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AN EFFICIENT AND PRACTICAL METHOD FOR THE SYNTHESIS OF THE 5-ACYLAMINO-4-(4-PYRIMIDINYL)ISOXAZOLE DERIVATIVE AKP-001, A POTENT P38 MAP KINASE INHIBITOR

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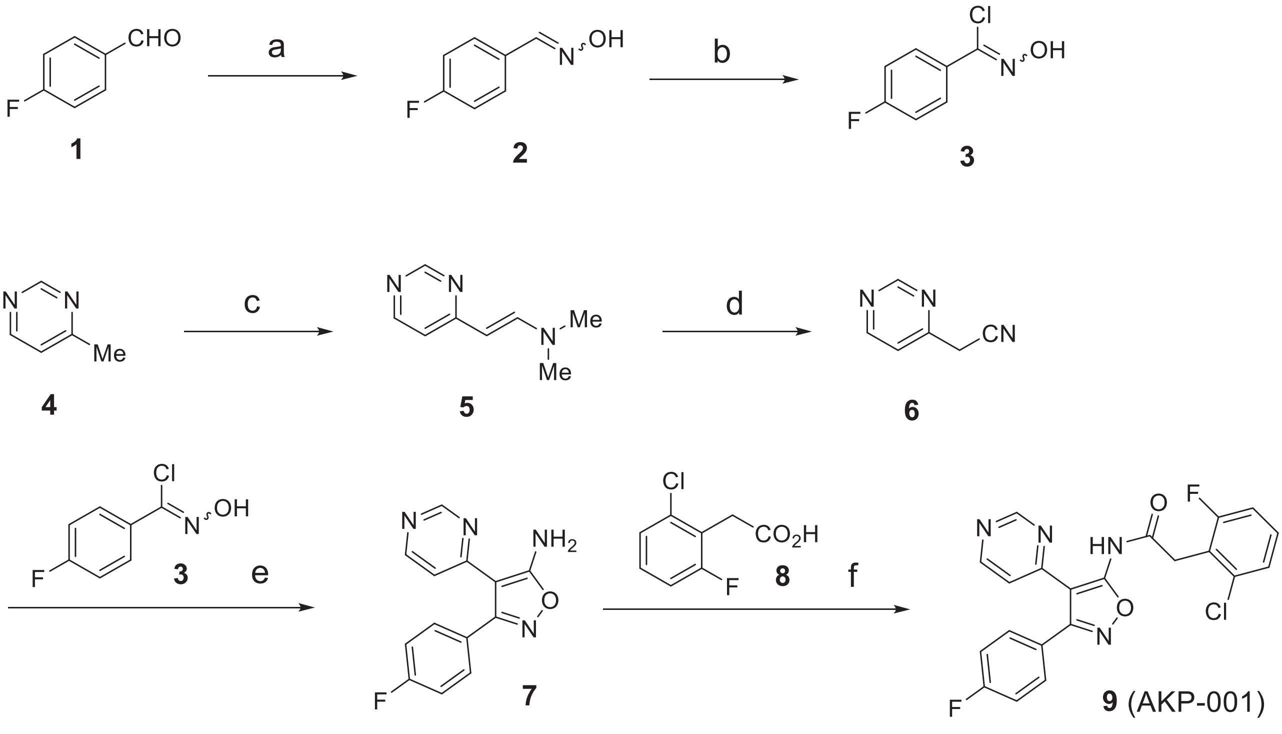
Abstract – 5-Acylamino-4-(4-pyrimidinyl)isoxazole derivative AKP-001 is a p38 mitogen-activated protein kinase inhibitor previously developed in our laboratory as an anti-inflammatory agent. Herein, we report our studies leading to the development of an improved synthetic route to AKP-001, which shows several advantages compared to the previously reported laboratory-scale process, namely, a lower number of steps, superior chemical yield and atom efficiency, and applicability at an industrial scale.

