organic layer was extracted by ethyl acetate (100 mL × 3), then dried, and recrystallized with ethyl acetate to obtain **1a** (39.2 g,[34] white sticky, yield 87.1%). **1b** (starting material 2-fluoro-4-methylbenzonitrile, white sticky, yield 85.0%), **1c**(starting material 3-fluoro-4-methylbenzonitrile, white sticky, yield 86.2%), **1d** (starting material 2,3-difluoro-4-methylbenzonitrile, white sticky, yield 84.5%), and **1e** (starting material 6-methylnicotinonitrile, white sticky, yield 84.5%) were synthesized using the same method.

*Synthesis of Intermediates **2a−2e***. To a solution of **1a** (39.2 g, 261 mmol) in THF (400 mL), trifluoroacetic anhydride (82.3 g, 392 mmol) was added dropwise at 0 °C and then heated to 20 °C for 3 h. The mixture was monitored with TLC. The reaction was quenched by saturated sodium bicarbonate solution. EA was extracted and dried by $MgSO_4$, giving white solid **2a**[35] (44.7 g, colorless oil, yield 75.2%). **2b** (starting material 2-fluoro-4-methylbenzonitrile, white solid, m.p. 60−61 °C, yield 85.5%), **2c** (starting material 3-fluoro-4-methylbenzonitrile, white solid, m.p. 63−64 °C, yield 86.8%), **2d** (starting material 2,3-difluoro-4-methylbenzonitrile, brown solid, m.p. 70−71 °C, yield 84.2%), and **2e** (starting material 6-methylnicotinonitrile, colorless oil, yield 80.2%) were synthesized using the same method.

*Synthesis of Intermediates **3a−3e***. To a solution of **2a** (44.7 g, 196 mmol) and NBS (36.2 g, 203 mmol) in CCl_4 (400 mL), AIBN (3.20 g, 19.5 mmol) was added dropwise at 65 °C and further stirred for 10 h. The mixture was detected by TLC. The reaction was quenched by saturated sodium bicarbonate solution. CH_2Cl_2 was extracted, dried by $MgSO_4$, and purified by column chromatography, giving white solid **3a**[35] (41.9 g, white solid, m.p. 60−61 °C, yield 70.5%). **3b** (starting material 2-fluoro-4-methylbenzonitrile, colorless oil, yield 68.8%), **1c** (starting material 3-fluoro-4-methylbenzonitrile, white solid, m.p. 70−71 °C, yield 64.7%), **1d** (starting material 2,3-difluoro-4-methylbenzonitrile, brown solid, m.p. 70−71 °C, yield 65.5%), and **1e**(starting material 6-methylnicotinonitrile, white solid, m.p. 64−65 °C, yield 65.3%) were synthesized using the same method.

*Synthesis of Intermediates **4a−4e***. To a solution of NaH (6.54 g, 273 mmol) in THF (400 mL), $(Boc)_2NH$ (35.6 g, 164 mmol) was added dropwise at room temperature. After further stirring for 30 min, the intermediate **3a** (41.9 g, 136 mmol) was added. The mixture was further stirred for 12 h. Then, the reaction was quenched by H_2O. EA was extracted and dried by $MgSO_4$ to give crude intermediate **4a**[36] (60.5 g), which was directly used in the next step without purification. Intermediates **4b**, **4c**, **4d**, and **4e** were synthesized using the same method.

*Synthesis of Intermediates **5a−5e***. Crude **4a** (60.5 g) was added into a 1000 mL three-port flask, and the prepared HCl gas was passed into the three-port flask for 3 h under ice bath conditions. Then, the mixture was stirred at room temperature for 12 h. After adding MTBE, the solid was precipitated and then filtered. After drying, the gray solid **5a**[36] (30.5 g) was obtained (m.p. 240−241 °C, yield 80.1%). **5b** (starting material 2-fluoro-4-methylbenzonitrile, white solid, m.p. 244−245 °C, yield 78.1%), **5c** (starting material 3-fluoro-4-methylbenzonitrile, white solid, m.p. 243−244 °C, yield 78.1%), **5d** (starting material 2,3-difluoro-4-methylbenzonitrile, white solid, m.p. 251−252 °C, yield 75.1%), and **5e** (starting material 6-methylnicotinonitrile, white solid, m.p. 250−251 °C, yield 60.1%) were synthesized using the same method.

Synthesis of Target Pyrimidinamine Derivatives U1−U29. The key intermediates of **5** were synthesized using substituted 4-methylbenzonitrile or substituted 6-methylnicotinonitrile as starting materials via five steps according to the reported references. Then, the intermediate **5** (0.500 mmol), substituted 4-chloropyrimidine (**d1−**

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d8, 0.600 mmol), and *i*-Pr_2EtN (1.25 mmol) were dissolved in *n*-BuOH (10 mL), and the mixture was refluxed for 8 h. After the reaction was completed, the solvent was removed and purified by column chromatography, and a white solid was obtained (Table 1).

Table 1. Substituents of Compounds U1−U29

no.	R^1	R^2	R^3	Z
U1	H	$(CH_3)_2CH$	Cl	C
U2	H	$(CH_3)_2CH$	Br	C
U3	H	CH_3CHF	Br	C
U4	H	CH_3CHF	Cl	C
U5	H	CH_3CHF	H	C
U6	2-F	CH_3CHF	H	C
U7	2-F	CH_3CHF	Cl	C
U8	2-F	CH_3CHF	Br	C
U9	3-F	CH_3CHF	Br	C
U10	3-F	CH_3CHF	Cl	C
U11	H	CH_3CHF	Br	N
U12	H	CH_3CHF	Cl	N
U13	2,3-diF	CH_3CHF	Cl	C
U14	2,3-diF	CH_3CHF	Br	C
U15	H	CHF_2	Br	C
U16	H	CHF_2	Cl	C
U17	H	CHF_2	H	C
U18	2-F	CHF_2	Br	C
U19	2-F	CHF_2	Cl	C
U20	2-F	CHF_2	H	C
U21	H	CHF_2	Br	N
U22	H	CHF_2	Cl	N
U23	H	CHF_2	H	N
U24	3-F	CHF_2	Br	C
U25	3-F	CHF_2	Cl	C
U26	3-F	CHF_2	H	C
U27	2,3-diF	CHF_2	Cl	C
U28	2,3-diF	CHF_2	Br	C
U29	2,3-diF	CHF_2	H	C

*5-Chloro-6-isopropyl-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U1**).* White solid, m.p. 90−91 °C, yield 53.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.26 (d, J = 7.2 Hz, 6H, -CH_3), 3.40−3.46 (m, 1H, -CH), 4.82 (d, J = 6.0 Hz, 2H, -CH_2), 5.77−5.85 (m, 1H, -NH), 7.50 (d, J = 7.8 Hz, 2H, Ar-H), 8.09 (d, J = 8.4 Hz, 2H, Ar-H), 8.49 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 20.5, 31.4, 44.7, 111.9, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.1, 128.1 (d, $^2J_{C\text{-}F}$ = 4.2 Hz), 143.0, 155.5, 157.6, 165.9, 168.3, 168.9. HRMS m/z (ESI): Calculated for $C_{17}H_{15}ClF_3N_5O$ ([M + H]$^+$): 398.0990, found 398.0999.

*5-Bromo-6-isopropyl-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U2**).* White solid, m.p. 117−118 °C, yield 57.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.26 (d, J = 6.6 Hz, 6H, -CH_3), 3.40−3.46 (m, 1H, -CH), 4.82 (d, J = 6.0 Hz, 2H, -CH_2), 5.92−5.99 (m, 1H, -NH), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 8.10 (d, J = 8.4 Hz, 2H, Ar-H), 8.50 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 20.6, 33.8, 45.0, 104.0, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, −CF_3), 124.1, 128.1 (d, $^2J_{C\text{-}F}$ = 2.1 Hz), 143.0, 156.1, 158.4, 165.9, 168.9, 169.9. HRMS m/z (ESI): Calculated for $C_{17}H_{15}BrF_3N_5O$ ([M + H]$^+$): 442.0485, found 442.0488.

