

LETTER

Synthesis, Crystal Structure, Fungicidal Activities and Molecular Docking of Acyl Urea Derivatives Containing 2-Chloronicotine Motif^①

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ABSTRACT Eighteen new acyl urea derivatives containing 2-chloronicotine moiety were synthesized using 2-chloronicotinic acid as starting material via 4 steps conveniently. These 2-chloronicotine acyl urea structures were confirmed by ¹H NMR, ¹³C NMR and HRMS. Compound **4r** was further confirmed by X-ray diffraction. It crystallizes in the orthorhombic system, space group *Pbca* with *a* = 7.2960(3), *b* = 14.8546(6), *c* = 25.2840(11) Å, *V* = 2740.3(2) Å³, *Z* = 8, the final *R* = 0.0442 and *wR* = 0.1033 for 4028 observed reflections with *I* > 2σ(*I*). The antifungal activity results demonstrate that some of these compounds possessed good activity against *B. cinerea*, *G. zeae*, *P. piricola*, and *P. Capsici* at 50 ppm. Further molecular docking results indicated that the key group is urea bridge and pyridine ring.

Keywords: acyl urea compounds, crystal structure, synthesis, antifungal activity, docking;

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1 INTRODUCTION

Nitrogen containing heterocycle is an important nucleus in synthetic compounds or natural products because of diversity activity^[1-5]. Among the nitrogen containing heterocycles, pyridine ring possessed various activities in synthetic small molecules or natural products, such as plant growth regulatory activities^[6], anti-HIV activity^[7], herbicidal activity^[8, 9], fungicidal activity^[10, 11], anti-inflammatory activity^[12], immunosuppressive activity^[13], antioxidant activity^[14], anticancer activity^[15], and so on. Some of pyridine compounds had been commercialized as pesticides or drugs. 2-Chloro-*N*-(4'-chloro-[1,1'-biphenyl]-2-yl)nicotinamide, whose commercial name is boscalid (SDH inhibitor), is a famous fungicide to protect crops, vegetables and fruits. In this fungicide, pyridine ring and

amide group are the key groups. Urea group also had the substrate of amide, which is always found in synthetic or natural compounds^[16-19]. For instance, the chitin synthetase inhibitor dimilin contains urea group, which is an insect growth regulatory.

In our previous work, lots of heterocyclic compounds with pesticidal activity or medicinal activity were designed and synthesized^[20-30], including the pyridine derivatives. A series of fluorine substituted pyridine acyl urea derivatives were synthesized from boscalid and some compounds exhibited moderate fungicidal activity^[31]. In this work, the fluorine substituted group was replaced by other non-fluorine substitution (Fig. 1). Some of the title pyridine acyl urea derivatives exhibited good fungicidal activity. The SAR was also studied using molecular docking method.

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2 EXPERIMENTAL

2.1 Instruments

Melting points (M.P.) were measured by an X-4 apparatus and uncorrected. ^1H NMR and ^{13}C NMR spectra were tested on a Bruker AV III-500 instrument. ESI-HRMS was measured on a JOEL AccuTOF instrument. Single crystal diffraction was done on a Bruker CCD area detector diffractometer.

2.2 General procedure

The intermediates **1**, **2** and **3** were synthesized according to the reported work^[32-35].

Syntheses of target compounds **4a~4r**

To a solution of intermediate **3** (0.36 g, 2 mmol) in dichloromethane (10 mL), the substituted aniline (2.2 mmol) was added dropwise and stirred at 20 °C for overnight. TLC monitor and then solvent dichloromethane were removed, and the crude products were purified by using flash chromatograph to give compounds **4a~4r**.

2-Chloro-*N*-((2,6-dichlorophenyl)carbamoyl)-nicotinamide **4a**

White solid, yield 71.2%, m.p. 185~187 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 7.25 (t, J = 6.4 Hz, 1H, Ph), 7.40~7.42 (m, 1H, Py), 7.42 (d, J = 6.4 Hz, 2H, Ph), 8.19~8.21 (m, 1H, Py), 8.57~8.59 (m, 1H, Py), 9.26 (s, 1H, NH), 10.09 (s, 1H, NH); HRMS (ESI) for $\text{C}_{13}\text{H}_8\text{Cl}_3\text{N}_3\text{O}_2$ m/z: calculated, 343.9755; found, 343.9768 $[\text{M}+\text{H}]^+$.

