Drug Discovery: Recent Progress and the Future

Regular Article

Discovery of 2-[(E)-2-(7-Fluoro-3-methylquinoxalin-2-yl)vinyl]-6-pyrroloidin-1-yl-N-(tetrahydro-2H-pyrano-4-yl)pyrimidin-4-amine Hydrochloride as a Highly Selective PDE10A Inhibitor

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Phosphodiesterase (PDE) 10A is a dual hydrolase of cAMP and cGMP and highly expressed in striatal medium spiny neurons. Inhibition of PDE10A modulates the activity of medium spiny neurons (MSN) via the regulation of cAMP and cGMP. Signal control of MSN is considered associated with psychotic symptoms. Therefore PDE10A inhibitor is expected as a therapeutic method for psychosis disease such as schizophrenia. Avanafil (I) is a PDE5 inhibitor (treatment for erectile dysfunction) discovered by our company. We paid attention to the homology of PDE10A and PDE5 and took advantage of PDE5 inhibitor library to discover PDE10A inhibitors, and found a series of compounds that exhibit higher potency for PDE10A than PDE5: We transformed the afforded derivatives, which had weak inhibitory activity against PDE10A, and discovered stilbene as a PDE10A inhibitor. Brain penetration of this compound was improved by further conversion of N-containing heterocycles and their substituents. The afforded dimethylaminopyrimidine was effective for rat conditioned avoidance response (CAR) test; however, it did not exhibit good brain penetration. We performed in-depth optimization focusing on substituents of the quinoxaline ring, and produced 3-methyl-7-fluoro quinoxaline. This compound was the most effective in rat CAR test due to its strong PDE10A inhibitory activity and good pharmacokinetics.

Key words phosphodiesterase (PDE) 10A; schizophrenia; conditioned avoidance response (CAR); stilbene; quinoxaline; pyrimidine

Phosphodiesterase (PDE) is an enzyme that hydrolyzes cyclic nucleotides (cAMP and cGMP). PDEs are classified into 11 families and 21 genes based on amino acid sequence and enzymatic chemistry.1) PDE10A was discovered as a single family member PDE in the 1990s.2,3) PDE10A is highly expressed in the striatum, and detected in testis and thyroid.4) PDE10A exists especially in 4-aminobutanoic acid (GABA)ergic medium spiny neurons (MSN) in the striatum, and it is speculated that PDE10A regulates GABAergic neuron systems via modulating cAMP and cGMP concentration.5) Signal disorder of MSN is considered associated with psychiatric disease. PDE10A inhibitor normalizes glutamatergic and dopaminergic neurons via activation of GABAergic neurons; therefore PDE10A inhibitor is expected as an antipsychotic. Indeed, it has been reported that administration of PDE10A inhibitor (Papaverine) or knockout of PDE10A gene is effective in antipsychotic model.6–9) For these reasons, PDE10A inhibitors have attracted attention of many academic and industrial researchers.10–12) Here, we report the discovery of stilbene 32 as a highly selective PDE10A inhibitor by producing a lead compound 3 from PDE5 inhibitor derivatives and optimization of the lead compound.

Results and Discussion

Avanafil (I)13–15) is a PDE5 selective inhibitor used as a treatment for erectile dysfunction, discovered by our company. We noticed the sequence homology between PDE5 and PDE10A (whole: 27%, catalytic domain: 46%)16) and searched our PDE5 inhibitor library to discover PDE10A inhibitors. In this work 2) was shown to exert weak PDE10A inhibitory activity (PDE10A IC50=280 nM) although it had higher inhibitory activity to PDE5 (PDE5 IC50=0.16 nM). We transformed 2 to PDE10A selective inhibitor 3 (PDE10A IC50=8.6 nM and PDE5 IC50>1000 nM) by focusing on substituents of pyrimidine ring (Chart 1).