*5-Bromo-6-(1-fluoroethyl)-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U3**).* White solid, m.p. 62−63 °C, yield 52.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.69 (dd, J = 24.0 Hz, 6.0 Hz, 3H, -CH_3), 4.84 (d, J = 6.0 Hz, 2H, -CH_2), 5.84−5.88 (m, 1H, -CH), 5.98−6.06 (m, 1H, -NH), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 8.10 (d, J = 8.4 Hz, 2H, Ar-H), 8.56 (s, H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.2 (d, $J_{C\text{-}F}$ = 24.3 Hz, −$CHFCH_3$), 45.0, 87.8, 89.0, 102.6, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.3, 128.2 (d, $^2J_{C\text{-}F}$ = 4.4 Hz), 142.5, 156.8, 158.6, 161.8, 166.7, 168.8. HRMS m/z (ESI): Calculated for $C_{16}H_{12}BrF_4N_5O$ ([M + H]$^+$): 446.0234, found 442.0444.

*5-Chloro-6-(1-fluoroethyl)-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U4**).* White solid, m.p. 55−56 °C, yield 55.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.69 (dd, J = 24.0 Hz, 6.6 Hz, 3H, -CH_3), 4.84 (d, J = 6.0 Hz, 2H, -CH_2), 5.85−5.90 (m, 1H, −CH), 5.93−5.99 (m, 1H, -NH), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 8.10 (d, J = 8.4 Hz, 2H, Ar-H), 8.57 (s, H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.2 (d, $J_{C\text{-}F}$ = 24.3 Hz, -$CHFCH_3$), 44.8, 86.2, 87.4, 111.4, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.3, 128.2 (d, $^2J_{C\text{-}F}$ = 4.4 Hz), 142.5, 156.0, 158.0, 159.9, 165.7, 168.8. HRMS m/z (ESI): Calculated for $C_{16}H_{12}ClF_4N_5O$ ([M + H]$^+$): 402.0739, found 402.0740.

*6-(1-Fluoroethyl)-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)-benzyl)pyrimidin-4-amine (**U5**).* White solid, m.p. 100−101 °C, yield 51.5%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.62 (dd, J = 24.6 Hz, 6.0 Hz, 3H, -CH_3), 4.69 (s, 2H, -CH_2), 5.38−5.52 (m, 1H, -CH), 6.53 (s, 1H, Pyrimidine-H), 7.48 (d, J = 7.8 Hz, 2H, Ar-H), 8.09 (d, J = 7.8 Hz, 2H, Ar-H), 8.49 (s, H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 20.9 (d, $J_{C\text{-}F}$ = 24.3 Hz, -$CHFCH_3$), 44.9, 89.6, 90.8, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.3, 128.2 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 142.5, 156.0, 158.0, 162.8, 165.9, 168.8. HRMS m/z (ESI): Calculated for $C_{16}H_{13}F_4N_5O$ ([M + H]$^+$): 368.1129, found 368.1130.

*N-(2-Fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U6**).* White solid, m.p. 98−99 °C, yield 50.7%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.63 (dd, J = 24.0 Hz, 6.0 Hz, 3H, -CH_3), 4.75 (d, J = 6.0 Hz, 2H, -CH_2), 5.65−5.75 (m, 1H, -CH), 5.88−5.93 (m, 1H, -NH), 6.45 (s, 1H, Pyrimidine-H), 7.46 (t, J = 7.8 Hz, 1H, Ar-H), 7.67 (dd, J = 10.2 Hz, 1.2 Hz, 1H, Ar-H), 7.72 (dd, J = 7.8 Hz, 1.2 Hz, 1H, Ar-H), 8.48 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 20.9 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 44.5, 87.8, 89.0, 101.5, 112.4, 115.3 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.4, 131.4 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 157.8, 157.5, 160.3, 161.9, 165.9, 168.1. HRMS m/z (ESI): Calculated for $C_{16}H_{12}F_5N_5O$ ([M + H]$^+$): 386.1035, found 368.1130.

*5-Chloro-N-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U7**).* White solid, m.p. 54−55 °C, yield 53.1%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.66 (dd, J = 24.0 Hz, 6.0 Hz, 3H, -CH_3), 4.87 (d, J = 6.0 Hz, 2H, -CH_2), 5.84−5.95 (m, 1H, -CH), 5.98−5.99 (m, 1H, -NH), 7.54 (t, J = 7.8 Hz, 1H, Ar-H), 7.83 (dd, J = 10.2 Hz, 1.2 Hz, 1H, Ar-H), 7.88 (dd, J = 7.8 Hz, 1.2 Hz, 1H, Ar-H), 8.55 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.0 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 39.0, 86.2, 87.3, 101.5, 111.5, 114.8 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.6, 131.4 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 155.9, 157.9, 160.3, 161.9, 165.9, 168.1. HRMS m/z (ESI): Calculated for $C_{16}H_{11}ClF_5N_5O$ ([M + H]$^+$): 420.0645, found 420.0645.

*5-Bromo-N-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U8**).* White solid, m.p. 58−59 °C, yield 59.5%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.66 (dd, J = 24.0 Hz, 6.0 Hz, 3H, -CH_3), 4.87 (d, J = 6.0 Hz, 2H, -CH_2), 5.82−5.93 (m, 1H, -CH), 6.05−6.06 (m, 1H, -NH), 7.53 (t, J = 7.8 Hz, 1H, Ar-H), 7.83 (dd, J = 10.2 Hz, 1.2 Hz, 1H, Ar-H), 7.88 (dd, J = 7.8 Hz, 1.2 Hz, 1H, Ar-H), 8.55 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.3 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 39.3, 87.8, 89.0, 102.5, 114.8 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.7, 126.1, 129.5, 130.8 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 156.7, 158.6, 160.3, 161.9, 168.1. HRMS m/z (ESI): Calculated for $C_{16}H_{11}BrF_5N_5O$ ([M + H]$^+$): 464.0140, found 464.0140.

*5-Bromo-N-(3-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U9**).* White solid, m.p. 87−88 °C, yield 58.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.69 (dd, J = 24.0 Hz, 6.6 Hz, 3H, -CH_3), 4.85 (t, J = 6.0 Hz, 2H, -CH_2), 5.84−5.97 (m, 1H, -CH), 6.06−6.11 (m, 1H, -NH), 7.25 (d, J = 10.8 Hz, 1H, Ar-H), 7.29 (d, J = 8.4 Hz, 1H, Ar-H), 8.07 (t, J = 7.2 Hz, 1H, Ar-H), 8.56 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.2 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 44.5, 87.8, 89.0, 102.5, 112.4, 115.7 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.4, 131.4 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 145.3, 156.7, 158.5, 160.1,

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162.0, 166.8. HRMS m/z (ESI): Calculated for $C_{16}H_{11}BrF_5N_5O$ ([M + H]$^+$): 464.0140, found 464.0138.

*5-Chloro-N-(3-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U10**).* White solid, m.p. 83−84 °C, yield 51.5%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.68 (dd, J = 24.0 Hz, 6.6 Hz, 3H, -CH_3), 4.84 (t, J = 6.0 Hz, 2H, -CH_2), 5.86−5.97 (m, 1H, -CH), 6.02 (t, J = 6.0 Hz, 1H, -NH), 7.24 (d, J = 10.8 Hz, 1H, Ar-H), 7.28 (d, J = 8.4 Hz, 1H, Ar-H), 8.06 (t, J = 7.2 Hz, 1H, Ar-H), 8.54 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.2 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 44.2, 86.2, 87.3, 111.5, 112.5, 115.6 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.5, 131.2 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 145.3, 155.9, 157.9, 160.1, 161.8, 165.8. HRMS m/z (ESI): Calculated for $C_{16}H_{11}ClF_5N_5O$ ([M + H]$^+$): 420.0645, found 420.0640.