INTRODUCTION

The mitogen-activated protein (MAP) kinase family consists of three major members: p38 MAP kinase, extracellular signal-related kinase, and c-Jun N-terminal kinase. The p38 MAP kinase is a serine/threonine kinase involved in the regulation of cellular functions such as proliferation, differentiation, cell death, and stress response, and plays a key role in signaling pathways modulating inflammatory cytokines including tumor necrosis factor-α and interleukin-1β. Thus, inhibitors of p38 MAP kinase have been considered potential targets for the treatment of rheumatoid arthritis, chronic obstructive respiratory disease, and inflammatory bowel disease (IBD).[1,2] However, there is a growing concern about their systemic side effects. In this context, we have recently developed a novel p38 MAP kinase inhibitor (**9**, AKP-001, p38α IC_{50} = 10.9 nM) based on the antedrug concept,[3] which was evaluated in clinical trials for the treatment of IBD.[4]

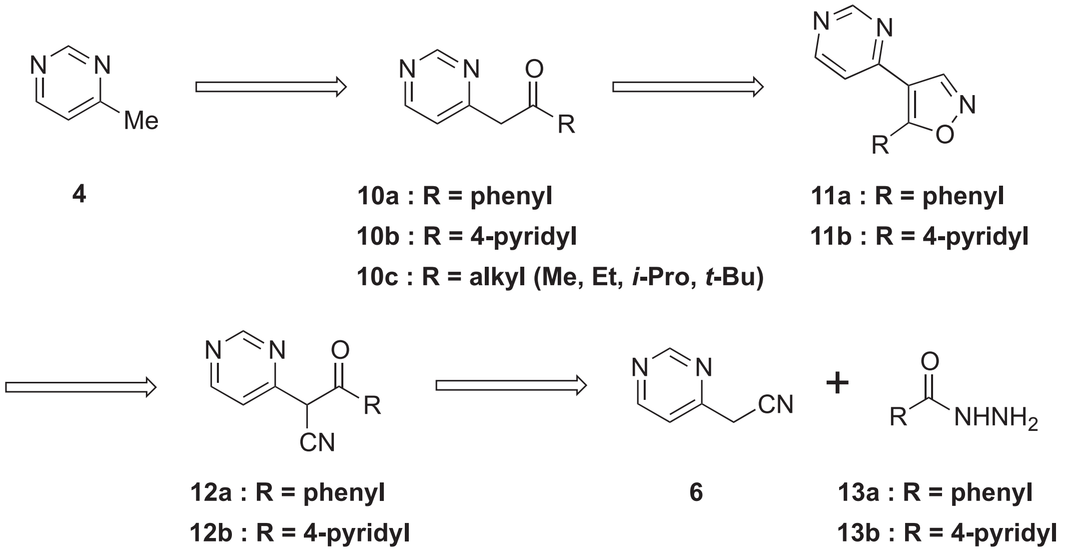


Scheme 1. Previous synthetic route of AKP-001. Reagents and conditions: (a) $NH_2OH \cdot HCl$, EtOH, H_2O, rt, 1 h, 87%; (b) NCS, DMF, rt, 1.5 h, 97%; (c) DMFDMA, DMF, 140 °C, 24 h, 95%; (d) H_2NOSO_3H, H_2O, 50 °C, 0.5 h, 39%; (e) NaOMe, MeOH, THF, rt, 7 h, 73%; (f) CDI, DBU, THF, rt, 2 h, 79%.

In the previously reported laboratory-scale synthesis of **9**, the annulation between nitrile (**6**) and chloride (**3**) is used as a key step,[5] as shown in **Scheme 1**.[4] Despite its utility in lead compound optimization, this method has several disadvantages. Most importantly, nitrile (**6**) is obtained from 4-methylpyrimidine (**4**) via enamine (**5**) using the expensive Bredereck's reagent[6] or *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) in a sealed tube, which prevents large-scale applications. Moreover, the reaction of enamine (**5**) with hydroxylamine-*O*-sulfonic acid provides **6** in low yield,[7] even after optimization of the reaction conditions and even though post-reaction operations, such as neutralization and column chromatography purification, proceeded smoothly. Thus, in order to promote further investigations and applications of **9** in the medicinal field, an improved synthetic strategy for its large-scale preparation is highly desirable. Herein, we describe our efforts to develop an efficient and practical synthesis approach for **9** through two phases of synthetic schemes, focusing on the improvement of each step for industrial applications.