We developed lead compound 3, and discovered compound 4 had approximately 1000 times selectivity for other PDEs (PDE1–9 and 11), and was effective for rat conditioned avoid-
ance response (CAR) test (ED$_{50}$ = 50 mg/kg, per os (p.o.)). To increase in vivo potency, we started optimization of the structure to improve metabolic stability and brain penetration. The pyrrolidine ring at 6-position of the pyrimidine ring was essential to express the selectivity to other PDEs. Based on that knowledge, we converted substituents of 4-position of pyrimidine ring (X-R) and nitrogen-containing hetero aromatic rings (Ar) (Table 1). First, we converted substituents on 4-position, and found that ether, amine, and amide compounds showed strong inhibitory activity. Amine 5 and amide 6 with 4-tetrahydropyranylamine were more potent than ether 4. Bicyclic 4-dimethylaminouinoxazine (7-10), 3-methyquinoline (11, 12), and 3-dimethylaminouinoxaline (13-17) were more potent than 4-dimethylaminopyrimidine (4-6). Especially, stilbene 14, containing 3-dimethylaminouinoxaline and 4-tetrahydropyranylamine, showed strong inhibitory activity (PDE10A IC$_{50}$=0.22 nM). Moreover, 14 was also improved in metabolic stability and more effective in rat CAR test (ED$_{50}$=2.0 mg/kg, p.o.) than 4, although it still had concern in brain penetration. For that reason we undertook further optimization of 14.

We focused on substituents at the 3-position of the quinoxaline ring (Table 2). First, 12 (R$^1$=NMe$_2$) was changed to 18 (R$^1$=N(Me)Et) or 19 (R$^1$=N(Me)c-Pr); however, the PDE10A IC$_{50}$ was not improved. Moreover, 20 (R$^1$=H) had significantly decreased PDE10A IC$_{50}$. When R$^1$ was substituted with methoxy (21) or ethoxy (22) group, the inhibitory activity was retained. PDE10A IC$_{50}$ of 21 (R$^1$=Me) was as potent as 14. And concentration ratio of unbound drug in brain to blood ($K_{pu}$) of 21 ($K_{pu}$=0.46) was about 5 times larger than that of 14 ($K_{pu}$=0.080). Furthermore, 23 had improved pharmacokinetics. Both 24 (R$^1$=Et) and 25 (R$^1$=CF$_3$) had almost the same activity as 14; however, brain penetration was not improved. It is thought that twisting occurs at the dihedral angle of the ole-
fin site due to the effect of the substituent at the 3-position of the quinoxaline ring, thereby reducing the energy difference between the most stable conformation and the active conformation, which leads to an improvement in PDE10A IC$_{50}$. These results are also supported by X-ray co-crystal structure analysis (Fig. 1). In this optimization it turned out that 23 (R=Me) was the most effective substituent.

We obtained 3-methylquinoxaline 23, which was active in vivo, so we conducted in-depth optimization of positions and substituents of quinoxaline further to improve the activity and...
pharmacokinetics \( (R^2-R^3) \) (Table 3). First, we synthesized mono or di-methylated 3-methylquinoxaline \((26-29)\). These compounds retained PDE10A IC\(_{50}\), but there were no other improvements. Next, fluorine substituted 3-methyl quinoxalines were synthesized \((30-33)\). These compounds also maintained or improved PDE10A IC\(_{50}\); especially, \(32\) (7-fluoro-3-methylquinoxaline) improved PDE10A IC\(_{50}\) (0.14 nM) with comparable \(K_{pu}\) to \(23\). The quinoxaline derivatives that were substituted on 7-position \((34: 7\text{-methoxy}, 35: 7\text{-trifluoromethyl and 36: 7-trifluoromethoxy})\) had improved PDE10A IC\(_{50}\); however, brain penetration was not improved. 7-Fluoroquinoxaline \(32\) was compatible with improvement of PDE10A IC\(_{50}\) and maintenance of brain penetration.

We examined 4 compounds \((4, 14, 23, 32)\) for rat CAR test (Chart 2). Lead compound 4 was effective in rat CAR test (ED\(_{50}=50\)mg/kg, p.o.) although weak. 4 was optimized to 14 by converting hetero-aromatics and the substituent of pyrimidine. 14 was more effective in rat CAR

### Table 3. SAR of Substituents at the 5,6,7-Positions of Quinoxalines

<table>
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<th>Ar</th>
<th>PDE10A IC(<em>{50}) (nM) K(</em>{pu})</th>
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test (ED_{50}=2.0 mg/kg, p.o.) than 4 probably due to improved PDE10A IC_{50} (0.22 nM); however, there was a concern in brain penetration (K_{pu}=0.080). 3-Methylquinoline 23 had better PDE10A IC_{50} and K_{pu} than 14; therefore rat CAR ED_{50} was improved to 1.6 mg/kg, p.o. 32 possessing 3-methyl-7-fluorquinoline showed better pharmacokinetic profile especially in bioavailability (BA) (23: 21%, 32: 64%), biological half-life (t_{1/2}) (23: 3.9 h, 32: 7.4 h), and good brain penetration (K_{pu}=0.34); therefore the effect of rat CAR was significantly improved (ED_{50}=0.60 mg/kg, p.o.).