2-Chloro-*N*-((3-isopropylphenyl)carbamoyl)-nicotinamide **4b**

White solid, yield 65.6%, m.p. 205~208 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 2.36 (s, 1H, CH_3), 7.16 (d, J = 6.6 Hz, 2H, Ph), 7.38 (d, J = 6.7 Hz, 2H, Ph), 7.42~7.45 (m, 1H, Py), 8.10~8.12 (m, 1H, Py), 8.60~8.62 (m, 1H, Py), 9.39 (s, 1H, NH), 10.37 (s, 1H, NH); HRMS (ESI) for $\text{C}_{14}\text{H}_{12}\text{ClN}_3\text{O}_2$ m/z: calculated, 290.0691; found, 290.0690 $[\text{M}+\text{H}]^+$.

2-Chloro-*N*-((2,6-dimethoxyphenyl)carbamoyl)-nicotinamide **4c**

White solid, yield 60.5 %, m.p. 138~141 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 1.27 (d, J = 5.6 Hz, 6H, CH_3), 2.88~2.95 (m, 1H, CH), 7.04 (d, J = 6.1 Hz, 1H, Ph), 7.25 (d, J = 6.0 Hz, 1H, Ph), 7.29 (s, 1H, Ph), 7.35 (t, J = 3.6 Hz, 1H, Ph), 7.41~7.44 (m, 1H, Py), 8.08~8.10 (m, 1H, Py), 8.59~8.60 (m, 1H, Py), 9.83 (s, 1H, NH), 10.47 (s, 1H, NH); HRMS (ESI) for $\text{C}_{16}\text{H}_{16}\text{ClN}_3\text{O}_2$ m/z: calculated, 318.1004; found, 318.1017 $[\text{M}+\text{H}]^+$.

2-Chloro-*N*-((2-methyl-4-nitrophenyl)carbamoyl)-

nicotinamide **4d**

White solid, yield 68.7%, m.p. 239~240 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 3.71 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.64~6.66 (m, 1H, Ph), 7.01 (d, J = 6.8 Hz, 1H, Ph), 7.56~7.58 (m, 1H, Py), 7.86 (s, 1H, Ph), 8.12~8.14 (m, 1H, Py), 8.55~8.56 (m, 1H, Py), 10.81 (s, 1H, NH), 11.44 (s, 1H, NH); HRMS (ESI) for $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4$ m/z: calculated, 336.0746; found, 336.0751 $[\text{M}+\text{H}]^+$.

2-Chloro-*N*-((2,4-dichlorophenyl)carbamoyl)-nicotinamide **4e**

White solid, yield 63.4%, m.p. 203~206 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 2.43 (s, 3H, CH_3), 7.58~7.61 (m, 1H, Py), 8.14~8.17 (m, 2H, Ph), 8.21 (s, 1H, Ph), 8.37~8.38 (m, 1H, Py), 8.57~8.59 (m, 1H, Py), 10.73 (s, 1H, NH), 11.74 (s, 1H, NH); HRMS (ESI) for $\text{C}_{14}\text{H}_{11}\text{ClN}_4\text{O}_4$ m/z: calculated, 335.0542; found, 335.0572 $[\text{M}+\text{H}]^+$.

2-Chloro-*N*-(phenylcarbamoyl)nicotinamide **4f**

White solid, yield 55.9%, m.p. 207~210 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 7.47~7.49 (m, 1H, Ph), 7.57~7.60 (m, 1H, Py), 7.74~7.76 (m, 1H, Ph), 8.15~8.17 (m, 1H, Py), 8.31 (d, J = 6.4 Hz, 1H, Ph), 8.57~8.58 (m, 1H, Py), 10.95 (s, 1H, NH), 11.70 (s, 1H, NH); HRMS (ESI) for $\text{C}_{13}\text{H}_8\text{Cl}_3\text{N}_3\text{O}_2$ m/z: calculated, 343.9755; found, 343.9775 $[\text{M}+\text{H}]^+$.