*5-Bromo-6-(1-fluoroethyl)-N-((5-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)pyridin-2-yl)methyl)pyrimidin-4-amine (**U11**).* White solid, m.p. 132−133 °C, yield 52.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.66 (dd, J = 24.0 Hz, 6.0 Hz, 3H, -CH_3), 4.89 (t, J = 4.8 Hz, 2H, -CH_2), 5.85−5.96 (m, 1H, -CH), 7.03 (s, 1H, -NH), 7.48 (d, J = 8.4 Hz, 1H, Py-H), 8.37 (dd, J = 8.4 Hz, 2.4 Hz, 1H, Py-H), 8.55 (s, 1H, Py-H), 9.32 (d, J = 1.8 Hz, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.4 (d, $J_{C\text{-}F}$ = 24.3 Hz, -$CHFCH_3$), 45.2, 87.8, 88.9, 102.7, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 120.3, 122.1, 135.7 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 148.3, 156.7, 158.4, 159.9, 161.7, 167.1. HRMS m/z (ESI): Calculated for $C_{15}H_{11}BrF_4N_6O$ ([M + H]$^+$): 447.0187, found 447.0186.

*5-Chloro-6-(1-fluoroethyl)-N-((5-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)pyridin-2-yl)methyl)pyrimidin-4-amine (**U12**).* White solid, m.p. 125−126 °C, yield 53.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.66 (dd, J = 24.0 Hz, 6.0 Hz, 3H, -CH_3), 4.90 (t, J = 4.8 Hz, 2H, -CH_2), 5.86−5.97 (m, 1H, -CH), 6.95 (s, 1H, -NH), 7.48 (d, J = 8.4 Hz, 1H, Py-H), 8.37 (dd, J = 8.4 Hz, 2.4 Hz, 1H, Py-H), 8.55 (s, 1H, Py-H), 9.31 (d, J = 1.8 Hz, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.1 (d, $J_{C\text{-}F}$ = 24.3 Hz, -$CHFCH_3$), 45.9, 86.1, 87.3, 111.7, 116.7 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 120.3, 122.1, 135.7 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 148.3, 155.9, 157.8, 159.9, 166.1, 167.1. HRMS m/z (ESI): Calculated for $C_{15}H_{11}ClF_4N_6O$ ([M + H]$^+$): 403.0692, found 403.0692.

*5-Chloro-N-(2,3-difluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U13**).* White solid, m.p. 120−121 °C, yield 57.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.67 (dd, J = 24.0 Hz, 6.6 Hz, 3H, -CH_3), 4.90 (d, J = 6.6 Hz, 2H, -CH_2), 5.84−5.96 (m, 1H, -CH), 6.00−6.04 (m, 1H, -NH), 7.32 (t, J = 7.2 Hz, 1H, Ar-H), 7.82 (t, J = 6.6 Hz, 1H, Ar-H), 8.56 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.2 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 38.8, 86.2, 87.3, 111.6, 114.5 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.9 (d, $J_{C\text{-}F}$ = 4.05 Hz, Ar-F), 131.8 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 148.2, 150.3, 155.9, 157.8, 160.2, 165.6, 165.9. HRMS m/z (ESI): Calculated for $C_{16}H_{10}ClF_6N_5O$ ([M + H]$^+$): 438.0551, found 438.0551.

*5-Bromo-N-(2,3-difluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(1-fluoroethyl)pyrimidin-4-amine (**U14**).* White solid, m.p. 128−129 °C, yield 58.6%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.67 (dd, J = 24.0 Hz, 6.6 Hz, 3H, -CH_3), 4.90 (d, J = 6.6 Hz, 2H, -CH_2), 5.83−5.96 (m, 1H, -CH), 6.00−6.04 (m, 1H, -NH), 7.32 (t, J = 7.2 Hz, 1H, Ar-H), 7.82 (t, J = 6.6 Hz, 1H, Ar-H), 8.56 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.3 (d, $J_{C\text{-}F}$ = 24.2 Hz, -$CHFCH_3$), 39.1, 87.8, 89.0, 102.5, 112.4, 114.5 (d, $J_{C\text{-}F}$ = 21.3 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.9 (d, $J_{C\text{-}F}$ = 4.05 Hz, Ar-F), 131.4 (d, $^2J_{C\text{-}F}$ = 31.8 Hz), 145.3, 150.0, 156.7, 158.6, 161.9, 165.3. HRMS m/z (ESI): Calculated for $C_{16}H_{10}BrF_6N_5O$ ([M + H]$^+$): 482.0046, found 482.0047.

*5-Bromo-6-(difluoromethyl)-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U15**).* White solid, m.p. 95−96 °C, yield 52.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.86 (d, J = 5.4 Hz, 2H, -CH_2), 6.12−6.19 (m, 1H, -NH), 6.74 (t, J = 53.4 Hz, 1H, -CHF_2), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 8.11 (d, J = 8.4 Hz, 2H, Ar-H), 8.59 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 45.1, 102.7, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.4, 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 142.0, 154.5, 156.8, 159.0, 165.7, 168.9. HRMS m/z (ESI): Calculated for $C_{15}H_9BrF_5N_5O$ ([M + H]$^+$): 449.9983, found 449.9993.

*5-Chloro-6-(difluoromethyl)-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U16**).* White solid, m.p. 87−88 °C, yield 51.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.86 (d, J = 6.0 Hz, 2H, -CH_2), 6.05−6.10 (m, 1H, -NH), 6.74 (t, J = 53.4 Hz, 1H, -CHF_2), 7.49 (d, J = 8.4 Hz, 1H, Ar-H), 8.11 (d, J = 7.8 Hz, 2H, Ar-H), 8.59 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 44.9, 109.9, 111.5 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 113.1, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.4, 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 142.0, 152.6, 156.1, 158.4, 166.0, 168.8. HRMS m/z (ESI): Calculated for $C_{15}H_9ClF_5N_5O$ ([M + H]$^+$): 406.0489, found 406.0492.

*6-(Difluoromethyl)-N-(4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U17**).* White solid, m.p. 120−121 °C, yield 58.4%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.73 (s, 2H, -CH_2), 6.72−5.77 (m, 1H, -NH), 6.41 (t, J = 53.4 Hz, 1H, -CHF_2), 6.66 (s, 1H, Pyrimidine-H), 7.48 (d, 2H, J = 8.4 Hz, Ar-H), 8.11 (d, 2H, J = 8.4 Hz, Ar-H), 8.61 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 44.9, 110.8, 112.4 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 114.0, 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.4, 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 142.0, 155.5, 157.6, 158.8, 166.0, 168.8. HRMS m/z (ESI): Calculated for $C_{15}H_{10}F_5N_5O$ ([M + H]$^+$): 372.0878, found 372.0878.

*5-Bromo-6-(difluoromethyl)-N-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U18**).* White solid, m.p. 93−94 °C, yield 53.2%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.89 (d, J = 6.0 Hz, 2H, -CH_2), 6.16−6.26 (m, 1H, -NH), 6.72 (t, J = 53.4 Hz, 1H, -CHF_2), 7.55 (t, J = 7.8 Hz, 1H, Ar-H), 7.85 (d, J = 9.6 Hz, 1H, Ar-H), 7.89 (d, J = 8.4 Hz, 1H, Ar-H), 8.59 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 39.4, 102.8, 110.7, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 114.8 (d, $J_{C\text{-}F}$ = 24.1 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.7, 126.3, 129.0, 130.8 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 142.0, 155.5, 157.6, 161.9, 168.0. HRMS m/z (ESI): Calculated for $C_{15}H_8BrF_6N_5O$ ([M + H]$^+$): 467.9889, found 467.9887.