Crystal structure of rat PDE10A with 32 was obtained, and X-ray crystal structure analysis revealed several interactions (9) (Fig. 1). Quinoline ring was located at the substrate (cAMP or cGMP)-binding site, and formed π–π interactions with Phe719 via face-to-face mode and Phe686 via edge-to-face mode. The nitrogen atom at 1-position of quinoxaline ring and NH of Glu716 side chain formed hydrogen bonding. The nitrogen atom at 1-position of pyrimidine ring and OH of Tyr683 side chain also formed hydrogen bonding. Quinoxaline ring and pyrimidine ring formed an active conformation by rotating form the olefin moiety as a linker. Pyrrolidine ring was positioned at the specificity pocket of PDE10A; therefore it is considered that the selectivity for other PDEs was expressed.

Stilbene 32 was synthesized by convergent route in which quinoxaline unit 43 and pyrimidine unit 47 were coupled in the last step (Chart 3). Quinoxaline 43 was synthesized from commercially available aniline 37 in 6 steps. 37 was acylated with ethyl malonyl chloride to afford 38. Amide 38 was cyclized via nucleophilic attack of acyl anion to nitro group under basic condition to give N-oxide 39, and N-oxyl moiety was reduced with phosphorus tribromide (PBr_{3}) to afford 40. Quinoxaline 40 was chlorinated with phosphorus (V) oxychloride (POCl_{3}), followed by methylation under Suzuki–Miyaura condition to give ester 42. Ester 42 was reduced to aldehyde 43 with diisobutylaluminium hydride (DIBAL-H) at low temperature. The pyrimidine 47 was synthesized from known dichloropyrimidine 44 via Michaelis–Arbuzov reaction. Dichlorophosphate 45 was treated successively with 4-aminotetrahydropyran and pyrrolidine to afford dianinophosphoryl 47 in one pot. Synthesized quinoxaline 43 and pyrimidine 47 were reacted in Honor–Wadsworth–Emmonds condition using lithium tert-butoxide (tBuOLi) as a base to produce predominantly E-alkene. This was treated with aqueous hydrochloric acid solution and recrystallized to afford stilbene 32 as hydrochloric acid salt, and 32 was used for various biological tests.

**Conclusion**

We have developed stilbene 32 as a highly selective PDE10A inhibitor. The lead compound 3 was discovered from PDE5 inhibitor library, and optimized in N-containing heterocycles and substituents at 4-position of pyrimidine. Structure–activity relationship (SAR) study revealed 4-aminotetrahydropyran as an effective substituent at 4-position of pyrimidine and 3-methyl-7-fluorquinoline as N-containing heterocycle. We successfully discovered 32, which was the most effective compound for rat CAR test due to strong PDE10A inhibitory activity and good pharmacokinetics and brain penetration. Further optimization of PDE10A inhibitors is currently under investigation for the production of novel antipsychotics.

**Experimental**

**General Method**

Melting points (mp: uncorrected) were determined by Büchi Melting Point B-545. IR spectra were recorded on a PerkinElmer, Inc. PARAGON1000 spectrometer. \(^1\)H-NMR spectra were taken on a JEOL JNM-ECX400P spectrometer (400 MHz) or Varian UNITY INOVA500 (500 MHz) in CDCl_{3} or dimethyl sulfoxide (DMSO)-d_{6}. IR spectra were recorded on a PerkinElmer, Inc. PARAGON1000 spectrometer. Elemental analyses were obtained with Finnigan MAT SSG7000C and ThermoQuest LCQ Advantage spectrometer (Atmospheric Pressure Chemical Ionization (APCI)). Elemental analyses were obtained with PerkinElmer, Inc. 2400 II CHN elemental analyzer and Dionex DX-320 ion chromatography.

