4-Hydroxypyridazin-3(2H)-one Derivatives as Novel \(\alpha\)-Amino Acid Oxidase Inhibitors

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Supporting Information

ABSTRACT: \(\alpha\)-Amino acid oxidase (DAAO) catalyzes the oxidation of \(\alpha\)-amino acids including \(\alpha\)-serine, a coagonist of the \(N\)-methyl-\(\alpha\)-aspartate receptor. We identified a series of 4-hydroxypyridazin-3(2H)-one derivatives as novel DAAO inhibitors with high potency and substantial cell permeability using fragment-based drug design. Comparisons of complex structures deposited in the Protein Data Bank as well as those determined with in-house fragment hits revealed that a hydrophobic subpocket was formed perpendicular to the flavin ring by flipping Tyr224 in a ligand-dependent manner. We investigated the ability of the initial fragment hit, 3-hydroxy-pyridine-2(1H)-one, to fill this subpocket with the aid of complex structure information. 3-Hydroxy-5-(2-phenylethyl)pyridine-2(1H)-one exhibited the predicted binding mode and demonstrated high inhibitory activity for human DAAO in enzyme- and cell-based assays. We further designed and synthesized 4-hydroxypyridazin-3(2H)-one derivatives, which are equivalent to the 3-hydroxy-pyridine-2(1H)-one series but lack cell toxicity. 6-[2-(3,5-Difluorophenyl)ethyl]-4-hydroxypyridazin-3(2H)-one was found to be effective against MK-801-induced cognitive deficit in the Y-maze.

INTRODUCTION

The flavoenzyme \(\alpha\)-amino acid oxidase (DAAO) catalyzes the stereospecific oxidative deamination of \(\alpha\)-amino acids to \(\alpha\)-keto acids, \(\text{NH}_3\), and \(\text{H}_2\text{O}_2\). \(\alpha\)-Serine, which is metabolized by DAAO, acts as an endogenous coagonist at the glycine site of the \(N\)-methyl-\(\alpha\)-aspartate receptor (NMDAR) as well as glycine. Noncompetitive NMDAR antagonists such as ketamine or phencyclidine have been shown to induce psychotic and neurocognitive symptoms similar to those observed in schizophrenia patients, including positive, negative, and cognitive symptoms.\(^1,2\) Additionally, hypofunction of DAAO expression and activity have been reported to be elevated in schizophrenia patients,\(^3\) while administration of high decreases of \(\alpha\)-serine in CSF and brain have been seen in schizophrenia patients,\(^4,5\) while administration of high decreases of \(\alpha\)-serine in combination with antipsychotic drugs was found to reduce positive, negative, and cognitive symptoms of schizophrenia.\(^6,7\) DAAO expression and activity have been reported to be elevated in schizophrenia patients,\(^8,9\) and mutant mice lacking functional DAAO are resistant to the effects of NMDAR antagonists.\(^10\)–\(^12\) Furthermore, the G72 gene on chromosome 13q, which is thought as a DAAO activator, has been significantly associated with schizophrenia.\(^13\)

Several compounds have been reported to exert inhibitory activity against DAAO (Figure 1), including benzoic acid,\(^1,4\) 5-methylpyrazole-3-carboxylic acid (AS057278)\(^2a\), 6-chlorobenzo[d]isoxazole-3-ol (CBOI)\(^3,16\), 4H-thieno[3,2-b]-pyrrole-5-carboxylic acid, and 4H-furo[3,2-b]pyrrole-5-carboxylic acid,\(^5,17\) which are all characterized as aryl carboxylic acids or corresponding acid-isosteres with small molecular weight. Although these compounds are reported to exhibit high inhibitory activity for human DAAO in cell-free systems and \(IC_{50}\) values in the submicromolar to nanomolar range (except benzoic acids), their high acidity and low hydrophobicity hamper cell permeability. Two recently reported inhibitors, 5-chloro-6-fluoro-3-hydroxy-1,8-naphthyridin-2(1H)-one,\(^6\) and 4,6-difluoro-1-hydroxy-1H-benzo[d]imidazol-2(3H)-one,\(^7\) overcome this drawback; however, their structures leave little room to introduce new chemical groups to either or both increase the activity or modify physicochemical properties for improving the pharmacokinetics profiles.\(^18\) While administration of DAAO inhibitors can ameliorate some NMDAR antagonist-induced deficits,\(^1,2\) such effects have not been observed across all behaviors related to NMDAR activity.\(^17\) Further, although NMDAR-mediated behaviors are indicative of activity in forebrain regions, the addition of DAAO inhibitors

Received: January 21, 2013
Published: April 8, 2013
increases D-serine levels in the cerebellum but not in the cortex.\textsuperscript{17,20} Therefore, compounds with more potent activity and better pharmacokinetics profiles than known inhibitors are preferred for testing the therapeutic value of DAAO inhibitors.

Here, we report 4-hydroxypyridazin-3(2H)-one derivatives as novel DAAO inhibitors using fragment-based drug discovery (FBDD), a recent and novel approach for the identification of small-molecular inhibitors which has already helped identify a number of clinical candidates by taking full advantage of structural information on a number of complex structures.\textsuperscript{21} Using X-ray crystallography, we designed and synthesized compounds that can utilize a subpocket formed by flipping Tyr224. The subpocket is considered unoccupied in complex structures with inhibitors 1–7, and using this pocket, we identified compounds with high potency and substantial cell permeability.

\section*{CHEMISTRY}

Compound 12 was prepared as illustrated in Scheme 1. 5-Bromo-3-hydroxypyridine-2(1H)-one 33 was first converted to the 2,3-bisprotected pyridine 34. Suzuki-coupling reaction using 2-phenylvinyl boronic acid with 34 and hydrogenation of the double bond and subsequent deprotection gave 12.

4-Hydroxypyridazin-3(2H)-one derivatives were prepared as illustrated in Scheme 2. 3-Chloro[1,4]benzodioxino[2,3-c]-pyridazine 35 was used as the starting material and converted by Suzuki or Sonogashira coupling to intermediates 36. Subsequent reduction of multiple bonds followed by deprotection gave 4-hydroxypyridazin-3(2H)-one derivatives 13, 14, 16–20, and 22–32. Compound 15 was directly deprotected after the coupling reaction.

