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Design and synthesis of potent and selective pyridazin-4(1*H*)-one-based PDE10A inhibitors interacting with Tyr683 in the PDE10A selectivity pocket

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ABSTRACT

Utilizing structure-based drug design techniques, we designed and synthesized phosphodiesterase 10A (PDE10A) inhibitors based on pyridazin-4(1*H*)-one. These compounds can interact with Tyr683 in the PDE10A selectivity pocket. Pyridazin-4(1*H*)-one derivative **1** was linked with a benzimidazole group through an alkyl spacer to interact with the OH of Tyr683 and fill the PDE10A selectivity pocket. After optimizing the linker length, we identified 1-(cyclopropylmethyl)-5-[3-(1-methyl-1*H*-benzimidazol-2-yl)propoxy]-3-(1-phenyl-1*H*-pyrazol-5-y l)pyridazin-4(1*H*)-one (**16f**) as having highly potent PDE10A inhibitory activity (IC₅₀ = 0.76 nM) and perfect selectivity against other PDEs (>13000-fold, IC₅₀ = >10000 nM). The crystal structure of **16f** bound to PDE10A revealed that the benzimidazole moiety was located deep within the PDE10A selectivity pocket and interacted with Tyr683. Additionally, a bidentate interaction existed between the 5-alkoxypyridazin-4(1*H*)-one moiety and the conserved Gln716 present in all PDEs.

Graphical abstract



Keywords: phosphodiesterase 10A; PDE10A; Inhibitors, schizophrenia; SBDD

1. Introduction

Phosphodiesterases (PDEs) constitute a superfamily of enzymes that metabolically inactivate the ubiquitous intracellular second messenger cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP).¹ The regulation of intracellular cAMP and/or cGMP levels can have various therapeutic outcomes depending on the expression site of each PDE.²⁻⁴ PDEs are classified into 11 families based on their amino acid sequences, substrate specificities, and pharmacological properties.¹ Phosphodiesterase 10A (PDE10A), which hydrolyzes both cAMP and cGMP, is expressed at high levels in the medium spiny neurons of the striatum, which is the main input station of the basal ganglia and is strongly associated with motor and cognitive functions.⁵⁻⁹ Based on this selective expression and its function, PDE10A inhibition is considered to be an attractive approach for the treatment of diseases related to dysfunctions of the basal ganglia, particularly schizophrenia and Huntington disease.¹⁰⁻¹²

Utilizing structure-based drug design (SBDD) techniques to optimize the key lead compound **1**, we previously identified

1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-o ne (TAK-063, Figure 1A),¹³ which shows highly potent PDE10A inhibitory activity (IC₅₀ = 0.30nM) and excellent selectivity against other PDEs (minimum selectivity: >15000-fold). This compound is currently being evaluated in Phase 2 clinical trials for the treatment of schizophrenia.¹⁴ The X-ray crystal structure of TAK-063 bound to PDE10A revealed that it binds to the active site, forming the following hydrogen bonding interactions (Figure 1B): (i) the methoxy and carbonyl groups on the pyridazin-4(1H)-one ring form a bidentate interaction with the carboxamide NH of the conserved Gln716 side chain; (ii) the nitrogen atom of the pyrazole ring interacts with Tyr514 through a water molecule. Furthermore, a pyrazole ring at the 4-position of the fluorine-containing aromatic ring contributed to the compound's high potency and excellent selectivity by effectively filling a large space adjacent to Leu625 and Ala626 at the N-terminus of helix H12.¹⁵ Interestingly, the X-ray crystal structure also disclosed that TAK-063 does not interact with the OH group of Tyr683 in the PDE10A selectivity pocket,¹⁶ which was utilized in previously reported PDE10A inhibitors to ensure PDE selectivity.^{16–30} As a part of a series of studies on PDE10A inhibitors, we decided to develop novel pyridazin-4(1H)-one-based PDE10A inhibitors capable of interacting with Tyr683 to explore a backup clinical candidate with a chemotype structurally different from that of TAK-063.



Figure 1. (A) Structures of compound **1** and TAK-063. (B) X-ray crystal structure of TAK-063 in the PDE10A catalytic domain.

To facilitate interaction with Tyr683 and access the PDE10A selectivity pocket, we focused on compound 2^{31} , which was also identified by high-throughput screening using our proprietary compound library. Compound 2 exhibited potent PDE10A inhibitory activity and excellent selectivity against other PDEs (PDE10A IC₅₀ = 0.46 nM, other PDEs IC₅₀ = >10000 nM, minimum selectivity: >20000-fold) (Figure 2A). The X-ray crystal structure of 2 in PDE10A is illustrated in Figure 2B. The benzimidazole moiety interacts with the OH of Tyr683 and fills the PDE10A selectivity pocket. Additionally, the nitrogen atom at the 3-position of the pyrrolo[3,5-*d*]pyrimidine ring interacts with the carboxamide NH of the conserved Gln716 side chain.



Figure 2. (A) Structure of compound 2. (B) X-ray crystal structure of 2 in PDE10A.

The design strategy used to develop novel pyridazin-4(1H)-one-based PDE10A inhibitors capable of interacting with Tyr683 is shown in Figure 3A. Based on an overlay study between TAK-063 and **2** (Figure 3B), the pyridazin-4(1H)-one moiety was linked with the benzimidazole ring via a flexible linker. Moreover, the linker was introduced into the alkoxy group at the 5-position of the pyridazin-4(1H)-one ring to maintain the bidentate interaction with the conserved Gln716. Herein, we describe the development of novel pyridazin-4(1H)-one derivatives capable of interacting with Tyr683 as potent and selective PDE10A inhibitors.