*5-Chloro-6-(difluoromethyl)-N-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U19**).* White solid, m.p. 85−86 °C, yield 52.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.90 (d, J = 6.0 Hz, 2H, -CH_2), 6.10−6.16 (m, 1H, -NH), 6.73 (t, J = 53.4 Hz, 1H, -CHF_2), 7.55 (t, J = 7.8 Hz, 1H, Ar-H), 7.85 (dd, J = 10.2 Hz, 1.2 Hz, 1H, Ar-H), 7.90 (dd, J = 8.4 Hz, 1.8 Hz, 1H, Ar-H), 8.58 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 39.2, 102.8, 109.8, 111.4, 114.8 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.0 (d, $J_{C\text{-}F}$ = 24.1 Hz, -CHF_2), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 126.3, 129.1, 130.9 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 152.7, 156.0, 158.4, 160.3, 161.9, 168.0. HRMS m/z (ESI): Calculated for $C_{15}H_8ClF_6N_5O$ ([M + H]$^+$): 424.0394, found 424.0387.

*6-(Difluoromethyl)-N-(2-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U20**).* White solid, m.p. 115−116 °C, yield 53.5%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.78 (s, 2H, -CH_2), 5.60−5.75 (m, 1H, -NH), 6.41 (t, J = 53.4 Hz, 1H, -CHF_2), 6.68 (s, 1H, Pyrimidine-H), 7.54 (t, J = 7.2 Hz, 1H, Ar-H), 7.84 (d, J = 10.2 Hz, 1H, Ar-H), 7.89 (d, J = 7.8 Hz, 1H, Ar-H), 8.64 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 39.1, 109.8, 111.4, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.0 (d, $J_{C\text{-}F}$ = 24.1 Hz, -CHF_2), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.7, 129.0, 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 142.0, 155.5, 157.6, 161.9, 168.0. HRMS m/z (ESI): Calculated for $C_{15}H_9F_6N_5O$ ([M + H]$^+$): 390.0784, found 390.0784.

*5-Bromo-6-(difluoromethyl)-N-((5-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)pyridin-2-yl)methyl)pyrimidin-4-amine (**U21**).* White solid, m.p. 148−149 °C, yield 55.4%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.92 (d, J = 4.8 Hz, 2H, -CH_2), 6.77 (t, J = 53.4 Hz, 1H, -CHF_2), 7.50 (d, J = 8.4 Hz, 1H, Py-H), 8.40−8.42 (m, 1H, Py-H), 8.60 (s, 1H, Pyrimidine-H), 9.35 (d, J = 1.8 Hz, 1H, Py-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 46.1, 103.1, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 120.4, 122.2, 135.7, 148.2, 154.0, 156.8, 159.3 (d, $^2J_{C\text{-}F}$ = 73.1 Hz), 161.9, 167.1. HRMS m/z (ESI): Calculated for $C_{14}H_8BrF_5N_6O$ ([M + H]$^+$): 450.9936, found 450.9945.

*5-Chloro-6-(difluoromethyl)-N-((5-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)pyridin-2-yl)methyl)pyrimidin-4-amine (**U22**).* White solid, m.p. 130−131 °C, yield 56.1%; ^{1}H NMR ($CDCl_3$, 600 MHz)

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δ: 4.93 (d, J = 4.8 Hz, 2H, -CH_2), 6.77 (t, J = 53.4 Hz, 1H, -CHF_2), 7.16−7.22 (m, 1H, -NH), 7.51 (d, J = 8.4 Hz, 1H, Py-H), 8.41 (dd, 1H, J = 8.4 Hz, 2.4 Hz, Py-H), 8.59 (s, 1H, Pyrimidine-H), 9.34 (d, J = 1.2 Hz, 1H, Py-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 45.8, 109.7, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 120.4, 122.2, 135.7, 148.2, 152.4, 156.1, 158.2, 159.3 (d, $^2J_{C\text{-}F}$ = 73.1 Hz), 161.9, 167.1. HRMS m/z (ESI): Calculated for $C_{15}H_8ClF_6N_5O$ ([M + H]$^+$): 450.9936, found 450.9945.

*6-(Difluoromethyl)-N-((5-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)pyridin-2-yl)methyl)pyrimidin-4-amine (**U23**).* White solid, m.p. 170−171 °C, yield 50.7%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.92 (d, J = 4.8 Hz, 2H, -CH_2), 6.77 (t, J = 53.4 Hz, 1H, -CHF_2), 7.16−7.22 (m, 1H, -NH), 7.05 (s, 1H, Pyrimidine-H), 7.51 (d, J = 8.4 Hz, 1H, Py-H), 8.41 (dd, 1H, J = 8.4 Hz, 2.4 Hz, Py-H), 8.59 (s, 1H, Pyrimidine-H), 9.34 (d, J = 1.2 Hz, 1H, Py-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 45.7, 109.7, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 120.4, 122.2, 135.7, 148.2, 152.4, 156.8, 158.2, 159.3 (d, $^2J_{C\text{-}F}$ = 73.1 Hz), 161.9, 167.1. HRMS m/z (ESI): Calculated for $C_{15}H_9F_6N_5O$ ([M + H]$^+$): 390.0784, found 390.0784.

*5-Bromo-6-(difluoromethyl)-N-(3-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U24**).* White solid, m.p. 110−111 °C, yield 56.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.86 (d, J = 6.0 Hz, 2H, -CH_2), 6.19−6.26 (m, 1H, -NH), 6.74 (t, J = 53.4 Hz, 1H, -CHF_2), 7.23−7.28 (m, 1H, Ar-H), 7.29 (d, 1H, J = 7.8 Hz, Ar-H), 8.08 (t, 1H, J = 7.8 Hz, Ar-H), 8.58 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 44.6, 102.7, 110.7, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.8 (d, $J_{C\text{-}F}$ = 24.1 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.5(d, $^2J_{C\text{-}F}$ = 7.8 Hz), 131.3, 144.7, 154.6, 156.8, 159.0, 160.0, 161.9, 165.7. HRMS m/z (ESI): Calculated for $C_{15}H_8BrF_6N_5O$ ([M + H]$^+$): 467.9889, found 467.9889.

*5-Chloro-6-(difluoromethyl)-N-(3-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U25**).* White solid, m.p. 95−96 °C, yield 55.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.87 (d, J = 6.0 Hz, 2H, -CH_2), 6.11−6.18 (m, 1H, -NH), 6.75 (t, J = 53.4 Hz, 1H, -CHF_2), 7.26 (d, J = 9.6 Hz, 1H, Ar-H), 7.29 (d, 1H, J = 8.4 Hz, Ar-H), 8.08 (t, 1H, J = 7.8 Hz, Ar-H), 8.58 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 44.3, 102.7, 109.9, 111.5 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.8 (d, $J_{C\text{-}F}$ = 24.15 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.7, 131.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 144.8, 152.8, 156.0, 158.4, 160.0, 161.9, 165.7. HRMS m/z (ESI): Calculated for $C_{15}H_8ClF_6N_5O$ ([M + H]$^+$): 424.0394, found 424.0400.

*6-(Difluoromethyl)-N-(3-fluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)pyrimidin-4-amine (**U26**).* White solid, m.p. 120−121 °C, yield 53.2%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.76 (s, 2H, -CH_2), 5.45−6.87 (m, 1H, -NH), 6.42 (t, J = 53.4 Hz, 1H, -CHF_2), 6.68 (s, 1H, Pyrimidine-H), 7.25 (d, 1H, J = 10.8 Hz, Ar-H), 7.29 (d, 1H, J = 8.4 Hz, Ar-H), 8.08 (t, 1H, J = 7.2 Hz, Ar-H), 8.65 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 44.3, 110.8, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.0(d, $J_{C\text{-}F}$ = 24.15 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 123.7, 131.3 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 144.8, 152.8, 156.0, 158.4, 160.0, 161.9, 165.7. HRMS m/z (ESI): Calculated for $C_{15}H_9F_6N_5O$ ([M + H]$^+$): 390.0784, found 390.0784.

*5-Chloro-N-(2,3-difluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(difluoromethyl)pyrimidin-4-amine (**U27**).* White solid, m.p. 128−129 °C, yield 58.0%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.93 (d, J = 6.0 Hz, 2H, -CH_2), 6.13−6.19 (m, 1H, -NH), 6.73 (t, J = 53.4 Hz, 1H, -CHF_2), 7.33 (t, J = 7.2 Hz, 1H, Ar-H), 7.83 (t, 1H, J = 6.6 Hz, Ar-H), 8.58 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 38.8, 110.8, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.0 (d, $J_{C\text{-}F}$ = 24.15 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.9 (d, $J_{C\text{-}F}$ = 4.05 Hz, Ar-F), 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 129.0, 142.0, 144.8, 152.8, 155.5, 157.6, 161.9, 166.7. HRMS m/z (ESI): Calculated for $C_{15}H_7ClF_7N_5O$ ([M + H]$^+$): 442.0300, found 442.0300.