Compound 21, which has an ether linkage, was prepared as illustrated in Scheme 3. 3-Chloro[1,4]benzodioxino[2,3-c]-pyridazine 35 was first converted to corresponding ester 38 by CO insertion. After the reduction of the methyl ester and conversion to the corresponding leaving group, alkylation with phenol yielded 40. Compound 21 was obtained from 40 in the same manner as shown in Scheme 2.

\section*{RESULTS AND DISCUSSION}

\subsection*{Fragment Screening and Complex Structure Determination.}

Six tertiary structures of human DAAO complexed with inhibitors or ligand analogues were deposited in the Protein Data Bank (PDB). The binding pocket, which is packed between Tyr224 and a flavin ring, appears to accommodate only small-sized compounds corresponding to a two-fused ring.

Comparisons of all structures revealed that Tyr224 adopts two conformations (Figure 2). In the first conformation, the phenyl ring of Tyr224 is oriented toward the side chain of Leu215 so that the overlap between the phenyl ring of Tyr224 and flavin ring is minimized (PDB ID: 2E49, 2E82, 3CUK, 3G3E; Tyr224-out). In the second conformation, Tyr224 moves so that a subpocket formed by flipping Tyr224 is oriented toward the side chain of Leu215 so that the overlap between the phenyl ring of Tyr224 and flavin ring is minimized (PDB ID: 2DU8, 2E4A; Tyr224-in). In the complex structure with substrate analogue imino-DOPA, the catechol moiety fills this subpocket.\textsuperscript{22} 3-(2-Phenylethyl)-1H-pyrazole-5-carboxylic acid 2b,\textsuperscript{23} which has a bulkier side chain than 2a, was also predicted to stretch into this subpocket in the recent review.\textsuperscript{25}

Because the subpocket faces an entry site and is large enough to introduce chemical groups, we carried out high-concentration screening of an in-house 3500 fragment library to find...
fragment hits that were expected to be elongated into the subpocket on the basis of complex structures. Most of the fragment hits belonged to small aryl carboxylic acids as predicted. One exception was 3-hydroxy-pyridine-2(1\textsubscript{H})-one \textsuperscript{8}, which is a substructure of \textsuperscript{6} (Figure 1). All fragment hits had a common interaction motif: \(\pi-\pi\) stacking with re-face of the flavin ring and the phenyl ring of Tyr224, and an electrostatic interaction with Arg283. Tyr224 residues adopted two conformations in the complex structures of fragment hits as well as those in the PDB. We found that hydrogen-bonding with Gly313 induces the Tyr224-out conformation. Among the fragment hits, we selected \textsuperscript{8} as a starting fragment because it did not contain highly acidic groups and was found to induce the Tyr224-out conformation, which helped us access the newly identified subpocket (Figure 3A). Introduction of a flexible linker into the 5-position of \textsuperscript{8} seemed to connect the original pocket and the subpocket without strain on the basis of the complex structure, indicating that \textsuperscript{8} is an appropriate starting fragment to fill the subpocket during subsequent elongation. This discovery motivated us to design and synthesize 3-hydroxy-pyridine-2(1\textsubscript{H})-one derivatives.

**Synthesis and SAR Analysis of 3-Hydroxypyridine-2(1\textsubscript{H})-one Derivatives.** Unsubstituted 3-hydroxy-pyridine-2(1\textsubscript{H})-one compound \textsuperscript{8} was much less potent than known inhibitor \textsuperscript{5} in the enzyme inhibitory assay. On the basis of the complex structure of \textsuperscript{8} and DAAO, we speculated that the 5-position was appropriate to elongate substituents into the subpocket as well as those in the PDB. We found that hydrogen-bonding with Gly313 induces the Tyr224-out conformation. Although the mechanism of this conformation switch is not clear, this rule held true for each complex structure (data not shown).

The only exception in the PDB and in-house structure data was the complex structure (PDB ID: 2E4A), in which the amino group of o-aminobenzoate makes a suboptimal hydrogen bond with the carbonyl atom of Gly313 when Tyr224 adopts the Tyr224-in conformation. Among the fragment hits, we selected \textsuperscript{8} as a starting fragment because it did not contain highly acidic groups and was found to induce the Tyr224-out conformation, which helped us access the newly identified subpocket (Figure 3A). Introduction of a flexible linker into the 5-position of \textsuperscript{8} seemed to connect the original pocket and the subpocket without strain on the basis of the complex structure, indicating that \textsuperscript{8} is an appropriate starting fragment to fill the subpocket during subsequent elongation. This discovery motivated us to design and synthesize 3-hydroxy-pyridine-2(1\textsubscript{H})-one derivatives.

**Scheme 3. Synthesis of 21**

\[ \begin{align*}
\text{35} & \rightarrow \text{38} \\
\text{38} & \rightarrow \text{39} \\
\text{40} & \rightarrow \text{21}
\end{align*} \]

\textsuperscript{a}Reagents and conditions: (a) Pd(OAc)\textsubscript{2}, dpff, CO, Et\textsubscript{3}N, DMSO–MeOH, 80 °C; (b) NaBH\textsubscript{4}, THF–MeOH, 0 °C; (c) SOCl\textsubscript{2}, DCE, 80 °C; (d) PhOH, K\textsubscript{2}CO\textsubscript{3}, DMF, rt; (e) PhCH\textsubscript{2}OH, tBuOK, toluene, 120 °C; (f) H\textsubscript{2}, Pd–BaSO\textsubscript{4}, EtOH, rt.

**Figure 2.** Binding sites of human DAAO in (upper) Tyr224-in conformation (PDB ID: 2DU8) and (lower) Tyr224-out conformation (PDB ID: 3CUK). Flavin ring of FAD (flavin adenine dinucleotide) and inhibitors are shown in orange and purple, respectively. Tyr224 residues are shown in yellow, and other residues are shown in green. The right panels are views in a different orientation to highlight the subpocket in Tyr224-out conformation indicated in dotted circle. The surfaces of the binding sites are represented in green. Figures were prepared using MOE2011 (Chemical Computing Group Inc., Quebec, Canada).