Figure 3. (A) Design of novel pyridazin-4(1*H*)-one derivatives possessing a Tyr683-binding motif. (B) Overlaid crystal structures of TAK-063 (yellow) and **2** (white) in the PDE10A catalytic domain.

2. Chemistry

Compound **9** was synthesized as described in Scheme 1. Diazotization of aniline **3** with NaNO₂ and HCl aq followed by treatment with methyl 4-methoxy-3-oxobutanoate yielded hydrazone **4**. The pyridazin-4(1*H*)-one ring was formed by treatment of **4** with dimethylformamide dimethylacetal (DMFDMA). Following hydrolysis under basic conditions, carboxylic acid **6** was converted to ketone **8** via the Weinreb amide **7**. Heating of the ketone **8** in DMFDMA and subsequent cyclization to a pyrazole ring afforded compound **9**.



Scheme 1. Synthesis of compound **9**. Reagents and conditions: (a) (1) NaNO₂, HCl aq, 0 °C, (2) methyl 4-methoxy-3-oxobutanoate, NaOAc, MeOH, 0 °C, 83%; (b) DMFDMA, 90 °C, 95%; (c) NaOH aq, MeOH, rt, 98%; (d) *N*,*O*-dimethylhydroxylamine hydrochloride,

1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt, TEA, DMA, rt, 84%; (e) MeMgBr, THF, -69 °C, 78%; (f) (1) DMFDMA, MeCN, reflux, (2) methylhydrazine, TFA, EtOH, rt, 55% in 2 steps.

The syntheses of compounds 14a-d are summarized in Scheme 2. After protection of 10 with a benzyl group, a Suzuki–Miyaura coupling³² of 11 with

1-phenyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole gave **12**. Deprotection and alkylation afforded compounds **14a**–**d**.



Scheme 2. Syntheses of compounds 14a–d. Reagents and conditions: (a) NaH, *n*-Bu₄NI, BnBr, DMF, rt, 53%; (b) 1-phenyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, PdCl₂[Pt-Bu₂(Ph-*p*-NMe₂)]₂, K₂CO₃, toluene, H₂O, reflux, 78%; (c) Pd(OH)₂/C, H₂, THF, MeOH, rt, 81%; (d) alkylating agent (R¹OSO₂Me, R¹Br, or R¹OSO₂CF₃), base (NaH or Cs₂CO₃), DMF, rt,

1-66%.

Demethylation of compound **14c** with chlorotrimethylsilane and sodium iodide gave hydroxypyridazine **15**. Compounds **16a**–**f** were then synthesized by either a Mitsunobu reaction³³ or alkylation (Scheme 3).



Scheme 3. Syntheses of compounds **16a**–**f**. Reagents and conditions: (a) chlorotrimethylsilane, NaI, MeCN, reflux, 80%; (b) R₃OH, di-tert-butyl azodicarboxylate or diethyl azodicarboxylate, triphenylphosphine, THF, toluene, rt, 46–73%; (c) R₃Cl, Cs₂CO₃, NaI, DMA, 80 °C, 62%.

3. Results and discussion

PDE10A inhibitory activities were measured using a scintillation proximity assay (SPA) based on the inhibition rate of cGMP hydrolysis. [³H] cGMP was used as a substrate for PDE10A2.

In the course of preparing compounds capable of interacting with the Tyr683 in the PDE10A selectivity pocket, the number of aromatic rings and the molecular weight of the compound are expected to increase. Therefore, to retain the physical properties required for CNS drugs,^{34,35} we initially tried to remove an aromatic ring. Results for the replacement of each phenyl group in compound **1** are shown in Table 1. Compound **14a** showed more potent PDE10A inhibitory activity than **9**. The phenyl group on the pyrazole ring, which occupied the ribose region of cAMP, made a bigger contribution to potency than the phenyl group on the pyridazin-4(1*H*)-one ring. To compensate for the dramatic loss of potency, we introduced small substituents into the 1-position of the pyridazin-4(1*H*)-one ring (substituent \mathbb{R}^1). Introduction of cyclopropyl (**14b**), (cyclopropyl)methyl (**14c**), or (trifluoromethyl)methyl (**14d**) groups improved the potency. These results suggested that potency strongly depends on the presence of a substituent that can occupy the hydrophobic region defined by the side chains of the Leu625 and Met703 residues (Figure 2A). From the view point of chemical tractability, the (cyclopropyl)methyl group (**14c**) was selected as the preferred group for further optimization because the synthetic yield of the cyclopropyl derivative **14b** was quite low (only 1%, Scheme 2).

Table 1. Optimization of substituents on the pyridazin-4(1H)-one and the pyrazole rings.

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	compound	\mathbb{R}^1	\mathbf{R}^2	PDE10A IC_{50}^{a} (nM)
	9	Ph	Me	2400 (1600-3400)
	14a	Me	Ph	1000 (880-1200)
	14b	cyclopropyl	Ph	130 (100-170)
	14c	(cyclopropyl)methyl	Ph	170 (130-210)
	14d	(trifluoromethyl)methyl	Ph	550 (380-790)

^{*a*} IC_{50} values are shown as the mean of duplicate experiments. 95% Confidence intervals are given in parentheses. All values were rounded to two significant figures.

