1－置換フェニル－3－（5－ハロベンゾイミダゾール－2－イル）アシル尿素の合成と抗真菌活性

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Note

Synthesis and antifungal activity of 1-substitutedphenyl-3-(5-halobenzimidazol-2-yl) acylurea

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Twelve novel compounds of 1-substitutedphenyl-3-(5-halobenzimidazol-2-yl) acylurea were synthesized by the reaction of 5-halobenzimidazol-2-yl acylisocyanate with substituted anilines in yields of 62.4–76.1%. The structures of the title compounds were characterized by IR, ^{1}H NMR spectra and elemental analysis. Antifungal activities against *Botrytis cinerea* and *Sclerotinia sclerotiorum* were also evaluated by the mycelium growth rate method in the lab. The results indicated that most of the title compounds exhibited excellent antifungal activities against *Sclerotinia sclerotiorum*, and their activities were higher than that of thiophanate-methyl. © Pesticide Science Society of Japan

Keywords: benzimidazole derivatives; acylurea; synthesis; antifungal activity.

Introduction

Benzimidazole derivatives exhibit broad spectrum of biological activities, such as antifungal,[1] antibacterial,[2] antiviral,[3] and antitumoral activities,[4] and thus play an important role in agricultural and pharmaceutical fields. In the pesticide field, benzimidazole fungicides with carbendazim, benomyl and thiabendazole, for example, are still used as fungicides due to their high efficiency, broad spectrum and good systemic property.[5] However, owing to enhancement of the resistance of fungal pathogens, their antifungal activities are decreasing. In order to reproduce the vigor and vitality of this classical fungicide, it is necessary to develop novel benzimidazole compounds.

On the other hand, many benzoylureas, such as hexaflumuron, diflubenzuron and teflubenzuron, show excellent insecticidal activity,[6] while others exhibit fungicidal,[7] and plant growth-regulating activities.[8] In order to seek new benzimidazole derivatives with highly efficient antifungal activity, we linked the *N*-phenyl acylurea moiety of benzoylurea to benzimidazole according to the sub-structure link method, and synthesized twelve novel 1-substitutedphenyl-3-(5-halobenzimidazol-2-yl) acylureas. The synthesis pathway is shown in Fig. 1.

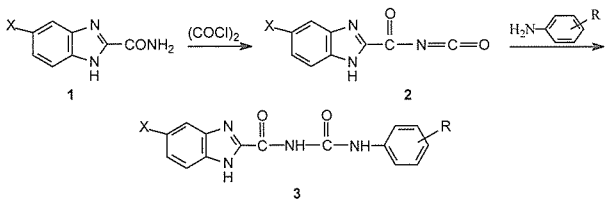


Fig. 1. Synthesis pathway of title compounds.

Materials and Methods

1. *Instruments and reagents*

The melting point was measured on an XRC-1 microscopic melting-point apparatus and was uncorrected. IR spectrum was recorded in KBr pellets on a Shimadzu IR-440 infrared spectrophotometer. ^{1}H NMR spectrum was measured on a Bruker AC-400 spectrophotometer (DMSO-d_6 as solvent, TMS as internal standard), and elemental analysis was performed with a Vario EL III Elemental Analyzer.

5-Chlorobenzimidazol-2-yl acylamide and 5-fluorobenzimidazol-2-yl acylamide (**1**) were prepared in our lab according to the reference.[9] 1,2-Dichloroethane and *N*,*N*-dimethyl formamide (DMF) were dried with molecular sieves before use.

2. *Chemical synthesis*

General procedure for synthesizing the title compounds.[8] Oxalyl chloride (1.5 g, 12 mmol) was slowly added to a mixture of 6 mmol 5-halobenzimidaol-2-yl acylamide in 15 ml anhydrous 1,2-dichloroethane with stirring in an ice-water bath. The reaction mixture was stirred at 50–55°C for 3 hr, and then refluxed for 6 hr. After removing the solvent and the excess oxalyl chloride under reduced pressure, a yellow solid of 5-halobenzimidaol-2-yl acylisocyanate (**2**) was obtained. The intermediate was directly used in the subsequent reaction without purification.