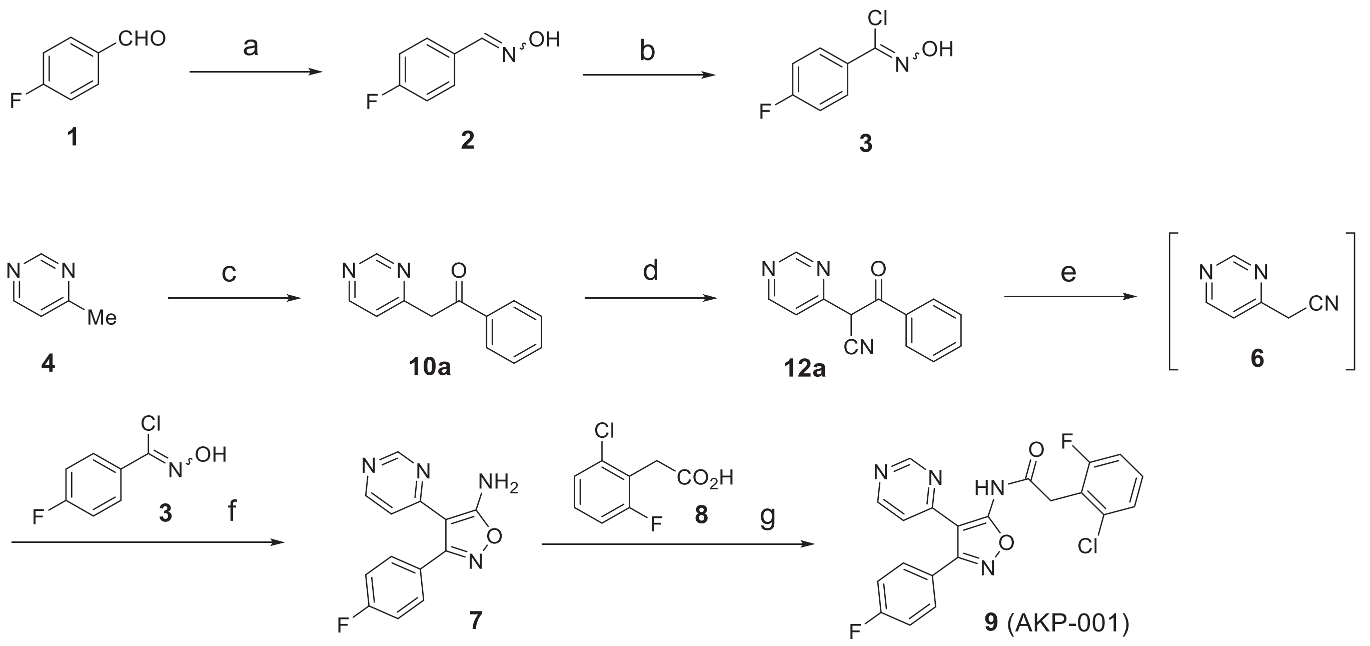
RESULTS AND DISCUSSION

To overcome the limitations of the laboratory-scale process, we sought to develop a new synthetic route to AKP-001 (**9**), in which nitrile (**6**) was prepared by a different approach (**Scheme 2**).