*5-Bromo-N-(2,3-difluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzyl)-6-(difluoromethyl)pyrimidin-4-amine (**U28**).* White solid, m.p. 131−132 °C, yield 58.2%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.93 (d, J = 6.0 Hz, 2H, -CH_2), 6.21−6.29 (m, 1H, -NH), 6.72 (t, J = 53.4 Hz, 1H, -CHF_2), 7.32 (t, J = 7.2 Hz, 1H, Ar-H), 7.83 (t, 1H, J = 6.6 Hz, Ar-H), 8.58 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 38.8, 110.8, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.0 (d, $J_{C\text{-}F}$ = 24.15 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.9 (d, $J_{C\text{-}F}$ = 4.05 Hz, Ar-F), 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 129.0, 142.0, 144.8, 152.8, 155.5, 157.6, 161.9, 166.7. HRMS m/z (ESI): Calculated for $C_{15}H_7BrF_7N_5O$ ([M + H]$^+$): 485.9795, found 485.9795.

*N-(2,3-Difluoro-4-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)-benzyl)-6-(difluoromethyl)pyrimidin-4-amine (**U29**).* White solid, m.p. 170−171 °C, yield 55.3%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 4.83 (s, 2H, -CH_2), 5.58−5.84 (m, 1H, -NH), 6.42 (t, J = 53.4 Hz, 1H, -CHF_2), 6.70 (s, 1H, Pyrimidine-H), 7.32 (t, 1H, J = 6.6 Hz, Ar-H), 7.29 (t, 1H, J = 6.6 Hz, Ar-H), 8.65 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 38.8, 110.8, 112.3 (t, $J_{C\text{-}F}$ = 240.0 Hz, -CHF_2), 115.0 (d, $J_{C\text{-}F}$ = 24.15 Hz, Ar-F), 116.5 (q, $J_{C\text{-}F}$ = 272.1 Hz, -CF_3), 124.9 (d, $J_{C\text{-}F}$ = 4.05 Hz, Ar-F), 128.2 (d, $^2J_{C\text{-}F}$ = 7.8 Hz), 129.0, 142.0, 144.8, 152.8, 155.5, 157.6, 161.9, 166.7. HRMS m/z (ESI): Calculated for $C_{15}H_7BrF_7N_5O$ ([M + H]$^+$): 485.9795, found 485.9795.

Bioassay for the Compounds U1−U29. The insecticidal activities of compounds **U1−U29** against *Mythimna separata*, *Aphis medicagini*, and *Tetranychus cinnabarinus* were evaluated by previously published methods.[3,37]

Insecticidal Activity. *M. separata.* Corn (*Zea mays*) leaves were fully infiltrated in the sample solution, then dried, placed in a 20 cm-diameter Petri dish with third instar larvae, covered, and placed in the observation room. Results were determined after 72 h. The test was repeated four times.

***A. medicagini*.** The foliar contact activities of compounds **U1−U29** and flufenerim against *A. medicagini* were tested. Stock solutions of compounds **U1−U29** were prepared in DMF at a concentration of 500 μg mL^{-1} and then diluted to 100, 20, 4, 0.8, and 0.16 μg mL^{-1} with water containing Tween-20. Fresh shoots of broad bean (*Vicia faba*) with 100 *A. medicagini* were dipped in the above solutions with different concentrations for 5 s, then the excess fluid was removed, and they were kept in the conditioned room for normal cultivation. Mortality was evaluated by the number of live larvae in the treated bottles relative to that in the untreated controls after 24 h. Controls were performed under the same conditions. Each test was performed in triplicate.

***T. cinnabarinus*.** Each of compounds **U1−U29** was dissolved in CH_3COCH_3 and then diluted with water containing Tween-20 to 500 or 100 mg/L. Fresh broad bean (*V. faba*) leaves infested with 100 adult *T. cinnabarinus* were dipped into the test solution and swirled for 3 s. Then, broad bean (*V. faba*) leaf was placed in a tube (10 cm inner diameter) lined with a piece of filter paper. The acaricidal activity was evaluated 4 days after treatment and repeated four times. Zero percent indicates no activity, and 100% indicates a total kill.

Greenhouse *In Vivo* Fungicidal Evaluation. Fungicidal activities of compounds **U1−U29** against *Pseudoperonospora cubensis* were tested with on potted cucumber in the greenhouse according to our previous work.[38,39] Concentrations of the positive control (azoxystrobin) and the compounds **U1−U29** were 50, 100, and 200 μg/mL. The fungi *P. cubensis* was inoculated when the cucumber was at the two-seed leaf stage. After inoculation, the plants were cultured in a climate chamber for 10 days, and the relative control efficacy was calculated by the following equation:

$$\text{relative control efficacy}(\%) = (\text{CK} - \text{PT})/\text{CK} \times 100\%$$

where CK is the average disease index during the blank assay and PT is the average disease index after treatment during testing. All experiments were replicated three times.

Acetylcholine Esterase (AChE) Activity *In Vivo* from *A. medicagini*. Stock solutions of compounds **U7**, **U8**, and positive control flufenerim were prepared in DMF at 10 μg mL^{-1} with water containing Tween-20. When the potted broad bean seedlings grew to a height of 4−5 cm, the adult aphids were inoculated on the broad bean seedlings and then the stock solution was sprayed. The adult aphids were collected and homogenized after 2 days, and then the enzyme activity test was performed with an acetylcholin esterase kit. The enzyme activity assay was performed according to the method reported by Ellman et al.[40]

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Table 2. Insecticidal Activity of Synthesized Compounds against *M. separata* and *T. cinnabarinus* (mg/L)[a]

	M. separata				*T. cinnabarinus*	
no.	500	100	20	4	500	100
U1	0	-	-	-	0	-
U2	0	-	-	-	0	-
U3	100 ± 0.0	80.3 ± 5.0	0	-	100 ± 0.0	100 ± 0.0
U4	100 ± 0.0	80.2 ± 3.1	0	-	95.2 ± 2.3	0
U5	0	-	-	-	0	-
U6	100 ± 0.0	30.3 ± 6.3	0	0	0	-
U7	100 ± 0.0	100 ± 0.0	79.8 ± 2.0	80.3 ± 7.0	90.1 ± 4.1	40.5 ± 5.0
U8	100 ± 0.0	100 ± 0.0	80.3 ± 1.3	60.2 ± 5.0	100 ± 0.0	30.4 ± 3.1
U9	70.3 ± 6.1	-	-	-	0	-
U10	100 ± 0.0	100 ± 0.0	0	0	0	-
U11	69.9 ± 4.2	-	-	-	0	-
U12	70.3 ± 5.0	-	-	-	0	-
U13	100 ± 0.0	100 ± 0.0	100 ± 0.0	0	0	-
U14	100 ± 0.0	100 ± 0.0	50.0 ± 10.0	0	0	-
U15	80.0 ± 2.0	0	0	0	0	-
U16	60.4 ± 6.1	-	-	-	0	-
U17	70.4 ± 8.2	-	-	-	0	-
U18	20.3 ± 10.1	-	-	-	0	-
U19	30.4 ± 7.0	-	-	-	80.0 ± 5.0	0
U20	20.0 ± 2.0	-	-	-	0	-
U21	0	-	-	-	0	-
U22	100 ± 0.0	0	0	0	0	-
U23	80.4 ± 3.0	0	0	0	0	-
U24	70.3 ± 5.2	-	-	-	0	-
U25	60.5 ± 4.5	-	-	-	0	-
U26	0	-	-	-	0	-
U27	100 ± 0.0	0	0	0	0	-
U28	0	-	-	-	0	-
U29	60.3 ± 5.0	-	-	-	0	-
flufenerim	100	100	100	70.0 ± 6.0	100	100
CK	0	0	0	0	0	0

[a]Note: "-" is not tested.