The above isocyanate was dissolved in 10 ml dry DMF, and a solution of 5.4 mmol (substituted) aniline in 5 ml DMF was then slowly added. After 4 hr of stirring at 60–70°C, the solvent was evaporated under reduced pressure to give the crude product, which was purified by recrystallization from a mixed solvent of DMF and toluene (V:V=1:2). Using this procedure, yellow powders of **3a**–**3l** were obtained.

1-Phenyl-3-(5-chlorobenzimidazol-2-yl) acylurea (3a). Yield: 73.2%. mp 224–226°C. IR (KBr) cm^{-1}: 3440, 3324, 3094, 1762, 1687. ^{1}H NMR, δ (DMSO-d_6): 14.01 (1H, s, Het–NH), 11.70 (1H, s, NH), 11.05 (1H, s, NH), 7.18–7.96 (8H, m, Ph–H). Anal. Found: C, 57.01; H, 3.74; N, 17.65%. Calcd. for $C_{15}H_{11}ClN_4O_2$: C, 57.25; H, 3.52; N, 17.80%.

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*1-(4-Fluorophenyl)-3-(5-chlorobenzimidazol-2-yl) acylurea (**3b**)*. Yield: 74.7%. mp 249–251°C. IR (KBr) cm^{-1}: 3426, 3329, 3082, 1762, 1688. ^1H NMR, δ (DMSO-d_6): 13.98 (1H, s, Het–NH), 11.67 (1H, s, NH), 11.10 (1H, s, NH), 7.22–7.96 (7H, m, Ph–H). Anal. Found: C, 53.87; H, 3.31; N, 17.02%. Calcd. for $C_{15}H_{10}ClFN_4O_2$: C, 54.15; H, 3.03; N, 16.83%.

*1-(4-Chlorophenyl)-3-(5-chlorobenzimidazol-2-yl) acylurea (**3c**)*. Yield: 70.3%. mp 272–274°C. IR (KBr) cm^{-1}: 3448, 3299, 3154, 1759, 1694. ^1H NMR, δ (DMSO-d_6): 13.93 (1H, s, Het–NH), 11.65 (1H, s, NH), 11.11 (1H, s, NH), 7.42–7.96 (7H, m, Ph–H). Anal. Found: C, 51.34; H, 3.03; N, 16.29%. Calcd. for $C_{15}H_{10}Cl_2N_4O_2$: C, 51.60; H, 2.89; N, 16.04%.

*1-(3,4-Dichlorophenyl)-3-(5-chlorobenzimidazol-2-yl) acylurea (**3d**)*. Yield: 64.8%. mp 257–259°C. IR (KBr) cm^{-1}: 3448, 3322, 3162, 1763, 1694 . ^1H NMR, δ (DMSO-d_6): 13.97 (1H, s, Het–NH), 11.63 (1H, s, NH), 11.34 (1H, s, NH), 7.38–8.18 (6H, m, Ph–H). Anal. Found: C, 46.64; H, 2.59; N, 15.00%. Calcd. for $C_{15}H_9Cl_3N_4O_2$: C, 46.97; H, 2.36; N, 14.60%.

*1-(4-Bromophenyl)-3-(5-chlorobenzimidazol-2-yl) acylurea (**3e**)*. Yield: 66.0%. mp 258–260°C. IR (KBr) cm^{-1}: 3448, 3299, 3163, 1762, 1694. ^1H NMR, δ (DMSO-d_6): 13.97 (1H, s, Het–NH), 11.66 (1H, s, NH), 11.15 (1H, s, NH), 7.42–7.95 (7H, m, Ph–H). Anal. Found: C, 45.53; H, 2.38; N, 14.60%. Calcd. for $C_{15}H_{10}BrClN_4O_2$: C, 45.77; H, 2.56; N, 14.23%.

