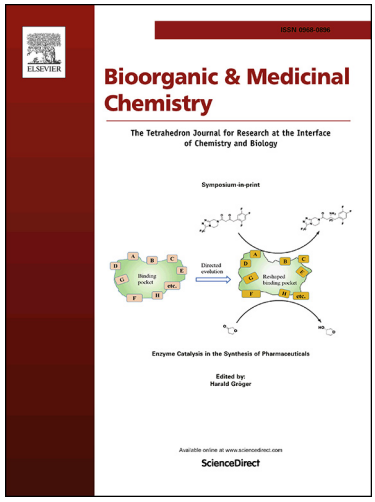
Accepted Manuscript



Synthesis, SAR study, and biological evaluation of novel 2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole derivatives as phosphodiesterase 10A inhibitors

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Abstract

Phosphodiesterase 10A (PDE10A) inhibitors were designed and synthesized based on the dihydro-imidazobenzimidazole scaffold. Compound **5a** showed moderate inhibitory activity and good permeability, but unfavorable high P-glycoprotein (P-gp) liability for brain penetration. We performed an optimization study to improve both the P-gp efflux ratio and PDE10A inhibitory activity. As a result, **6d** was identified with improved P-gp liability and high PDE10A inhibitory activity. Compound **6d** also showed satisfactory brain penetration, suppressed phencyclidine-induced hyperlocomotion and improved MK-801-induced working memory deficit.

Keywords

PDE10A inhibitor; schizophrenia; brain penetration; P-gp liability; Y-maze

1. Introduction

Schizophrenia is a chronic and debilitating psychiatric disorder that affects approximately 1% of the world's population.[1] Schizophrenia is characterized by a combination of positive symptoms, negative symptoms and cognitive impairments.[2] Current anti-psychotics are marginally effective for treating positive symptoms but are less effective against negative symptoms and cognitive dysfunction. Therefore, novel drug types that are effective against not only positive symptoms but also negative symptoms and cognitive impairments would represent a significant advancement in schizophrenia treatment.

The phosphodiesterases (PDEs) consist of an 11-membered family of enzymes that catalyze the hydrolysis of secondary signal messengers, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). PDE10A is a dual substrate PDE that hydrolyzes both cAMP and cGMP. PDE10A is highly expressed in the brain, particularly in 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.[3] The distribution of PDE10A expression is conserved across mammalian species.[3] Inhibition of PDE10A is thought to be effective for the treatment of schizophrenia and a range of neurological, psychotic, anxiety, and movement disorders that benefit from increased levels of cAMP and cGMP in neurons. Therefore,

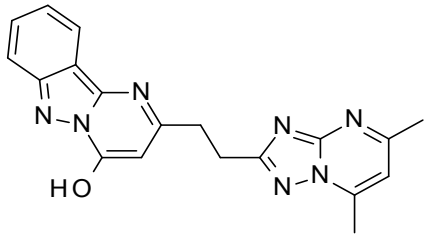
PDE10A inhibitors might prove useful for the treatment of neurological and psychiatric disorders.[4]

At present, several PDE10A inhibitors are undergoing clinical investigation[5] and there are a large number of studies on PDE10A inhibitors.[6] Preclinical evidence suggests that these inhibitors may demonstrate anti-psychotic, pro-cognitive, and anti-negative symptom efficacy.[7]

We previously reported that the triazolopyrimidine analogue (**1**) had high inhibitory activity with in vitro efficacy (Fig. 1).[8] From the X-ray co-crystal structure of the pyrimidoazaindazole derivative (**2**) with PDE10A enzyme (Fig. 2), it was revealed that the 1-nitrogen of pyrimidoazaindazole ring of **2** accepted a hydrogen bond from Tyr693 and 4-nitrogen of triazolopyrimidine ring accepted it from Gln726. Unfortunately, **1** displayed a poor brain penetration profile in mice as shown in Table 1 making it unsuitable as a tool compound for in vivo studies despite its low P-gp liability (Net Efflux Ratio (NER) = 1.8). Compound **1** showed low blood to brain permeability (Pint = 3.2×10^{-6} cm/sec).

Given that permeability is correlated with lipophilicity or the number of hydrogen bond donors (HBDs),[9] we speculated that the hydroxyl group of **1** was the cause of the poor permeability and low brain penetration because of the high polarity and the HBD ability. However, the hydroxyl group was crucial for maintaining high PDE10A

inhibitory activity.[8] For example, compound **3**, in which the hydroxyl group was removed from **1**, showed significantly decreased inhibitory activity (Table 2, entry 1). Therefore, further research was conducted to find novel scaffolds other than pyrimidoindazole without a hydroxyl group that showed high inhibitory activity.



1

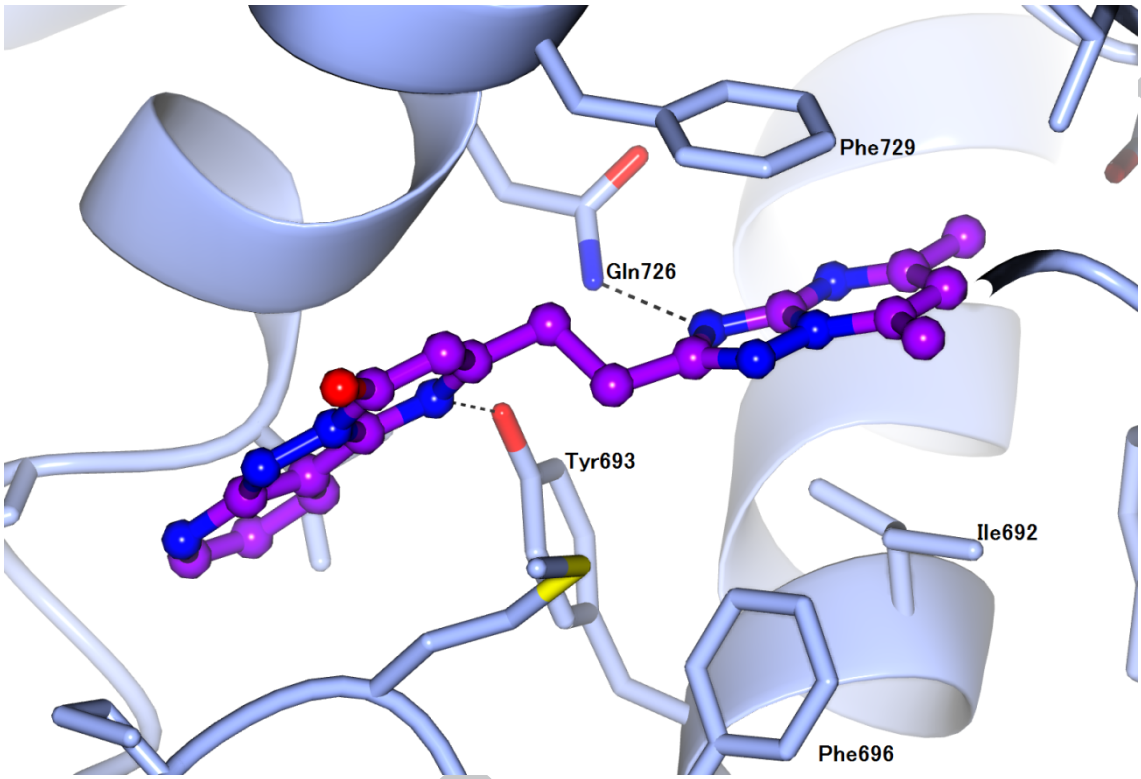
PDE10A IC_{50}: 2.0 nM
$logD_{7.4}$: 0.3
P-gp NER: 1.8
Pint: 3.2×10^{-6} cm/sec

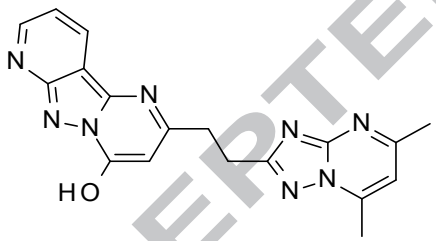
Figure 1. Structure of compound **1**.

Table 1. Plasma and brain concentration of **1** at 1 h after oral administration to mice

Dose (mg/kg)	**Plasma (ng/mL)**	**Brain (ng/g)**	K_p**,brain** [a]
0.1	0.59	ND [b]	ND [b]

[a] Average value of individual Kp (brain/plasma) (n=3).
[b] Not Detected





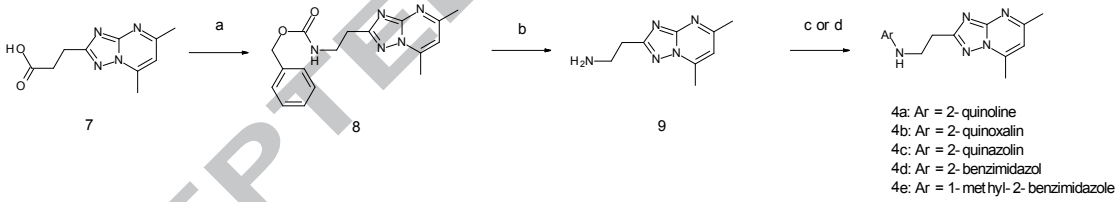
2

PDE10A IC_{50}: 2.9 nM

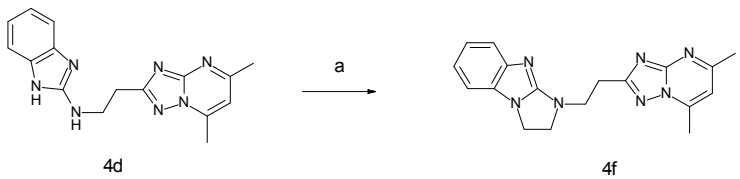
Figure 2. Crystal structure of compound **2** (purple) bound to PDE10A (PDB code: 6KDZ).

2. Chemistry

The synthesis of target compounds is shown in Schemes 1–6. The synthetic route of 5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4a**–**e** is outlined in Scheme 1. A Curtius rearrangement of (5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)acetic acid **7** with benzyl alcohol gave the corresponding carbamate **8**. Catalytic hydrogenation of **8** yielded amine **9**. Buchwald-Hartwig cross coupling reaction or ipso substitution of aryl chlorides with **9** gave **4a**–**e**. Compound **4f** was prepared by alkylation of **4d** with 1,2-dibromoethane as shown in Scheme 2.

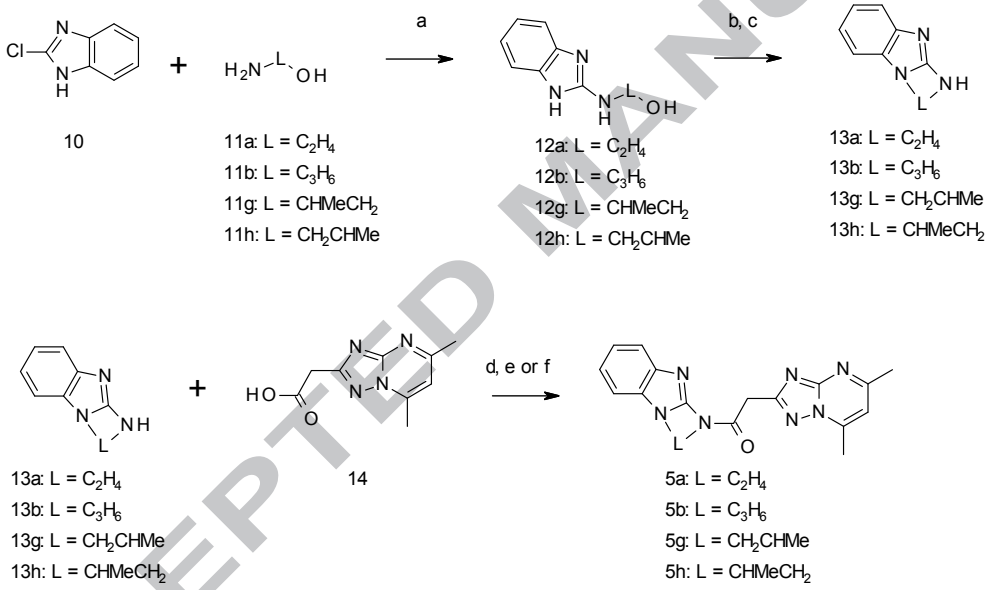


Scheme 1. Reagents and conditions: (a) BnOH, diphenylphosphorylazide, triethylamine, toluene, 69%; (b) H_2, Pd-C, MeOH, quant.; (c) Ar-Cl, $Pd(OAc)_2$, (*S*)-1-[(*R*)-2-(dicyclohexylphosphino)ferrocenyl]ethyldi-*tert*-butylphosphine ((*S*)-(*R*)-JOSIPHOS), *t*-BuONa, DME, 13%; (d) Ar-Cl, NMP, 14–96%.



Scheme 2. Reagents and conditions: (a) 1,2-dibromoethane, tetrabuthylammonium bromide, NaOH aq., DCE, 31%.