**Figure 3.** Binding sites of human DAAO in the complex structures with \textsuperscript{8} (A) and \textsuperscript{12} (B). Hydrogen bonds are shown in dotted lines. Color schemes and the software used to generate the figures are the same as those in Figure 2.
enhanced activity compared with 8 (Table 1). Quinolone analogue 11, in which the 5- and 6-positions of 3-hydroxypyridin-2(1H)-one were converted to cyclic structures, showed very strong inhibitory activity as previously reported. The improved activity might be due to a \( \pi \)-\( \pi \) stacking effect between the flavin and quinolone rings, as this effect would have been improved by the increased \( \pi \)-surface of the bicyclic structure. To investigate whether or not occupation of the subpocket increased affinity, we introduced a 2-phenylethyl group at the 5-position of 3-hydroxypyridin-2(1H)-one. The inhibitory activity of 12 was found to be more than 30 times as potent as that of compound 10, which was comparable to that of 11. The complex structure of DAAO with 12 revealed that 12 was able to bind to DAAO as expected (Figure 3B). The binding mode of the 3-hydroxypyridine-2(1H)-one scaffold of 12 closely resembled that of 8, in that hydrogen bonds with Arg283 and \( \pi \)-\( \pi \) stacking with the flavin ring were retained. The introduced 2-phenylethyl group connected by a flexible linker occupied the subpocket perpendicular to the pyridin-2(1H)-one ring, and the benzene ring was involved in T-shaped stacking with Tyr224. Of note, 12 was much more potent than 5 or 9 when evaluated in cell lines, possibly due to an increase in lipophilicity and cell permeability. These results demonstrate that the subpocket provides a preferable region not only for enhancement of inhibitory affinity but also for modulation of the molecular properties. To the best of our knowledge, this is the first finding to raise a new possibility of this subpocket.

**Synthesis and SAR Analysis of 4-Hydroxy-pyridazin-3(2H)-one Derivatives.** While 12 was highly potent DAAO inhibitor in the evaluation of enzymes and cell lines, it was found to induce cell toxicity at high concentrations. In an effort to identify new compounds with reduced cell toxicity, we designed compounds that are predicted to conserve the interaction equivalent to 3-hydroxyypyridine-2(1H)-one derivatives: a stacking interaction with flavin and Tyr224, an electrostatic interaction with Arg283, and hydrogen bonding with Gly313, which induces the Tyr224-out conformation as mentioned above. 4-Hydroxy-pyridazin-3(2H)-one derivative 13 provided the expected properties (Figure 4). Compound 13 retained potent inhibitory activity (Table 2) in both enzymes (IC\(_{50} = 3.8 \text{ nM}\)) and cell lines (IC\(_{50}\_\text{human} = 2.4 \text{ nM}\), IC\(_{50}\_\text{mouse} = 3.3 \text{ nM}\), IC\(_{50}\_\text{rat} = 4.6 \text{ nM}\)) without cytotoxicity even at high

### Table 1. Preliminary SAR Studies of Analogues of Fragment Hit 3-Hydroxypyridin-2(1H)-one 8 for Human DAAO

<table>
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<th>compd.</th>
<th>structure</th>
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<th>cell IC(_{50} ) (nM)</th>
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<td>12</td>
<td></td>
<td>3.9</td>
<td>&gt;30</td>
<td>20(^d)</td>
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\( ^{a} \)Effective permeability measured by parallel artificial membrane permeability assay (PAMPA) method. \( ^{b} \)pH of donor buffer. \( ^{c} \)ION membrane lipid was used. \( ^{d} \)N.T.: not tested. \( ^{e} \)Cytotoxicity was observed at high concentration conditions.

### Table 2. In Vitro Inhibitory Activities of 4-Hydroxy-pyridazin-3(2H)-one Derivatives 13–21 for Human, Mouse, and Rat DAAOs

<table>
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<th>cell(h) ( \text{IC}_{50} ) (nM)</th>
<th>cell(m) ( \text{IC}_{50} ) (nM)</th>
<th>cell(r) ( \text{IC}_{50} ) (nM)</th>
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concentrations. Because the 4-hydroxypyrudazin-3(2H)-one scaffold is confirmed to be less toxic than that of 3-hydroxy-pyridine-2(1H)-one, the SAR and substituent tolerance at the 5-position was investigated with this new scaffold.

Comparison of the data for 13–16 shows that the subpocket can tolerate a series of one to three methylene linkers and loss of aromacity. This property clearly indicates that the T-shaped stacking interaction between the benzene ring of 13 and Tyr224 observed in the complex structure is not essential for the high affinity. Compound 17 shows the introduction of a chloro group at the p-position was acceptable for inhibitory activity. A significant decrease in the activity of 18 compared to 17 suggests the importance of linker flexibility, as the rigid linker of 18 may fail to provide the correct twist needed to allow the benzene ring to locate perpendicular to the pyridazine ring, instead creating a steric clash. Low potency of 19 in which the cyclohexyl group is directly bonded to the 5′-position may also be attributed to a lack of flexibility. According to the complex structure with 13, the space sandwiched between Tyr224 and Leu215 of the subpocket is too narrow to accept bulky substituents. The inter-residue distance between Tyr224 and Leu215 is 7.1 Å for Tyr224 CE1 and Leu215 CD1 and 5.1 Å for Tyr224 HE1 and Leu215 HD1. This narrow space is consistent with the reduced activity of 20, which has a t-Bu group instead of a benzene ring. The subpocket can accommodate not only a lipophilic moiety but also the polar linkers introduced in 21.

We further examined the effect of substituents on the benzene ring of compound 13 (Table 3). These data indicate that the introduction of small groups to the benzene ring is tolerated regardless of the electron-donating or electron-withdrawing properties and effect likely due to (i) small substituents not being expected to interact with DAAO residues and (ii) T-shaped stacking interactions between the benzene ring of 13 and Tyr224 not being essential, as mentioned above. However, the SAR of disubstituted compounds (30–32) suggests that large groups introduced in the benzene ring cannot be accommodated by the subpocket. While compound 30, which is the smallest of the three, is as potent as 13, the inhibitory activity decreases with increasing size of the substituent; 32 is 3-fold less potent than 13, and 31, which is the largest of the three, is 15-fold less potent than 13.