Molecular Docking. The structure of AChE (PDB ID: 1ODC) was downloaded from protein data bank (PDB). The protein crystal structure AChE and the small molecule **U7**, **U8**, and flufenerim were prepared by standard methods using the discovery studio 2.5. After molecular docking, the best binding mode was selected according to the results of docking energy.[41]

Density Functional Theory (DFT-B3LYP) Analysis. According to the insecticidal and fungicidal results, flufenerim and highly active compound **U7** were drawn in Gaussview 5.0 and then were optimized using DFT-B3LYP/6-31G methods according to our previous work.[42]

RESULTS AND DISCUSSION

Synthesis and Spectra. The synthetic route of pyrimidin-4-amine derivatives is illustrated in Scheme 2. There are many references on natural products and synthetic compounds about the pyrimidine derivatives. Many classic pyrimidine synthetic methods were reported, such as Pinner synthesis, Remfry-Hull synthesis, Bredereck synthesis, Beginelli synthesis, and so on. In this work, the pyrimidine ring was synthesized (Pinner synthesis) using 1,3-diketone and amidine as reaction materials. For the final target compounds, if R_3 is Cl or Br, the compounds can be synthesized easily under Et_3N conditions. When R_3 is H, the yield is lower. If the base Et_3N is changed to DIPEA, the yield is increased but still lower than when R_3 is Cl or Br.

All the pyrimidine-4-amine derivatives were confirmed and characterized by ^1H NMR, ^{13}C NMR, and HRMS. In the ^1H NMR spectra of these pyrimidines, the difluoromethyl proton signals of the compounds **U15**−**U29** can be found around 6.5 ppm as a triple peak with the coupling constant 54 Hz. The appearance of signals at 8.5 ppm is assigned to pyrimidine. The NH peaks were found at 5.5−5.8 ppm as multipeaks. The single peak at 4.8 ppm was assigned as the NCH_2 peak. In the ^{13}C NMR spectra of these pyrimidines, some carbons were split by the fluorine atom. Meanwhile, all of these pyrimidine compounds exhibited the M + H^+ peak in the ESI-HRMS results.

Biological Activity. *Insecticidal Activity.* For *M. separata*, most of the title compounds possessed good activity (>70.0%) at 500 mg/L (Table 2). Among them, compounds **U3**, **U4**, **U6**, **U7**, **U8**, **U10**, **U13**, **U14**, **U22**, and **U27** exhibited excellent activity (100%). At 100 mg/L, these compounds even possessed good activity (>80.0%), except for compounds **U6**, **U22**, and **U27**. Even at 20 mg/L, compounds **U7**, **U8**, and **U13** still exhibited good activity (>80.0%), which is the same as the positive control flufenerim (100%). Compound **U14** exhibited moderate activity (50.0%) against *M. separata* at 20 mg/L. With the concentration further reduced to 4 mg/L, the activity of compounds **U13** and **U14** disappeared, and only compounds **U7** (80.3%) and **U8** (60.2%) still exhibited good activity, which is still similar to the positive control flufenerim (70.0%) at 4 mg/L. The LC_{50} values of compounds **U7** (3.57 ± 0.42 mg/L) and **U8** (4.22 ± 0.47 mg/L) are the same as

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that of the positive control flufenerim (3.14 ± 0.73 mg/L) (Table 3).

Table 3. LC_{50} of Compounds U7, U8, and Flufenerim against *M. separata*

compound	LC_{50} (mg/L)	regression equation	r
U7	3.57 ± 0.42	$Y = 1.64X + 4.10$	0.971
U8	4.22 ± 0.47	$Y = 1.64X + 3.98$	0.993
flufenerim	3.14 ± 0.73	$Y = 0.94X + 4.50$	0.968

For *T. cinnabarinus* (Table 2), it is just the opposite of the activity against *M. separata*. Most of the compounds exhibited no activity at 500 mg/L, except for compounds **U3**, **U4**, **U7**, and **U8**. These four compounds displayed excellent activity (>90.0%) at 500 mg/L. Compound **U3** still exhibited good activity (100%) at 100 mg/L, which is similar to the positive control flufenerim (100%). However, the other three compounds **U4**, **U7**, and **U8** exhibited moderate activity (<50.0%) at 100 mg/L.

For *A. medicagini* (Table 4), compounds **U3**, **U4**, **U5**, **U6**, **U7**, **U8**, **U9**, **U10**, **U13**, **U14**, **U15**, **U16**, **U18**, and **U19** displayed good activity (>90.0%) at 500 ppm. Compounds **U3**, **U4**, **U7**, **U8**, **U9**, **U10**, and **U19** still maintained excellent activity (100%) at 100 mg/L. Surprisingly, compounds **U3**, **U7**, and **U8** still possessed excellent activity (100%) at 20 mg/L, which is still the same as the positive control flufenerim (100%). Notably, compounds **U7** and **U8** still exhibited excellent activity (100%) at 4 mg/L. When the concentration was reduced to 0.8 mg/L, the activity of compounds **U7** (80.2%) and **U8** (60.4%) was still good, which is a little weaker than that of the positive control flufenerim (100%). However, the two compounds still possessed good activity (>60.0%) at 0.16 mg/L, which is better than that of flufenerim (10.0%).

Fungicidal Activity against *P. cubensis In Vivo*. Most of the compounds (**U1**, **U2**, **U3**, **U4**, **U7**, **U8**, **U10**, **U13**, **U15**, **U16**, **U24**, **U27**, and **U28**) possessed good activity (>80.0%) against *P. cubensis* at 200 mg/L (Table 5), which is similar to the positive control azoxystrobin (95.0%). Compounds **U1**, **U2**, **U3**, **U4**, **U7**, and **U8** were selected for further screening. Among them, compounds **U3**, **U4**, **U7**, and **U8** exhibited a good control effect (>80.0%) at 100 mg/L, which is better than that of azoxystrobin (65.0%) at the same concentration. At 50 mg/L, **U7** and **U8** exhibited a good control effect (~70.0%), which is better than that of azoxystrobin (54.0%). The EC_{50} values (Table 6) were 24.94 ± 2.13, 30.79 ± 2.21, and 3.18 ± 0.21 mg/L for **U7**, **U8**, and azoxystrobin against *P. cubensis*, respectively.

Structure–Activity Relationship. Structure–activity relationship analysis indicated that both R_1 and R_2 are important

Table 4. Insecticidal Activity against *A. medicagini* (mg/L)[a]

	A. medicagini					
no.	500	100	20	4	0.8	0.16
U1	0	-	-	-	-	-
U2	0	-	-	-	-	-
U3	100 ± 0.0	100 ± 0.0	100 ± 0.0	80.3 ± 3.2	0	0
U4	100 ± 0.0	100 ± 0.0	90.0 ± 4.1	89.9 ± 6.0	0	0
U5	90.3 ± 5.2	0	0	0	-	-
U6	90.1 ± 7.2	70.2 ± 3.0	30.3 ± 10.3	0	-	-
U7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	80.2 ± 4.1	60.2 ± 5.0
U8	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	60.4 ± 2.0	60.3 ± 5.0
U9	90.2 ± 1.0	100 ± 0	40.4 ± 5.2	0	-	-
U10	100 ± 0	100 ± 0	80.0 ± 3.0	40.2 ± 4.2	0	-
U11	80.3 ± 5.0	0	0	0	-	-
U12	80.4 ± 5.2	79.7 ± 2.3	20.5 ± 1.0	0	-	-
U13	100 ± 0.0	90.2 ± 4.9	90.4 ± 2.0	60.1 ± 8.2	59.6 ± 4.4	-
U14	100 ± 0.0	70.3 ± 5.0	40.3 ± 7.9	0	-	-
U15	90.2 ± 6.2	40.1 ± 2.2	0	0	-	-
U16	100 ± 0.0	80.0 ± 4.0	0	0	-	-
U17	0	-	-	-	-	-
U18	100 ± 0.0	59.9 ± 4.0	0	0	-	-
U19	100 ± 0.0	100 ± 0.0	79.9 ± 5.1	70.2 ± 3.1	-	-
U20	80.2 ± 3.0	0	0	0	-	-
U21	0	-	-	-	-	-
U22	80.3 ± 6.2	0	0	0	-	-
U23	60.3 ± 5.3	-	-	-	-	-
U24	90.4 ± 2.9	30.1 ± 2.0	0	0	-	-
U25	0	-	-	-	-	-
U26	0	-	-	-	-	-
U27	0	-	-	-	-	-
U28	0	-	-	-	-	-
U29	0	-	-	-	-	-
flufenerim	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	10.0 ± 5.0
CK	0	0	0	0	0	0

[a]Note:"-" is not tested.