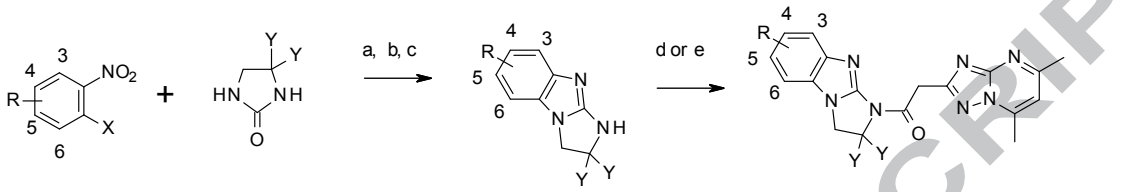
The synthesis of [1,2,4]triazolo[1,5-*a*]pyrimidine analogues **5a, b, g** and **h** is illustrated in Scheme 3. Ipso substitution of 2-chloro-1*H*-benzimidazole **10** with amino alcohols **11a, b, g** and **h** gave alcohols **12a, b, g** and **h**, which were chlorinated with $SOCl_2$. Intramolecular alkylation gave **13a, b, g** and **h**, and subsequent condensation with (5,7-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-acetic acid **14** gave **5a, b, g** and **h**.



Scheme 3. Reagents and conditions: (a) neat, 24–97%; (b) $SOCl_2$, DCE; (c) xylene, 14–97% (2 steps); (d) WSC•HCl, HOBt, triethylamine, DMF, 54–58%; (e) WSC•HCl, HOBt, DMF, 44%; (f) HATU, DIPEA, CH_2Cl_2, 59%.

Imidazo[1,2-*a*]benzimidazolyl derivatives **5c**–**f** and **5i** were synthesized in accordance with the procedures outlined in Scheme 4. Buchwald-Hartwig cross coupling reaction of imidazolidinones **16a**–**b** with 2-nitrohalobenzene **15c**–**f** and **15i**, reduction of nitro substitution with hydrazine using an iron-based catalyst, and chlorination and

cyclization gave imidazo[1,2-*a*]benzimidazols **17c**–**f** and **17i**. Amine **17c**–**f** and **17i** were condensed with carboxylic acid **14** to give **5c**–**f** and **5i**.



15c: R = 3- Me, X = Br
15d: R = 4- Me, X = I
15e: R = 5- Me, X = I
15f: R = 6- Me, X = Br
15i: R = H, X = I

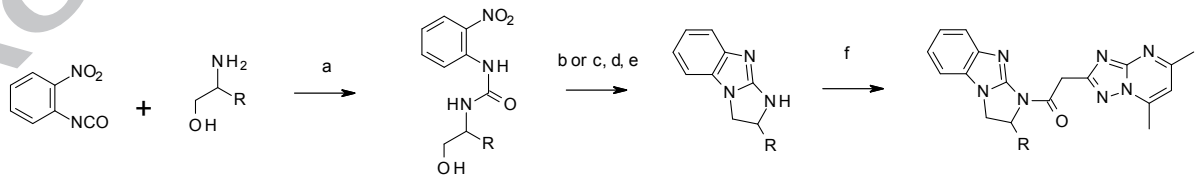
16a: Y = H
16b: Y = Me

17c: R = 3- Me, Y = H
17d: R = 4- Me, Y = H
17e: R = 5- Me, Y = H
17f: R = 6- Me, Y = H
17i: R = H, Y = Me

5c: R = 3- Me, Y = H
5d: R = 4- Me, Y = H
5e: R = 5- Me, Y = H
5f: R = 6- Me, Y = H
5i: R = H, Y = Me

Scheme 4. Reagents and conditions: (a) $Pd_2(dba)_3$, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos), Cs_2CO_3, 1,4-dioxane; (b) $FeCl_3$•$6H_2O$, N_2H_4•H_2O, activated carbon, EtOH, H_2O; (c) $POCl_3$, 2.0–63% (3 steps); (d) **14**, WSC•HCl, HOBt, DMF, 41–84%; (e) **14**, HATU, DIPEA, CH_2Cl_2, 44%.

Imidazo[1,2-*a*]benzimidazolyl derivatives **5j–l** were synthesized as depicted in Scheme 5. An ipso substitution of 2-nitrophenyl isocyanate **18** with alkyl amine **19j–l** gave the urea analogues **20j–l**. Subsequently, imidazo[1,2-*a*]benzimidazolyl derivatives **5j–l** were prepared in a manner similar to that described for **5c–f** and **5i**.



18

19j: R = (2*S*)- Ph
19k: R = (2*R*)- Ph
19l: R = (2*S*)- Me

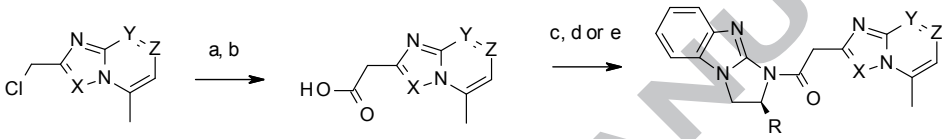
20j: R = (2*S*)- Ph
20k: R = (2*R*)- Ph
20l: R = (2*S*)- Me

21j: R = (2*S*)- Ph
21k: R = (2*R*)- Ph
21l: R = (2*S*)- Me

5j: R = (2*S*)- Ph
5k: R = (2*R*)- Ph
5l: R = (2*S*)- Me

Scheme 5. Reagents and conditions: (a) THF, quant.; (b) $FeCl_3$•$6H_2O$, N_2H_4•H_2O, activated carbon, EtOH, H_2O; (c) H_2, Pd-C, EtOH, THF; (d) $POCl_3$; (e) xylene, 13–24% (3 steps); (f) **14**, WSC•HCl, HOBt, DMF, 46–73%.

As shown in Scheme 6, synthesis of imidazo[1,2-*a*]benzimidazolyl derivatives **6a–d** started with cyanation of methyl chlorides **22a–c**. Subsequently, hydrolysis with NaOH aqueous solution yielded carboxylic acids **23a–c**. Amidation of **23a–c** with amines **13a** or **21l** gave **6a–d**.



22a: X = CH, Y = N, Z = CMe
22b: X = N, Y = CH, Z = CMe
22c: X = N, Y = CMe, Z = N

23a: X = CH, Y = N, Z = CMe
23b: X = N, Y = CH, Z = CMe
23c: X = N, Y = CMe, Z = N

6a: R = H, X = CH, Y = N, Z = CMe
6b: R = H, X = N, Y = CH, Z = CMe
6c: R = H, X = N, Y = CMe, Z = N
6d: R = Me, X = N, Y = CMe, Z = N

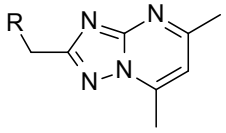
Scheme 6. Reagents and conditions: (a) NaCN, DMF, H_2O; (b) NaOH aq., 1,4-dioxane, 40–81% (2 steps).; (c) **13a**, WSC•HCl, HOBt, DIPEA, DMF 53%; (d) **13a**, HATU, DIPEA, DMF, 71%; (e) **13a** or **21l**, WSC•HCl, HOBt, DMF, 55–68%.

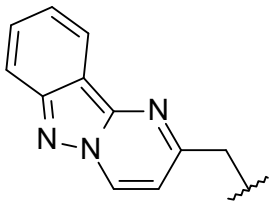
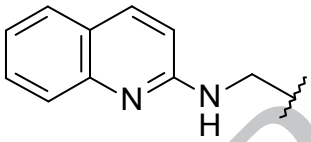
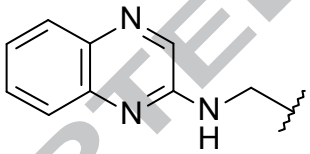
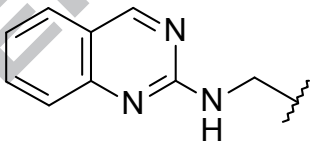
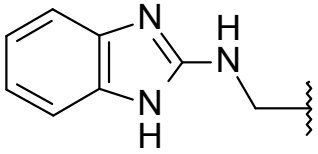
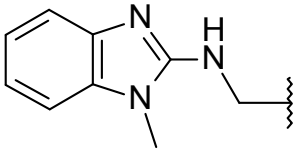
3. Results and Discussion

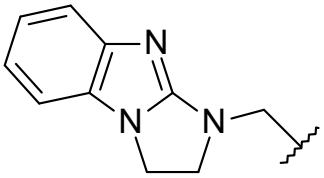
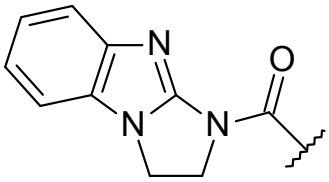
We first changed the pyrimidoindazole scaffold to contain a small number of other hetero aromatics that could interact with Tyr693, and assayed the resulting compounds. Some of the results are shown in Table 2. The 2-amino quinoline derivative **4a** showed moderate inhibitory activity, and the binding mode of **4a** with the PDE10A enzyme was similar to that of **1** (Fig. 3). The quinoxaline derivative **4b** and quinazoline derivative **4c** had dramatically lower potency, whereas the benzimidazole derivatives **4d** and **4e** had higher potency than **4a**.

In the view of the brain penetration, it would be better that the NH of benzimidazole is capped with substituent to reduce the number of HBDs. We synthesized 2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole derivative **4f** and found that it had a similar PDE10A inhibitory to **4d**. Furthermore, **4f** showed much better permeability (Pint = 19.2 x 10^{-6} cm/sec) than **1**, but had high P-glycoprotein (P-gp) liability (NER = 3.6). Our target NER value was less than 2.0. While compound **5a**, in which a carbonyl substituent was introduced into the linker for the immobilization of the structure, showed more potent inhibitory activity while maintaining similar permeability to **4f** (Pint = 16.8 x 10^{-6} cm/sec). Compound **5a** showed lower P-gp liability than **4f**, though the value was still high (NER = 2.3). We therefore moved to further optimize **5a** to improve potency and reduce P-gp liability.

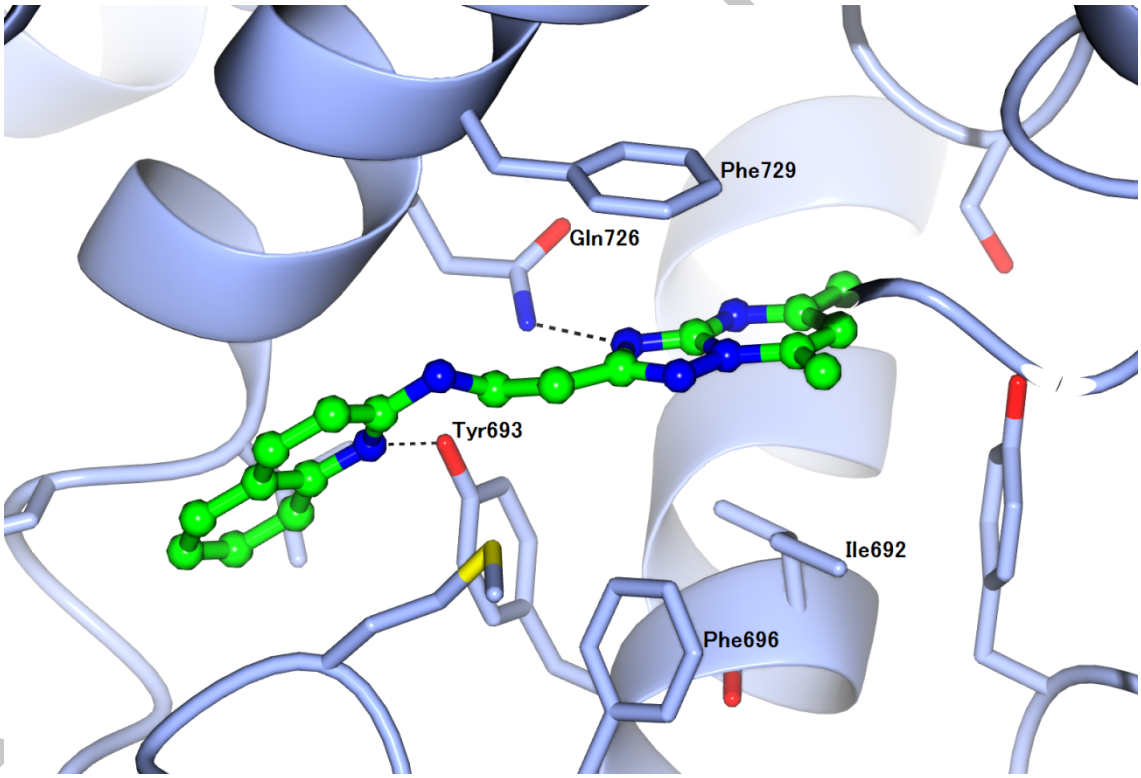
Table 2. PDE10A inhibitory activity and P-gp net efflux ratio of heterocycles



Compds	R	PDE10A IC_{50} (nM)	P-gp NER
3		78	NT [a]
4a		294	NT [a]
4b		1219	NT [a]
4c		2083	NT [a]
4d		105	NT [a]
4e		88	NT [a]

4f		138	3.6
5a		52	2.3

[a] Not tested



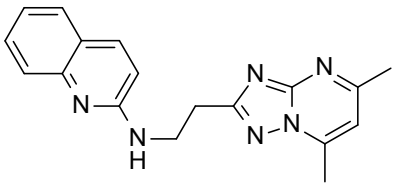


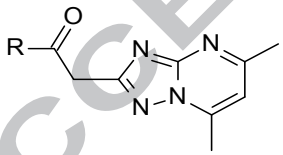
Figure 3. Crystal structure of compound **4a** (green) bound to PDE10A (PDB code: 6KDX).

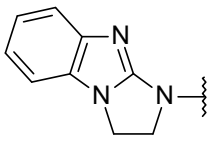
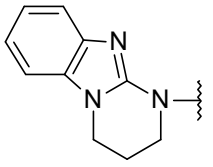
Table 3 describes the SAR of substituents on the imidazo[1,2-*a*]benzimidazole ring for improving the inhibitory activity. First, replacement of 2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole with 1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (**5b**) resulted in a 20-fold loss of affinity, indicating that the 2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole core was suitable for further optimization.