In total, the data in Tables 2 and 3 demonstrate that the inhibitory activities of enzyme and cell lines can be improved by the use of the newly identified subpocket. In addition, this subpocket exhibits broad preference for size, aromaticity, and hydrophobicity of substituents. Further, amino acid residues around the subpocket are well-conserved among human, mouse, and rat DAAOs. Consistent with this, 17 and 22–32 showed comparable activities among three species. Taken together, these findings suggest the possibility that the physical properties such as hydrophobicity can be controlled to improve the pharmacokinetic profile by changing substituents at the 5′-position of 4-hydroxy-3(2H)-one without loss of enzymatic and cellular activities in humans, mice, and rats.

Indeed, the 4-hydroxy-3(2H)-one derivatives 13–32 take a wide range (0.13–33.5) of ACDlogP values.

Effects on MK-801-Induced Working Memory Deficit in Mice. For further evaluation, we selected those compounds from 13–32 expected to be present in high concentrations in plasma and brain. Because this series of compounds exhibited low sensitivity in mass spectroscopy, we first selected compounds using the parallel artificial membrane permeation assay (PAMPA) for blood–brain barrier as indices of cell permeability and brain penetration (Supporting Information Table S1). Brain concentrations of the resulting compounds were then measured after oral administration (30 mg/kg) to mice. Compound 30 exhibited the most preferable combination of in vitro activities and brain concentrations as early as 30 min; (Cbrain, t=0.5 = 460 ng/mL, Cplasma, t=0.5 = 640 ng/mL). We next evaluated the effect of 30 on the MK-801-induced spontaneous alternation deficit using the mouse Y-maze task, which is a model of cognitive impairment associated with schizophrenia (CIAS) based upon the NMDA hypothesis.

The alternation rate and the number of total arm entries were measured. While 30 significantly increased the alternation rate at doses of 0.03 and 0.1 mg/kg, po (Figure 5), it did not affect total number of arm entries in MK801-treated mice (data not shown), suggesting that 30 ameliorated the cognitive deficit induced by MK-801. We were unable to reduce the dose range of compound 30 when determining plasma and brain levels (30 mg/kg, po) to that for the Y-maze study (0.003–0.3 mg/kg, po) because of the low sensitivity of the mass spectroscopy. The brain concentration of compound 30 after administration (0.1 mg/kg, po 0.5 h) was expected to be 6.1 nM, which was 1.4-fold the IC50 determined in the cell-based assay, assuming linearity between the dose and brain level. This in vivo result supports the hypothesis that DAAO inhibitors are effective for the treatment of CIAS. To our knowledge, this is the second demonstration of efficacy in animal models of schizophrenia by oral administration of a DAAO inhibitor.15 We also note that the dose–response curve of 30 was an inverted U-shape in the Y-maze, a characteristic shared by many other compounds when investigated in cognitive tests despite different modes of action.27–29 For instance, previous studies have suggested that proper cognitive function requires dopamine levels be

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Table 3. In Vitro Inhibitory Activities of 4-Hydroxypyrudazin-3(2H)-one Derivatives 22–32 for Human, Mouse, and Rat DAAOs

<table>
<thead>
<tr>
<th>compd</th>
<th>R1-</th>
<th>enzyme IC50 (nM)</th>
<th>cell(h) IC50 (nM)</th>
<th>cell(m) IC50 (nM)</th>
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optimum, otherwise cognition is impaired. Similarly, DAAO inhibition by agents such as 30 may also have an optimum level. D-Serine levels in the brain after administration of 30 were not measured, preventing us from drawing definitive conclusions about the mode of action of this compound. Previous studies indicate that the administration of DAAO inhibitors increases D-serine levels in the cerebellum and plasma but not in the frontal cortex. If 30 had similar effects on D-serine levels in the cortex, then the mode of action could be explained by other substrates for DAAO like D-alanine, which is also known to function as a coagonist of NMDAR. In contrast to D-serine, D-alanine levels were reported to increase across multiple brain regions not only in the cerebellum in mice lacking DAAO activity. Another possibility was that improvement of MK-801 induced working memory deficit was mediated by regions other than forebrain regions via an unknown mechanism. The third possible mode of action involves off-target of 30. It is noted that 30 exhibited no significant activity against a panel of 57 receptors, ion channels, transporters, and enzymes (Supporting Information Table S2). Detailed analysis using microdialysis is required to address the mode of action.

A new series of DAAO inhibitors reported after the submission of this manuscript has a chemical structure similar to that of 30 and is therefore expected to show high affinity and good cell permeability. The compounds in that series as well as 30 could prove useful in evaluating the importance of DAAO inhibitors. Furthermore, such compounds are also expected to be useful in studying the effects of coadministration of D-serine and a DAAO inhibitor, which has been reported to be effective even in cases when DAAO inhibitors alone did not exert efficacy, potentially representing new therapeutic possibilities.

CONCLUSION

We identified a series of novel DAAO inhibitors, 4-hydroxy- pyridazin-3(2H)-one derivatives, using FBDD from fragment 3- hydroxyypyridine-2(1H)-one 1 and successfully established SARs in a series of DAAO inhibitors that showed no cytotoxicity in 4-hydroxy-pyridazin-3(2H)-one derivatives. Among the compounds prepared in this series, compound 30 demonstrated improved DAAO inhibitory activity over 2–7, showed oral activity and brain penetrability, and ameliorated MK-801-induced cognitive deficit in mice.

EXPERIMENTAL SECTION

Protein Production and Purification. The recombinant human DAAO was produced and purified using the same procedures described previously. The purified enzyme was dialyzed against buffer containing 10 mM Na citrate (pH 8.0), 20 μM FAD, and 400 μM Na benzoate at 4 °C and then concentrated to 10 mg/mL.

Cell Line Establishment and Cell Culture. Cell lines and cell cultures were made using previous methods with some modifications. Briefly, full-length human, rat, and mouse DAAO were cloned by PCR using first strand cDNA synthesized from human kidney RNA, rat kidney RNA, and mouse kidney RNA (Clontech. Inc. Palo Alto, CA, USA), respectively. Primer sets used were: 5′-AAG GTG GGA TGG TGG CA-3′ for human DAAO, 5′-AAG GTG ATG CGT GTG GTG ATT GG-3′ for rat DAAO, and 5′-AAG GTT ATG CGT GTG GCC GTG ATT GG-3′ for mouse DAAO. The PCR products were cloned into a pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA), and the sequences were confirmed. The confirmed plasmids were then digested with restricted enzymes, HindIII/BamHI, and the digested products were inserted into a pcDNA3.1(+) vector (Invitrogen). A human embryonic kidney (HEK)-293 cell line was cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C, 5% CO2. To obtain human, rat, or mouse DAAO-overexpressing HEK293 cell lines, we transfected the vectors into HEK293 cell lines using Lipofectamine 2000 (Invitrogen) and maintained the transfected cell lines in the above medium with 800 μg/mL of Geneticin (G418).