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Table 5. Control Effect of Title Compounds against *P. cubensis* (%)[a]

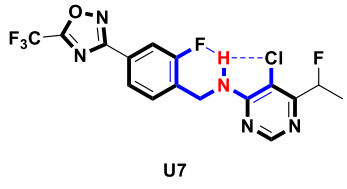
	P. cubensis		
no.	200 mg/L	100 mg/L	50 mg/L
U1	80.0 ± 8.1	5.1 ± 2.2	-
U2	100 ± 0.0	10.2 ± 3.0	-
U3	100 ± 0.0	80.2 ± 5.1	-
U4	100 ± 0.0	80.1 ± 9.9	-
U5	45.2 ± 8.1	-	-
U6	0	-	-
U7	100 ± 0.0	90.0 ± 5.0	70.2 ± 3.0
U8	100 ± 0.0	89.4 ± 2.3	67.4 ± 3.1
U9	50.0 ± 4.0	-	-
U10	90.1 ± 2.1	-	-
U11	59.8 ± 6.2	-	-
U12	29.9 ± 5.1	-	-
U13	90.2 ± 7.1	-	-
U14	60.1 ± 5.1	-	-
U15	100 ± 0.0	-	-
U16	80.3 ± 3.4	-	-
U17	20.2 ± 2.1	-	-
U18	69.8 ± 5.2	-	-
U19	100 ± 0.0	-	-
U20	70.1 ± 4.2	-	-
U21	10.3 ± 2.2	-	-
U22	30.2 ± 5.2	-	-
U23	0	-	-
U24	100 ± 0.0	-	-
U25	40.0 ± 4.0	-	-
U26	15.2 ± 2.9	-	-
U27	84.8 ± 5.2	-	-
U28	100 ± 0.0	-	-
U29	15.0 ± 3.0	-	-
azoxystrobin	90.0 ± 5.0	65.0 ± 3.0	54.0 ± 2.0
CK	0	0	0

[a]Note: "-" is not tested.

Table 6. EC_{50} of Compounds U7, U8, and Azoxystrobin against *P. cubensis*

compound	EC_{50} (mg/L)	regression equation	*r*
U7	24.94 ± 2.13	$Y = 2.34X + 1.73$	0.980
U8	30.79 ± 2.21	$Y = 2.47X + 1.33$	0.974
azoxystrobin	3.18 ± 0.21	$Y = 2.92X + 3.53$	0.983

for the activity. For the R_1 substitution, when R_1 is H or 2-F, the compounds displayed the best insecticidal and fungicidal activity. While with 2,3-diF substitution on the compounds, the activity decreased. The activity of 3-F substitution on the compounds gave the lowest activity. Hydrogen bonds may have formed between the halogen atom and the NH group (Figure 3). The intramolecular three-center hydrogen bond is



U7

Figure 3. Intramolecular hydrogen bonds between the NH and halogen atoms.

regarded as an efficient and reliable control for molecular conformation and influence for bioactivity. Therefore, the activity trend of R_1 is 2-F > H > 3-F > 2,3-diF. For the R_2 substitution, when R_2 is a CH_3CHF group, compounds always possessed excellent insecticidal and fungicidal activity (e.g., **U3**−**U14**). If the CH_3CHF group was replaced by CHF_2, the activity decreased. Furthermore, if the CH_3CHF group was changed to an iso-Pr group, the activity disappeared. The activity trends of R_2 is CH_3CHF > CHF_2 > $(CH_3)_2CH$. The halogen on the pyrimidine ring had no impact on the activity. For example, whether compounds had a Cl atom or Br atom on the pyrimidine ring, they still exhibited good insecticidal and fungicidal activity (e.g., **U3**, **U4**, **U7**, and **U8**). The activity trend of halogens is Cl > Br > H.

Mode of Action. Although IRAC did not report the mode of action of flufenerim, Ghanim[9] and co-workers reported that the mode of action of flufenerim may target AChE, so compounds **U7**, **U8**, and the positive control flufenerim were selected as representative compounds to test their inhibitory activity against AChE in vivo. The enzyme activities of compounds **U7**, **U8**, and flufenerim are 0.215, 0.184, and 0.184 U/mg prot, respectively. The results that we obtained here showed that the compounds **U7** and **U8** may have the same mode of action as flufenerim. Further biochemical and genetic assays are in progress.

Molecular Docking of U7, U8, and Flufenerim. From the reference and bioassay, the compounds **U7** and **U8** may have the same mode of action as flufenerim (AChE inhibitors), so we conducted molecular modeling analysis of compounds **U7**, **U8**, and flufenerim with *Tc*AChE. As shown in Figure 4, there were two $\pi-\pi$ interactions for compounds **U7** and **U8** between Trp84 and the 1,2,4-oxadiazole ring with distances of 4.5 and 4.6 Å, respectively. On the other hand, there was $\pi-\pi$ interaction between Tyr334 and the pyrimidine ring with distances of 5.2 Å (**U8**) or 4.8 Å (**U8**). Furthermore, there was a strong hydrogen bonding interaction (2.4 and 2.3 Å) between Tyr121 of *Tc*AChE and NH in the compounds **U7** and **U8**, respectively. For the positive control flufenerim, there were also two $\pi-\pi$ interactions between Trp84 and the pyrimidine ring with distances of 5.2 and 6.2 Å, respectively. It was also observed in many co-crystals of *Tc*AChE with Tacrine.[43] Meanwhile, there was $\pi-\pi$ interaction between Tyr334 and the benzene ring with a distance of 5.3 Å. Furthermore, there were two strong hydrogen bonds existing between Tyr121 of *Tc*AChE and the nitrogen atom of the pyrimidine ring and Asp72 of *Tc*AChE and NH of flufenerim with distances of 2.6 and 2.2 Å, respectively.

DFT Calculation of High-Activity U7 and Flufenerim. Molecular total energy (MTE), frontier orbital energy (FMO), energy gap between the HOMO and LUMO, ClogP, and TPSA of high-activity compound **U7** and flufenerim are calculated using the B3LYP method, and the results are listed in Table 7.

According to the FMO theory, LUMO and HOMO are the most important factors that affect the electron transition. LUMO can accept electrons, while HOMO can provide electrons. Hence, the frontier orbital energy, HOMO, LUMO, and energy gap can provide useful information about the biological mechanism. From Figure 5, the geometries of compound **U7** and flufenerim contain two parts: the pyrimidine ring and substituted phenyl ring. The HOMO of the compound **U7** is mainly located on the pyrimidine ring, while the LUMO of compound **U7** is located on the phenyl

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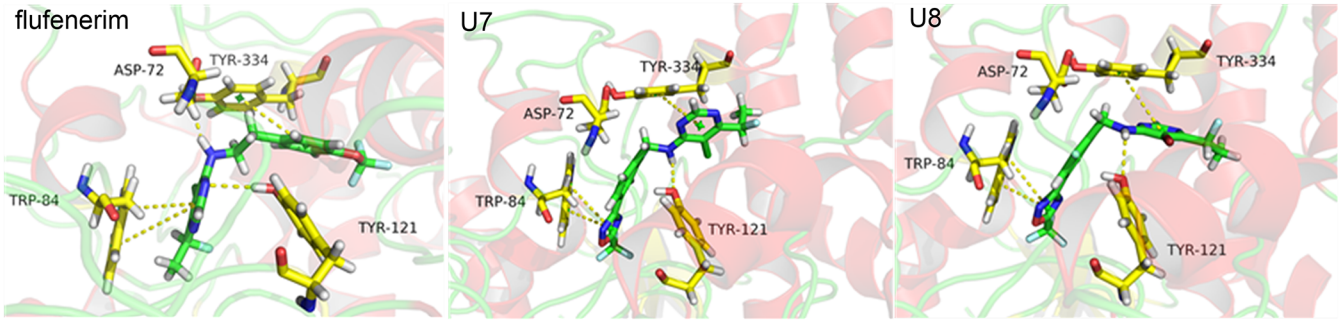

Figure 4. Simulated binding modes of compounds **U7**, **U8**, and flufenerim with *Tc*AChE.