2-Chloro-*N*-((2-chlorophenyl)carbamoyl)nicotinamide **4g**

White solid, yield 58.9%, m.p. 166~169 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 7.17 (t, J = 5.9 Hz, 1H, Ph), 7.36 (t, J = 6.4 Hz, 2H, Ph), 7.43~7.45 (m, 1H, Py), 7.51 (d, J = 6.4 Hz, 2H, Ph), 8.11~8.13 (m, 1H, Py), 8.60~8.62 (m, 1H, Py), 9.34 (s, 1H, NH), 10.45 (s, 1H, NH); HRMS (ESI) for $\text{C}_{13}\text{H}_{10}\text{ClN}_3\text{O}_2$ m/z: calculated, 276.0534; found, 276.0527 $[\text{M}+\text{H}]^+$.

N*-((3-(*tert*-butyl)phenyl)carbamoyl)-2-chloronicotinamide **4h*

White solid, yield 48.9%, m.p. 176~179 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 7.09~7.12 (m, 1H, Ph), 7.31~7.32 (m, 1H, Py), 7.44~7.47 (m, 2H, Ph), 8.19~8.21 (m, 1H, Py), 8.24~8.26 (m, 1H, Ph), 8.61~8.62 (m, 1H, Py), 9.24 (s, 1H, NH), 11.00 (s, 1H, NH); HRMS (ESI) for $\text{C}_{13}\text{H}_9\text{Cl}_2\text{N}_3\text{O}_2$ m/z: calculated, 310.0145; found, 310.0162 $[\text{M}+\text{H}]^+$.

N*-((2,6-diethylphenyl)carbamoyl)nicotinamide **4i*

Yellow solid, yield 62.3%, m.p. 183~186 °C; ^1H NMR (CDCl_3 , 500 MHz), δ : 1.34 (s, 9H, CH_3), 7.34~7.36 (m, 1H, Py), 7.37 (s, 1H, Ph), 7.40~7.43 (m, 3H, Ph), 8.07~8.09 (m, 1H, Py), 8.59~8.61 (m, 1H, Py), 9.90 (s, 1H, NH), 10.41 (s, 1H, NH); HRMS (ESI) for $\text{C}_{17}\text{H}_{18}\text{ClN}_3\text{O}_2$ m/z: calculated,

332.1160; found, 332.1172[M+H]⁺.

2-Chloro-*N*-((4-methyl-2-nitrophenyl)carbamoyl)nicotinamide 4j

White solid, yield 61.3%, m.p. 193~195 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 1.24 (t, *J* = 6.1 Hz, 6H, CH₃), 2.64~2.69 (m, 4H, CH₂), 7.16 (d, *J* = 6.1 Hz, 2H, Ph), 7.28 (t, *J* = 6.0 Hz, 1H, Ph), 7.32~7.35 (m, 1H, Py), 8.11~8.13 (m, 1H, Py), 8.54~8.55 (m, 1H, Py), 9.33 (s, 1H, NH), 9.76 (s, 1H, NH); ¹³C NMR (CDCl₃, 125 MHz) δ: 14.93, 24.96, 123.51, 125.68, 127.97, 131.91, 133.05, 138.67, 141.73, 146.49, 151.52, 151.90, 167.86; HRMS (ESI) for C₁₇H₁₈ClN₃O₂ m/z: calculated, 332.1160; found, 332.1157[M+H]⁺.

2-Chloro-*N*-((2-methyl-3-nitrophenyl)carbamoyl)nicotinamide 4k

White solid, yield 57.1%, m.p. 215~218 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 2.44 (s, 3H, CH₃), 7.47~7.49 (m, 1H, Py), 7.50 (d, *J* = 5.6 Hz, 1H, Ph), 8.04 (s, 1H, Ph), 8.29~8.31 (m, 1H, Py), 8.50 (d, *J* = 6.8 Hz, 1H, Ph), 8.61~8.62 (m, 1H, Py), 8.78 (s, 1H, NH), 12.32 (s, 1H, NH); HRMS (ESI) for C₁₄H₁₁ClN₄O₄ m/z: calculated, 335.0542, found, 335.0572[M+H]⁺.