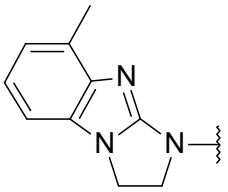
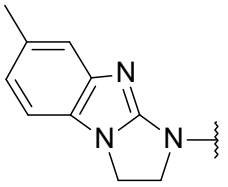
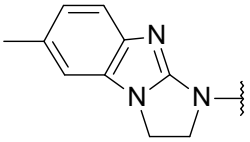
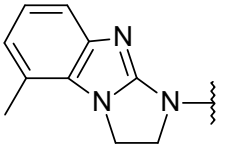
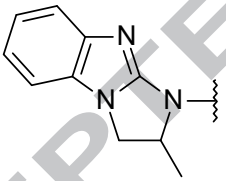
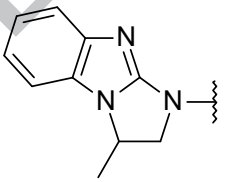
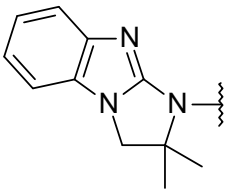
A methyl group was introduced into the phenylene ring of the 2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole core to increase inhibitory activity. Unfortunately, it resulted in a decrease in inhibitory activity. This result indicates that any substituent could not be tolerated on the phenylene ring. (**5c**, **5d**, **5e** and **5f**) In particular, a methyl substituted next to the 8-nitrogen atom (**5c**) resulted in a loss of inhibitory activity, suggesting that the 8-nitrogen atom is important for interaction with the PDE10A enzyme. We then shifted our efforts to introducing a methyl group onto the imidazoline moiety. Racemic 2-methyl derivative (**5g**) had the same potent activity as **5a**, while 3-methyl derivative (**5h**) showed weaker activity. As additional substituents to the 2-position seemed tolerable, we introduced other substituents to this position. Changing the mono methyl substituent to dimethyl substituents (**5i**) did not substantially change the activity. We subsequently synthesized chiral phenyl substituent compounds **5j** and **5k**. (*S*)-phenyl analogue **5j** showed significantly higher inhibitory activity than (*R*)-

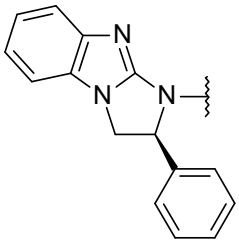
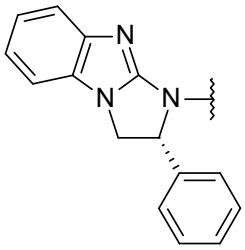
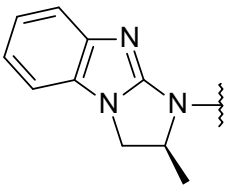
phenyl group **5k**, although **5j** showed similar potency to **5g**. This indicates that the (*S*)-isomer is crucial for inhibitory activity, while larger 2-substituents may not have an appreciable contribution to potency because the 2-substituent is exposed to the solvent. As a result, (*S*)-methyl analogue **5l** displayed higher potency than its racemate **5g**. The X-ray co-crystal structure of **5l** and PDE10A enzyme revealed that the nitrogen atom at the 9-position of the imidazo[1,2-*a*]benzimidazol unit binds to Tyr693 (Fig. 4). The (*S*)-methyl substituent of **5l** underwent van der Waals interactions with Gly725, which might underlie the enhanced potency. While we were able to enhance the PDE10A inhibitory activity, **5l** was still suffered from high P-gp liability (NER = 2.4). To address this issue, we turned our attention to optimizing the triazolopyrimidine ring.

Table 3. PDE10A inhibitory activity and P-gp net efflux ratio of heterocycles

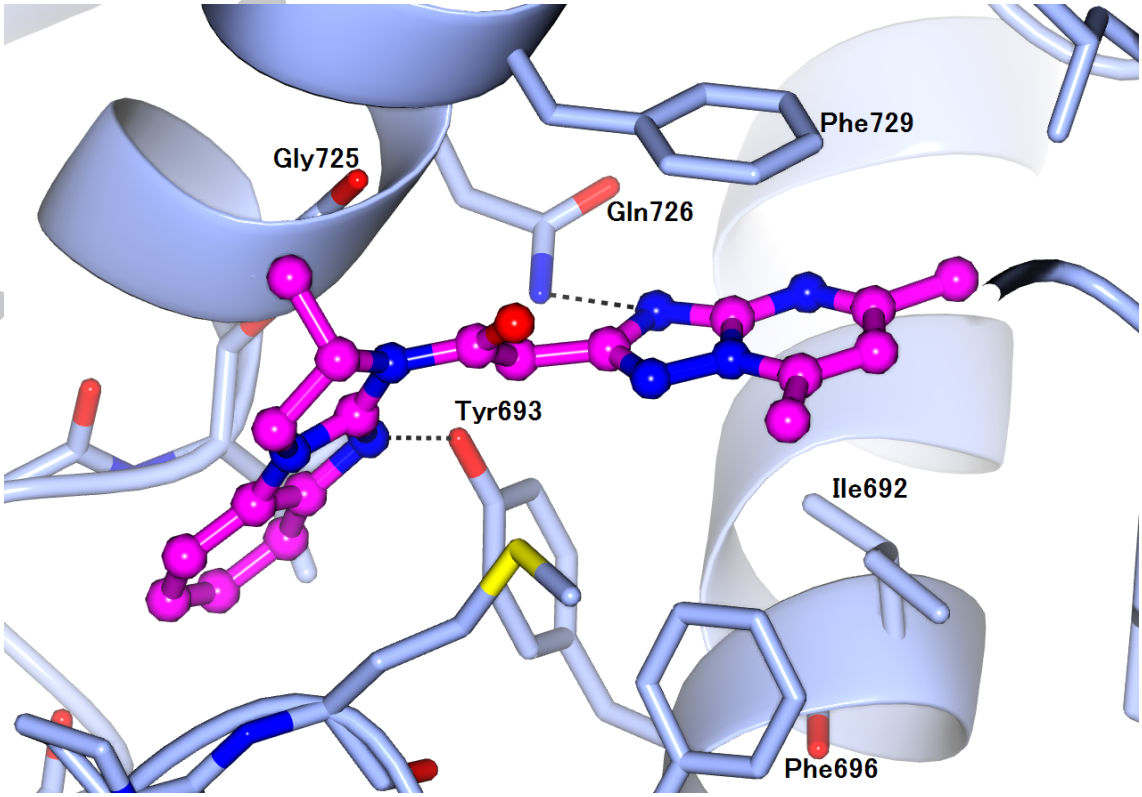


Compds	R	PDE10A IC_{50} (nM)	P-gp NER
5a		52	2.3
5b		1476	NT [a]

5c		>10000	NT [a]
5d		441	NT [a]
5e		416	NT [a]
5f		279	NT [a]
5g		69	2.4
5h		292	NT [a]
5i		74	2.1

5j		75	3.8
5k		1920	NT [a]
5l		28	2.4

[a] Not tested



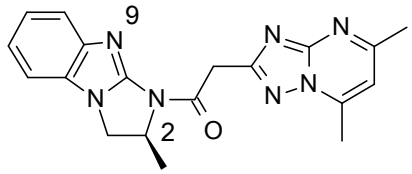
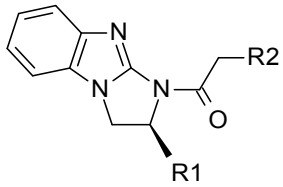


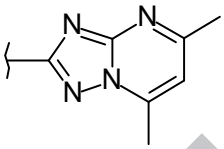
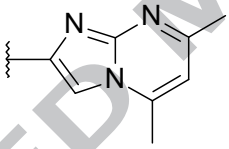
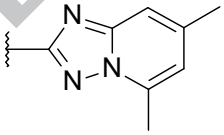
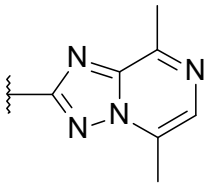
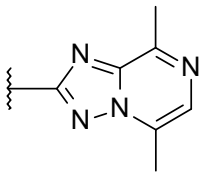
Figure 4. Crystal structure of compound **5l** (magenta) bound to PDE10A (PDB code: 6KE0).

Previous reports suggest that the number and position of heteroatoms are correlated with P-gp liability.[10] We therefore focused on changing the number and position of nitrogen atoms on the triazolopyrimidine ring to reduce the P-gp liability. (Table 4)

Although the P-gp NER was improved in imidazopyrimidine (**6a**) and triazolopyridine (**6b**), they showed a loss of PDE10A inhibitory activity, indicating that the 1- and 4-nitrogen atoms on the triazolopyrimidine ring were important for the inhibitory activity and were crucial for P-gp liability. Therefore, triazolopyrazine (**6c**), which contained a nitrogen atom at the 5-position instead of the 4-position, was evaluated. Changing the scaffold of **5a** to that of **6c** improved the P-gp NER while maintaining the potency observed in **5a**. We then introduced 2-methyl substituent on the imidazobenzimidazol moiety of compound **6c** to enhance the potency. As a result, **6d** exhibited single-digit nanomolar inhibitory activity while maintaining low P-gp liability.

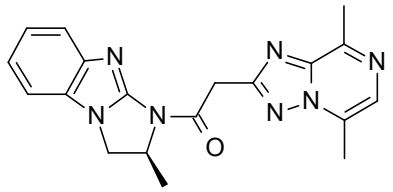
Table 4. PDE10A inhibitory activity and P-gp NER of heterocycles



Compds	R1	R2	PDE10A IC_{50} (nM)	P-gp NER
5a	**H**		52	2.3
6a	**H**		275	1.0
6b	**H**		135	1.2
6c	**H**		44	1.3
6d	**Me**		8.4	1.2

Additional profiles of **6d** are shown in Fig. 5. Compound **6d** exhibited good solubility,

metabolic stability and permeability, and low CYP1A2, 2C8, 2C9, 2C19, 2D6 and CYP3A4 inhibition.



PDE10A IC_{50}: 8.4 nM
log$D_{7.4}$: 2.0
Solubility [b]: ≥ 100 μM
CYP inhibition (1A2, 2C8, 2C9, 2C19, 2D6, 3A4); residual activity [a]: 98%, 99%, 96%, 90%, 102%, 90%
CL_{int} [c] (mL/min/kg) (human, mouse): No Dep.[d], 175.3
P-gp NER: 1.2
Pint: 27.5 x 10^{-6} cm/sec

Figure 5. Profiles of compound **6d**.

[a] Residual activities of HLM for metabolism of each substrate in the presence of test compounds were determined following pre-incubation for 30 min.
[b] Aqueous solubility in the Japanese Pharmacopoeia 2nd fluid for disintegration test (JP2; pH = 6.8).
[c] intrinsic clearance
[d] No depletion in this condition

Table 5. Plasma and brain concentration of **6d** at 1 h after oral administration to mice

Dose (mg/kg)	**Plasma (ng/mL)**	**Brain (ng/g)**	K_p**,brain** [a]
0.1	0.91	2.2	2.3

[a] Average value of individual Kp (brain/plasma) (n=3).

We next examined the pharmacokinetic profile of **6d** in mice at a single dose of 0.1 mg/kg. As shown in Table 5, compound **6d** showed good intracerebral transferability in mice (Kp, brain=2.3). The good brain permeability of **6d** prompted us to assess its in vivo effect on hyperlocomotion induced by phencyclidine (PCP) in mice, an animal model for positive symptoms of schizophrenia. As shown in Fig. 6, compound **6d** dose-dependently attenuated the PCP-induced hyperlocomotion after oral administration.

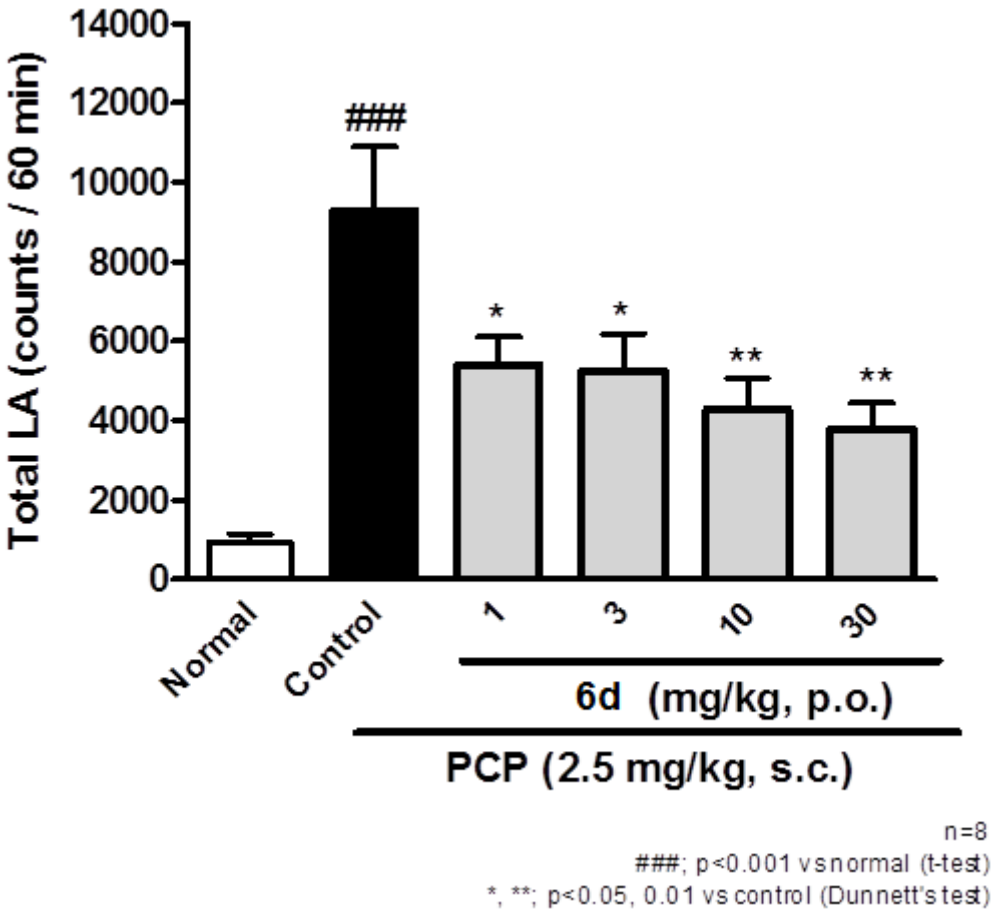


Figure 6. Effect of oral administration of **6d** on PCP-induced hyperlocomotion in

mice. PCP was administered subcutaneously (sc). The data represent the mean ± SEM: ### p <0.001 versus normal group (Student's t test); **p < 0.01 vs control, *p < 0.05 vs control (Dunnett's test).