Crystallography. Small crystals of human DAAO were obtained using the sitting-drop vapor-diffusion method under previously reported conditions. After optimization of the conditions, ligand-free holoenzyme crystals suitable for X-ray diffraction experiments were grown at 293 K using the small crystals as seeds. Proteins were mixed with an equal volume of reservoir solution including 10–15% (w/v) PEG 4000, 0.1 M sodium citrate, pH 8.0, 0.2 M ammonium dihydrogen phosphate, and 10% (v/v) glycerol. Plate-shaped yellow crystals appeared within a day and reached average dimensions of 0.2 × 0.2 × 0.05 mm3 after 3 days. Crystals in complex with inhibitors were prepared by the soaking method. Soaking solutions were prepared by mixing stabilization solution (20% PEG 4000, 0.1 M sodium citrate pH 8.0, 0.2 M ammonium dihydrogen phosphate, and 10% (v/v) glycerol) with 100 mM compound solution dissolved in DMSO at a ratio of 10 to 1. The ligand-free holoenzyme crystal was soaked in the soaking solution for 12 h at 293 K. X-ray diffraction data were collected using a synchrotron beamline AR-NE3A at the Photon Factory. All data sets were processed and scaled with HKL2000. Structure solution and model refinement were carried out using CCP4 suite. The structures were solved by molecular replacement using a previously reported human DAAO structure (PDB ID: 2D8U) as a model. Automated ligand fitting into initial fo–fc map were performed using AFPI-T-CL41 followed by model rebuilding using COOT. Data collection and refinement statistics are presented in Supporting Information.

Animals. Mice were housed in groups of 10 in temperature- and humidity-controlled colony rooms (23 ± 1 °C and 55 ± 5%) under a 12 h light/dark cycle with water and laboratory chow supplied ad libitum. All experiments were conducted in accordance with the Astellas Pharma Inc. guidelines for the care and use of animals and

Figure 5. Effects of 30 on MK-801-induced working memory deficit in the mouse Y-maze test. The alternation rate was measured. Compound 30 was administrated orally, followed 10 min later by intraperitoneal injection of MK-801 (0.15 mg/kg). Then 20 min after administration of MK-801, each mouse was placed at the end of one arm and allowed to freely explore the apparatus for 8 min. Each value shows the mean ± SEM (n = 8). N, normal group treated with saline; C, control group treated with MK-801. **p < 0.01: statistically significant compared with normal group, as assessed by Student’s t test. ***p < 0.05: statistically significant compared with control group, as assessed by Dunnett’s multiple comparison test.
under approved protocols from the Institutional Animal Care and Use Committee of Astellas Pharma Inc.

**Human DAAO Inhibition Enzyme Assay.** All compounds were screened in human DAAO inhibition assays as described below. Amplex Red reagent (catalogue code: A-12222, Invitrogen) was used to measure H₂O₂ produced by the DAAO reaction. Briefly, a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.5), 0.02% CHAPS, 5 mM DAAO, 6 μM FAD, 0.75 mM d-α-tyrosine, 0.5 U/mL HRP, and 50 μM Amplex Red in the presence or absence of the compounds was incubated at room temperature for 30 min. The fluorescence signal was then detected in a plate reader (Envision, Perkin-Elmer Inc., MA, USA) with excitation at 544 nm and emission at 590 nm. Each IC₅₀ value was comprised of at least two measurements of inhibition per data point.

**DAAO Inhibition Cell Assays.** DAAO inhibition cell assays were conducted as previously described with some modification. 5 Briefly, the reaction mixture containing 50 mM potassium phosphate buffer (pH 7.5), 0.02% CHAPS, HEK293/human (mouse, rat) DAAO cells (30000 cells/well), 6 μM FAD, 50 mM d-α-tyrosine, 0.5 U/mL HRP, and 80 μM Amplex Red in the presence or absence of compounds was incubated at room temperature for 30 min. The fluorescence signal was then detected in a plate reader (Envision, Perkin-Elmer Inc.) with excitation at 544 nm and emission at 590 nm. Each IC₅₀ value was comprised of at least two measurements of inhibition per data point.

**Cytoxicity Evaluation.** HEK293/human DAAO cells viability in the presence of the compounds was measured using alamarBlue (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Parallel Artificial Membrane Permeability Assay (PAMPA).** The PAMPA Evolution instrument from pION Inc. (Woburn, MA, USA) was used in this study. In PAMPA, a “sandwich” is formed from a 96-well microtiter plate (PN 110163; pION Inc.) and a 96-well filter plate (IPVH; Milipore, Bedford, MA, USA), such that each composite well is divided into two chambers: a donor at the bottom and an acceptor at the top. The two were separated by a 125 μm thick microfilter disc (0.45 μm diameter pores) and coated with a 20% (w/v) dodecanol solution of a lecithin mixture (PN 110669; pION Inc.). Drug samples were introduced as 10 mM DMSO stock solutions in a mixture containing 50 mM potassium phosphate buffer (pH 3.58) and 5% MeOH in H₂O₂ with 0.1% formic acid for 3 min at 0.7 mL/min and UV detection at 254 nm.

5, 8, and 9 are commercially available and were obtained from MAYBRIDGE (catalogue code: CC39001DA), Aldrich (catalogue code: 122505) and WAKO (catalogue code: 32S-72092), respectively.

**5.0 and 6.5 (NaOH-treated universal buffer) were used.** The donor solutions were adjusted in pH while the acceptor solutions were kept at pH 7.4 (PN 110139; pION Inc.). The blood and permeability (Pe) was calculated using PAMPA Evolution software (pION Inc.). The blood and permeability (Pe) was calculated using PAMPA Evolution software (pION Inc.).