Methyl 4-(3-(2-chloronicotinoyl)ureido)benzoate 4l

White solid, yield 49.2%, m.p. 195~198 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 2.51 (s, 3H, CH₃), 7.39 (t, *J* = 6.5 Hz, 1H, Ph), 7.47~7.50 (m, 1H, Py), 7.65 (d, *J* = 6.2 Hz, 1H, Ph), 8.21~8.23 (m, 1H, Py), 8.26 (d, *J* = 6.1 Hz, 1H, Ph), 8.63~8.64 (m, 1H, Py), 9.00 (s, 1H, NH), 10.64 (s, 1H, NH); HRMS (ESI) for C₁₄H₁₁ClN₄O₄ m/z: calculated, 335.0542, found, 335.0557[M+H]⁺.

2-Chloro-*N*-((2,5-dichlorophenyl)carbamoyl)nicotinamide 4m

White solid, yield 65.5%, m.p. 206~209 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 3.94 (s, 3H, CH₃), 7.46~7.48 (m, 1H, Py), 7.61 (d, *J* = 6.8 Hz, 2H, Ph), 8.05 (d, *J* = 6.8 Hz, 2H, Ph), 8.13~8.15 (m, 1H, Py), 8.63~8.65 (m, 1H, Py), 9.28 (s, 1H, NH), 10.70 (s, 1H, NH); ¹³C NMR (CDCl₃, 125MHz) δ: 52.42, 119.66, 123.48, 125.07, 130.88, 131.70, 138.78, 142.39, 146.48, 150.62, 151.66, 166.20, 167.73; HRMS (ESI) for C₁₅H₁₂ClN₃O₄ m/z: calculated, 334.0589; found, 334.0610[M+H]⁺.

2-Chloro-*N*-((3,4-dimethylphenyl)carbamoyl)nicotinamide 4n

White solid, yield 65.7%, m.p. 168~171 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 2.40 (s, 3H, CH₃), 7.09~7.12 (m, 1H, Ph), 7.23 (t, *J* = 6.2 Hz, 2H, Ph), 7.42~7.44 (m, 1H, Py),

7.91 (t, *J* = 6.4 Hz, 1H, Ph), 8.13~8.15 (m, 1H, Py), 8.60~8.61 (m, 1H, Py), 9.45 (s, 1H, NH), 10.41 (s, 1H, NH); HRMS (ESI) for C₁₄H₁₂ClN₃O₂ m/z: calculated, 290.0691, found, 290.0648[M+H]⁺.

2-Chloro-*N*-((4-phenoxyphenyl)carbamoyl)nicotinamide 4o

White solid, yield 67.9%, m.p. 189~191 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 2.26 (s, 3H, CH₃), 2.27 (s, 3H, CH₃), 7.10 (d, *J* = 6.5 Hz, 1H, Ph), 7.23 (d, *J* = 6.4 Hz, 1H, Ph), 7.27 (s, 1H, Ph), 7.40~7.42 (m, 1H, Py), 8.09~8.11 (m, 1H, Py), 8.59~8.61 (m, 1H, Py), 9.49 (s, 1H, NH), 10.35 (s, 1H, NH); HRMS (ESI) for C₁₅H₁₄ClN₃O₂ m/z: calculated, 304.0847; found, 304.0837[M+H]⁺.

2-Chloro-*N*-((4-ethylphenyl)carbamoyl)nicotinamide 4p

White solid, yield 65.0%, m.p. 172~175 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 7.01~7.03 (m, 4H, Ph), 7.13 (t, *J* = 6.0 Hz, 1H, Ph), 7.34~7.38 (m, 2H, Ph), 7.43~7.45 (m, 1H, Py), 7.47~7.49 (m, 2H, Ph), 8.12~8.14 (m, 1H, Py), 8.59~8.60 (m, 1H, Py), 9.24 (s, 1H, NH), 10.61 (s, 1H, NH); HRMS (ESI) for C₁₉H₁₄ClN₃O₃ m/z: calculated, 368.0796; found, 368.0775 [M+H]⁺.