We examined the effect of **6d** on working memory disruption in mice by observing spontaneous alternation behavior in a Y-maze following treatment with MK-801. This model is generally used to screen for agents that improve cognitive deficits because working memory deficit is a common symptom of disorders involving cognitive dysfunction, such as dementia and schizophrenia. MK-801 is a noncompetitive antagonist of the NMDA receptor, and animals treated with MK-801 show various memory and learning deficits caused by inhibition of signaling through NMDA receptors. [11]

Alternation was defined as consecutive entries into each of the three arms. In this study, the alternation rate in the normal group was approximately 70%, which significantly decreased to approximately 50% after administration of MK-801 (Fig. 7). Doses of 0.1mg/kg and 0.3mg/kg of **6d** significantly attenuated the MK-801-induced decrease in alternation rate ($p < 0.01$ and $p < 0.05$, respectively). It was reported that PDE10A plays a pivotal role in regulating the tone of dopamine D1 receptor signaling, [12] and an "inverted-U" relationship between prefrontal dopamine transmission and cortical

efficacy that varies as a function of working memory load was also suggested,[13] which may explain the inverted "U-shaped" dose–response curve of **6d** in MK-801-induced working memory deficit in mice during Y-maze test. The mean number of arm entries was not affected by **6d** administration except at a dose of 1 mg/kg compared with the control group (data not shown). Compound **6d** ameliorated MK-801-induced decreases in alternation behavior, suggesting that **6d** would be effective for treating symptoms of cognitive disorders.

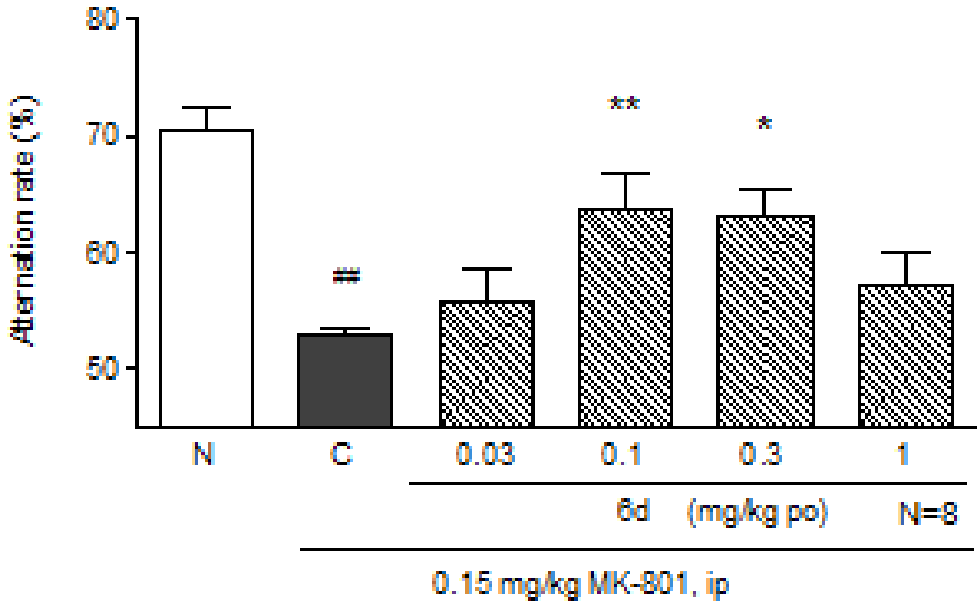


Figure 7. Effect of **6d** on MK-801-induced disruption of spontaneous alternation behavior in mice. Data represent the mean ± S.E.M. alternation rate in mice. ##p < 0.01

vs normal group (Student's *t*-test); **p < 0.01 vs control, *p < 0.05 vs control (Dunnett's test). N: normal (vehicle/saline-treated group), C: control (vehicle/MK801-treated group).

Existing antipsychotic drugs are known to induce catalepsy in rodents [16], which is an index of extrapyramidal side effects (EPS).[14] We therefore evaluated whether **6d** induces catalepsy in mice. Compound **6d** did not significantly induce catalepsy at up to 100 mg/kg, p.o. (Fig. 8).

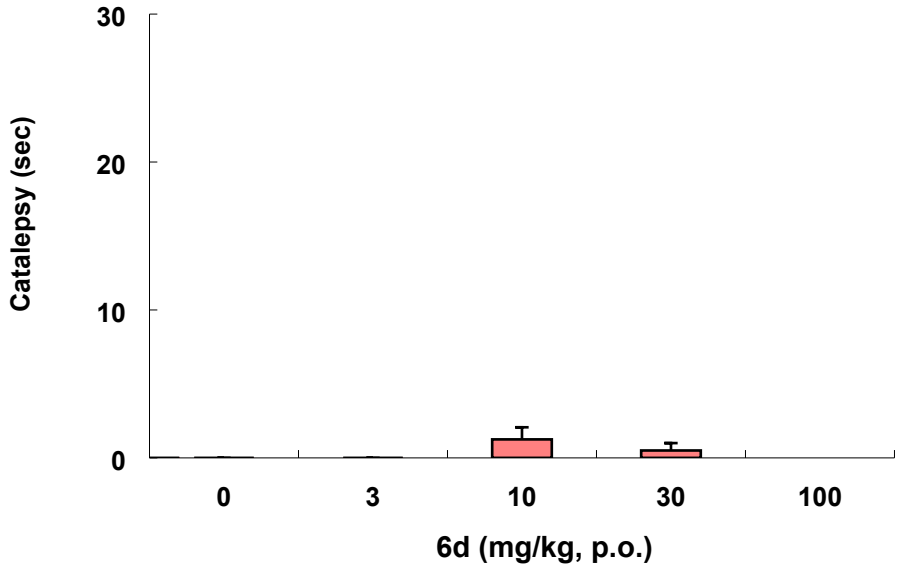


Figure 8. Effects of **6d** on catalepsy in mice Catalepsy time (sec) (max = 120 sec) of **6d**.

Data represent the mean ± S.E.M. (n = 6–8 for each group).

4 Conclusion

In this study, we investigated the SAR of various dihydro-imidazobenzimidazole derivatives and their PDE10A inhibitory activity and P-gp liability to obtain potent PDE10A inhibitors with good CNS penetration. The 2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole derivative **5a**, which was chosen as a new scaffold, showed moderate inhibitory activity and good permeability but unfavorable high P-gp liability. Substitution of a 2-methyl group into imidazo[1,2-*a*]benzimidazole resulted in improved PDE10A inhibitory activity. Moreover, replacement of the triazolopyrimidine core of **6c** with a triazolopyrazine core (**6d**) resulted in potent PDE10A inhibitory activity and acceptable P-gp liability. PK study indicated that **6d** exhibited good brain penetration. Further, **6d** attenuated the hyperlocomotor activity induced by PCP after oral administration at 1 mg/kg to 30 mg/kg, and significantly improved MK-801-induced working memory deficit in the Y-maze test following oral administration at 0.1 and 0.3 mg/kg. These effects suggest that **6d** may satisfy current unmet medical needs for the treatment of schizophrenia.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were recorded on a Varian 400-MR and BRUKER AV-III HD500, and chemical shifts were expressed as δ (ppm) values with tetramethylsilane as an internal reference (s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, m=multiplet, dd=double doublet, dt=double triplet, ddd=double double doublet, and br=broad peak). Mass spectra (MS) were recorded on a Waters UPLC/SQD and Waters Acquity UPLC/ZQ. Elemental analyses were performed using a Yanaco JM10 (C, H, N) and were within ±0.4% of theoretical values. Electrospray ionization positive high-resolution mass spectra (HRMS) were obtained using a Thermo EXACTIVE-Plus Waters LCT Premier. Specific rotation was obtained using Horiba SEPA-500 and OHM Electric OCE-TCR12075WL. Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification.

5.1.1. 2-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]pyrido[2',3',3,4]pyrazolo[1,5-*a*]pyrimidin-4-ol (2)

To a mixture of 3-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)propanoic acid (2.20 g, 10.0 mmol) in THF (33 mL) was added 1,1'-carbonyldiimidazole (1.95 g, 12.0 mmol), and the mixture was stirred at 50 °C for 1 h. To the reaction mixture were added ethyl potassium malonate (3.40 g, 20.0 mmol), magnesium chloride (1.90 g, 20.0 mmol), triethylamine (3.48 mL, 25.0 mmol), and the mixture was stirred at 50 °C for 12 h. After cooling to room temperature, to the reaction mixture was added 1 M HCl aqueous solution (50 mL), and the mixture was stirred at room temperature for 1 h. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. To a half of the residue were added 1,4-dioxane (15 mL), 1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (615 mg, 4.58 mmol) and AcOH (4 mL), and stirred at 90 °C for 36 h. After cooling to room temperature, the precipitate was collected by filtration. The solid was washed with EtOAc and saturated $NaHCO_3$ aqueous solution to give **2** (201 mg, 11%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.70 (d, 3H, J = 0.88 Hz), 2.99–3.17 (m, 2H), 3.21–3.28 (m, 2H), 5.86 (s, 1H), 6.84 (dd, 1H, J = 7.9, 4.4 Hz), 7.10 (d, 1H, J = 0.66 Hz), 8.31 (dd, 1H, J = 7.9, 1.8 Hz), 8.54 (dd, 1H, J = 4.2, 1.8 Hz); MS (ESI) m/z 361 $[M+H]^+$; HRMS

(ESI) calcd for $C_{18}H_{17}N_8O$ $[M+H]^+$ 361.1520; found, 361.1519.

5.1.2. 2-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]pyrimido[1,2-*b*]indazole (3)

To a suspension of 4-chloro-2-[2-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]pyrimido[1,2-*b*]indazole [8] (104 mg, 0.275 mmol) in EtOH (1.0 mL) was added 5% Pd-C (29.3 mg) and stirred at room temperature for 24 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo.* The residue was purified by flash column chromatography (silica gel, 0–10% MeOH in $CHCl_3$) to give **3** (3.3 mg, 3%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H), 2.68 (s, 3H), 3.39–3.53 (m, 4H), 7.10 (s, 1H), 7.22–7.28 (m, 1H), 7.51 (d, 1H, J = 7.3 Hz), 7.60 (ddd, 1H, J = 8.4, 6.9, 0.99 Hz), 7.76 (d, 1H, J = 8.8 Hz), 8.21 (d, 1H, J = 8.2 Hz), 9.32 (d, 1H, J = 7.1 Hz); MS (ESI) m/z 344 $[M+H]^+$.

5.1.3. Benzyl [2-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]carbamate (8)

To a suspension of 3-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)propanoic acid **7** (3.00 g, 13.6 mmol) in toluene (50 mL) were added benzyl alcohol (7.00 mL, 67.6

mmol), triethylamine (3.00 mL, 21.5 mmol) and diphenylphosphoryl azide (4.46 g, 16.2 mmol) at room temperature. The mixture was stirred at 120 °C for 2 h. After cooling to room temperature, H_2O was added to the residue followed by extraction with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–4% MeOH in $CHCl_3$) to give **8** (3.05 g, 69%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.69 (s, 3H), 2.96 (t, 2H, J = 7.3 Hz), 3.40–3.50 (m, 2H), 5.02 (s, 2H), 7.14 (s, 1H), 7.26–7.39 (m, 6H); MS (ESI) m/z 326 $[M+H]^+$.

5.1.4. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethanamine (9)

To a solution of **8** (521 mg, 1.60 mmol) in MeOH (20 mL) was added 5% Pd-C (200 mg) and stirred at room temperature for 9 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to give **9** (310 mg, quant.) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.70 (d, 3H, J = 0.78 Hz), 2.89–2.96 (m, 2H), 2.99–3.06 (m, 2H), 7.11 (d, 1H, J = 0.98 Hz); MS (ESI) m/z 192 $[M+H]^+$.