The protocols for animal experiments described in this paper were approved by the Institutional Animal Care and Use Committee, Mitsubishi Tanabe Pharma Corporation.

**Preparation of Compounds**

Spectroscopic data were described as new compounds. Compounds 3, 5, 7, 8, 12, 15, 17–31, 33–36, and 43–44 were prepared following the reported procedures.

2-\{(E)-2-\{4-(1-Imidazolyl)quinazoline-2-yl|vinyl]-6-(3-methyl-2-oxo-imidazol-1-yl)-pyrrolidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine Hydrochloride (3)\} \(^1\)H-NMR (300 MHz, CDCl_{3}) \(\delta\): 2.82 (3H, s), 3.51 (2H, t, J=8.2 Hz), 3.82 (s, 3H), 3.89 (s, 3H), 4.07 (2H, t, J=7.3 Hz), 7.12 (1H, d, J=8.4 Hz), 7.66 (1H, dd, J=2.2, 9.2 Hz), 7.70 (1H, d, J=1.8 Hz), 7.85–7.93 (1H, m), 7.95 (1H, t, J=1.7 Hz), 7.99 (2H, d, J=2.0 Hz), 8.15–8.26 (3H, m), 8.25 (1H, s), 8.41 (1H, t, J=1.8 Hz), 9.70 (1H, s). MS (APCI): \(m/z\) 535 \([M+H]^+\).

2-\{(E)-2-\{4-(N,N-Dimethylamino)-5,6-dimethyl-
pyrimidin-2-yl[vinyl]-6-pyrroloidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine Hydrochloride (5)

$^{1}$H-NMR (300 MHz, CDCl$_3$): $\delta$: 1.46–1.62 (2H, m), 1.90–2.09 (6H, m), 2.18 (3H, s), 2.41 (3H, s), 3.00 (6H, s), 3.40–3.61 (6H, s), 3.71–3.86 (1H, m), 3.99 (2H, ddd, $J$ = 11.7, 3.7, 3.7 Hz), 5.10 (s, 1H), 7.58 (1H, d, $J$ = 15.4 Hz), 7.72 (1H, d, $J$ = 15.4 Hz). MS (APCI): $m/z$ 424 [M + H$^+$].

2-{[(E)-2-[4-(N,N-Dimethylamino)-quinzaolin-2-yl]-vinyl]-6-pyrroloidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine 3 Hydrochloride (7)

$^{1}$H-NMR (500 MHz, DMSO-$d_6$): $\delta$: 1.44–1.55 (2H, m), 1.84–1.92 (2H, m), 1.98 (4H, brs), 3.57 (6H, s), 3.59–3.74 (7H, m), 3.86–3.92 (2H, m), 5.53 (1H, s), 7.63–8.05 (5H, m), 8.40 (1H, d, $J$=7.7 Hz). MS (APCI): $m/z$ 446 [M + H$^+$].

2-{[(E)-2-[4-(N,N-Dimethylamino)-quinzaolin-2-yl]-vinyl]-6-pyrroloidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine 2 Hydrochloride (8)

$^{1}$H-NMR (500 MHz, DMSO-$d_6$): $\delta$: 1.51–1.59 (2H, m), 1.79–1.90 (2H, m), 1.98 (4H, s), 2.96 (3H, s), 3.30–3.59 (7H, m), 3.67 (6H, s), 3.91–3.99 (2H, m), 5.52 (1H, s), 7.70 (1H, dd, $J$=8.0, 8.0 Hz), 7.85–8.06 (4H, m), 8.42 (1H, d, $J$=8.3 Hz). MS (APCI): $m/z$ 460 [M + H$^+$].

Ethyl 3-[4-Fluoro-2-nitrophenyl]amino]-3-oxopropanoate (38)

To a solution of 4-fluoro-2-nitroaniline 37 (100 g, 0.641 mol) in toluene (1260 mL) was added ethyl malonyl chloride (106 g, 0.704 mol) at 0°C. After being refluxed for 1.5 h, the reaction mixture was concentrated in vacuo. The residue was purified by recrystallization from ethyl acetate to give the title compound as a pale yellow powder (155 g, 90%). mp 93–95°C. IR (Nujol): 3353, 1739, 1682, 1594, 1555, 1527 cm$^{-1}$.