2-Chloro-*N*-(mesitylcarbamoyl)nicotinamide 4q

White solid, yield 65.7%, m.p. 133~136 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 1.25 (t, *J* = 6.1 Hz, 3H, CH₃), 2.63~2.68 (m, 2H, CH₂), 7.18 (d, *J* = 6.7 Hz, 2H, Ph), 7.42 (d, *J* = 7.2 Hz, 2H, Ph), 7.43~7.45 (m, 1H, Py), 8.11~8.13 (m, 1H, Py), 8.60~8.62 (m, 1H, Py), 9.29 (s, 1H, NH), 10.37 (s, 1H, NH); ¹³C NMR (CDCl₃, 125 MHz) δ: 16.10, 28.02, 120.46, 123.47, 128.68, 131.79, 135.46, 138.79, 139.91, 146.52, 150.66, 151.60, 167.73; HRMS (ESI) for C₁₅H₁₄ClN₃O₂ m/z: calculated, 304.0847; found, 304.0871[M+H]⁺.

2-Chloro-*N*-((2,5-dichlorophenyl)carbamoyl)nicotinamide 4r

Yellow solid, yield 54.9%, m.p. 172~175 °C; ¹H NMR (CDCl₃, 500 MHz), δ: 2.25 (s, 6H, CH₃), 2.31 (s, 3H, CH₃), 6.93 (s, 2H, Ph), 7.34~7.36 (m, 1H, Py), 8.10~8.12 (m, 1H, Py), 8.54~8.55 (m, 1H, Py), 9.40 (s, 1H, NH), 9.68 (s, 1H, NH); ¹³C NMR (CDCl₃, 125 MHz) δ: 18.49, 20.96, 123.50, 128.87, 130.00, 131.74, 135.26, 136.31, 138.70, 146.51, 151.30, 151.50, 167.69; HRMS (ESI) for C₁₆H₁₆ClN₃O₂ m/z: calculated, 318.1004; found, 318.1007[M+H]⁺.

2.3 Structure determination

A colorless crystal suitable for X-ray diffraction study was cultivated in the test tube from EtOH by self-volatilization. A

crystal with dimensions of 0.28 mm \times 0.22 mm \times 0.14 mm was mounted on a Bruker APEX-II CCD diffractometer equipped with a graphite-monochromatic MoK α radiation (λ = 0.71073 Å). Intensity data were collected at 296(2) K by using a multi-scan mode in the range of $5.48 \leq \theta \leq 60.23^\circ$ with the following index ranges: $-10 \leq h \leq 10$, $-20 \leq k \leq 20$ and $-35 \leq l \leq 35$. A total of 58508 reflections were collected and 4028 were independent (R_{int} = 0.0532). The crystal structure was solved by direct methods with SHELXS-97^[44] and refined by full-matrix least-squares refinements based on F^2 with SHELXL-97. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were located in the calculated positions and refined with a riding model. The final refinement converged at R = 0.0442 and wR = 0.1033.

2.4 Fungicidal activity

Antifungal activities of 2-chloronicotinic acyl urea compounds **4a–4r** were determined according to our previous work^[36–38].

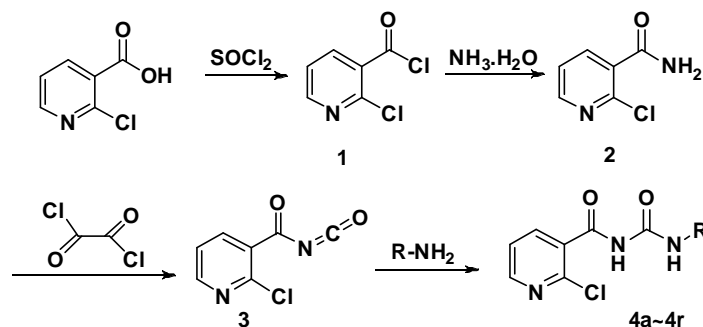
2.5 Molecular docking

Molecular docking was carried out by using DS 2.5. The active sites were generated from the crystal of SDH (PDB code: 2FBW). The detailed method was according to our previous work^[39–42].