5.1.5. *N*-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]quinolin-2-

amine (4a)

To a mixture of **9** (310 mg, 1.62 mmol) in 1,2-dimethoxyethane (10 mL) were added 2-chloroquinoline (300 mg, 1.83 mmol), *t*-BuONa (220 mg, 2.29 mmol), $Pd(OAc)_2$ (40.0 mg, 0.178 mmol) and (*S*)-1-[(*R*)-2-(dicyclohexylphosphino)ferrocenyl]ethyldi-*tert*-butylphosphine ((*S*)-(*R*)-JOSIPHOS; 90.0 mg, 0.162 mmol) at room temperature. The mixture was stirred at 80 °C for 3 h under argon atmosphere. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, MeOH in $CHCl_3$ then NH silica gel, EtOAc in n-hexane), and concentrated *in vacuo*. The residue was precipitated from EtOAc with Et_2O, and the resulting precipitate was collected by filtration to give **4a** (65.6 mg, 13%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.69 (d, 3H, J = 0.78 Hz), 3.17 (t, 2H, J = 7.0 Hz), 3.81–3.93 (m, 2H), 6.76 (d, 1H, J = 8.8 Hz), 7.10 (d, 1H, J = 0.78 Hz), 7.16 (br-t, 1H, J = 6.9 Hz), 7.41–7.57 (m, 2H), 7.62 (d, 1H, J = 7.4 Hz), 7.85 (br-d, 1H, J = 8.8 Hz); MS (ESI) *m*/*z* 319 $[M+H]^+$; HRMS (ESI) calcd for $C_{18}H_{19}N_6$ $[M+H]^+$ 319.1666; found, 319.1666.

5.1.6. *N*-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]quinoxalin-2-amine (4b)

To a solution of 2-chloroquinoxaline (100 mg, 0.608 mmol) in NMP (1.00 mL) was added **9** (250 mg, 1.31 mmol) under argon atmosphere. The mixture was stirred at 160 °C for 3 h under microwave irradiation. After cooling to room temperature, H_2O was added to the residue followed by extraction with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–100% EtOAc in hexane), and concentrated *in vacuo*. The residue was washed with hexane/EtOAc to give **4b** (58.0 mg, 30%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.68 (s, 3H), 3.18 (t, 2H, J = 7.1 Hz), 3.83–3.92 (m, 2H), 7.10 (s, 1H), 7.32 (ddd, 1H, J = 8.2, 5.8, 2.3 Hz), 7.50–7.59 (m, 2H), 7.68–7.78 (m, 2H), 8.27 (s, 1H); MS (ESI) m/z 320 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{18}N_7$ $[M+H]^+$ 320.1618; found, 320.1619.

5.1.7. *N*-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]quinazolin-2-amine (4c)

Compound **4c** was prepared from 2-chloroquinazoline in a manner similar to that described for compound **4b**, with a yield of 14% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.68 (d, 3H, J = 0.66 Hz), 3.15 (t, 2H, J = 7.2 Hz), 3.77–3.88 (m, 2H),

7.10 (d, 1H, J = 0.88 Hz), 7.23 (ddd, 1H, J = 7.9, 6.8, 1.1 Hz), 7.39–7.50 (m, 2H), 7.69 (ddd, 1H, J = 8.5, 7.0, 1.5 Hz), 7.79 (dd, 1H, J = 8.1, 0.99 Hz), 9.11 (br-s, 1H); MS (ESI) m/z 320 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{18}N_7$ $[M+H]^+$ 320.1618; found, 320.1620.

5.1.8. *N*-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]-1*H*-benzimidazol-2-amine (4d)

Compound **4d** was prepared from 2-chlorobenzimidazole **10** in a manner similar to that described for compound **4b**, with a yield of 96% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H), 2.70 (d, 3H, J = 0.66 Hz), 3.15 (t, 2H, J = 7.2 Hz), 3.75 (q, 2H, J = 6.8 Hz), 6.59 (br-t, 1H, J = 6.1 Hz), 6.77–6.94 (m, 2H), 7.05–7.19 (m, 3H), 10.75 (s, 1H); MS (ESI) m/z 308 $[M+H]^+$; HRMS (ESI) calcd for $C_{16}H_{18}N_7$ $[M+H]^+$ 308.1618; found, 308.1619; Anal. Calcd for $C_{16}H_{17}N_7 \cdot 1.5H_2O$: C, 57.47; H, 6.03; N, 29.32. Found: C, 57.51; H, 6.09; N, 29.19.

5.1.9. *N*-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]-1-methyl-1*H*-benzimidazol-2-amine (4e)

Compound **4e** was prepared from 2-chloro-1-methylbenzimidazole in a manner similar to that described for compound **4b**, with a yield of 89% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H), 2.70 (d, 3H, J = 0.88 Hz), 3.19 (t, 2H, J = 7.4 Hz), 3.47 (s, 3H), 3.73–3.84 (m, 2H), 6.80 (t, 1H, J =5.7 Hz), 6.88–6.99 (m, 2H), 7.09–7.16 (m, 2H), 7.19–7.25 (m, 1H); MS (ESI) m/z 322 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{20}N_7$ $[M+H]^+$ 322.1775; found, 322.1776.

5.1.10. 1-[2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (4f)

To a mixture of **4d** (95.0 mg, 0.309 mmol) in 1,2-dichloroethane (1.0 mL) were added 1,2-dibromoethane (0.100 mL, 1.16 mmol), 1M NaOH aqueous solution (1.00 mL, 1.00 mmol) and tetrabutylammonium bromide (18.0 mg, 55.8 μmol). The mixture was stirred at room temperature for 2 h. Subsequently, 1,2-dibromoethane (0.100 mL, 1.16 mmol) and 1 M NaOH aqueous solution (1.00 mL, 1.00 mmol) were added. The reaction mixture was stirred at 60 °C for 20 h. After cooling to room temperature, H_2O was added to the residue followed by extraction with $CHCl_3$/MeOH co-solvent. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–5% MeOH in $CHCl_3$), and

concentrated *in vacuo*. The solid was washed with EtOAc, and the resulting precipitate was collected by filtration to give **4f** (32.0 mg, 31%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.68 (d, 3H, J = 0.88 Hz), 3.23 (t, 2H, J = 7.4 Hz), 3.77 (t, 2H, J = 7.4 Hz), 3.94–4.03 (m, 2H), 4.06–4.14 (m, 2H), 6.87–6.97 (m, 2H), 7.08–7.13 (m, 2H), 7.16–7.20 (m, 1H); MS (ESI) m/z 334 $[M+H]^+$; HRMS (ESI) calcd for $C_{18}H_{20}N_7$ $[M+H]^+$ 334.1775; found, 334.1775; Anal. Calcd for $C_{18}H_{19}N_7 \cdot 0.5H_2O$: C, 63.14; H, 5.89; N, 28.64. Found: C, 63.09; H, 5.85; N, 28.49.

5.1.11. 2-[(1*H*-Benzimidazol-2-yl)amino]ethan-1-ol (12a)

A mixture of 2-chlorobenzimidazole **10** (10.0 g, 65.5 mmol) and 2-aminoethanol **11a** (12.0 mL, 199 mmol) was stirred at 140 °C for 14 h. After cooling to room temperature, saturated $NaHCO_3$ aqueous solution and H_2O were added to the reaction mixture with stirring. The resulting precipitate was collected by filtration, washed with H_2O and dried to give **12a** (11.2 g, 97%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 3.35 (t, 3H, J = 5.9 Hz), 3.54–3.59 (m, 2H), 4.96 (br-s, 1H), 6.48 (br-s, 1H), 6.81–6.88 (m, 2H), 7.08–7.15 (m, 2H); MS (ESI) m/z 178 $[M+H]^+$.

5.1.12. 2,3-Dihydro-1*H*-imidazo[1,2-*a*]benzimidazole hydrogen chloride (13a)

To a mixture of **12a** (1.77 g, 9.99 mmol) in 1,2-dichloroethane (20 mL) was added thionyl chloride (0.850 mL, 11.7 mmol). The mixture was stirred under reflux for 50 min. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Saturated $NaHCO_3$ aqueous solution was added to the residue followed by extraction with $CHCl_3$/MeOH co-solvent. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue and xylene (20 mL) was stirred under reflux for 21 h. After cooling to room temperature, the resulting precipitate was collected by filtration, washed with hexane and dried to give **13a** (1.78 g, 91% in 2 steps) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 4.20–4.38 (m, 4H), 7.17–7.30 (m, 2H), 7.35–7.43 (m, 2H), 9.46 (br-s, 1H), 13.44 (br-s, 1H); MS (ESI) *m*/*z* 160 $[M+H]^+$.

5.1.13. 1-(2,3-Dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)-2-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethan-1-one (5a)

To a mixture of **13a** (149 mg, 0.762 mmol) and (5,7-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-acetic acid **14** (169 mg, 0.820 mmol) in DMF (3.0 mL) were added WSC·HCl (161 mg, 0.840 mmol), HOBt (55.0 mg, 0.407 mmol) and triethylamine (160 μL, 1.15 mmol). The mixture was stirred at room temperature for 18 h. Saturated

$NaHCO_3$ aqueous solution and H_2O were added to the reaction mixture. The resulting precipitate was collected by filtration. The filtered solid was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$), and concentrated *in vacuo*. The residue was washed with EtOAc, and the resulting precipitate was collected by filtration to give **5a** (143 mg, 54%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H), 2.72 (d, 3H, J = 0.88 Hz), 4.28–4.36 (m, 2H), 4.47–4.57 (m, 2H), 4.80 (s, 2H), 7.10–7.19 (m, 3H), 7.39–7.45 (m, 1H), 7.46–7.53 (m, 1H); MS (ESI) *m*/*z* 348 $[M+H]^+$; HRMS (ESI) calcd for $C_{18}H_{18}ON_7$ $[M+H]^+$ 348.1567; found, 348.1569; Anal. Calcd for $C_{18}H_{17}N_7O$: C, 62.24; H, 4.93; N, 28.23. Found: C, 61.88; H, 4.95; N, 27.92.

5.1.14. 3-[(1*H*-Benzimidazol-2-yl)amino]propan-1-ol (12b)

A mixture of 2-chlorobenzimidazole **10** (2.00 g, 13.1 mmol) and 3-aminopropanol **11b** (5.00 mL, 65.8 mmol) was stirred at 140 °C for 15.5 h. After cooling to room temperature, saturated $NaHCO_3$ aqueous solution was added to the reaction mixture followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give **12b** (198 mg, 8%) as a colorless solid. A small amount of the solid was added to the aqueous layer with stirring. The resulting precipitate was collected by filtration to give **12b** (624 mg, 25%) as a gray solid. ^{1}H

NMR (DMSO-d_6) δ 1.70 (quint., 2H, J = 6.4 Hz), 3.29–3.38 (m, 2H), 3.42–3.53 (m, 2H), 4.77 (br-s, 1H) 6.49 (t, 1H, J = 5.8 Hz), 6.74–6.94 (m, 2H), 7.02–7.17 (m, 2H), 10.69 (br-s, 1H); MS (ESI) m/z 192 $[M+H]^+$.

5.1.15. 1,2,3,4-Tetrahydropyrimido[1,2-*a*]benzimidazole (13b)

To a mixture of **12b** (300 mg, 1.57 mmol) in 1,2-dichloroethane (5.0 mL) was added thionyl chloride (0.150 mL, 2.06 mmol). The mixture was stirred under reflux for 2 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Saturated $NaHCO_3$ aqueous solution was added to the residue followed by extraction with $CHCl_3$/MeOH. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. A mixture of the residue and xylene (5.0 mL) was stirred under reflux for 14 h. After cooling to room temperature, the resulting precipitate was collected by filtration and washed with hexane. The filtered solid was dissolved with MeOH. Saturated $NaHCO_3$ aqueous solution was added to the solution followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$) to give **13b** (148 mg, 54% in 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.19–2.28 (m, 2H), 3.49–3.55 (m, 2H), 4.02 (t, 2H, J = 6.2 Hz),

5.26 (br-s, 1H), 6.99–7.14 (m, 3H), 7.38 (dt, 1H, J = 7.8, 0.88 Hz); MS (ESI) m/z 174 $[M+H]^+$.

5.1.16. 1-(3,4-Dihydropyrimido[1,2-*a*]benzimidazol-1(2*H*)-yl)-2-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethan-1-one (5b)

To a mixture of **13b** (73.0 mg, 0.421 mmol) and **14** (95.0 mg, 0.461 mmol) in DMF (1.0 mL) were added WSC·HCl (99.0 mg, 0.516 mmol) and HOBt (34.0 mg, 0.252 mmol). The mixture was stirred at room temperature for 18 h. H_2O was added to the reaction mixture. The resulting precipitate was collected by filtration. The filtered solid was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$), and concentrated *in vacuo*. The resulting solid was washed with EtOAc and collected by filtration to give **5b** (67.0 mg, 44%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.18–2.28 (m, 2H), 2.56 (s, 3H), 2.69 (d, 3H, J = 0.88 Hz), 3.99–4.07 (m, 2H), 4.18 (t, 2H, J = 6.2 Hz), 4.96 (s, 2H), 7.12 (d, 1H, J = 0.88 Hz), 7.15–7.21 (m, 2H), 7.40–7.47 (m, 1H), 7.48–7.54 (m, 1H); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1725; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N, 27.13. Found: C, 63.04; H, 5.28; N, 26.97.