**Determination of Plasma and Brain Levels in Mice.** The pharmacokinetic characterization of test compounds was conducted in 6-week-old male ddY mice (Japan SLC, Inc., Hamamatsu, Japan). Test compounds were orally administrated as 0.5% methylcellulose suspensions at a dose of 30 mg/kg. Blood samples for the determination of the test compound concentration were obtained at 0.5 and 2 h after single dose administration of the compound. Concentrations of the unchanged compound were analyzed using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Groups contained three animals each.

**MK-801-Induced Working Memory Deficit in the Mouse Y-Maze Test.** The mouse Y-maze test was performed as previously described, and male ddY mice (5 weeks old; Japan SLC, Inc.) were used. The maze was made of gray polycarbonate with arms (40 cm long, 13 cm tall, and 3 and 10 cm wide at the bottom and top, respectively) converging at equal angles. Ten minutes after oral administration of 30 or vehicle, 0.15 mg/kg of MK-801 (+)-MK-801 hydrogen maleate; Sigma Aldrich, St. Louis, MO, USA) was administered intraperitoneally to each animal. Normal animals were administered with vehicle and saline instead of MK-801 or saline, each instead of one arm and explore the apparatus freely for 8 min. Alternation was defined as entries into all three arms on consecutive occasions. The alternation percentage was calculated using the following formula:

\[
\text{alternation(%) = } \frac{100 \times \text{number of alternations}}{\text{number of total arm entries} - 2}
\]

All values are given as the mean ± standard error of the mean. Statistical analysis of the normal (vehicle + saline) group vs control (vehicle + MK-801) was conducted using the unpaired Student’s t-test. Control (vehicle + MK-801) vs drug + MK-801 groups were compared using one-way ANOVA followed by Dunnett’s multiple comparison test. Groups contained eight animals each.

**Chemistry.** Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. 1H NMR spectra were recorded on a Varian 400 MHz, BRUKER 400 MHz, or JEOL 400 MHz spectrometer, and the chemical shifts were expressed in δ (ppm) values with trimethylsilane as an internal reference (δ = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and b = broad peak). Mass spectra were recorded on an Agilent 6140 Quadrupole LC/MS, Waters UPLC/SGD-LC/MS system, or JEOL JMS-LX2000 spectrometer. High-resolution mass spectroscopy (HRMS) values were recorded on a Waters Q-ToF Premier or Waters LCT Premier XI. The purities of the tested compounds were found to be above 95%, as determined using the Waters UPLC LC/MS system and UPLC HSS T3 2.1 mm × 50 mm column, 1.8 μm, 5–95% MeOH in H₂O with 0.1% formic acid for 3 min at 0.7 mL/min and UV detection at 254 nm.

5, 8, and 9 are commercially available and were obtained from MAYBRIDGE (catalogue code: CC39001DA), Aldrich (catalogue code: 122505) and WAKO (catalogue code: 32S-72092), respectively.

**10** (CAS Registry no.: 856952-50-6) was prepared in a similar manner to 12, described below. 1H NMR (DMSO-d₆, δ 1.95 (s, 3H), 6.57 (s, 1H), 6.61 (s, 1H), 8.84 (br, 1H), 11.39 (br, 1H), MS (ESI/APCI⁺) m/z = 126 (MH⁺). 11 (CAS Registry no.: 26386-86-7) was prepared from 2-aminobenzoaldehyde as described in the literature. 1H NMR (DMSO-d₆, δ 7.08–7.14 (m, 2H), 7.24–7.48 (m, 2H), 7.49 (d, J = 7.6 Hz, 4H), 9.44 (br, 1H), 12.00 (br, 1H), MS (ESI⁻) m/z = 162 (MH⁻). 5-Hydroxy-5-(2-phenylethyl)pyridin-2(1H)-one (12). Step 1: To a suspension of NaH (60% with oil, 253 mg, 5.80 mmol) in DMF was added 5-bromo-2,3-dihydroxypropionate (500 mg, 2.63 mmol) at 0 °C. The mixture was stirred for 1 h at room temperature, and then chromanol methyl ether (0.44 mL, 5.85 mmol) was added at 0 °C. The mixture was stirred for 3 h at room temperature and poured into water (20 mL). The aqueous layer was extracted with AcOEt, and the organic layer was dried over MgSO₄ and concentrated in vacuo. Purification via column chromatography on silica gel (CHCl₃/MeOH gave 5-bromo-2,3-bis(methoxymethoxy)pyridine (14) (149 mg, 20%) as a colorless oil. 1H NMR (CDCl₃, δ 3.52 (s, 3H), 3.54 (s, 3H), 5.24 (s, 2H), 5.57 (s, 2H), 7.53 (d, J = 2.4 Hz, 1H), 7.88 (d, J = 2.4 Hz, 1H). MS ((ESI/APCI⁻) m/z = 278, 280 (MH⁻) along with 5-bromo-3- (methoxymethoxy)-1-(methoxymethyl)pyridin-2(1H)-one (363 mg, 50%) as a colorless oil. 1H NMR (CDCl₃, δ 3.41 (s, 3H), 3.50 (s, 3H), 5.23 (s, 2H), 5.33 (s, 2H), 7.07 (d, J = 2.4 Hz, 1H), 7.22 (d, J = 2.4 Hz, 1H). MS (ESI/APCI⁺) m/z = 278, 280 (MH⁺).

Step 2: To a solution of 5-bromo-2,3-bis(methoxymethoxy)pyridine (34 (149 mg, 0.536 mmol) in dioxane (10 mL) and water (2 mL) was added [(3)-2-phenylvinyl]boronic acid (119 mg, 0.804 mmol), K₂PO₄ (341 mg, 1.61 mmol), and Pd(PPh₃)₄ (62 mg, 0.054 mmol), and the mixture was stirred at 100 °C for 6 h under an argon atmosphere. The reaction mixture was cooled to room temperature, the residue partitioned between AcOEt and water, and the organic layer was washed with brine, dried over MgSO₄, and concentrated in vacuo.
Purification via column chromatography on silica gel (Hex/ACOEt) gave 2,3-bis(methoxymethoxy)-5-[(E)-2-phenylvinyl]pyridine (114 mg, 71%) as a pale-yellow oil. 