Scheme 2. New synthetic approach of the key intermediate nitrile **6**

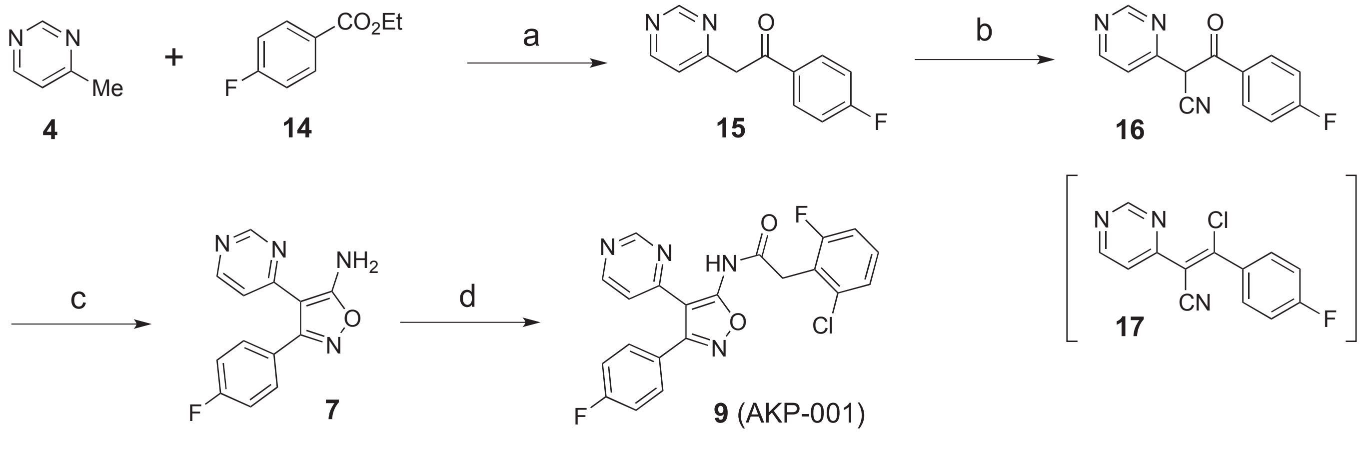
The newly designed strategy to assemble compound **6** required a higher number of synthetic steps than the laboratory-scale procedure did as shown in **Scheme 1**; however, each step consisted of basic transformations. Moreover, in order to enable large-scale applications, the isolation/purification procedures could be simplified by controlling the physical properties of each intermediate through careful choice of substituent **R**, which is ultimately not part of the final product. At first, we considered the use of compact alkyl **R** substituents; however, these compounds proved to be not suitable for industrial processes because of their difficult handling, due to their oily nature, and the low yield (14-69%, unpublished data) of the corresponding ketones (**10c**). However, when an aromatic ring was introduced as the **R** substituent, the yield of **10** increased, and the intermediates, i.e., ketone (**10**) as well as isoxazole (**11**) and/or cyanoketone (**12**), were obtained as crystals. We first attempted to use a 4-pyridyl substituent, which would simplify the isolation of the final product based on the different solubilities of nitrile (**6**) and the salt of byproduct (**13b**), specifically, the forming the HCl salt of **13b** was followed by the extraction of only **6** with ethyl acetate. However, the reaction resulted in a low yield of **11b** and incomplete separation of **6** and **13b** salt. Hence, we switched to a phenyl **R** substituent, the simplest and very inexpensive aromatic ring, and the reaction sequence was optimized to establish the improved route shown in **Scheme 3**.



Scheme 3. Improved synthetic route of AKP-001. Reagents and conditions: (a) $NH_2OH \cdot HCl$, NaOH aq., 2-PrOH, 0 °C, 1 h, 93%; (b) NCS, DMF, 50 °C, 1 h, 96%; (c) methyl benzoate, *t*-BuOK, DMF, 50 °C, 1 h, 88%; (d) 1) DMFDMA, DMF, 70 °C, 3.5 h; 2) $NH_2OH \cdot HCl$, MeOH, rt, 1 h; 3) NaOH aq., 50 °C, 1.5 h, 83%; (e) 1) $NH_2NH_2 \cdot H_2O$, MeCN, reflux, 2 h; 2) 4, 4'-Methylenebis (phenylisocyanate), 50 °C, 15 min; (f) NaOH aq., MeOH, rt, 0.5 h, 81% from **12a**; (g) CDI, DBU, DMF, rt, 1 h, 96%.

Several aspects have been considered in the systematic improvement of the laboratory-scale procedure. At first, in order to minimize the costs, the solvent used in the oximation of **1** was optimized. When the reaction was performed in methanol instead of ethanol, oxime (**2**) did not crystallize because of its high solubility in the reaction solvent, and an extraction procedure was required; thus, 2-propanol was chosen as the solvent for this transformation. In the subsequent conversion of **2** into chloride (**3**), the reaction temperature was increased from room temperature to 50 °C because of the suppression of a rapid onset of fever by warming the reaction system in advance. 5-Aminoisoxazole (**7**) was synthesized from commercially available 4-methylpyrimidine (**4**) according to the improved sequence shown in **Scheme 2**. 4-Methylpyrimidine (**4**) was reacted with methyl benzoate in the presence of potassium *tert*-butoxide to afford ketone (**10a**). Subsequent reaction with DMFDMA provided the corresponding enamine, which was treated with hydroxylammonium chloride to afford isoxazole (**11a**) (**Scheme 2**). Because of its oily nature, intermediate (**11a**) was not isolated and was directly subjected to ring opening under alkaline conditions to yield cyanoketone (**12a**), which was conveniently isolated as a crystalline solid. Next, **12a** was reacted with hydrazine monohydrate to form nitrile (**6**) and hydrazide by-products (**13a**) (**Scheme 2**). Several separation techniques suitable for pilot-scale applications were investigated. Initially, we examined the removal of hydrazide (**13a**) by the formation of the hydrochloride salt with HCl or by washing with aqueous potassium hydrogen sulfate; however, in both cases, **6** was obtained in low yield due to the low extraction efficiency. We then attempted the derivatization of hydrazide (**13a**) using 4,4′-methylenebis(phenyl isocyanate) (MDI).[8] As a result, a water-insoluble urea derivative was obtained, which could be easily removed by filtration, leaving highly pure nitrile (**6**) in aqueous solution. Thus, the following reaction of nitrile (**6**) with **3** was performed using NaOH aqueous solution as a base in methanol to provide aminoisoxazole (**7**) in good yield. In the next step, in order to reduce the amount of solvent, tetrahydrofuran (THF) was replaced by *N,N*-dimethylformamide (DMF) for the condensation of **7** with carboxylic acid (**8**). Moreover, 1-propanol was selected as the optimal recrystallization solvent in terms of purification efficiency, recovery yield, and safety. Gratifyingly, highly pure product (**9**) was obtained in 56% overall yield from starting material (**4**), and could be used for the synthesis of new potential drugs on the basis of the clinical trials of AKP-001 (**9**).