1-(4-Nitrophenyl)-3-(5-chlorobenzimidazol-2-yl) acylurea *(**3f**)*. Yield: 62.7%. mp 286–288°C. IR (KBr) cm^{-1}: 3452, 3317, 3096, 1761, 1697. ^1H NMR, δ (DMSO-d_6): 14.00 (1H, s, Het–NH), 11.68 (1H, s, NH), 11.58 (1H, s, NH), 7.41–8.30 (7H, m, Ph–H). Anal. Found: C, 50.29; H, 3.10; N, 19.68%. Calcd. for $C_{15}H_{10}ClN_5O_4$: C, 50.08; H, 2.80; N, 19.46%.

*1-Phenyl-3-(5-fluorobenzimidazol-2-yl) acylurea (**3g**)*. Yield: 73.6%. mp 228–230°C. IR (KBr) cm^{-1}: 3460, 3328, 3145, 1766, 1690. ^1H NMR, δ (DMSO-d_6): 13.97 (1H, s, Het–NH), 11.76 (1H, s, NH), 11.06 (1H, s, NH), 7.20–7.96 (8H, m, Ph–H). Anal. Found: C, 60.65; H, 4.00; N, 18.57%. Calcd. for $C_{15}H_{11}FN_4O_2$: C, 60.40; H, 3.72; N, 18.78%.

*1-(4-Fluorophenyl)-3-(5-fluorobenzimidazol-2-yl) acylurea (**3h**)*. Yield: 76.1%. mp 237–238°C. IR (KBr) cm^{-1}: 3452, 3331, 3129, 1760, 1694. ^1H NMR, δ (DMSO-d_6): 13.92 (1H, s, Het–NH), 11.67 (1H, s, NH), 11.10 (1H, s, NH), 7.22–7.96 (7H, m, Ph–H). Anal. Found: C, 57.13; H, 3.42; N, 17.28%. Calcd. for $C_{15}H_{10}F_2N_4O_2$: C, 56.97; H, 3.19; N, 17.71%.

*1-(4-Chlorophenyl)-3-(5-fluorobenzimidazol-2-yl) acylurea (**3i**)*. Yield: 71.8%. mp 255–257°C. IR (KBr) cm^{-1}: 3453, 3297, 3129, 1760, 1694. ^1H NMR, δ (DMSO-d_6): 13.91 (1H, s, Het–NH), 11.66 (1H, s, NH), 11.15 (1H, s, NH), 7.25–7.95 (7H, m, Ph–H). Anal. Found: C, 54.11; H, 3.33; N, 16.97%.Calcd. for $C_{15}H_{10}FClN_4O_2$: C, 54.15; H, 3.03; N, 16.83%.

*1-(3,4-Dichlorophenyl)-3-(5-fluorobenzimidazol-2-yl) acylurea (**3j**)*. Yield: 62.4%. mp 248–250°C. IR (KBr) cm^{-1}: 3432, 3319, 3125, 1763, 1693. ^1H NMR, δ (DMSO-d_6): 13.95 (1H, s, Het–NH), 11.64 (1H, s, NH), 11.32 (1H, s, NH), 7.26–8.18 (6H, m, Ph–H). Anal. Found: C, 48.97; H, 2.75; N, 15.52%. Calcd. for $C_{15}H_9FCl_2N_4O_2$: C, 49.06; H, 2.47; N, 15.25%.

*1-(4-Bromophenyl)-3-(5-fluorobenzimidazol-2-yl) acylurea (**3k**)*. Yield: 65.9%. mp 266–268°C. IR (KBr) cm^{-1}: 3465, 3328, 3155, 1763, 1694. ^1H NMR, δ (DMSO-d_6): 13.91 (1H, s, Het–NH), 11.66 (1H, s, NH), 11.14 (1H, s, NH), 7.26–7.95 (7H, m, Ph–H). Anal. Found: C, 47.53; H, 2.96; N, 15.01%. Calcd. for $C_{15}H_{10}BrFN_4O_2$: C, 47.77; H, 2.67; N, 14.85%.