To fill the selectivity pocket and target the interaction with Tyr683, a benzimidazole ring was introduced into the pyridazin-4(1H)-one moiety via a linker. In view of the brain penetration,^{34,35} the NH of benzimidazole was capped with a methyl group to reduce a hydrogen-bonding donor. The results of the linker optimization study are summarized in Table 2. First, the optimal linker length was investigated. Compound **16a**, linked with a methylene spacer between the oxygen and 1-methylbenzimidazole showed weak PDE10A inhibitory activity. Compound **16b**, a two-carbon-linked analogue, exhibited increased potency. Introduction of a three-carbon propyl linker (**16c**) resulted in dramatically enhanced PDE10A inhibitory activity. Finally, a four-carbon-linked analogue (**16d**) showed a decreased potency compared to **16c**. We identified the three-carbon propyl linker as the most suitable link between the 5-hydroxypyridazin-4(1*H*)-one and the benzimidazole rings. Heteroatoms were then introduced into the linker. Introduction of an oxygen atom (**16e**) resulted in a 5-fold decrease in potency, whereas cyclization between the linker and the benzimidazole ring (**16f**) slightly increased PDE10A inhibitory acceptor (HBA) in the anchor might play an important role in the ability of the compound to inhibit PDE10A.

Table 2. Optimization of the linker



 ${}^{a}IC_{50}$ values are shown as the mean of duplicate experiments. 95% Confidence intervals are given in parentheses. All values were rounded to two significant figures.

The crystal structure of compound **16f** bound to PDE10A is illustrated in Figure 4. As with the crystal structure of the complex formed between PDE10A and TAK-063,¹³ π – π stacking interactions between the phenyl rings of Phe719 and Phe686 and the pyridazin-4(1*H*)-one moiety were observed. Additionally, the pyrazole ring made a hydrogen bond with Tyr514 via a water molecule. A lone pair of the propoxy oxygen was directed toward the NH of the Gln716 side chain, resulting in a bidentate interaction between Gln716 and the 5-alkoxypyridazin-4(1*H*)-one moiety. The benzimidazole ring was positioned deep within the PDE10A selectivity pocket, interacting with Tyr683 as expected. Furthermore, the binding mode of compound **16f** resulted in perfect selectivity against other PDEs (IC₅₀ = >10000 nM , >13000-fold), as shown in Figure 5.



Figure 4. X-ray crystal structure of compound 16f in the PDE10A catalytic domain.



Figure 5. Inhibitory activities of compound 16f against human PDEs

 ${}^{a}IC_{50}$ values are shown as the mean of duplicate experiments. 95% Confidence intervals are given in parentheses. All values were rounded to two significant figures.

Conclusion

Utilizing SBDD techniques, we have designed and synthesized novel PDE10A inhibitors based on pyridazin-4(1*H*)-one; these inhibitors interact with the Tyr683 residue in the PDE10A selectivity pocket. A benzimidazole group was selected as the anchor to interact with the OH group of Tyr683 and fill the PDE10A selectivity pocket. The 5-position of the pyridazin-4(1*H*)-one ring was chosen to introduce the linker required to access to the PDE10A selectivity pocket. After the optimization effort, compound **16f** was found to display highly potent PDE10A inhibitory activity and perfect selectivity against other PDEs. The crystal structure of compound **16f** with PDE10A revealed that the benzimidazole moiety was positioned deep within the PDE10A selectivity pocket and interacted with the OH group of Tyr683. Additionally, the bidentate interaction between Gln716 and the 5-alkoxypyridazin-4(1*H*)-one moiety was also observed. We succeeded in identifying a highly potent and selective PDE10A inhibitor with different binding modes based on TAK-063 after the hybridization of two distinct chemotypes.

5. Experimental procedure

All commercially available solvents and reagents were used without further purification. Yields were not optimized. Melting points were determined on a Büchi melting point apparatus B-545 or an OptiMelt melting point apparatus MPA100 and were not corrected. ¹H NMR spectra were recorded on Varian Mercury-300 (300 MHz) or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane (TMS) of the indicated organic solution. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; td, triplet of doublets; ddd, doublet of doublet of doublets; quin, quintet; brs, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). LC-MS was performed on a Waters liquid chromatography-mass spectrometer system, using a CAPCELL PAK UG-120 ODS column (2.0 mm i.d. × 50 mm, Shiseido Co., Ltd.) with a 5–95% gradient of CH₃CN in water containing 0.04% TFA and an HP-1100 (Agilent Technologies) apparatus for monitoring at 220 nm. Elemental analyses were carried out by Takeda Analytical Laboratories and Sumika Chemical Analysis Service, Ltd., and the results were within 0.4% of theoretical values. All of the final products undergoing biological testing were >95% pure as demonstrated by analysis carried out using analytical high-performance liquid chromatography (HPLC). The HPLC analyses were performed using a Shimadzu ultra-fast liquid chromatography (UFLC) instrument, equipped with an L-column 2 ODS (3.0×50 mm, 2 µm), eluting with a gradient of 5–90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile), at a flow rate of 1.2 mL/min, with UV detection at 220 nm. Reaction progress was determined by thin-layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Column chromatography was performed using silica gel (Merck Kieselgel 60, 70-230 mesh), basic silica gel (Chromatorex NH-DM 1020, 100-200 mesh, Fuji Silysia Chemical, Ltd.), or Purif-Pack (SI ϕ 60 μ M or NH ϕ 60 μ M, Fuji Silysia Chemical, Ltd.). Preparative HPLC purification was performed by using a Waters Corporation UV purification system equipped with a Develosil ODS-UG-10 (4.6×150 mm, 5µm) column, and eluted with a gradient of 5–90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile), at a flow rate of 150 mL/min, with UV detection at 220 nm. Abbreviations of solvents are used as follows: CDCl₃, deuterated chloroform; DMSO-*d*₆, dimethyl sulfoxide-d₆; EtOAc, ethyl acetate; DMF, N,N-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; DMSO, dimethyl sulfoxide; DMA, N,N-dimethylactamide; AcOH, acetic acid; DMFDMA, N,N-dimethylformamide dimethylacetal; MeCN, acetonitrile; DIPEA, *N*,*N*-diisopropylethylamine; TEA, triethylamine; diisopropyl ether, IPE; dichloromethane, DCM; 2-propanol, IPA; trifluoroacetic acid, TFA; 1-hydroxy-1H-benzotriazole, HOBt.