5.1.17. 8-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (17c)

To a mixture of 3-bromo-2-nitrotoluene **15c** (2.13 g, 9.86 mmol) and 1,4-dioxane (50 mL) were added 2-imidazolidone **16a** (4.01 g, 46.6 mmol), Cs_2CO_3 (4.71 g, 14.5 mmol), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$; 400 mg, 0.437 mmol), and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (XantPhos; 503 mg, 0.869 mmol) under argon atmosphere. The mixture was stirred at 100 °C for 19.5 h. After cooling to room temperature, the mixture was filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–5% MeOH in $CHCl_3$), and concentrated *in vacuo*. To a mixture of the residue in EtOH (12 mL) and H_2O (3.0 mL) were added $FeCl_3 \cdot 6H_2O$ (99.0 mg, 0.366 mmol), $N_2H_4 \cdot H_2O$ (1.10 mL, 22.7 mmol), and activated carbon (102 mg). The mixture was stirred under reflux for 14 h. After cooling to room temperature, the mixture was filtered through a pad of Celite. The filtrate was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. A mixture of the residue and $POCl_3$ (2.00 mL, 21.8 mmol) was stirred at 100 °C for 5 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. 1 M NaOH aqueous solution was added to the residue followed by extraction with $CHCl_3$/MeOH co-solvent. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*.

The residue was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$) to give **17c** (345 mg, 20% in 3 steps) as a brown solid. ^{1}H NMR ($CDCl_3$) δ 2.52 (s, 3H), 4.07–4.20 (m, 4H), 4.97–5.66 (m, 1H), 6.89–7.05 (m, 3H); MS (ESI) *m*/*z* 174 $[M+H]^+$.

5.1.18. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-(8-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)ethan-1-one (5c)

To a mixture of **17c** (98.0 mg, 0.566 mmol) and **14** (128 mg, 0.621 mmol) in DMF (2.0 mL) were added WSC·HCl (133 mg, 0.694 mmol) and HOBt (37.0 mg, 0.274 mmol). The mixture was stirred at room temperature for 3.5 h. H_2O was added to the reaction mixture. The resulting precipitate was collected by filtration. The filtered solid was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$), and concentrated *in vacuo*. The residue was washed with EtOAc (2 mL)/MeOH (1 mL) co-solvent, and the resulting precipitate was collected by filtration to give **5c** (83.0 mg, 41%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 3H), 2.57 (s, 3H), 2.72 (d, 3H, J = 0.88 Hz), 4.26–4.32 (m, 2H), 4.47–4.55 (m, 2H), 4.84 (s, 2H), 6.94–7.00 (m, 1H), 7.05 (t, 1H, J = 7.7 Hz), 7.15 (d, 1H, J = 0.88 Hz), 7.23 (d, 1H, J = 7.7 Hz); MS (ESI) *m*/*z* 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found,

362.1725; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N, 27.13. Found: C, 62.78; H, 5.21; N, 26.98.

5.1.19. 7-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (17d)

Compound **17d** was prepared from 4-iodo-3-nitrotoluene **15d** in a manner similar to that described for compound **17c**, with a yield of 15% in 3 steps as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 4.07–4.18 (m, 4H), 4.51 (br-s, 1H), 6.83–6.88 (m, 1H), 6.95 (d, 1H, J = 8.0 Hz), 7.23 (s, 1H); MS (ESI) m/z 174 $[M+H]^+$.

5.1.20. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-(7-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)ethan-1-one (5d)

To a mixture of **17d** (44.0 mg, 0.254 mmol) and **14** (59.0 mg, 0.286 mmol) in DMF (1.0 mL) were added WSC·HCl (56.0 mg, 0.292 mmol) and HOBt (17.0 mg, 0.126 mmol). The mixture was stirred at room temperature for 3.5 h. H_2O was added to the reaction mixture. The resulting precipitate was collected by filtration to give **5d** (47.0 mg, 51%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H), 2.57 (s, 3H), 2.72 (d, 3H, J = 0.88 Hz), 4.24–4.32 (m, 2H), 4.46–4.54 (m, 2H), 4.79 (s, 2H), 6.95–7.00 (m, 1H), 7.15

(d, 1H, J = 0.88 Hz), 7.27–7.32 (m, 2H); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1725.

5.1.21. 6-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (17e)

Compound **17e** was prepared from 3-iodo-4-nitrotoluene **15e** in a manner similar to that described for compound **17c**, with a yield of 2% in 3 steps as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 4.08–4.18 (m, 4H), 4.58 (br-s, 1H), 6.89–6.94 (m, 2H), 7.30 (d, 1H, J = 8.0 Hz); MS (ESI) m/z 174 $[M+H]^+$.

5.1.22. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-(6-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)ethan-1-one (5e)

Compound **5e** was prepared from **17e** in a manner similar to that described for compound **5c**, with a yield of 84% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H), 2.57 (s, 3H), 2.71 (d, 3H, J = 0.88 Hz), 4.24–4.32 (m, 2H), 4.46–4.54 (m, 2H), 4.78 (s, 2H), 6.94–7.00 (m, 1H), 7.15 (d, 1H, J = 0.88 Hz), 7.21–7.25 (m, 1H), 7.37 (d, 1H, J = 8.2 Hz); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$

362.1724; found, 362.1725; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N, 27.13. Found: C, 62.75; H, 5.32; N, 27.05.

5.1.23. 5-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (17f)

Compound **17f** was prepared from 2-bromo-3-nitrotoluene **15f** in a manner similar to that described for compound **17c**, with a yield of 10% in 3 steps as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H), 4.09–4.16 (m, 2H), 4.36–4.43 (m, 2H), 6.81 (d, 1H, J = 7.4 Hz), 6.99 (t, 1H, J = 7.7 Hz), 7.23–7.25 (m, 1H); MS (ESI) m/z 174 $[M+H]^+$.

5.1.24. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-(5-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)ethan-1-one (5f)

Compound **5f** was prepared from **17f** in a manner similar to that described for compound **5d**, with a yield of 60% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H), 2.57 (s, 3H), 2.72 (d, 3H, J = 0.66 Hz), 4.46–4.60 (m, 4H), 4.79 (s, 2H), 6.91 (d, 1H, J = 7.3 Hz), 7.02 (t, 1H, J = 7.7 Hz), 7.15 (d, 1H, J = 0.88 Hz), 7.30 (d, 1H, J = 7.7 Hz); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1725; ; Anal. Calcd for $C_{19}H_{19}N_7O \cdot 0.5H_2O$: C, 61.61; H, 5.44; N, 26.47.

Found: C, 61.80; H, 5.18; N, 26.47.

5.1.25. 2-[(1*H*-Benzimidazol-2-yl)amino]propan-1-ol (12g)

Compound **12g** was prepared from 2-chlorobenzimidazole **10** and 2-amino-1-propanol **11g** in a manner similar to that described for compound **12a**, with a yield of 91% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 1.17 (d, 3H, J = 6.7 Hz), 3.40 (dd, 1H, J = 10.4, 5.7 Hz), 3.50 (dd, 1H, J = 10.5, 5.2 Hz), 3.75–3.88 (m, 1H), 4.93 (br-s, 1H), 6.26 (br-d, 1H, J = 7.8 Hz), 6.85 (br-s, 2H), 7.08–7.14 (m, 2H), 10.53 (br-s, 1H); MS (ESI) m/z 192 $[M+H]^+$.

5.1.26. 2-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (13g)

Compound **13g** was prepared from **12g** in a manner similar to that described for compound **13b**, with a yield of 14% in 2 steps as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.49 (d, 3H, J = 6.5 Hz), 3.70 (dd, 1H, J = 9.2, 6.7 Hz), 4.27 (dd, 1H, J = 9.1, 8.3 Hz), 4.52–4.61 (m, 1H), 5.19 (br-s, 1H), 7.00–7.12 (m, 3H), 7.38–7.44 (m, 1H); MS (ESI) m/z 174 $[M+H]^+$.

5.1.27. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-(2-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)ethan-1-one (5g)

To a mixture of **13g** (103 mg, 0.595 mmol) and **14** (146 mg, 0.708 mmol) in dichloromethane (2.0 mL) were added *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'* -tetramethyluronium hexafluorophosphate (HATU; 248 mg, 0.652 mmol) and *N*,*N*-diisopropylethylamine (DIPEA; 300 μL, 1.75 mmol). The mixture was stirred at room temperature for 20 h. 1 M NaOH aqueous solution and H_2O were added to the reaction mixture followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$), and concentrated *in vacuo*. The residue was washed with EtOAc (1 mL)/MeOH (1 mL), and the resulting precipitate was collected by filtration to give **5g** (127 mg, 59%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.51 (d, 3H, J = 6.4 Hz), 2.57 (s, 3H), 2.71 (d, 3H, J = 0.88 Hz), 3.99 (dd, 1H, J = 9.9, 3.1 Hz), 4.42 (dd, 1H, J = 9.8, 8.5 Hz), 4.74 (d, 1H, J = 16.5 Hz), 4.83 (d, 1H, J = 16.5 Hz), 5.10–5.19 (m, 1H), 7.12–7.19 (m, 3H), 7.38–7.44 (m, 1H), 7.47–7.53 (m, 1H); MS (ESI) *m*/*z* 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1725; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N,

27.13. Found: C, 63.18; H, 5.26; N, 27.09.

5.1.28. 1-[(1*H*-Benzimidazol-2-yl)amino]propan-2-ol (12h)

Compound **12h** was prepared from **10** and 1-amino-2-propanol **11h** in a manner similar to that described for compound **12a**, with a yield of 88% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 1.09 (d, 3H, J = 6.3 Hz), 3.12–3.30 (m, 2H), 3.76–3.87 (m, 1H), 4.99 (br-d, 1H, J = 4.3 Hz), 6.43 (br-t, 1H, J = 5.9 Hz), 6.76–6.93 (m, 2H), 7.11 (br-d, 2H, J = 7.6 Hz), 10.60 (br-s, 1H); MS (ESI) m/z 192 $[M+H]^+$.

5.1.29. 3-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole hydrogen chloride (13h)

Compound **13h** was prepared from **12h** in a manner similar to that described for compound **13a**, with a yield of 97% in 2 steps as a brown solid. ^{1}H NMR (DMSO-d_6) δ 1.57 (d, 3H, J = 6.5 Hz), 3.82 (dd, 1H, J = 10.0, 6.5 Hz), 4.39 (t, 1H, J = 9.6 Hz), 4.83–4.98 (m, 1H), 7.18–7.31 (m, 2H), 7.35–7.44 (m, 1H), 7.48–7.54 (m, 1H), 9.58 (br-s, 1H), 13.52 (br-s, 1H); MS (ESI) m/z 174 $[M+H]^+$.

5.1.30. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-(3-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)ethan-1-one (5h)

To a mixture of **13h** (127 mg, 0.606 mmol) and **14** (137 mg, 0.664 mmol) in DMF (3.0 mL) were added WSC·HCl (139 mg, 0.725 mmol), HOBt (47.0 mg, 0.348 mmol) and triethylamine (150 μL, 1.08 mmol). The mixture was stirred at room temperature for 22 h. H_2O was added to the reaction mixture. The resulting precipitate was collected by filtration. The residue was washed with EtOAc, and the resulting precipitate was collected by filtration to give **5h** (126 mg, 58%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 1.60 (d, 3H, J = 6.4 Hz), 2.57 (s, 3H), 2.71 (d, 3H, J = 0.66 Hz), 4.07 (dd, 1H, J = 11.3, 5.5 Hz), 4.67 (dd, 1H, J = 11.1, 8.7 Hz), 4.74–4.90 (m, 3H), 7.11–7.20 (m, 3H), 7.47–7.54 (m, 2H); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1725; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N, 27.13. Found: C, 63.00; H, 5.31; N, 27.08.

5.1.31. 2,2-Dimethyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole（17i）

Compound **17i** was prepared from 1-iodo-2-nitrobenzene **15i** and 4,4-dimethylimidazolidin-2-one **16b** in a manner similar to that described for compound

17c, with a yield of 63% in 3 steps as a brown solid. ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 6H), 3.88 (s, 2H), 6.99–7.12 (m, 3H), 7.41 (dt, 1H, J = 7.7, 0.93 Hz); MS (ESI) m/z 188 $[M+H]^+$.

5.1.32. 1-(2,2-Dimethyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)-2-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)ethan-1-one (5i)

To a mixture of **17i** (155 mg, 0.828 mmol) and **14** (194 mg, 0.941 mmol) in dichloromethane (3.0 mL) were added HATU (357 mg, 0.939 mmol) and DIPEA (300 μL, 1.75 mmol). The mixture was stirred at room temperature for 6 days. The reaction mixture was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$), and concentrated *in vacuo*. The solid was washed with EtOAc (3 mL), and the resulting precipitate was collected by filtration to give **5i** (137 mg, 44%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.76 (s, 6H), 2.57 (s, 3H), 2.71 (d, 3H, J = 0.88 Hz), 4.16 (s, 2H), 4.83 (s, 2H), 7.11–7.18 (m, 3H), 7.36–7.42 (m, 1H), 7.46–7.52 (m, 1H); MS (ESI) m/z 376 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{22}ON_7$ $[M+H]^+$ 376.1880; found, 376.1881; Anal. Calcd for $C_{20}H_{21}N_7O$: C, 63.98; H, 5.64; N, 26.12. Found: C, 63.81; H, 5.63; N, 26.00.