*1-(4-Nitrophenyl)-3-(5-fluorobenzimidazol-2-yl) acylurea (**3l**)*. Yield: 68.4%. mp 271–273°C. IR (KBr) cm^{-1}: 3461, 3318, 3121, 1763, 1697. ^1H NMR, δ (DMSO-d_6): 13.94 (1H, s, Het–NH), 11.67 (1H, s, NH), 11.56 (1H, s, NH), 7.27–8.29 (7H, m, Ph–H). Anal. Found: C, 52.31; H, 3.09; N, 20.09%. Calcd. for $C_{15}H_{10}FN_5O_4$: C, 52.49; H, 2.94; N, 20.39%.

3. *Antifungal activity assays*

The *in vitro* antifungal activities of **3a–3l** against *Botrytis cinerea* and *Sclerotinia sclerotiorum*, which were both collected from tomato and cucumber plants, respectively, in Xintai city, China, and were susceptible to thiophanate-methyl,[10] were evaluated using the mycelium growth rate method in the lab.[11a] Culture media, with known concentrations of the test compounds (10, 25, 50, 100, 200 mg/l), were obtained by mixing the solution of **3a–3l** with potato dextrose agar (PDA), on which fungus cakes were placed. The cultures were carried out at 25±1°C for 96 hr. Thiophanate-methyl, a commercial fungicide, was used as a control, and sterile water was used as a blank. Three replications were performed in antifungal activity assays. The inhibition rate was expressed as the mean of values obtained in three independent experiments. Lastly, effective concentrations (EC_{50}) that inhibited mycelium growth by 50% and toxicity regression equations were obtained according to Finney's probability value method,[11b] and given satisfactory regression equations with a correlation coefficient of 0.9505–0.9973. The EC_{50} values were shown in Table 1. The inhibition rate was calculated according to the formula:

$$\text{Inhibition rate} = \frac{D_0 - D_1}{D_0} \times 100\ (\%)$$

In which D_0 is the expansion diameter of mycelia in the blank test, and D_1 is the expansion diameter of mycelia in the presence of tested compounds.

Results and Discussion

1. *Synthesis*

Acylisocyanate is usually prepaired in the lab by the reaction of acylamide with oxalyl chloride; however, some by-products, such as *N*-acyl oxalylamide and a polymer of isocyanate, will be produced if the dosage of oxalyl chloride is insufficient or the reaction temperature is inappropriate.[12,13] To reduce *N*-acyl oxalylamide, the amount of oxalyl chloride was doubled and a lower temperature (0°C) was maintained during the dropwise addition. Moreover, 5-halobenzimidaol-2-yl acylisocynate (**2**) was directly used in the subsequent reaction without purification in order to prevent its polymerization or decomposition.

2. *Antifungal activity*

As shown in Table 1, title compounds **3e** and **3i** exhibited good antifungal activities against *B. cinerea*, in which EC_{50} values

Table 1. Antifungal activities of compounds **3a–3l** against *Botrytis cinerea* and *Sclerotinia sclerotiorum* (*in vitro*)

Compd.	X	R	EC_{50} (μg/ml), 95% CL	
			Botrytis cinerea	*Sclerotinia sclerotiorum*
3a	Cl	H	22.40 (5.79–329.30)	11.65 (5.77–48.70)
3b	Cl	4-F	8.76 (3.54–204.89)	6.23 (4.78–8.69)
3c	Cl	4-Cl	18.87 (9.43–65.11)	6.05 (4.27–9.76)
3d	Cl	3,4-Cl_2	12.65 (4.12–340.11)	5.50 (4.39–7.23)
3e	Cl	4-Br	3.94 (2.85–6.59)	9.28 (4.70–39.55)
3f	Cl	4-NO_2	30.72 (11.40–350.00)	9.14 (5.65–19.76)
3g	F	H	19.94 (6.35–129.86)	6.55 (4.14–13.79)
3h	F	4-F	10.94 (5.04–53.10)	10.94 (9.87–13.98)
3i	F	4-Cl	4.65 (2.21–31.32)	14.61 (10.96–20.77)
3j	F	3,4-Cl_2	11.55 (2.93–32.08)	9.03 (5.3–21.71)
3k	F	4-Br	13.63 (4.57–489.10)	8.66 (6.86–11.52)
3l	F	4-NO_2	10.38 (5.08–34.75)	8.71 (7.29–10.70)
Thiophanate-methyl			1.03 (0.81–1.32)	10.46 (5.41–40.50)