5.1. Methyl 4-methoxy-3-oxo-2-(phenylhydrazono)butanoate (4).

A solution of sodium nitrite (55.6 g, 803 mmol) in water (142 mL) was added dropwise to a solution of **3** (50 g, 539 mmol) in 6 M HCl aq (536 mL) at 0°C. The mixture was added to a suspension of methyl 4-methoxyacetoacetate (78.5 g, 537 mmol) and sodium acetate (264 g, 3220

mmol) in MeOH (1400 mL) and water (350 mL) at 0°C. After the mixture was stirred at room temperature for 2 h, the mixture was diluted with water and stirred for 30 min. The formed precipitate was collected by filtration, washed with water, EtOH/IPE, and IPE, successively, and dried to give **4** (111.2 g, 83%) as a yellow solid. ¹H NMR (CDCl₃) δ 3.51 (3H, s), 3.88 (3H, s), 4.69 (2H, s), 7.18-7.25 (1H, m), 7.37-7.48 (4H, m), 14.99 (1H, brs).

5.2. Methyl 5-methoxy-4-oxo-1-phenyl-1,4-dihydropyridazine-3-carboxylate (5).

A mixture of **4** (25.0 g, 100 mmol) and DMFDMA (120 mL) was stirred at 90 °C for 1 h and at room temperature for 1 h. The formed precipitate was then collected by filtration, washed with diethyl ether and hexane, and dried to give **5** (24.7 g, 95%) as a pale yellow solid. LC-MS (ESI) m/z 261.1 (M+H)⁺. ¹H NMR (CDCl₃) δ 3.96 (3H, s), 3.98 (3H, s), 7.43-7.48 (1H, m), 7.51-7.56 (2H, m), 7.61-7.64 (2H, m), 7.95 (1H, s).

5.3. 5-Methoxy-4-oxo-1-phenyl-1,4-dihydropyridazine-3-carboxylic acid (6).

1 M NaOH aq (300 mL) was added dropwise to a solution of **5** (39.0 g, 150 mmol) in MeOH (663 mL) at 0 °C. After the mixture was stirred at room temperature for 1 h, 1 M HCl aq (300 mL) was added to the mixture at 0 °C. The mixture was stirred at 0 °C for 1 h, and the formed precipitate was collected by filtration. The obtained solid was washed with water, EtOH/IPE, and IPE, successively, and dried to give **6** (36.1 g, 98%) as a pale yellow solid. LC-MS (ESI) m/z 247.1 (M+H)⁺. ¹H NMR (DMSO-*d*₆) δ 3.99 (3H, s), 7.55-7.68 (3H, m), 7.87 (2H, d, *J* = 8.4 Hz), 8.94 (1H, s), 15.25 (1H, s).

5.4. N,5-Dimethoxy-N-methyl-4-oxo-1-phenyl-1,4-dihydropyridazine-3-carboxamide (7).

To a solution of **6** (40 g, 162 mmol) in DMA (600 mL) were added *N*,*O*-dimethylhydroxylamine hydrochloride (23.7 g, 243 mmol) and HOBt (21.9 g, 162 mmol),

1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (46.6 g, 243 mmol), and TEA (81.4 mL, 584 mmol) at 0 °C. After being stirred at room temperature for 96 h, the mixture was diluted with EtOAc and the insoluble was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography with EtOAc/MeOH to give **7** (39.2 g, 84%) as a white solid. LC-MS (ESI) m/z 290.1 (M+H)⁺. ¹H NMR (CDCl₃) δ 3.40 (3H, s), 3.71 (3H, s), 3.96 (3H, s), 7.39-7.44 (1H, m), 7.49-7.54 (2H, m), 7.59-7.63 (2H, m), 7.98 (1H, s).

5.5. 3-Acetyl-5-methoxy-1-phenylpyridazin-4(1*H*)-one (8).

To a solution of **7** (20.0 g, 69.1 mmol) in THF (1000 mL) was added dropwise methylmagnesium bromide in THF (1 mol/L, 138 mL) at -69 °C, and the mixture was stirred at -69 °C for 1.5 h. The mixture was quenched with saturated ammonium chloride solution and warmed to room temperature. The organic layer was washed with brine, dried over MgSO₄. The aqueous layer was extracted with EtOAc. The extract dried over MgSO₄ and the organic layer were combined and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography with EtOAc/MeOH to give **8** (13.2 g, 78%) as a yellow solid. LC-MS (ESI) m/z

245.1 (M+H)⁺. ¹H NMR (CDCl₃) δ 2.70 (3H, s), 3.96 (3H, s), 7.44-7.49 (1H, m), 7.53-7.58 (2H, m), 7.61-7.65 (2H, m), 7.94 (1H, s).

5.6. 5-Methoxy-3-(1-methyl-1*H*-pyrazol-5-yl)-1-phenylpyridazin-4(1*H*)-one (9).

To a suspension of **8** (6.0 g, 24.6 mmol) in MeCN (18 mL) was added DMFDMA (49 mL). After refluxing for 4.5 h, the mixture was concentrated under reduced pressure. The residue was dissolved in EtOH (9 mL), and then to the solution was added dropwise a solution of methylhydrazine (2.6 mL, 49.1 mmol) in 10% TFA/EtOH (85 mL) at below 15 °C. After the mixture was stirred at room temperature for 18 h, the formed precipitate was collected by filtration, washed with EtOH and IPE, and dried under reduced pressure at 50 °C to give **9** (3.8 g, 55%) as a pale yellow solid. ¹H NMR (CDCl₃) δ 3.97 (3H, s), 4.12 (3H, s), 7.24 (1H, d, *J* = 2.3 Hz), 7.42-7.49 (1H, m), 7.52-7.60 (3H, m), 7.60-7.66 (2H, m), 8.02 (1H, s). LC-MS (ESI) m/z 283.2 (M+H)⁺. Anal. Calcd for C₁₅H₁₄N₄O₂: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.69; H, 5.04; N, 19.94.