3 RESULTS AND DISCUSSION

3.1 Synthesis and spectra analysis

The synthetic route of acyl urea compounds containing nicotinic motif is illustrated in Scheme 1. In the first step, SOCl₂ is used as solvent and reactant. A gas absorption device was used in this reaction in order to avoid environment pollution. During the second synthesis step, the diluted 2-chloronicotinoyl chloride was dropwise added into ammonia water under an ice bath slowly. Then the Py-CONCO was synthesized easily and used without purification. Finally, the target compounds were gotten as white solid under mild condition.



Scheme 1. Synthesis route of 2-chloro-N-(phenylcarbamoyl)nicotinamide compounds

These nicotinic acyl urea compounds have two amino groups, and two single peaks can be found around 9.5 and 10.5 ppm respectively. For the pyridine and benzene rings, these protons are in aromatic 6–8 ppm. For ESI-HR-MS, the measured value is according to the theoretical value within 0.003 errors.

3.2 Crystal structure

The compound 2-chloro-N-((2,5-dichlorophenyl)carbamoyl)nicotinamide **4r** was further characterized by X-ray diffraction single crystal analysis and is illustrated in Table 1 and Figs. 2 and 3. As shown in Fig. 2, the phenyl ring (C(8)–C(13)) is nearly in the same plane with pyridine ring (C(1), C(2), C(3), C(4), C(5), N(8)), in which the dihedral angle (θ) is 15.1° with plane equation $6.991x - 0.591y + 7.169z = 3.9984$ and $6.214x - 0.467y + 13.226z = 8.0225$ respectively, and the largest deviations from the least-

squares plane are 0.0062 and 0.0091 nm. The torsion angles of C(2)–C(6)–N(1)–N(2), C(6)–N(1)–C(7)–N(2) and N(1)–C(7)–N(2)–C(8) are $176.43(15)^\circ$, $-1.2(3)^\circ$ and $-175.91(15)^\circ$ respectively, which showed the acyl urea bridge is nearly in the same plane. The torsion angles of O(1)–C(6)–N(1)–C(7) and O(2)–C(7)–N(1)–C(6) are $-5.3(3)^\circ$ and $3.5(3)^\circ$ respectively, which indicated the two C=O groups are in the opposite orientations and nearly in the same plane. Compound **4r** has four intermolecular hydrogen bonds and one intramolecular hydrogen bond. The parameters of five hydrogen bonds are listed in Table 2. They are linked together by N–H \cdots N, C–H \cdots Cl, C–H \cdots O and N–H \cdots O. From Fig. 1, the intramolecular N(2)–H(2) \cdots O(1) hydrogen bond formed a six-membered ring in molecule **4r**. The hydrogen bonding interactions strengthen the integration of the 3D networks.