EC_{50}: Concentration that inhibited mycelium growth by 50%; 95% CL: 95% confidence interval of EC_{50}.

were 3.94 and 4.65 μg/ml, respectively, but their activities were still lower than that of the control fungicide (thiophanate-methyl). On the other hand, except **3a**, **3h** and **3i**, EC_{50} values of the other nine compounds were from 5.50 to 9.28 μg/ml against *S. sclerotiorum*, all less than that of thiophanate-methyl (EC_{50} 10.46 μg/ml), which means that they displayed significant antifungal activities against *S. sclerotiorum*.

In addition, we synthesized analogues of the title compounds, 1-substitutedphenyl-3-benzimidazol-2-yl acylurea,[14] in which EC_{50} values ranged from 28.46 to 73.74 μg/ml against *B. cinerea* and from 17.93 to 87.37 μg/ml against *S. sclerotiorum*. Compared to their analogues, EC_{50} values of the title compounds were lower, which revealed the title compounds exhibited higher antifungal activities. From this result, it was deduced that the activity of this type of compound is increased when chlorine or fluorine atom is introduced into the 5-position of the benzimidazole ring.

References

1) H. M. F. Madkour, A. A. Farag, S. S. Ramses and N. A. A. Ibrahiem: *Phosphorus, Sulfur, and Silicon* **181**, 255–264 (2006).
2) Z. A. Kaplancikli, G. Turan-Zitouni, G. Revial and K. Guven: *Arch. Pharm. Res.* **27**, 1081–1084 (2004).
3) P. L. Beaulieu, J. Gillard and D. Bykowski: *Bioorg. Med. Chem. Lett.* **16**, 4987–4993 (2006).
4) A. Seaton, C. Higgins and J Mann: *Eur. J. Cancer* **39**, 2548–2555 (2003).
5) Y. Zhang, S. Yang, B. A. Song and D. Y. Hu: *Agrochemicals* **47**, 164–170 (2008) (in Chinese).
6) D. S. Mao, J. Lin and F. C. Liu: *Pestcides* **40**(4), 6–12 (2001) (in Chinese).
7) X. L. Yang, Y. Ling, D. Q. Wang and F. H. Chen: *Chin. J. Synth. Chem.* **10**, 510–512 (2002) (in Chinese).
8) X. J. Song, G. R. Feng, C. B. Chen, Z. Y. Zhang and Y. G. Wang: *Chin. J. Org. Chem.* **25**, 1587–1590 (2005) (in Chinese).
9) J. H. Du, J. W. Yin, S. Q. Xia and Y. Jiang: *J. Chin. Pharm. Univ.* **29**, 243–246 (1998) (in Chinese).
10) Z. Ding, F. Liu, L.Y. Mu: *Chin. J. Pesticide Sci.* **3**(4), 59–63 (2001) (in Chinese).
11) L. Y. Mu: "*Research Method of Plant Chemical Protection*," China Agriculture Press, Beijing, China. 11a: pp. 76–82, 11b: pp. 41–45 (1994) (in Chinese).
12) A. J. Speziale and L. R. Smith: *J. Org. Chem.* **28**, 1805–1811 (1963).
13) J. T. Wei, X. S. Tan, D. M. Tian and Z. F. Zhu: *Hunan Chem. Ind.* **30**(5), 17–19 (2000) (in Chinese).
14) D. K. Zhang, F. Liu, W. W. Ji and L. Jiang: *Chem. Res. Appl.* **20**, 286–289 (2008) (in Chinese).