5.7. 1-Benzyl-3-chloro-5-methoxypyridazin-4(1*H*)-one (11). To a solution of 10 (10.0 g, 62.3 mmol) in DMF (300 mL) were added NaH (55 wt%, 3.26 g, 74.7 mmol) and ^{*n*}Bu₄NI (4.60 g, 12.5 mmol) at 0 °C. The mixture was stirred for 10 min at 0 °C, and then benzyl bromide (12.3 g, 71.6 mmol) was added to it at 0 °C. The mixture was stirred for 20 h at room temperature and then quenched with water and extracted with DCM (twice). The combined organic layer was washed with water and brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residual solid was recrystallized from EtOAc/hexane to give 11 (19.8 g, 53%) as a white solid. ¹H NMR (CDCl₃) δ 3.82 (3H, s), 5.31 (2H, s), 7.34-7.42 (5H, m), 7.89 (1H, s).

5.8. 1-Benzyl-5-methoxy-3-(1-phenyl-1*H***-pyrazol-5-yl)pyridazin-4(1***H***)-one (12). A mixture of 11** (13.6 g, 54.3 mmol), 1-phenyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (22.0 g, 81.0 mmol), K₂CO₃ (51.0 g, 109 mmol) and PdCl₂[Pt-Bu₂(Ph-*p*-NMe₂)]₂ (1.92 g, 2.71 mmol) in toluene (330 mL) and water (33 mL) was refluxed for 24 h under N₂ atmosphere. The mixture was diluted with water and saturated NaHCO₃ aqueous solution. The mixture was extracted with EtOAc, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was recrystallized from EtOAc/hexane to give **12** (15.1 g, 78%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 3.81 (3H, s), 5.10 (2H, s), 6.95 (1H, d, *J* = 1.6 Hz), 7.05-7.07 (2H, m), 7.24-7.38 (8H, m), 7.74 (1H, d, *J* = 1.6 Hz), 8.33 (1H, s).

5.9. 5-Methoxy-3-(1-phenyl-1*H***-pyrazol-5-yl)pyridazin-4-ol (13)**. A mixture of **12** (15.0 g, 41.9 mmol) and Pd(OH)₂/C (Pd: 20%, 50% wet, 5.88 g, 4.19 mmol) in THF (500 mL) and MeOH (300 mL) was stirred at room temperature for 2 days under H₂ atmosphere. After filtration through a Celite pad, the filtrate was concentrated under reduced pressure. The residual solid was washed with EtOH/hexane to give **13** (10 g, 81%) as a brown solid. ¹H NMR (DMSO-*d*₆) δ 3.74 (3H, s), 6.79 (1H, d, *J* = 1.6 Hz), 7.27-7.40 (5H, m), 7.76 (1H, d, *J* = 2.0 Hz), 8.15 (1H, s), 13.4 (1H, brs).

LC-MS (ESI) m/z 269 (M+H)⁺.

5.10. 5-Methoxy-1-methyl-3-(1-phenyl-1*H*-pyrazol-5-yl)pyridazin-4(1*H*)-one (14a).

To a solution of **13** (135 mg, 0.5 mmol) in DMF (2 mL) was added cesium carbonate (361 mg, 1,11 mmol), then, to the mixture was added dimethyl sulfate (0.071 mL, 0.75 mmol) at room temperature. After being stirred at the same temperature for 16 h, the mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with EtOAc/MeOH and recrystallized from MeOH/EtOAc to give **14a** (47 mg, 33%) as a pale yellow solid. ¹H NMR (CDCl₃) δ 3.66 (3 H, s), 3.84 (3 H, s), 7.12 (1H, d, *J* = 1.9 Hz), 7.28 - 7.37 (5H, m), 7.43 (1H, s), 7.76 (1H, d, *J* = 1.9 Hz). LC-MS (ESI) m/z 283.2 (M+H)⁺. Anal. Calcd for C₁₅H₁₄N₄O₂: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.74; H, 5.01; N, 19.95.

5.11. 1-Cyclopropyl-5-methoxy-3-(1-phenyl-1*H*-pyrazol-5-yl)pyridazin-4(1*H*)-one (14b).

To a mixture of **13** (1.0 g, 3.7 mmol) and cesium carbonate (2.4 g, 7.5 mmol) in DMF (20 mL) was added bromocyclopropane (0.60 mL, 7.5 mmol) at room temperature. After stirring at 150 °C in an autoclave for 30 h, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with EtOAc/MeOH and basic silica gel column chromatography with EtOAc/MeOH to give **14b** (15 mg, 1.3%) as a white solid. ¹H NMR (CDCl₃) δ 0.51-0.59 (2H, m), 0.69-0.78 (2H, m), 3.37 (1H, tt, *J* = 7.3, 3.7 Hz), 3.89 (3H, s), 7.24 (1H, d, *J* = 1.9 Hz), 7.29-7.42 (5H, m), 7.58 (1H, s), 7.75 (1H, d, *J* = 2.3 Hz). LC-MS (ESI) m/z 309.1 (M+H)⁺.

5.12. 1-(Cyclopropylmethyl)-5-methoxy-3-(1-phenyl-1*H*-pyrazol-5-yl)pyridazin-4(1*H*)-one (14c).