Table 7. Total Energy and Frontier Orbital Energy

energy	flufenerim	U7
E_{total}/Hartree	−1678.635	−1924.137
E_{HOMO}/Hartree	−0.232	−0.226
E_{LUMO}/Hartree	−0.033	−0.105
ΔE^a/Hartree	0.199	0.121
TPSA	47.1	76.7
CLogP	5.04	3.87

ring and 5-(trifluoromethyl)-1,2,4-oxadiazole ring. The electron transition occurs from the pyrimidine ring to 5-(trifluoromethyl)-1,2,4-oxadiazole ring via the phenyl ring. The energy gap is 0.121 Hartree. On the other hand, the HOMO of flufenerim is mainly located on the pyrimidine ring, phenyl ring, and NCH_2CH_2 bridge, and the LUMO of flufenerim is located on the pyrimidine ring. The electron transition is from the phenyl ring to the pyrimidine ring via the NCH_2CH_2 bridge, and the energy gap is 0.199 Hartree. From Figure 5, the electron transition orientation is opposed. The total energies of the compound **U7** and flufenerim are different: compound **U7** (−1924.137 Hartree) and lead compound flufenerim (−1678.635 Hartree).

Physiochemical properties of molecules play key roles in pesticide properties.[44] The topological polar surface areas (TPSA) were calculated from the website (http://www.molinspiration.com/cgi-bin/properties), and the octanol−water partition coefficients (ClogP) were calculated by Chemdraw. It is reported[45] that the ClogP value of insecticides is 4.00 ± 2.30. The ClogP value of our positive control flufenerim is 5.04, which is higher than the average value of ClogP for commercial insecticides. The ClogP value of optimized candidate compound **U7** is 3.87, which is similar to that of commercial insecticides. From Table 7, the most suitable TPSA value may be around 50, and the TPSA of positive control flufenerim is 47.1, which was very close to 50. However, the TPSA of compound **U7** is 76.7, which is much higher. Therefore, when designing more potent insecticidal compounds in the future, perhaps keeping the ClogP values of the designed compounds around 5.0 and maintaining TPSA values near 50 will improve insecticidal activity.

Molecular Electrostatic Potential (MEP) of Flufenerim and Compound U7. Knowing the molecular electrostatic potential, mainly the van der Waals (vdW) surface of a molecule, is critical for predicting intermolecular interactions,[46,47] such as electrophilic and nucleophilic reactions, hydrogen bonding interactions, and molecular recognition processes. Besides the ESP surface, this model can help visualize charged regions (negative or positive) of a molecule in order to check the interactions between receptors and other compounds.[48] Hence, we selected flufenerim and the highly active compound **U7** to conduct ESP analysis. As shown in Figure 6, the positive regions (blue) are the phenyl ring or alkyl group of **U7** or flufenerim. The negative regions (red and yellow) mainly around the fluorine atom or the nitrogen atoms on the pyrimidine ring may play an important role between the compound and its pesticide target site receptor.

Toxicity. Acute oral toxicities were carried out at Zhejiang Changsanjiao Chemicals Safety Evaluation Co., Ltd. The compounds **U7** and **U8** were selected for testing according to the pesticide standard procedure. Unfortunately, the compounds **U7** and **U8** possessed high toxicity against rat (LD_{50}:

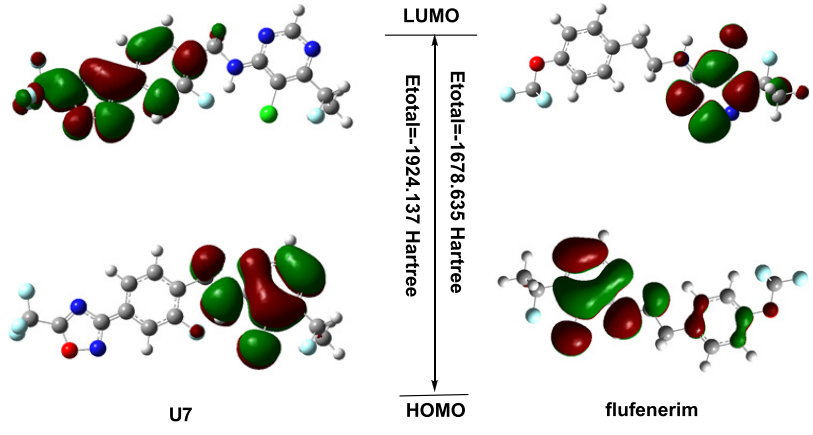

Figure 5. HOMO and LUMO of **U7** and flufenerim.

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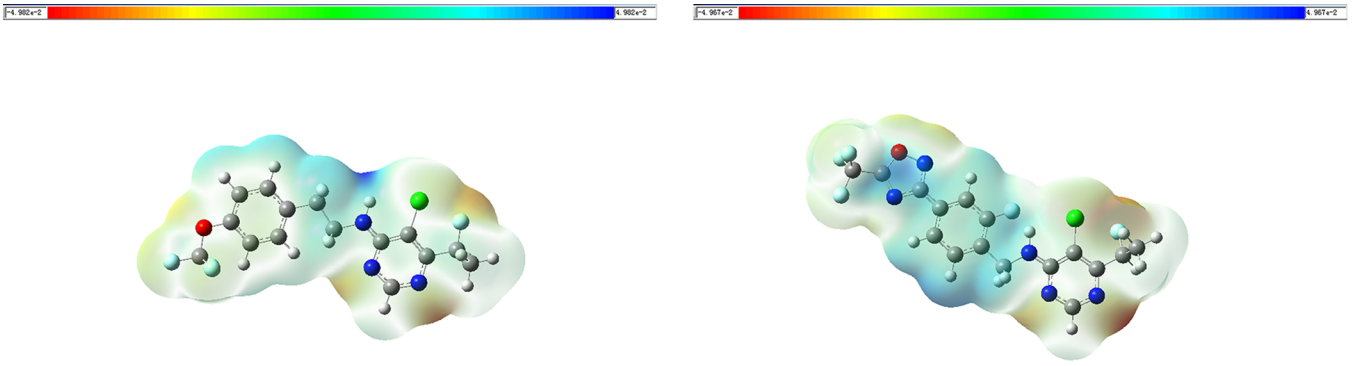

Figure 6. Molecular electrostatic potential (MEP) of flufenerim (left) and compound **U7** (right).

oral, <500 mg/kg), while the positive control flufenerim has low toxicity. Their different toxicity may be attributable to the different $\pi-\pi$ interactions and different hydrogen bonds, which had the opposite docking mode between compound **U7** or **U8** and positive control flufenerim in the active site of AChE. Also, from the results of DFT calculations of compound **U7** and flufenerim, the molecular total energy (MTE), energy gap between the HOMO and LUMO, ClogP, and TPSA of compound **U7** and flufenerim are different. Hence, the docking results and DFT calculation results provided meaningful information to design potential low-toxic pyrimidin-4-amine insecticides. Further structure optimization is undergoing according to the molecular docking model and DFT calculation results, which will be reported in the near future.

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Notes

The authors declare no competing financial interest.

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