的で，OTFP処理を解除することによって，EGA反応の強度および再分極時間はともに回復した．非フッ素化類縁体3-オクチルチオプロパン-2-オン（3-octhylthiopropan-2-one, OTP）は，フェロモンに対するEGA反応の強度にも2/3 RTにも影響が無く，この種の化合物におけるフッ素原子の重要性を示唆した．本研究の結果は，将来に向けてFAW制御におけるOTFPの応用に結び付くであろう．

（文責：編集事務局）

インド各地におけるネッタイイエカ *Culex quinquefasciatus* の殺虫剤抵抗性

Dev: Shankar Suman, Sachin N. Tikar, Brahma Dutta Parashar, Shri Prakash

ネッタイイエカ *Culex quinquefasciatus* は，リンパフィラリア症の主要媒介体として知られている．ここでは，インドにおけるネッタイイエカ *Culex pipiens quinquefasciatus* Say, 1823の4種，Jodhpur（JD），Bikaner（BKN），Jamnagar（JMN）およびBathinda（BTH）を用いて，実験室で飼育している感受性種と比較しながら，テメホス，フェンチオン，*Bacillus thuringiensis israilensis* とNeemarin（主成分アザディラクチン）などの殺幼虫剤，およびシペルメトリン（cypermethrin），アルファシペルメトリン（alpha cypermethrin）とラムダシハロトリン（lambda cyhalothrin）などの殺成虫剤に対する抵抗性について報告する．野外種と実験室種の間のLC_{50}について抵抗性比（RR）を表した．JD種は，テメホス，フェンチオン，Neemarinおよびシペルメトリンに対して，それぞれ10.8，6.94，5.29と2.82倍の抵抗性を示した．BTH種は，テメホス，フェンチオン，ラムダシハロトリン，アルファシペルメトリンとシペルメトリンに対して抵抗性を示した（それぞれ9.06，2.06，3.33，4.96と3.19倍）．BKN種において，テメホス，フェンチオン，NeemarinおよびアルファシペルメトリンのRRはそれぞれ5.17，4.12，4.33と3.04倍であった．しかし，JMN種においては，アルファシペルメトリンとフェンチオンを除いたほとんどの殺虫剤においてRRは低かった．本研究の結果は，フェンチオンとテメホス抵抗性種の *C. quinquefasciatus* に対して，幼虫の制御に *B. thuringiensis israilensis* とNeemarinは効果的で，成虫の制御にはラムダシハロトリンとシペルメトリンの有効であることを示した．

（文責：編集事務局）

1-置換フェニル-3-(5-ハロベンゾイミダゾール-2-イル）アシル尿素の合成と抗真菌活性

Jiang Lin, Liu Feng, Zhang Dengke, Wang Haibo

5-ハロベンゾイミダゾール-2-イルアシルイソシアネートと置換アニリンとの反応により，62.4%~76.1%の収率で，12種の新規な1-置換フェニル-3-(5-ハロベンゾイミダゾール-2-イル）アシル尿素を合成し，それらの構造解析を，IR，^{1}H NMRスペクトルおよび元素分析で行った．菌糸生育試験により，*Botrytis cinerea* と *Sclerotinia sclerotiorum*（*S. s*）に対する抗真菌活性を評価したところ，表題のほとんどの化合物は *S. s* に対して優れた抗真菌活性を示し，チオファネートメチルの活性を上回った．

（文責：編集事務局）

2-ブロモ-および2,2-ジブロモ-1-エトキシ-(2′,4′-ジクロロ）スチレン：工場製造されたブロムフェンビンホスに存在する2種の不純物の同定・合成および構造の完全解析

Bogumiła Huras, Anna Kiełczawska, Jerzy Zakrzewski, Danuta Bombińska, Agnieszka Łysik

殺虫剤ブロムフェンビンホスは，工業的に94~96%の純度で製造されるが，主成分のブロムフェンビンホスの他に10種類以上の不純物の存在が確認されている．その中の，2種の不純物が，2-ブロモ-1-エトキシ-(2′,4′-ジクロロ）スチレン（幾何異性体混合物）および2,2-ジブロモ-1-エトキシ-(2′,4′-ジクロロ）スチレンであることを，MS解析により新たに同定した．そして，この2種の不純物を，2′,4′-ジクロロアセトフェノンから別途に合成し，分光学的に解析したところ，工業製造されたブロムフェンビンホス中の不純物として以前に同定されていた化合物と同一であることが判明した．

（文責：編集事務局）