To a solution of **13** (135 mg, 0.50 mmol) in DMF (2 mL) under argon atmosphere was added 60% oil suspension of sodium hydride (40.3 mg, 1.01 mmol), and the suspension was stirred for 30 min at 0 °C. (Bromomethyl)cyclopropane (0.073 mL, 0.75 mmol was added to the mixture, which was then stirred at the same temperature for 7 h, before MeOH (5 mL) was added to the mixture at below 15 °C. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography with EtOAc/MeOH and recrystallized from MeOH/EtOAc to give **14c** (103 mg, 63%) as a white solid. ¹H NMR (CDCl₃) δ 0.17 (2H, q, *J* = 4.9 Hz), 0.52 (2H, q, *J* = 6.2 Hz), 0.83-0.96 (1H, m), 3.59 (2H, d, *J* = 7.2 Hz), 3.88 (3H, s), 7.18 (1H, d, *J* = 1.9 Hz), 7.27-7.33 (1H, m), 7.33-7.37 (4H, m), 7.50 (1H, s), 7.76 (1H, d, *J* = 1.9 Hz). LC-MS (ESI) m/z 323.3 (M+H)⁺. Anal. Calcd for C₁₈H₁₈N₄O₂: C, 67.07; H, 5.63; N, 17.38. Found: C, 67.05; H, 5.58; N, 17.36.

5.13. 5-Methoxy-3-(1-phenyl-1*H***-pyrazol-5-yl)-1-(2,2,2-trifluoroethyl)pyridazin-4(1***H***)-one (14d).**

To a solution of **13** (2.0 g, 7.5 mmol) in DMF (40 mL) under argon atmosphere was added 60% oil suspension of sodium hydride (0.596 g, 14.91 mmol), and the suspension was stirred for 30 min at 0 °C. Then, trifluoromethanesulfonic acid 2,2,2-trifluoroethyl ester (2.6 g, 11.2 mmol) was added. After the mixture was stirred at the same temperature for 18 h, MeOH (5 mL) was added at below 15°C. This mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography with EtOAc/hexane to give **14d** (1.7 g, 66%) as a white solid. ¹H NMR (CDCl₃) δ 3.84 (3H, s), 4.26 (2H, q, *J* = 8.2 Hz), 7.17 (1H, d, *J* = 1.9 Hz), 7.30-7.39 (5H, m), 7.44 (1H, s), 7.76 (1H, d, *J* = 1.9 Hz). LC-MS (ESI) m/z 351.0 (M+H)⁺. Anal. Calcd for C₁₆H₁₃F₃N₄O₂: C, 54.86; H, 3.74; N, 15.99. Found: C, 54.78; H, 3.87; N, 15.89.

5.14. 1-(Cyclopropylmethyl)-5-hydroxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one (15)

TMSCl (3.2 mL, 25.0 mmol) was added at room temperature to a solution of NaI (3.8 g, 25.0 mmol) in CH₃CN (150 mL). After the mixture was stirred for 30 min, **14c** (1.6 g, 5.0 mmol) was added to the resulting suspension. The mixture was stirred for 30 min at room temperature and then refluxed for 4 h. After being cooled to room temperature, the mixture was poured into water and stirred for 30 min. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was triturated with EtOAc, and collected by filtration. The crystals were washed with EtOAc and IPE, and dried under reduced pressure at 50 °C to give **15** (1.2 g, 80 %) as a pale brown solid. ¹H NMR (CDCl₃) δ 0.18-0.26 (2H, m), 0.35-0.44 (2H, m), 0.85-0.98 (1H, m), 3.74 (2H, d, *J* =7.2 Hz), 6.91 (1H, d, *J* =1.9 Hz), 7.25-7.34 (3H, m), 7.35-7.43 (2H, m), 7.77 (1H, d, *J* =1.9 Hz), 8.22 (1H, s). LC-MS (ESI) m/z 309 (M+H)⁺.

5.15.

1-(Cyclopropylmethyl)-5-[(1-methyl-1*H*-benzimidazol-2-yl)methoxy]-3-(1-phenyl-1*H*-pyrazol-5-yl)pyridazin-4(1*H*)-one (16a).

To a solution of **15** (100 mg, 0.32 mmol), 2-(1-methyl-1H-benzo[d]imidazol-2-yl)methanol (79 mg, 0.49 mmol) and triphenylphosphine (149 mg, 0.57 mmol) in THF (10 mL) was added (E)-di-tert-butyl diazene-1,2-dicarboxylate (20% toluene solution, 0.64 mL, 0.49 mmol) at rt. After stirring at room temperature overnight, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with EtOAc/hexane and recrystallized from EtOAc to give **16a** (72 mg, 49 %) as a white solid. ¹H NMR (CDCl₃) δ 0.12-0.21 (2H, m), 0.40-0.50 (2H, m), 0.74-0.91 (1H, m), 3.53 (2H, d, *J* = 7.2 Hz), 3.97 (3H, s), 5.55 (2H, s), 7.18 (1H, d, *J* = 2.3 Hz), 7.27-7.43 (8H, m), 7.71-7.79 (2H, m), 8.17 (1H, s). LC-MS (ESI) m/z 453.1 (M+H)⁺. Anal. Calcd for C₂₆H₂₄N₆O₂ + 0.2 H₂O: C, 68.47; H, 5.39; N, 18.43. Found: C, 68.51; H, 5.35; N, 18.59.

5.16.

1- (Cyclopropylmethyl) - 5- [2-(1-methyl-1H-benzimidazol-2-yl)ethoxy] - 3-(1-phenyl-1H-pyrazol-2-yl)ethoxy] - 3-(1-phenyl-2-yl)ethoxy] - 3-(1-phenyl-2-yl)ethoxy]

5-yl)pyridazin-4(1*H*)-one (16b).