$^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$: 1.34 (3H, t, $J$=7.1 Hz), 3.56 (2H, s), 4.31 (2H, q, $J$=7.1 Hz), 7.40 (1H, ddd, $J$=9.5, 9.4, 3.0 Hz), 7.93 (1H, dd, $J$=8.5, 3.0 Hz), 8.73 (1H, dd, $J$=9.4, 5.1 Hz), 11.15 (1H, brs). MS (APCI): $m/z$ 271 [M + H$^+$].

Anal. Calcd for C$_{11}$H$_{11}$FN$_2$O$_5$: C, 48.89; H, 4.10; N, 10.37. Found: C, 48.87; H, 4.04; N, 10.28.

Ethyl 7-Fluoro-3-hydroxyquinoxaline-2-carboxylate 1-Oxide (39)

To a solution of sodium ethoxide (21 wt% in denatured ethanol, 152 mL, 0.407 mol) in tetrahydrofuran (500 mL) and N,N-dimethylformamide (500 mL) was added a solution of ethyl 3-[4-fluoro-2-nitrophenyl]amino]-3-oxopropanoate 38 (50.0 g, 0.185 mol) in tetrahydrofuran (100 mL) and N,N-dimethylformamide (100 mL) dropwise at -30°C over 60 min. After being stirred at the same temperature for 15 min, the reaction mixture was poured into cold water (2000 mL), then 4 N aqueous hydrochloric acid solution (120 mL) was added. The mixture was extracted with ethyl acetate (1500 mL), and the organic layer was washed with saturated brine (1500 mL),
dried over magnesium sulfate, filtrated, and concentrated in vacuo. The residue was purified by trituration with disopropyl ether (300 mL) to give the title compound as a yellow powder (35.0 g, 75%). mp 210–212°C. IR (Nujol): 3299, 1729, 1685, 1612, 1539 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 1.47 (3H, t, J=7.1 Hz), 4.57 (2H, q, J=7.1 Hz), 7.44–7.51 (2H, m), 8.04 (1H, ddd, J=8.4, 2.3, 0.8 Hz), 12.66 (1H, br). MS (APCI): m/z 253 [M+H⁺]. Anal. Calcd for C₁₁H₈FN₂O₃: C, 61.53; H, 4.73; F, 8.11; N, 11.96. Found: C, 61.15; H, 4.61; F, 8.09; N, 11.90.

Ethyl 7-Fluoro-3-hydroxyquinoxaline-2-carboxylate (40) To a solution of ethyl 7-fluoro-3-hydroxyquinoxaline-2-carboxylate 1-oxide 39 (41.8 g, 0.166 mol) in N,N-dimethylformamide (480 mL) was added phosphorus tribromide (89.7 g, 0.331 mol) portionwise at a rate to keep the internal temperature at 65°C for 40 min. After being stirred at room temperature for 15 min, the reaction mixture was poured into cold water (1000 mL) and extracted with ethyl acetate. The filtrate was combined and concentrated in vacuo. The residue was purified by trituration with ethyl acetate (800 mL×2). The organic layer was washed with water (1000 mL×3)] and concentrated in vacuo) and saturated brine (1000 mL), dried over potassium carbonate (144.1 g, 1.04 mol) in 1,4-dioxane (1800 mL) was heated at 115°C for 2 h, then trimethylboroxine (32.5 g, 0.260 mol) was added and again heated at 115°C for 30 min. After being cooled to ambient temperature, the reaction mixture was filtered through celite with ethyl acetate. The filtrate was combined and concentrated in vacuo. The residue was purified by silica gel column chromatography (3.5 kg, n-hexane–ethyl acetate=4:1) to give title compound as a colorless solid (119 g, 97%). mp 79–81°C from n-hexane. IR (Nujol): 1722, 1621, 1565, 1554 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 1.49 (3H, t, J=7.2 Hz), 2.93 (3H, s), 4.56 (2H, q, J=7.2 Hz), 7.61 (1H, ddd, J=9.3, 8.1, 2.9 Hz), 7.80 (1H, ddd, J=9.1, 2.9 Hz), 8.06 (1H, dd, J=9.3, 5.6 Hz) MS (APCI): m/z 223 [M+H⁺]. Anal. Calcd for C₁₁H₁₀F₂N₂O₃: C, 62.36; H, 2.88; F, 9.14; N, 14.54. Found: C, 62.16; H, 2.89; F, 9.09; N, 14.28.