5.1.33. *N*-[(1*S*)-2-Hydroxy-1-phenylethyl]-*N′*-(2-nitrophenyl)urea (20j)

To a mixture of 2-nitrophenyl isocyanate **18** (1.00 g, 6.09 mmol) in THF (15 mL) was added (2*S*)-2-amino-2-phenylethan-1-ol **19j** (850 mg, 6.20 mmol). The mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated *in vacuo* to give **20j** (1.86 g, quant.) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.53–3.67 (m, 2H), 4.73–4.81 (m, 1H), 4.96 (t, 1H, J = 5.5 Hz), 7.11 (ddd, 1H, J = 8.4, 7.2, 1.3 Hz), 7.20–7.28 (m, 1H), 7.30–7.37 (m, 4H), 7.61 (ddd, 1H, J = 8.6, 7.2, 1.5 Hz), 8.04 (dd, 1H, J = 8.4, 1.4 Hz), 8.08 (br-d, 1H, J = 8.0 Hz), 8.31 (dd, 1H, J = 8.6, 0.98 Hz), 9.49 (s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.34. (2*S*)-2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (21j)

To a mixture of **20j** (1.85 g, 6.14 mmol) in EtOH (20 mL) and H_2O (5.0 mL) were added $FeCl_3 \cdot 6H_2O$ (189 mg, 0.699 mmol), $N_2H_4 \cdot H_2O$ (1.20 mL, 24.7 mmol), and activated carbon (172 mg). The mixture was stirred under reflux for 2 h. After cooling to room temperature, the mixture was filtered through a pad of Celite. The filtrate was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. A mixture of the residue and

$POCl_3$ (5.00 mL, 54.6 mmol) was stirred at 100 °C for 1.5 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. 1 M NaOH aqueous solution was added to the residue followed by extraction with $CHCl_3$/MeOH co-solvent. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–5% MeOH in $CHCl_3$), and concentrated *in vacuo*. A mixture of the residue and xylene (10 mL) was stirred under reflux for 17 h. After cooling to room temperature, the resulting precipitate was collected by filtration, washed with hexane. The filtered solid was solved with MeOH (5 mL). H_2O and saturated $NaHCO_3$ aqueous solution were added to the solution followed by extraction with $CHCl_3$/MeOH co-solvent. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$) to give **21j** (191 mg, 13% in 3 steps) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.97 (dd, 1H, J = 9.4, 7.4 Hz), 4.55 (dd, 1H, J = 9.4, 8.8 Hz), 5.09–5.30 (m, 1H), 5.48 (dd, 1H, J = 8.7, 7.5 Hz), 7.01–7.08 (m, 2H), 7.08–7.15 (m, 1H), 7.33–7.47 (m, 6H); MS (ESI) m/z 236 $[M-H]^-$.

5.1.35. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-[(2*S*)-2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl]ethan-1-one (5j)

Compound **5j** was prepared from **21j** in a manner similar to that described for compound **5b**, with a yield of 53% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.70 (d, 3H, J = 0.88 Hz), 4.18 (dd, 1H, J = 10.1, 3.1 Hz), 4.69–4.79 (m, 2H), 4.98 (d, 1H, J = 16.3 Hz), 6.16 (dd, 1H, J = 8.8, 2.9 Hz), 7.12–7.20 (m, 3H), 7.29–7.45 (m, 6H), 7.51–7.57 (m, 1H); MS (ESI) m/z 424 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{22}N_7O$ $[M+H]^+$ 424.1880; found, 424.1882; Anal. Calcd for $C_{24}H_{21}N_7O$: C, 68.07; H, 5.00; N, 23.15. Found: C, 68.05; H, 5.17; N, 23.16; $[\alpha]_D^{23}$ +48.0 (c 0.375, MeOH).

5.1.36. *N*-[(1*R*)-2-Hydroxy-1-phenylethyl]-*N′*-(2-nitrophenyl)urea (20k)

To a mixture of **18** (1.00 g, 6.09 mmol) in THF (15 mL) was added (2R)-2-amino-2-phenylethan-1-ol **19k** (854 mg, 6.23 mmol). The mixture was stirred at room temperature for 1 h. After concentrated *in vacuo*, the reaction mixture was purified by flash column chromatography (silica gel, 0–5% MeOH in $CHCl_3$), and concentrated *in vacuo* to give **20k** (1.89 g, quant.) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.26 (br-s, 1H), 3.88–4.03 (m, 2H), 4.95–5.06 (m, 1H), 5.91 (br-s, 1H), 7.00–7.07 (m, 1H), 7.28–7.35 (m, 1H), 7.35–7.42 (m, 4H), 7.50–7.59 (m, 1H), 8.15 (dd, 1H, J = 8.5, 1.5 Hz), 8.59 (d, 1H, J = 8.6 Hz), 9.85 (s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.37. (2*R*)-2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (21k)

Compound **21k** was prepared from **20k** in a manner similar to that described for compound **21j**, with a yield of 24% in 3 steps as a beige solid. ^{1}H NMR ($CDCl_3$) δ 3.97 (dd, 1H, J = 9.4, 7.4 Hz), 4.51–4.58 (m, 1H), 5.31 (br-s, 1H), 5.46–5.51 (m, 1H), 7.04–7.06 (m, 2H), 7.08–7.14 (m, 1H), 7.32–7.48 (m, 6H); MS (ESI) *m*/*z* 236 $[M-H]^-$.

5.1.38. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-[(2*R*)-2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl]ethan-1-one (5k)

Compound **5k** was prepared from **21k** in a manner similar to that described for compound **5b**, with a yield of 46% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.70 (d, 3H, J = 0.88 Hz), 4.18 (dd, 1H, J = 10.3, 3.0 Hz), 4.69–4.79 (m, 2H), 4.98 (d, 1H, J = 16.3 Hz), 6.16 (dd, 1H, J = 8.8, 2.9 Hz), 7.12–7.20 (m, 3H), 7.29–7.45 (m, 6H), 7.50–7.58 (m, 1H); MS (ESI) *m*/*z* 424 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{22}N_7O$ $[M+H]^+$ 424.1880; found, 424.1883; Anal. Calcd for $C_{24}H_{21}N_7O$: C, 68.07; H, 5.00; N, 23.15. Found: C, 68.17; H, 5.06; N, 23.07; $[\alpha]_D^{23}$ -55.8 (c 0.375, MeOH).

5.1.39. *N*-[(2*S*)-1-Hydroxypropan-2-yl]-*N'*-(2-nitrophenyl)urea (20l)

Compound **20l** was prepared from **18** and (2S)-2-aminopropan-1-ol **19l** in a manner similar to that described for compound **20j**, with a quantitative yield as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.08 (d, 3H, J = 6.7 Hz), 3.36–3.43 (m, 1H), 3.62–3.76 (m, 1H), 4.75 (t, 1H, J = 5.5 Hz), 7.11 (ddd, 1H, J = 8.4, 7.1, 1.3 Hz), 7.43 (br-d, 1H, J = 7.6 Hz), 7.63 (ddd, 1H, J = 8.5, 7.1, 1.6 Hz), 8.04 (dd, 1H, J = 8.4, 1.6 Hz), 8.32 (dd, 1H, J = 8.6, 1.2 Hz), 9.31 (s, 1H); MS (ESI) m/z 240 $[M+H]^+$.

5.1.40. (2*S*)-2-Methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (21l)

To a solution of **20l** (1.45 g, 6.06 mmol) in EtOH (15 mL) and THF (15 mL) was added 5% Pd-C (160 mg) and stirred at room temperature for 2.5 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. A mixture of the residue and $POCl_3$ (4.00 mL, 43.7 mmol) was stirred at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. 1M NaOH aqueous solution and $CHCl_3$/MeOH co-solvent were added to the reaction mixture. The resulting precipitate was collected by filtration. A mixture of the residue and xylene (10 mL) was stirred under reflux for 37 h. After cooling to room temperature, the resulting precipitate was collected by filtration, and

washed with hexane. The filtered solid was dissolved with MeOH (2 mL). H_2O and saturated $NaHCO_3$ aqueous solution were added to the solution followed by extraction with $CHCl_3$/MeOH co-solvent. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–3% MeOH in $CHCl_3$) to give **21l** (229 mg, 22% in 3 steps) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.49 (d, 3H, J = 6.3 Hz), 3.70 (dd, 1H, J = 9.2, 6.9 Hz), 4.28 (dd, 1H, J = 9.2, 8.2 Hz), 4.51–4.64 (m, 1H), 4.89 (br-s, 1H), 7.01–7.12 (m, 3H), 7.42 (dt, 1H, J = 7.8, 0.90 Hz); MS (ESI) m/z 174 $[M+H]^+$.

5.1.41. 2-(5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-1-[(2*S*)-2-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl]ethan-1-one (5l)

Compound **5l** was prepared from **21l** in a manner similar to that described for compound **5b**, with a yield of 73% as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.51 (d, 3H, J = 6.4 Hz), 2.57 (s, 3H), 2.71 (d, 3H, J = 0.66 Hz), 3.99 (dd, 1H, J = 9.9, 3.1 Hz), 4.42 (dd, 1H, J = 9.9, 8.6 Hz), 4.74 (d, 1H, J = 16.5 Hz), 4.83 (d, 1H, J = 16.5 Hz), 5.10–5.19 (m, 1H), 7.12–7.19 (m, 3H), 7.38–7.44 (m, 1H) 7.47–7.53 (m, 1H); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1723; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N, 27.13. Found: C, 63.22; H,

5.46; N, 27.25; $[\alpha]_D^{23}$ +45.5 (c 0.375, MeOH).

5.1.42. (5,7-Dimethylimidazo[1,2-*a*]pyrimidin-2-yl)acetic acid (23a)

To a solution of 2-(chloromethyl)-5,7-dimethylimidazo[1,2-*a*]pyrimidine **22a** (1.00 g, 5.11 mmol) in DMF (10 mL) were added sodium cyanide (500 mg, 10.2 mmol) and H_2O (2.0 mL). The mixture was stirred at 80 °C for 6 h. After cooling to room temperature, saturated $NaHCO_3$ aqueous solution was added to the solution followed by extraction with EtOAc. The aquaous layer was concentrated with toluene. H_2O was added to the residue followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography and concentrated *in vacuo*. To a mixture of the residue in 1,4-dioxane (4.4 mL) was added NaOH (374 mg, 9.36 mmol) in H_2O (4.4 mL). The mixture was stirred under reflux for 3 h. The reaction mixture was cooled at room temperature. To the mixture was added 1M HCl aqueous solution (9.4 mL) under ice-bath cooling. The resulting precipitate was collected by filtration. The filtered solid was washed with $CHCl_3$/MeOH (4:1) co-solvent and diisopropyl ether successively to give **23a** (418 mg, 40% in 2 steps) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 2.66 (s, 3H), 2.76 (s, 3H), 4.00 (s, 2H), 7.45–7.49 (m, 1H), 8.12 (s, 1H); MS (ESI) *m*/*z* 206 $[M+H]^+$.

5.1.43. 1-(2,3-Dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)-2-(5,7-dimethylimidazo[1,2-*a*]pyrimidin-2-yl)ethan-1-one (6a)

To a mixture of **13a** (100 mg, 0.628 mmol) and **23a** (215 mg, 0.943 mmol) in DMF (3.0 mL) were added WSC·HCl (145 mg, 0.756 mmol), HOBt (51.0 mg, 0.377 mmol) and DIPEA (323 μL, 1.89 mmol). The mixture was stirred at room temperature for 2 days. H_2O was added to the reaction mixture followed by extraction with EtOAc. The aqueous layer was concentrated *in vacuo* with toluene. H_2O was added to the residue followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, MeOH in $CHCl_3$), and concentrated *in vacuo* to give **6a** (115 mg, 53%) as a pale brown solid. ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 6H), 4.19–4.32 (m, 2H), 4.63 (br-t, 2H, J = 7.5 Hz), 4.82 (br-s, 2H), 6.54 (s, 1H), 7.14–7.24 (m, 3H), 7.46 (s, 1H), 7.57–7.67 (m, 1H); MS (ESI) *m*/*z* 347 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_6O$ $[M+H]^+$ 347.1615; found, 347.1614; Anal. Calcd for $C_{19}H_{18}N_6O·2.2H_2O$: C, 59.12; H, 5.85; N, 21.77. Found: C, 58.99; H, 5.83; N, 21.72.

5.1.44. (5,7-Dimethyl[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)acetic acid (23b)

To a solution of 2-(chloromethyl)-5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyridine **22b** (564 mg, 2.88 mmol) in DMF (6.0 mL) was added sodium cyanide (298 mg, 6.08 mmol). The mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo*. H_2O was added to the residue followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0–5% MeOH in $CHCl_3$) and concentrated *in vacuo*. To a mixture of the residue in 1,4-dioxane (6.0 mL) was added NaOH (480 mg, 12.0 mmol) in H_2O (6.0 mL). The mixture was stirred under reflux for 3 h. The reaction mixture was cooled at room temperature. To the mixture was added 1M HCl aqueous solution (12 mL) under ice-bath cooling. The mixture was concentrated *in vacuo*. The residue was dissolved with $CHCl_3$/MeOH (9:1) co-solvent and filtered. The filtrated was concentrated *in vacuo*. The residue was washed with diisopropyl ether to give **23b** (480 mg, 81% in 2 steps) as a brown solid. MS (ESI) *m*/*z* 206 $[M+H]^+$.