To a solution of **15** (100 mg, 0.32 mmol), 2-(1-methyl-1H-benzo[d]imidazol-2-yl)ethanol (86 mg, 0.49 mmol) and triphenylphosphine (149 mg, 0.57 mmol) in THF (10 mL) was added (E)-diethyl diazene-1,2-dicarboxylate (0.221 mL, 0.49 mmol) at rt. After being stirred at room temperature for 3 h, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with EtOAc/MeOH to give **16b** (71 mg, 47 %) as an off-white solid. ¹H NMR (DMSO-*d*₆) δ 0.15-0.24 (2H, m), 0.30-0.42 (2H, m), 0.78-0.94 (1H, m), 3.38 (2H, t, *J* = 6.6 Hz), 3.72 (2H, d, *J* = 7.2 Hz), 3.83 (3H, s), 4.44 (2H, t, *J* = 6.6 Hz), 6.89 (1H, d, *J* = 1.9 Hz), 7.10-7.44 (7H, m), 7.47-7.59 (2H, m), 7.75 (1H, d, *J* = 1.9 Hz), 8.37 (1H, s). LC-MS (ESI) m/z 467.1 (M+H)⁺. Anal. Calcd for C₂₇H₂₆N₆O₂ + 0.8 H₂O: C, 67.43; H, 5.78; N, 17.47. Found: C, 67.45; H, 5.76; N, 17.22.

5.17.

1-(Cyclopropylmethyl)-5-[3-(1-methyl-1*H*-benzimidazol-2-yl)propoxy]-3-(1-phenyl-1*H*-pyrazo l-5-yl)pyridazin-4(1*H*)-one (16c).

Compound **16c** was obtained (46%) as a white solid in a manner similar to that described for compound **16a**. ¹H NMR (CDCl₃) δ 0.10-0.18 (2H, m), 0.43-0.51 (2H, m), 0.80-0.93 (1H, m), 2.47 (2H, quin, *J* =6.5 Hz), 3.13 (2H, t, *J* =6.8 Hz), 3.55 (2H, d, *J* =7.2 Hz), 3.75 (3H, s), 4.22 (2H, t, *J* =6.2 Hz), 7.17 (1H, d, *J* =1.9 Hz), 7.20-7.28 (2H, m), 7.29-7.32 (2H, m), 7.32-7.36 (4H, m), 7.65-7.70 (1H, m), 7.71 (1H, s), 7.76 (1H, d, *J* =1.9 Hz). LC-MS (ESI) m/z 481.3 (M+H)⁺. Anal. Calcd for C₂₈H₂₈N₆O₂ + H₂O: C, 67.95; H, 6.29; N, 16.39. Found: C, 68.21; H, 6.26; N, 16.26.

5.18.

1-(Cyclopropylmethyl)-5-[4-(1-methyl-1*H*-benzimidazol-2-yl)butoxy]-3-(1-phenyl-1*H*-pyrazol-5-yl)pyridazin-4(1*H*)-one (16d).

Compound **16d** was obtained (54%) as a white solid in a manner similar to that described for compound **16b**. ¹H NMR (CDCl₃) δ 0.14-0.25 (2H, m), 0.31-0.43 (2H, m), 0.80-0.96 (1H, m), 1.77-1.97 (4H, m), 2.94 (2H, t, *J* = 7.2 Hz), 3.65-3.81 (5H, m), 3.94-4.09 (2H, m), 6.89 (1H, d, *J* = 1.9 Hz), 7.08-7.58 (9H, m), 7.75 (1H, d, *J* = 1.9 Hz), 8.28 (1H, s). LC-MS (ESI) m/z 495.2 (M+H)⁺. Anal. Calcd for C₂₉H₃₀N₆O₂ + 2 H₂O: C, 65.10; H, 6.24; N, 16.27. Found: C, 65.33; H, 6.20; N, 16.26.

5.19.

1-(Cyclopropylmethyl)-5-(2-((1-methyl-1*H*-benzimidazol-2-yl)oxy)ethoxy)-3-(1-phenyl-1*H*-pyr azol-5-yl)pyridazin-4(1*H*)-one (16e).

Compound **16e** was obtained (73%) as a white solid in a manner similar to that described for compound **16a**. ¹H NMR (CDCl₃) δ 0.09-0.16 (2H, m), 0.43-0.51 (2H, m), 0.76-0.91 (1H, m), 3.50 (2H, d, *J* = 7.6 Hz), 3.54 (3H, s), 4.67 (2H, dd, *J* = 5.7, 3.8 Hz), 4.86 (2H, dd, *J* = 5.7, 3.8 Hz), 7.13-7.18 (3H, m), 7.19 (1H, d, *J* = 1.9 Hz), 7.27-7.37 (5H, m), 7.46-7.53 (1H, m), 7.75 (1H, s),

7.77 (1H, d, J = 1.9 Hz). LC-MS (ESI) m/z 483.1 (M+H)⁺. Anal. Calcd for $C_{27}H_{26}N_6O_3 + 0.6$ H₂O: C, 65.73; H, 5.56; N, 17.03. Found: C, 65.60; H, 5.55; N, 16.81.

5.20.

1-(Cyclopropylmethyl)-5-[2-(2,3-dihydro-1*H*-imidazo[1,2-a]benzimidazol-1-yl)ethoxy]-3-(1-ph enyl-1*H*-pyrazol-5-yl)pyridazin-4(1*H*)-one (16f).