**3** To a solution of 2,3-bis(methoxymethoxy)-5-[(E)-2-phenylvinyl]pyridine (114 mg, 0.378 mmol) in EtOH (10 mL) was added 10% Pd–C (50 mg) with EtOH (10 mL). The mixture was stirred at room temperature for 4 h under a hydrogen atmosphere and then passed through a Celite pad, where the filtrate was concentrated in vacuo to give 3-(methoxymethoxy)-5-[(E)-2-phenylvinyl]pyridin-2(1H)-one (94 mg, 96%) as a colorless oil. 

**4** The solid was recrystallized from CHCl₃ to give 3-[(E)-2-phenylvinyl]boronic acid in 15% yield (3 steps) as a white solid. 

**5** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([(E)-2-phenylvinyl]boronic acid in 15% yield (3 steps) as a white solid. 

**6** To a solution of benzyl alcohol (0.50 mL, 4.83 mmol) in toluene (10 mL) was added 1 M BBr₃ in CH₂Cl₂ (5.0 mL, 50 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h and concentrated in vacuo, and the residue was washed with water and purified by column chromatography on silica gel (CHCl₃/MEOH) to give a solid. 

**7** To a solution of benzyl alcohol (0.50 mL, 4.83 mmol) in toluene (10 mL) was added 1 M BBr₃ in CH₂Cl₂ (5.0 mL, 50 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h and concentrated in vacuo, and the residue was washed with water and purified by column chromatography on silica gel (CHCl₃/MEOH) to give a solid. 

**8** The solid was recrystallized from CHCl₃ to give 3-[(E)-2-phenylvinyl]boronic acid in 15% yield (3 steps) as a white solid.

**9** MS (ESI+) calcd for [C₁₀H₁₀N₂O₂ +H]⁺, 216.1025; found, 216.1019.

**10** MS (ESI+) calcd for [C₁₀H₁₀N₂O₂ +H]⁺, 216.1025; found, 216.1019.

**11** MS (ESI+) calcd for [C₁₀H₁₀N₂O₂ +H]⁺, 216.1025; found, 216.1019.

**12** MS (ESI+) calcd for [C₁₀H₁₀N₂O₂ +H]⁺, 216.1025; found, 216.1019.

**13** MS (ESI+) calcd for [C₁₀H₁₀N₂O₂ +H]⁺, 216.1025; found, 216.1019.

**14** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([(E)-1-phenethyl]pyridin-2(1H)-one). 

**15** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([(E)-1-phenethyl]pyridin-2(1H)-one).

**16** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid. 

**17** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**18** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**19** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**20** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**21** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**22** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**23** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.

**24** The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material ([E]-3-phenylprop-1-en-1-yl)boronic acid in place of [[(E)-2-phenylvinyl]boronic acid in 10% yield (3 steps) as a white solid.
dimethylbut-1-en-1-yl]boronic acid in place of [(E)-2-phenylvinyl]boronic acid in 13% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 0.90 (s, 9H), 1.54 (s, 3H), 2.37–2.44 (m, 2H), 6.53 (s, 1H), 10.66 (br, 1H). 12.63 (s, 1H). MS (ESI+) m/z = 197 (MH+). HRMS (ESI+) calculated for [C9H11NO2 + H]+, 197.1290; found, 197.1292.

4-Hydroxy-6-(phenoxymethyl)pyridazin-3(2H)-one (21). Step 1: The mixture of 3-chloro-[1,4]benzodioxino[2,3-c]pyridazine 35 (2.21 g, 10.0 mmol), Pd(OAc)2 (449 mg, 2.00 mmol), DPPF (2.22 g, 4.00 mmol), MeOH (30 mL), DMSO (30 mL), and Et3N (2.8 mL, 20.1 mmol) was stirred at 80 °C for 19 h under a N2 atmosphere. The reaction mixture was cooled to room temperature and partitioned between AcOEt and water. The organic layer was washed with brine, dried over MgSO4, and concentrated in vacuo. Purification by column chromatography on silica gel (Hex/ACOEt) gave methyl [1,4]-benzodioxino[2,3-c]pyridazine-3-carboxylic acid (1.46 g, 60% as a white solid. 1H NMR (DMSO-d6) δ 3.92 (s, 3H), 7.09–7.14 (m, 3H), 7.18–7.22 (m, 1H), 7.65 (s, 1H). MS (ESI/APCI+) m/z = 245 (MH+).

Step 2: To a mixture of methyl [1,4]-benzodioxino[2,3-c]pyridazine-3-carboxylic acid (1.46 g, 5.97 mmol), MeOH (15 mL), and THF (30 mL) was added sodium borohydride (677 mg, 17.9 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. The reaction mixture was partitioned between AcOEt and water. The organic layer was washed with brine, dried over MgSO4, and concentrated in vacuo. Purification via column chromatography on silica gel (Hex/ACOEt) gave methyl [1,4]-benzodioxino[2,3-c]pyridazine-3-carboxylate (1.46 g, 60% as a white solid. 1H NMR (DMSO-d6) δ 4.59 (d, J = 6.0 Hz, 2H), 5.63 (s, t = 6.0 Hz, 1H), 7.03–7.13 (m, 3H), 7.14–7.19 (m, 1H), 7.20 (s, 1H). MS (ESI (APCI)+) m/z = 217 (MH+).

Step 3: The mixture of [1,4]-benzodioxino[2,3-c]pyridazine-3-ylmethanol (8.10 g, 37.5 mmol), 1,2-dichloroethane (80 mL), and thionyl chloride (38.9 g, 186 mmol) was stirred at 80 °C for 2 h. The reaction mixture was diluted with chloroform (500 mL), and the organic layer was neutralized with saturated sodium bicarbonate aq (250 mL). The organic layer was dried over MgSO4 and concentrated in vacuo. The residue was triturated with diethyl ether to give 3-(bromomethyl)[1,4]-benzodioxino[2,3-c]pyridazine (8.0 g). 1H NMR (DMSO-d6) δ 4.73 (s, 2H), 7.06–7.19 (m, 4H), 7.44 (s, 1H). MS (ESI+) m/z = 279, 281 (M+H+).