Purification of nitrile (**6**) using MDI is a key process in the described synthetic route to **9**, which makes it applicable to large-scale production. However, the method still had several drawbacks. Firstly, it is a multistep process and is inefficient in terms of atom economy because of the production of large amounts of the urea derivative of undesired hydrazide byproduct (**13a**), which correspond to about half quantity of molecular weight at synthesis of nitrile (**6**). In addition, the use of MDI is associated with occupational safety and health concerns, mainly related to the respiratory system.[9] Thus, in order to overcome these issues, we directed our efforts to further improve the synthetic route to AKP-001 (**9**).



Scheme 4. Final synthetic route of AKP-001. Reagents and conditions: (a) NaHMDS, diglyme, 0 °C, 2 h, 92%; (b) 1) DMFDMA, DMF, 65 °C, 2 h; 2) $NH_2OH\cdot HCl$, MeOH, rt, 1 h; 3) NaOH aq., 55 °C, 2 h, 85%; (c) 1) $POCl_3$, DMF, MeCN, rt, 2 h; 2) NH_2OH aq., 1-PrOH, H_2O, 20-40 °C, 1 h, 82%; (d) **8**, CDI, DBU, DMF, rt, 2 h, 89%.

The new sequence started with the synthesis of **15**[10] by a reaction similar to that previously described for the preparation of **10a** (**Scheme 3**), except that sodium bis(trimethylsilyl)amide was used as the base and diglyme as the solvent to avoid the formation of byproduct, which was 4-fluorobenzoic acid afforded by an ester degradation in the presence of *t*-butoxy anion due to the effect of the para-fluoro substituent (**Scheme 4**). Next, cyanoketone (**16**) was prepared combining three steps in a one-pot procedure: **15** was reacted with DMFDMA to give the corresponding enamine, followed by treatment with hydroxylammonium chloride to obtain the isoxazole intermediate and final cleavage under alkaline conditions. The key step of this newly devised strategy was inspired by the conventional isoxazole synthesis via condensation of cyanoketones with hydroxylamines;[11] however, this reaction has been previously incomplete for a cyanoketone (**16**) due to its poor reactivity by the effect of the pyrimidine ring. Thus, in order to increase its reactivity, **16** was converted into the corresponding 3-chloroacrylonitrile (**17**), which was then coupled with hydroxylamine to obtain the desired aminoisoxazole (**7**).[12] Specifically, high yields of **7** were obtained by reacting **16** with phosphoryl chloride in the presence of DMF in acetonitrile to form **17**, which was treated with hydroxylamine in 1-propanol followed by recrystallization of the final product. The final condensation and recrystallization procedures were performed under the same conditions used in the previous approach. Hence, using this synthetic route, the desired compound (**9**) was obtained in four steps in 57% overall yield, and showed purity and impurity profiles comparable to those of **9** obtained by the synthetic strategy shown in **Scheme 3**.