Ethyl 3-Chloro-7-fluoroquinoxaline-2-carboxylate (41) A mixture of ethyl 7-fluoro-3-hydroxyquinoxaline-2-carboxylate 40 (77.1 g, 0.326 mol) and phosphorus (V) oxychloride (77.1 g, 0.326 mol) and phosphorus (V) oxychloride (133 g, 0.522 mol) and phosphorus (V) oxychloride (500 mL) was heated at 115°C for 1 h. After being cooled to ambient temperature, the reaction mixture was concentrated in vacuo. The residue was poured into cold water (1000 mL) and extracted with ethyl acetate (2000 mL×2). The organic layer was washed with water (1000 mL×3) and saturated brine (1000 mL), dried over magnesium sulfate, filtrated, and concentrated in vacuo. The residue was purified by trituration with diisopropyl ether (3000 mL). The organic layer was separated and aqueous layer extracted with ethyl acetate–tetrahydrofuran (20:3, 1150 mL) dropwise at −78°C over 2.5 h. Then, methanol (180 mL) was added dropwise at the same temperature over 5 min. The mixture was diluted ethyl acetate (3000 mL) and aqueous saturated potassium sodium tartrate (1800 mL) was added. The resulting mixture was stirred at room temperature for 1 h. After being cooled toambient temperature, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (2.0 kg, n-hexane–ethyl acetate=4:1) followed by trituration with disopropyl ether to give the title compound as a yellow solid (79.9 g, 83%). mp 165–167°C. IR (Nujol): 1715, 1623, 1567, 1547 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 3.03 (3H, s), 7.68 (1H, ddd, J=9.3, 8.1, 2.9 Hz), 7.83 (1H, dd, J=8.8, 2.7 Hz), 8.10 (1H, dd, J=9.3, 5.4 Hz), 10.31 (1H, s) MS (APCI): m/z 225 [M+MeOH+H⁺], 191 [M+H⁺]. Anal. Calcd for C₁₁H₁₀F₂N₂O₃: C, 63.16; H, 3.71; F, 9.99; N, 14.73. Found: C, 62.76; H, 3.63; F, 9.85; N, 14.69.

Diethyl [4,6-Dichloropyrimidin-2-yl]methylphosphonate (45) A mixture of 4,6-dichloro-2-(chloromethyl)pyrimidine 44 (73.6 g, 0.373 mol) and triethylphosphate (186 g, 1.12 mol) was heated at 90°C for 23 h. After being cooled to ambient temperature, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (800 g, n-hexane–ethyl acetate=3:1 to 1:9) to give the title compound as a pale yellow oil (79.9 g, 72%). IR (Neat): 2984, 1530 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 1.34 (6H, t, J=7.1 Hz), 3.57 (2H, d, J=22.4 Hz), 4.08–4.27 (4H, m), 7.30 (1H, d, J=1.8 Hz). MS (APCI): m/z 299/301 [M+H⁺]⁺. Anal. Calcd for C₁₁H₁₀Cl₂N₂O₃P (adherent H₂O, 0.25 mol): C, 35.61; H, 4.48; Cl, 23.36; N, 9.23; P, 10.20. Found: C, 35.62; H, 4.22; Cl, 23.26; N, 8.95; P, 10.48.