A mixture of 15 (231 mg, 0.75 mmol),

1-(2-chloroethyl)-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (249 mg, 1.13 mmol), cesium carbonate (428 mg, 1.31 mmol), sodium iodide (253 mg, 1.69 mmol) in DMA (10 mL) was stirred at 80 °C for 13 h. The precipitate was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography with EtOAc/hexane to give **16f** (231 mg, 62%) as a white solid. ¹H NMR (CDCl₃) δ 0.11 (2H, q, *J* = 5.0 Hz), 0.45 (2H, q, *J* = 6.2 Hz), 0.74-0.87 (1H, m), 3.47 (2H, d, *J* = 7.2 Hz), 3.84 (2H, t, **J** = 5.1 Hz), 4.06-4.14 (2H, m), 4.22-4.30 (2H, m), 4.43 (2H, t, *J* = 5.1 Hz), 7.00-7.11 (3H, m), 7.19 (1H, d, *J* = 1.9 Hz), 7.27-7.34 (5H, m), 7.38 (1H, d, *J* = 7.6 Hz), 7.72 (1H, s), 7.77 (1H, d, *J* = 1.9 Hz). LC-MS (ESI) m/z 494.4 (M+H)⁺. Anal. Calcd for C₂₈H₂₇N₇O₂ + 1.7 H₂O: C, 64.16; H, 5.85; N, 18.70. Found: C, 64.13; H, 5.86; N, 18.64.

5.21. Crystallization and Structure Determination. Human PDE10A catalytic domain for crystallographic study was prepared as previously described.¹³ Crystals were obtained using the sitting-drop vapor-diffusion method by mixing 50 nL of protein solution (5-10 mg/mL PDE10A catalytic domain in TBS (pH 7.4), 0.5 mM DTT, 1 mM EDTA, and 10% glycerol) and 50 nL of reservoir solution containing 0.1 M HEPES (pH 7.5-8.0), 24-32% PEG 3350, and 200 mM MgCl2 at 4 °C. Complex crystals for compound 2 were obtained by immersing apo crystals into the corresponding reservoir solution containing 0.25 mM inhibitor for 48 hours. As for complex crystals with compound 16f, apo crystals were soaked into 0.2 mM compound 16f solution for 40 hours. Prior to data collection, complex crystals were treated with the reservoir solution containing 25% ethylene glycol as a cryoprotectant and were flash-frozen in liquid nitrogen. Diffraction data of 2 and 16f were collected from each single crystal at the Advanced Light Source beamline 5.0.2 and The data were processed using the program HKL2000.³⁶ The 5.0.3 (Berkeley, CA), respectively. structures were determined by molecular replacement using MOLREP,³⁷ utilizing the previously reported coordinate of PDE10A with the PDB accession code 3WYM.¹³ Subsequently, the structures were refined through an iterative procedure utilizing REFMAC,³⁸ followed by model building with WinCoot.³⁹ The dictionary files for the inhibitors were prepared using AFITT (OpenEye Scientific Software). Crystallographic processing and refinement statistics are summarized in Table 3. The coodinates and structure factors have been deposited in PDB with accession codes 5B4K and 5B4L for 2 and 16f, respectively.

Table 3. X-ray data collection and refinement statistics

	2	16f	
Data Collection			
X-ray source	ALS BL5.0.2	ALS BL5.0.3	
Wavelength (Å)	1.0000	0.97645	
Space group	P2 ₁ 2 ₁ 2 ₁	$P2_{1}2_{1}2_{1}$	
Unit cell dimensions (Å)	a=49.7, b=81.7, c=161.7	a=49.1, b=81.5, c=159.2	
Resolution (Å)	2.90	2.40	
Unique reflections	13970	26587	
Redundancy	6.0	5.1	
Completeness (%)	90.9 (53.1)	99.3 (98.4)	
Ι/σ(Ι)	18.3 (5.3)	10.0 (1.1)	
$\mathbf{R}_{\mathrm{sym}}^{a}$	0.082	0.123	
Refinement			
Reflections used	13872	25650	
RMS Bonds (Å)	0.004	0.005	
RMS Angles (°)	0.914	1.013	
Average B value (Å ²)	51.110	37.221	
R-value ^b	0.181	0.235	
R free b	0.261	0.282	

^aRsym = $\Sigma h\Sigma j$ |<I(h)> - I(h)j | / $\Sigma h\Sigma j$ <I(h)>, where <I(h)> is the mean intensity of symmetry-related reflections. ^bR-value = Σ | |Fobs| - |Fcalc| | / Σ |Fobs|. Rfree for 5% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

5.22. Enzyme assay protocol. Human PDE10A2 was generated from COS-7 cells transfected with the full-length gene. The enzyme was stored at -70 °C until use. PDE10A activity was measured using a SPA (PerkinElmer). To evaluate the inhibitory activity, 10 µL of serial diluted compounds were incubated with 20 µL of PDE enzyme in assay buffer (50 mM HEPES-NaOH, 8.3 mM MgCl₂, 1.7 mM EGTA, 0.1% BSA (pH 7.4)) for 30 min at rt. Final concentration of DMSO in the assay was 1 % as compounds were tested in duplicate or triplicate in 96-well half-area plates (Corning). To start the reaction, 10 µL of substrate [³H]cGMP (PerkinElmer) for PDE10A2 was added for a final assay volume of 40 µL. After 60 minutes incubation at rt, 20 µL of 20 mg/mL yttrium SPA beads containing ZnSO₄ was added to terminate the PDE reaction. After being settled for more than 120 min, the assay plate was counted in a scintillation counter (PerkinElmer) to allow calculation of inhibition rate. Inhibition rate was calculated on the basis of 0% control wells with DMSO and 100% control wells with enzyme plus 10 µM papaverine (Wako Pure Chemical Industries) for

PDE10A2. IC₅₀ values were determined by the least squares non-linear regression with logistic. The calculation was carried out using XLfit software (ID Business Solusions, Guildford, UK).

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