In summary, we established an efficient synthetic route to AKP-001 (**9**), which featured excellent atom economy and required only four steps, that is, two steps less than the laboratory-scale process. In view of the interesting bioactivities of 5-aminoisoxazole derivatives including AKP-001 (**9**), we envision that this strategy will find applications in the large-scale synthesis of these derivatives.

EXPERIMENTAL

General

^{1}H NMR spectra were recorded on a JEOL JNM-ECP 400 spectrometer. Chemical shifts (δ) are expressed in parts per million using tetramethylsilane as the internal standard. Mass (MS) spectra were obtained on a Shimadzu GC/MS QP-5000 spectrometer equipped with an electrospray ionization (ESI) source. Fourier transform infrared (FT/IR) spectra were recorded on a JASCO FT/IR-470 spectrometer. Electrospray ionization high-resolution mass spectra (ESI-HRMS) were obtained on a Waters Xevo Q-TOF apparatus. The purity of the final compounds was determined by high-performance liquid chromatography (HPLC) on a Waters HPLC system (separations module: Waters 2695, UV detector: Waters 2487, column oven: Waters SMC, computer: DELL GX620, software: Waters Empower 2). Elemental analyses (C, H, and N) were performed using an Elementar vario Micro cube analyzer.

4-Fluorobenzaldoxime (2)

To a solution of 4-fluorobenzaldehyde (**1**) (157.0 g, 1.265 mol) and hydroxylammonium chloride (96.7 g, 1.392 mol) in 2-propanol (246 mL) was added dropwise 2 mol/L NaOH aqueous solution (1400 mL, 2.800 mol) at 10 °C or less over 10 min, and the mixture was further stirred at 0 °C for 1 h. The reaction mixture was poured into water (625 mL), and 2 mol/L HCl aqueous solution (732 mL) was added. Next, 2 mol/L NaOH aqueous solution (270 mL) was added to adjust the pH at 6~7, and the resulting precipitate was collected by filtration. The precipitate was washed with water (625 mL) and forced-air dried at room temperature to afford the title compound **2** (164.0 g, 93%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.11 (s, 1H), 7.60-7.53 (m, 2H), 7.41 (s, 1H), 7.11-7.04 (m, 2H); MS (EI): *m/z* 139 (M^+).

4-Fluoro-*N*-hydroxybenzimidoyl chloride (3)

To a solution of 4-fluorobenzaldoxime (**2**) (140.0 g, 1.006 mol) in DMF (692 mL) was added *N*-chlorosuccinimide (147.7 g, 1.106 mol) portionwise at an outer temperature of 50 °C and an inner temperature of 90 °C or lower. After stirring at 50 °C for 1 h, the reaction mixture was cooled to 10 °C or less and poured into water (1400 mL). The solution was extracted with *t*-butyl methyl ether (840 mL × 2), washed with water (840 mL × 2), dried over $MgSO_4$, and evaporated. The resulting residue was dried under reduced pressure at room temperature to afford the title compound **3** (168.3 g, 96%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 7.87-7.81 (m, 2H), 7.69 (s, 1H), 7.14-7.06 (m, 2H); MS (EI): *m/z* 173 (M^+).

1-Phenyl-2-(pyrimidin-4-yl)ethan-1-one (10a)

To a solution of 4-methylpyrimidine (**4**) (128.0 g, 1.360 mol) and methyl benzoate (222.2 g, 1.632 mol) in DMF (640 mL) was added potassium *t*-butoxide (229.1 g, 2.042 mol), and the mixture was stirred at 50 °C for 1 h under dry conditions. After cooling to 10 °C or less, 2 mol/L HCl aqueous solution (1000 mL) was added until pH 5~6. The mixture was poured into ice-cold water (2550 mL), and the resulting precipitate was collected by filtration. The precipitate was washed with 0.8 mol/L $NaHCO_3$ aqueous solution (1280 mL) and water (1280 mL), and forced-air dried at 60 °C to provide the title compound **10a** (237.4 g, 88%) as a yellow solid. ^{1}H-NMR ($CDCl_3$) exists as an enol tautomer: δ: 14.99 (s, 1H), 8.85 (s, 1H), 8.44 (d, *J* = 5.6 Hz, 1H), 7.88-7.83 (m, 2H), 7.44 (d, *J* = 2.2 Hz, 2H), 7.43 (d, *J* = 1.7 Hz, 1H), 6.95 (dd, *J* = 1.3, 5.6 Hz, 1H), 6.03 (s, 1H); MS (EI): *m/z* 197 (M^+-1).