5.1.45. 1-(2,3-Dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)-2-(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyridin-2-yl)ethan-1-one (6b)

To a mixture of **13a** (100 mg, 0.628 mmol) and **23b** (167 mg, 0.814 mmol) in DMF (3.0 mL) were added HATU (310 mg, 0.815 mmol) and DIPEA (323 μL, 1.89 mmol). The mixture was stirred at room temperature for 2 days. After concentrated *in vacuo*, the reaction mixture was purified by flash column chromatography (silica gel, 0–5% MeOH in $CHCl_3$) and concentrated *in vacuo* to give **6b** (155 mg, 71%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.42 (d, 3H, J = 0.66 Hz), 2.73 (s, 3H), 4.24–4.31 (m, 2H), 4.61–4.70 (m, 2H), 4.95 (s, 2H), 6.63 (br-s, 1H), 7.16–7.25 (m, 3H), 7.30–7.34 (m, 1H), 7.60–7.64 (m, 1H); MS (ESI) *m*/*z* 347 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_6O$ $[M+H]^+$ 347.1615; found, 347.1614; Anal. Calcd for $C_{19}H_{18}N_6O \cdot 0.2H_2O$: C, 65.20; H, 5.30; N, 24.01. Found: C, 65.18; H, 5.28; N, 23.82.

5.1.46. (5,8-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrazin-2-yl)acetic acid (23c)

To a solution of 2-(chloromethyl)-5,8-dimethyl[1,2,4]triazolo[1,5-*a*]pyrazine **22c** (1.00 g, 5.09 mmol) in DMF (10 mL) was added sodium cyanide (499 mg, 10.2 mmol). The mixture was stirred at 80 °C for 3 h. H_2O (2.0 mL) was added to the mixture and stirred at 80 °C for 30 min. After cooling to room temperature, H_2O was added to the reaction mixture followed by extraction with EtOAc. The organic layer was washed with H_2O

and brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* To a half of the residue in 1,4-dioxane (10 mL) was added NaOH (793 mg, 19.8 mmol) in H_2O (10 mL). The mixture was stirred under reflux for 1.5 h. After cooling to room temperature, H_2O was added to the reaction mixture followed by extraction with EtOAc. To the aqueous layer was added 1M HCl aqueous solution (20 mL) under ice-bath cooling. The mixture was concentrated *in vacuo*. To the residue was added $CHCl_3$/MeOH (20 mL/5 mL). The mixture was filtered, and the filtrate was concentrated *in vacuo* to give **23c** (555 mg, 86% in 2 steps) as an orange solid. ^{1}H NMR (DMSO-d_6) δ 2.66 (s, 3H), 2.75 (s, 3H), 3.93 (s, 2H), 7.97–8.00 (m, 1H).

5.1.47. 1-(2,3-Dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl)-2-(5,8-dimethyl[1,2,4]triazolo[1,5-*a*]pyrazin-2-yl)ethan-1-one (6c)

Compound **6c** was prepared from **13a** and **23c** in a manner similar to that described for compound **5d**, with a yield of 55% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.67 (s, 3H), 2.75 (s, 3H), 4.27–4.36 (m, 2H), 4.48–4.56 (m, 2H), 4.92 (s, 2H), 7.13–7.19 (m, 2H), 7.40–7.46 (m, 1H), 7.48–7.54 (m, 1H), 8.01 (d, 1H, J = 0.88 Hz); MS (ESI) m/z 348 $[M+H]^+$; HRMS (ESI) calcd for $C_{18}H_{18}N_7O$ $[M+H]^+$ 348.1567; found, 348.1568; Anal. Calcd for $C_{18}H_{17}N_7O$: C, 62.24; H, 4.93; N, 28.23. Found: C, 62.44; H, 4.96; N,

28.44.

5.1.48. 2-(5,8-Dimethyl[1,2,4]triazolo[1,5-*a*]pyrazin-2-yl)-1-[(2S)-2-methyl-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazol-1-yl]ethan-1-one (6d)

Compound **6d** was prepared from **21l** and **23c** in a manner similar to that described for compound **5b**, with a yield of 68% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.51 (d, 3H, J = 6.6 Hz), 2.66 (s, 3H), 2.75 (d, 3H, J = 0.66 Hz), 3.99 (dd, 1H, J = 9.9, 3.1 Hz), 4.42 (dd, 1H, J = 9.9, 8.4 Hz), 4.87 (d, 1H, J = 16.8 Hz), 4.94 (d, 1H, J = 16.8 Hz), 5.10–5.20 (m, 1H), 7.12–7.20 (m, 2H), 7.38–7.45 (m, 1H), 7.48–7.55 (m, 1H), 8.00 (d, 1H, J = 1.1 Hz); MS (ESI) m/z 362 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{20}N_7O$ $[M+H]^+$ 362.1724; found, 362.1724; Anal. Calcd for $C_{19}H_{19}N_7O$: C, 63.14; H, 5.30; N, 27.13. Found: C, 63.03; H, 5.40; N, 27.11; $[\alpha]_D^{23}$ +55.3 (c 0.750, MeOH).

5.2. PDE10A enzyme assay protocol

Inhibitory activity of compounds on human PDE10A2 was assessed by measuring the residual amount of cAMP, substrate for PDE10A2, by the Homogeneous Time-Resolved Fluorescence (HTRF) detection method (cisbio).[15] The obtained results were converted to activity relative to an uninhibited control (100 %) and IC_{50} values were calculated using Prism software (GraphPad Software, Inc.).

5.3. Animal experiments

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Further, Astellas Pharma Inc. Tsukuba Research Center was awarded Accreditation Status by the AAALAC International. All efforts were made to minimize the number of animals used and to avoid suffering and distress.

5.3.1. In vivo behavioral assay in mice

Phencyclidine-induced hyperlocomotion: ICR mice aged 5 weeks were used to evaluate the effect of PDE10A inhibitor on hyper-locomotion induced by the NMDA antagonist phencyclidine (PCP). Immediately after oral administration of either vehicle or agent as pre-treatment, mice were placed into individual plastic test cages (30 × 35

× 17.5 cm) of a SUPERMEX system (PAT.P; Muromachi Kikai Co., Ltd), and measurement of locomotor activity was started. After 1 h, the mice were injected with a post-treatment of saline or PCP (2.5 mg/10 mL/kg, sc), and locomotor activity was measured for a further 60 min. Total locomotor activity for 60 min post-treatment was calculated.

MK-801-induced working memory deficit in mice during Y-maze test: Spatial working memory performance was assessed by recording spontaneous alternation behavior of male ddY mice (aged 5-6 weeks, Japan SLC, Inc.) in a Y-maze as described previously.[16–17] The maze was constructed from gray polyvinyl chloride, with arms (length, 40 cm; height, 13 cm; width at bottom, 3 cm; and width at top, 10 cm) converging at equal angles. 40 minutes after p.o. administration of **6d** or vehicle, 0.15 mg/kg of MK-801 was administered intraperitoneally (i.p.) to each animal. Control animals were administered vehicle instead of **6d** and saline instead of MK-801 (i.p.). Twenty minutes after administration of MK-801 or saline, each mouse was placed at the end of one arm and allowed to freely explore the apparatus for 8 min. The total number of arm entries was recorded for each animal throughout the period. Alternation was defined as entries into all three arms on consecutive occasions. The alternation rate was calculated using the following formula:

$$\text{Alternationrate}(\%)=100\times\text{Numberofalternations}/(\text{Numberoftotalarmentries}-2)$$

Catalepsy in mice: Eight-week-old male ICR mice were administered **6d** or vehicle (p.o.) as previously described.[16] After 60 min, each mouse was assessed for catalepsy for 120 s. Catalepsy was measured via the bar method, which consists of placing an animal following drug administration, with its front legs resting on a bar suspended above the floor of the test apparatus. Intensity of catalepsy was measured as the length of time the test subject maintains this abnormal posture. Catalepsy time (duration of catalepsy) was calculated. 100 mg/kg of **6d** became jelly like it was impossible to administer, so we excluded this group.

5.3.2. Mouse pharmacokinetic study

The mice were treated orally with compound **1** or **6d** suspended in 0.5% methylcellulose aqueous solution. Blood samples were collected using syringes containing heparin sodium at 1 h after oral administration. Blood samples were kept on ice and centrifuged, which was then frozen stored prior to analysis. Whole brain samples were collected at 1 h after administration, and frozen stored and homogenized in 4-fold volume of phosphate buffered saline (pH 7.4) before extraction processing. Extraction and analysis of compound concentrations were performed via LC-MS/MS

with a ACQUITY UPLC(Waters) and QTRAP5500_Nexera.

5.4. Crystallization and structural analysis

PDE10A crystals were obtained as previously reported.[8] Compounds were soaked into the apo PDE10A crystal. An apo crystal was transferred to a mother liquor containing compound (1 or 2 mM final concentration) and incubated at 4 °C for 1 h or overnight. X-ray diffraction data for **2** were collected using a Rigaku FR-E+ Superbright rotating-anode X-ray source equipped with a Rigaku R-AXIS VII X-ray detector (Rigaku, Tokyo, Japan). X-ray diffraction data for **4a** and **5l** were collected at AR-NE3A beamline [18] at the Photon Factory in the National Laboratory for High Energy Physics (KEK), Tsukuba, Japan. Diffraction data were indexed, integrated, and scaled using HKL2000 [19]. Crystal structures of PDE10A were determined by the molecular replacement method using AMoRe [20] with previously our determined PDE10A structure [8] as a search model. An initial refinement was performed using REFMAC [21]. Compounds were fitted into electron densities observed in initial Fo-Fc maps using AFITT (OpenEye Scientific Software, NM, USA). Water placements and further refinements were performed using Coot [22] and REFMAC, and the final models were determined. Data collection and refinement statistics are summarized in Table S1.

Table S1. Data processing and refinement statistics.
values for the outer shell are given in parentheses

Compound	**2**	**4a**	**5l**
PDB ID	6KDZ	6KDX	6KE0
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a (Å)	50.2	50.4	50.4
b (Å)	81.0	81.4	81.6
c (Å)	162.1	160.0	160.8
α (°)	90	90	90
β (°)	90	90	90
γ (°)	90	90	90
Resolution (Å)	50.0-3.10 (3.18-3.10)	50.0-2.44 (2.50-2.44)	50.0-2.95 (3.03-2.95)
Multiplicity	2.9 (3.0)	5.4 (5.5)	5.0 (5.3)
Average $I/\sigma(I)$	5.4 (1.8)	33.5 (5.0)	19.7 (3.8)
R_{merge} [a] (%)	14.1 (50.9)	7.3 (40.1)	13.5 (54.5)
No. of reflections	10821 (660)	21947 (1439)	12737 (801)
Completeness (%)	90.2 (82.5)	91.1 (89.5)	91.9 (90.8)
R_{work} [b] (%)	25.2	24.1	25.1
R_{free} [c] (%)	32.5	30.1	30.2
Average *B* factor (Å^2)	72.9	56.9	50.9
RMSD bond length (Å)	0.009	0.014	0.010
RMSD bond angle (°)	1.540	1.823	1.589

[a] $R_{merge}=\Sigma_{hkl}\Sigma_i|I_i-I|/\Sigma_{hkl}\Sigma_i I_i$, where I_i is the intensity of an individual reflection and I is the mean intensity obtained from multiple observations of symmetry related reflections. [b] $R_{work}=\Sigma_{hkl}||F_{obs}|-|F_{calc}||/\Sigma_{hkl}|F_{obs}|$ [c] 5% randomly omitted reflections were used for R_{free}

5.5. Transcellular transport study in LLC-PK1-MDR1 cells

Wild type or MDR1-expressing LLC-PK1 cells (LLC-PK1-WT or LLC-PK1-MDR1, respectively) cultured for 5 days on a Millicell-96 Cell Culture Insert Plate (Millipore) were pre-incubated with transport buffer (HBSS, pH 7.4, for the apical and basolateral sides) for 1 h. After aspiration of the transport buffer, the donor solution (transport buffer (0.5% DMSO) containing the test compound (1 μM) and Texas Red (1 μM)) was added to the apical or basolateral side for the influx or efflux transport study, respectively, and the receiver solution (transport buffer (0.5% DMSO)) was added to the opposite side. After incubation for 3 h, the test compound in both sides was analyzed by LC/MS/MS and the apparent permeability was determined. Efflux ratio (ER) was calculated by dividing the apparent permeability in the direction from the basolateral to the apical side by that in the opposite direction. Net efflux ratio (NER) was the ratio of ER of LLC-PK1-MDR1 to LLC-PK1-WT. Texas Red was used for the estimation of the apparent permeability via para cellular transport. Pint was expressed as a mean value of apparent permeability from apical to basal and from basal to apical in a wild-type cells.

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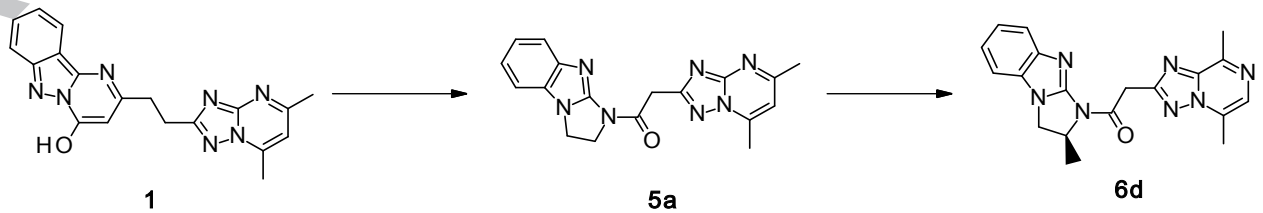

1

PDE10A: IC_{50} = 2.0 nM
P-gp NER: 1.8
Pint: 3.2 x 10^{-6} cm/sec
Kp, brain: Not Detected

5a

PDE10A: IC_{50} = 52 nM
P-gp NER: 2.3
Pint: 16.8 x 10^{-6} cm/sec

6d

PDE10A: IC_{50} = 8.4 nM
P-gp NER: 1.2
Pint: 27.5 x 10^{-6} cm/sec
Kp, brain: 2.3