Step 4: The mixture of 3-(bromomethyl)[1,4]-benzodioxino[2,3-c]pyridazine (500 mg, 1.79 mmol), DMF (10 mL), potassium iodide (30 mg, 181 mmol), K2CO3 (620 mg, 4.49 mmol), and phenol (250 mg, 2.66 mmol) was stirred at room temperature for 24 h. The reaction mixture was partitioned between AcOEt and water. The organic layer was washed with 1N NaOH aq and brine, dried over Na2SO4, and concentrated in vacuo. Puri

4-Hydroxy-6-(6-(3-trifluoromethyl)phenyl)ethyl)pyridazin-3(2H)-one (25). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material [(E)-2-(3-fluorophenyl)vinyl]boronic acid in place of [(E)-2-phenylvinyl]boronic acid in 35% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.73–2.80 (m, 2H), 2.86–2.94 (m, 2H), 6.59 (s, 1H), 6.97–7.11 (m, 3H), 7.26–7.34 (m, 1H), 10.72 (br, 1H). MS (ESI+) m/z = 235 (M+H+). HRMS (ESI+) calculated for [C11H9F3N2O4 + H]+, 235.0883; found, 235.0892.

4-Hydroxy-6-(6-(2-(fluorophenyl)vinyl)ethyl)pyridazin-3(2H)-one (26). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material [(E)-2-(4-fluorophenyl)vinyl]boronic acid in place of [(E)-2-phenylvinyl]boronic acid in 18% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.70–2.79 (m, 2H), 2.80–2.88 (m, 2H), 3.72 (s, 3H), 6.59 (s, 1H), 6.71–6.80 (m, 3H), 7.14–7.21 (m, 1H), 10.69 (br, 1H), 12.67 (s, 1H). MS (ESI+) m/z = 324 (M+H+). HRMS (ESI+) calculated for [C12H7F2N2O4 + H]+, 324.0832; found, 324.0833.

4-Hydroxy-6-(6-(4-fluorophenyl)ethyl)4-hydroxypyridazin-3(2H)-one (27). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material [(E)-2-(4-fluorophenyl)vinyl]boronic acid in place of [(E)-2-phenylvinyl]boronic acid in 4% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.69–2.78 (m, 2H), 2.82–2.91 (m, 2H), 6.58 (s, 1H), 7.04–7.13 (m, 2H), 7.20–7.28 (m, 2H), 10.71 (br, 1H), 12.67 (s, 1H). MS (ESI+) m/z = 235 (M+H+). HRMS (ESI+) calculated for [C11H9F3N2O4 + H]+, 235.0883; found, 235.0887.

4-Hydroxy-6-(6-(2-(4-trifluoromethyl)phenyl)ethyl)pyridazin-3(2H)-one (28). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material [(E)-2-(4-trifluoromethyl)phenyl]boronic acid in place of [(E)-2-phenylvinyl]boronic acid in 6% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.67–2.74 (m, 2H), 2.76–2.84 (m, 2H), 3.71 (s, 3H), 6.75 (s, 1H), 6.80 (d, J = 8.8 Hz, 2H), 7.11 (d, J = 8.8 Hz, 2H), 10.68 (br, 1H), 12.65 (s, 1H). MS (ESI+) m/z = 247 (M+H+). HRMS (ESI+) calculated for [C12H7F4N2O4 + H]+, 247.1083; found, 247.1090.
6-[2-(3,5-Difluorophenyl)ethyl]-4-hydroxy pyridazin-3(2H)-one (30). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material 2-[(E)-2-(3,5-difluorophenyl)vinyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane in place of [(E)-2-phenylvinyl]boronic acid in 26% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.72–2.81 (m, 2H), 2.87–2.95 (m, 2H), 6.59 (s, 1H), 6.94–7.06 (m, 3H), 10.74 (br, 1H), 12.68 (s, 1H). MS (ESI+) m/z = 253 (MH+). HRMS (ESI+) calc for [C14H10N2O2F6 +H]+, 353.0725; found, 353.0784.

6-[2-(3,5-Bis(trifluoromethyl)phenyl)ethyl]-4-hydroxy pyridazin-3(2H)-one (31). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material [(E)-2-[3,5-bis(trifluoromethyl)phenyl]vinyl]boronic acid in place of [(E)-2-phenylvinyl]boronic acid in 6% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.79–2.87 (m, 2H), 3.05–3.14 (m, 2H), 6.63 (s, 1H), 7.90 (s, 1H), 7.96 (s, 2H), 10.75 (br, 1H), 12.68 (s, 1H). MS (ESI+) m/z = 353 (MH+). HRMS (ESI+) calc for [C14H10N2O2F6 +H]+, 353.0725; found, 353.0717.

6-[2-(3,5-Dimethoxyphenyl)ethyl]-4-hydroxy pyridazin-3(2H)-one (32). The title compound was prepared in a manner similar to that used to prepare 13, with the substitution of starting material [(E)-2-(3,5-dimethoxyphenyl)vinyl]boronic acid in 18% yield (3 steps) as a white solid. 1H NMR (DMSO-d6) δ 2.69–2.84 (m, 4H), 3.70 (s, 6H), 6.28–6.32 (m, 1H), 6.35–6.40 (m, 2H), 6.59 (s, 1H), 10.70 (br, 1H), 12.67 (s, 1H). MS (ESI+) m/z = 277 (MH+). HRMS (ESI+) calc for [C14H10N2O2F6 +H]+, 277.1188; found, 277.1181.

**ASSOCIATED CONTENT**

**Supporting Information**

Permeability of compounds 13–32 measured by PAMPA and panel screening of compound 30 for 57 targets. This material is available free of charge via the Internet at http://pubs.acs.org.

**Accession Codes**

All coordinates have been deposited in the PDB with accession codes 3W4J (8), 3W4J (12), and 3W4K (13).

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank Katsuhiko Gato, Masahiro Fujii, and Mitsuru Hosaka for evaluating the physical chemistries, drug metabolisms, and pharmacokinetics. We also thank Dr. Atsushi Suzuki and Dr. Takahiko Tobe for helpful discussions.

**ABBREVIATIONS USED**

DAAO, D-amino acid oxidase; NMDA, N-methyl-D-aspartate; CBIO, 6-chlorobenzoi[δ]isoxazol-3-ol; FBDD, fragment-based drug design; PDB, Protein Data Bank; CIAS, cognitive impairment associated with schizophrenia; PAMPA, parallel artificial membrane permeability assay; BBB, blood–brain barrier

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