Diethyl [4-Pyrrolidin-1-yl-6-((tetrahydro-2H-pyrany-4-ylamino)pyrimidin-2-yl)methyl]phosphonate (47) A mixture of diethyl [4,6-dichloropyrimidin-2-yl]methylphosphonate 45 (76.0 g, 0.254 mol), 4-aminotetrahydro-2H-pyran acetate (45.0 g, 0.279 mol), and triethylamine (64.3 g, 0.635 mol) in N,N-dimethylformamide (500 mL) was stirred at room temperature for 23 h. The reaction mixture was poured into saturated brine (500 mL), dried over potassium carbonate (1404 mol) in 1,4-dioxane (1800 mL) was heated at 115°C for 2 h, then trimethylboroxine (32.5 g, 0.260 mol) was added and again heated at 115°C for 30 min. After being cooled to ambient temperature, the reaction mixture was filtered through celite with ethyl acetate. The filtrate was combined and concentrated in vacuo to give diethyl [4-chloro-6-(tetrahydro-2H-pyran-4-ylamino)pyrimidin-2-yl]methyl]phosphonate 46 as a pale yellow solid (73.5 g), which was used for the next reaction without further purification. A mixture of 46 (73.5 g) and pyrroline (181.0 g, 0.254 mol) in N,N-dimethylacetamide (500 mL) was stirred at 70°C for 3 h. After being cooled to ambient temperature, the reaction mixture was...
poured into saturated brine (500 mL), and extracted with ethyl acetate–tetrahydrofuran (2:1, 1000 mL×4). The organic layer was dried over magnesium sulfate, filtrated and concentrated in vacuo. The residue was purified by trituration with ethyl acetate–diethyl ether to give the title compound as a colorless powder (55.2 g, 55%). mp 122–123°C. IR (Nujol): 3301, 1605, 1577, 1506 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 1.31 (6H, t, J =7.1 Hz), 1.46–1.56 (2H, m), 1.93–2.01 (6H, m), 3.23 (2H, δ, J =21.81 Hz), 3.41 (4H, br), 3.51 (2H, ddd, J =11.4, 11.4, 2.3 Hz), 3.68 (1H, m), 3.97 (2H, ddd, J =11.9, 3.7, 3.7 Hz), 4.10–4.22 (4H, m), 4.51 (1H, d, J =7.61 Hz), 5.03 (1H, d, J =1.5 Hz). MS (APCI): m/z 399 [M+H]⁺. Anal. Calcd for C₃₄H₃₄ClN₄O₄P: C, 58.95; H, 6.18; Cl, 7.27; F, 3.89; N, 17.19. Found: C, 58.65; H, 6.05; Cl, 7.09; F, 3.69; N, 17.01.

2-[(E)-2-(7-Fluoro-3-methylquinoxalin-2-yl)vinyl]6-pyrroldin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrindle-4-amine Hydrochloride (32) To a solution of diethyl [[4-pyrroldin-1-yl-6-(tetrahydro-2H-pyran-4-ylamino)pyridin-2-yl]methyl]phosphonate 47 (2.57 g, 6.37 mmol) in toluene (65 mL) was added lithium tert-butoxide (540 mg, 6.69 mmol) at 0°C. After 30 min, 7-fluoro-3-methylquinoxaline-2-carboaldehyde 43 (1.21 g, 6.37 mmol) was added, and the reaction mixture was refluxed for 2h. After being cooled to ambient temperature, the reaction mixture was poured into water (70 mL). The mixture was extracted with chloroform (70 mL×3), and the organic layer was washed with saturated brine (50 mL), dried over magnesium sulfate, filtrated, and concentrated in vacuo. The crude mixture was dissolved in ethanol (30 mL) and 2 N aqueous hydrochloric acid (3.0 mL), and the resulting precipitate was collected and washed with ethanol (30 mL) to give the title compound as a yellow powder (1.82 g, 54.26%). mp 267–268°C. IR (KBr): 3444, 3234, 3079, 2955, 2871, 1653, 1588, 1568, 1490, 1456 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ: 1.19–1.23 (2H, m), 1.46–1.56 (4H, m), 1.65–1.77 (4H, m), 1.80–1.88 (2H, m), 2.09–2.15 (2H, m), 2.32–2.44 (2H, m), 2.57 (2H, t, J =6.78 Hz), 2.97–3.02 (2H, m), 3.31 (2H, ddd, J =11.9, 9.2, 2.7 Hz), 3.72 (1H, m), 3.82 (2H, δ, J =7.67 Hz), 4.06 (2H, ddd, J =11.9, 4.4, 4.2 Hz), 5.07 (1H, s), 7.51 (2H, ddd, J =9.2, 8.2, 2.9 Hz), 7.68 (1H, d, J =15.7 Hz), 7.69 (1H, dd, J =9.2, 2.9 Hz), 8.07 (1H, dd, J =9.2, 5.7 Hz), 8.79 (1H, d, J =15.7 Hz). MS (APCI): m/z 435 [M+H]⁺. Anal. Calcd for C₂₄H₂₇FN₄O·HCl·H₂O: C, 58.65; H, 6.18; Cl, 7.09; F, 3.69; N, 17.01.

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References and Notes