3-Oxo-3-phenyl-2-(pyrimidin-4-yl)propanenitrile (12a)

A mixture of ketone (**10a**) (233.0 g, 1.175 mol), DMFDMA (168.2 g, 1.412 mol), and DMF (233 mL) was stirred at 70 °C for 3.5 h under dry conditions. After cooling at 30 °C or less, hydroxylammonium chloride (122.6 g, 1.764 mol) and MeOH (2330 mL) were added, and the mixture was stirred at room temperature for 1 h. Next, 5 mol/L NaOH aqueous solution (470 mL, 2.35 mol) was added, and the mixture was stirred at 50 °C for 1.5 h. The reaction solution was poured into ice-cold water (2330 mL), and 2 mol/L HCl aqueous solution (1100 mL) was added to reach pH 4~5. The resulting precipitate was collected by filtration, washed with water (2330 mL), and forced-air dried at 60 °C to provide the title compound **12a** (218.4 g, 83%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) exists as an enol tautomer: δ: 17.01 (s, 1H), 8.66 (s, 1H), 8.49 (d, *J* = 5.8 Hz, 1H), 7.94-7.90 (m, 2H), 7.57-7.46 (m, 3H), 7.43 (d, *J* = 6.3 Hz, 1H); MS (EI): *m/z* 222 (M^+-1); Anal. Calcd for $C_{13}H_9N_3O$: C 69.95%, H 4.06%, N 18.82%. Found: C 70.0%, H 4.0%, N 18.8%.

3-(4-Fluorophenyl)-4-(pyrimidin-4-yl)isoxazol-5-amine (7)

Method A (from **12a**): To a solution of cyanoketone (**12a**) (200.0 g, 0.896 mol) in MeCN (900 mL) was added hydrazine monohydrate (53.8 g, 1.075 mol), and the mixture was heated to reflux for 2 h. After cooling to 47 °C, pulverized MDI (224.2 g, 0.896 mol) was added portionwise to the solution at 50 °C for 15 min, and the mixture was stirred at 50 °C for 1 h. Then, water (2700 mL) was added, and the mixture was stirred at the same temperature for 30 min. The resulting insoluble matter was removed by filtration, and the residue was washed with MeCN-water (1:3, v/v) (3600 mL). The filtrate and washing were combined, 5 mol/L NaOH aqueous solution (270 mL, 1.350 mol) was added, and the mixture was stirred at room temperature for 10 min. A solution of chloride (**3**) (155.6 g, 0.896 mol) in MeOH (900 mL) was added dropwise with stirring at room temperature over 20 min, and the mixture was further stirred at room temperature for 30 min. The resulting precipitate was collected by filtration, washed with water (2000 mL), and forced-air dried at 60 °C to obtain the title compound **10** (186.8 g, 81%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 9.02 (d, *J* = 1.3 Hz, 1H), 8.32 (d, *J* = 5.7 Hz, 1H), 7.54-7.49 (m, 2H), 7.24-7.18 (m, 2H), 6.87 (bs, 2H), 6.70 (dd, *J* = 1.3, 5.7 Hz, 1H); MS (EI): *m/z* 256 (M^+).

Method B (from **16**): To a solution of cyanoketone (**16**) (10.0 g, 41.45 mmol) and DMF (0.310 g, 4.24 mmol) in MeCN (50 mL) was added phosphoryl chloride (6.37 g, 41.54 mmol), and the mixture was stirred at room temperature for 2 h under dry conditions. The reaction solution was transferred to a dropping funnel and added dropwise to a mixture of 50% hydroxylamine aqueous solution (41.15 g, 622.9 mmol), 1-PrOH (50 mL), and water (50 mL) over 15 min. The mixture was further stirred at room temperature for 30 min, and 1 mol/L NaOH aqueous solution (300 mL) was added, followed by stirring at room temperature for 1 h and at 0 °C for 30 min. The resulting precipitate was collected by filtration, washed with water (410 mL), and dried under reduced pressure at 40 °C to provide the title compound **7** (8.727 g, 82.3%) as a pale yellow solid.