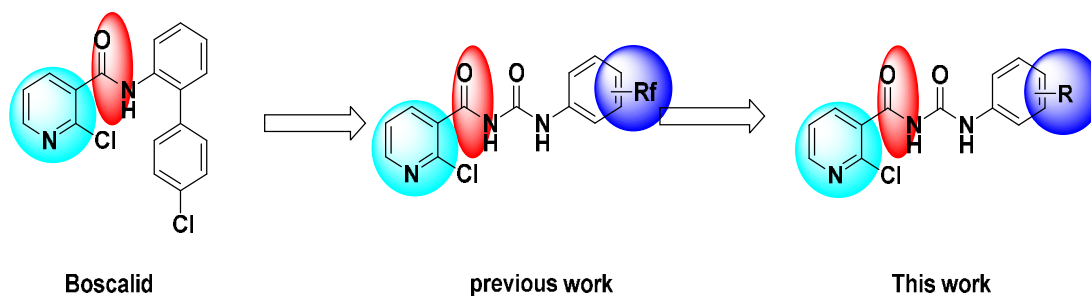


Fig. 1. Design method of nicotinic acyl urea derivatives

Table 1. Selected Bond Lengths (Å) and Bond Angles (°) for the Title Compound 4r

Bond	Dist.	Angle	(°)
Cl(1)–C(1)	1.7302(16)	C(1)–N(1)–C(7)	128.30(13)
Cl(2)–C(12)	1.7359(19)	C(7)–N(2)–C(8)	123.34(14)
N(1)–C(6)	1.358(2)	N(8)–C(1)–Cl(1)	114.20(12)
O(1)–C(6)	1.217(2)	N(8)–C(1)–C(2)	124.59(14)
O(2)–C(7)	1.208(2)	O(2)–C(7)–N(1)	119.04(14)
N(1)–C(7)	1.4060(19)	O(2)–C(7)–N(2)	125.53(15)
N(2)–C(7)	1.352(2)	O(1)–C(6)–N(1)	124.04(14)
N(2)–C(8)	1.4055(19)	O(1)–C(6)–N(2)	120.1(2)
N(8)–C(1)	1.321(2)	C(3)–C(2)–C(1)	116.33(14)
C(1)–C(2)	1.393(2)	C(8)–C(9)–Cl(3)	119.90(13)
C(2)–C(3)	1.390(2)	C(8)–C(9)–C(10)	121.01(17)

Table 2. Hydrogen-bond Parameters (Å) of the Title Compound 4r

D–H⋯A	d(D–H)	d(H⋯A)	d(D⋯A)	∠(DHA)
N(1)–H(1)⋯N(8) ⁱ	0.86	2.23	3.024(19)	153
N(2)–H(2)⋯O(1)	0.86	1.98	2.661(18)	135
C(4)–H(4)⋯Cl(2) ⁱⁱ	0.93	2.95	3.868(18)	169
C(5)–H(5)⋯Cl(1) ⁱⁱⁱ	0.93	2.98	3.855(17)	157
C(5)–H(5)⋯O(2) ^{iv}	0.93	2.51	3.196(2)	131

Symmetry transformations used to generate the equivalent atoms: ⁱ $-1/2+x, 3/2-y, 1-z$; ⁱⁱ $y, 1+y, z$; ⁱⁱⁱ $1/2-x, 1/2+y, z$; ^{iv} $1/2+x, 3/2-y, 1-z$

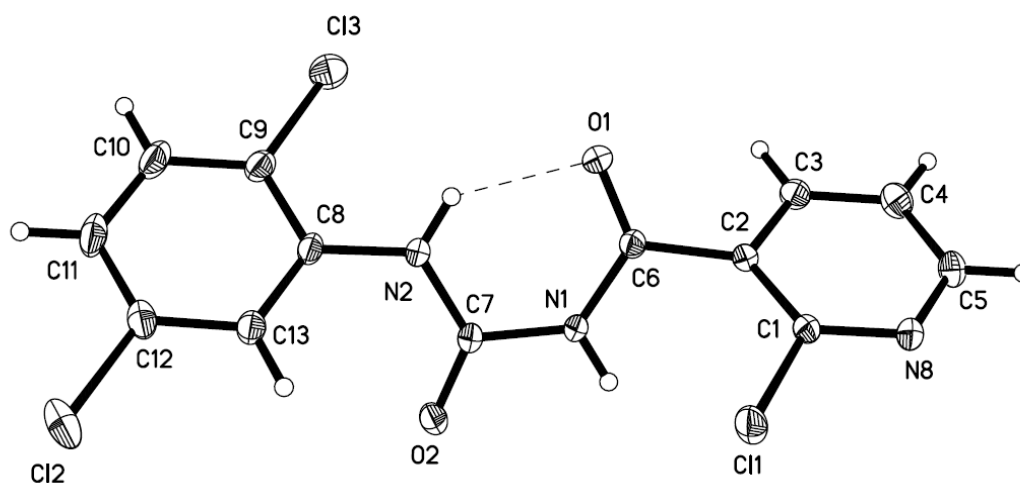


Fig. 2. Molecular structure of the title compound 4r

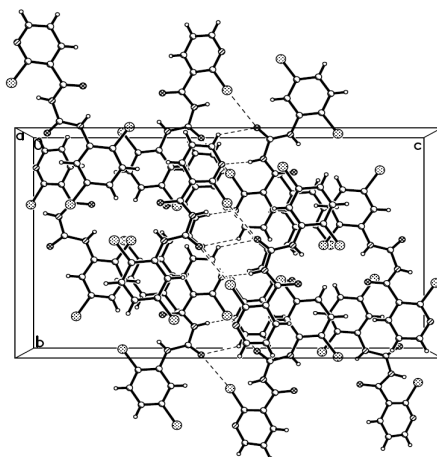


Fig. 3. Packing of the title compound **4r**

3.3 Fungicidal activity

Antifungal activities of compounds **4a~4r** and positive control fluxapyroxad against *Sclerotinia sclerotiorum* (SS), *Phytophthora infestans* (PI), *Fusarium oxysporum* (FO), *Phytophthora capsici* (PC), *Gibberella zeae* (GZ), *Rhizoctonia solani* (RS), *Cercospora arachidicola* (CA), *Alternaria solani* (AS), *Physalospora piricola* (PP) and *Botrytis cinerea* (BC) were tested at 50 ppm according to published method^[42], with the results shown in Table 3. The antifungal activity results showed some compounds exhibited good inhibitory against *B. Cinerea*, *G. zeae*, *P. capsici*, and *P. piricola*. Compounds **4e**, **4f** and **4i** still possessed good inhibitory (> 63%) against *B. cinerea*, which is the same as fluxapyroxad. Most of the title compounds

show good inhibitory against *P. piricola*, such as compounds **4b**, **4d**, **4h**, **4q**, **4r** and **4s**. For the *G. zeae*, four compounds (**4f**, **4g**, **4i**, and **4q**) exhibited good activity (57.1%). But the control displayed weak activity (28.6%) against *G. zeae*. There is no significant fungicidal activity against *A. solani*, *F. oxysporum*, *P. infestans*, *R. solani*, and *C. arachidicola*. For *F. oxysporum*, only compounds **4g** (40%) and **4r** (40%) exhibited moderate activity, which is the same as the positive control (29.4%). As the same as *C. arachidicola*, most of these compounds exhibited low activity (< 40%), which are weaker than the positive control (100%). For the *P. capsici*, compound **4b** (70%) exhibited good activity, while the others exhibited low activity.

Table 3. Fungicidal Activity of the Title Compounds 4a~4r at 50 $\mu\text{g/mL}$

[illegible]

3.4 Docking study

In order to study the mode of action of these compounds, the molecular docking was carried out between compound **4i** and the enzyme SDH (PDB:2FBW) using DS 2.5. The docking results indicated that compound **4i** can well occupy active site of SDH (Fig. 4). From Fig. 2, a π -cation interaction

exists between compound **4i** and Arg 43 amino acid residue of SDH with the distance of 3.9 Å. There is another hydrogen bond (distance 2.0 Å) between the C=O of compound **4i** and Trp 173 amino acid residue of SDH. From the docking, the pyridine ring and amide group are key active group in this fungicide, which is the same as the lead compound boscalid.

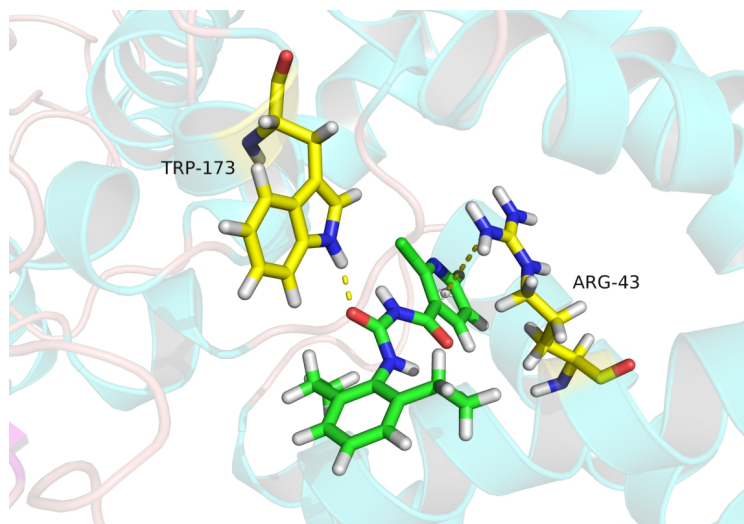


Fig. 4. Docking mode of compound **4i** and SDH

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