2-(2-Chloro-6-fluorophenyl)-*N*-(3-(4-fluorophenyl)-4-(pyrimidin-4-yl)isoxazol-5-yl)acetamide (9, AKP-001)

To a solution of 2-chloro-6-fluorophenylacetic acid (153.6 g, 0.814 mol) in DMF (530 mL) was added 1,1'-carbonyldiimidazole (132.2 g, 0.815 mol) portionwise, and the mixture was stirred at room temperature for 30 min under dry conditions. Then, 5-aminoisoxazole (**7**) obtained by **method A** (174.0 g, 0.679 mol) was added, followed by dropwise addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (124.1 g, 0.815 mol) at such a rate that the inner temperature does not exceed 50 °C, and the mixture was stirred at room temperature for 1 h. Next, 2 mol/L HCl aqueous solution was added dropwise with stirring, ensuring that the inner temperature does not exceed 40 °C. The resulting precipitate was collected by filtration, washed with water (1040 mL), and forced-air dried at 60 °C to afford the crude compound **9** (287.6 g, 99%). The crude compound (281.0 g) was recrystallized from 1-PrOH (4215 mL) and forced-air dried at 60 °C to provide pure **9** (272.8 g, 97%, 99.87% purity as determined by HPLC) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 11.55 (s, 1H), 8.65 (s, 1H), 8.41 (d, *J* = 5.7 Hz, 1H), 7.52-7.45 (m, 2H), 7.43-7.34 (m, 2H), 7.26-7.13 (m, 3H), 6.78 (dd, *J* = 1.3, 5.7 Hz, 1H), 4.14 (d, *J* = 1.5 Hz, 2H); MS (EI): *m/z* 426 (M^+).

When compound **7** obtained by **method B** was used, **9** (99.81% purity as determined by HPLC) was obtained in 89% yield.

1-(4-Fluorophenyl)-2-(pyrimidin-4-yl)ethan-1-one (15)

A mixture of $NaNH_2$ (46.1 g, 1.182 mol), hexamethyldisilazane (171.5 g, 1.063 mol), and diglyme (200 mL) was stirred at 0 °C for 2 h. Then, a mixture of 4-methylpyrimidine (**4**) (40.0 g, 0.425 mol), 4-fluorobenzoic acid ethyl ester (**14**) (71.5 g, 0.425 mol), and diglyme (200 mL) was added dropwise at 0 °C, and the reaction mixture was stirred at the same temperature for 2 h. Next, 2 mol/L HCl aqueous solution (1360 mL) was added to reach pH 7~9, and the mixture was diluted with water (600 mL). The resulting precipitate was collected by filtration, washed with water (1600 mL), and dried under reduced pressure at 40 °C to provide the title compound **15** (84.16 g, 92%) as a yellow solid. ^{1}H-NMR ($CDCl_3$) exists as an enol tautomer: δ: 15.06 (s, 1H), 8.81 (d, *J* = 1.2 Hz, 1H), 8.41 (d, *J* = 5.5 Hz, 1H), 7.88-7.81 (m, 2H), 7.14-6.97 (m, 2H), 6.92 (dd, *J* = 1.2, 5.5 Hz, 1H), 5.95 (s, 1H); MS (EI): *m/z* 216 (M^+).

3-(4-Fluorophenyl)-3-oxo-2-(pyrimidin-4-yl)propanenitrile (16)

A mixture of ketone (**15**) (80.0 g, 0.370 mol), DMFDMA (66.1 g, 0.555 mol), and DMF (80 mL) was stirred at 65 °C for 4 h under dry conditions. Hydroxylammonium chloride (38.6 g, 0.555 mol) and MeOH (800 mL) were added, and the mixture was stirred at room temperature for 1 h. Then, 5 mol/L NaOH aqueous solution (152 mL) was added, and the mixture was stirred at 55 °C for 2 h. The solution was then poured into water (960 mL), and 2 mol/L HCl aqueous solution (320 mL) was added dropwise to reach pH 6~7. The resulting precipitate was collected by filtration, washed with water (1200 mL), and dried under reduced pressure at 40 °C to provide the title compound **16** (76.63 g, 85%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) exists as an enol tautomer: δ: 16.95 (s, 1H), 8.64 (s, 1H), 8.49 (d, *J* = 6.3 Hz, 1H), 7.99-7.93 (m, 2H), 7.42 (d, *J* = 6.3 Hz, 1H), 7.19-7.13 (m, 2H); MS (EI): *m/z* 241 (M^+).

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