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Discovery of (1*R*,2*S*)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)-*N*-(5-fluoropyridin-2-yl)cyclopropanecarboxamide (E2006): A Potent and Efficacious Oral Orexin Receptor Antagonist

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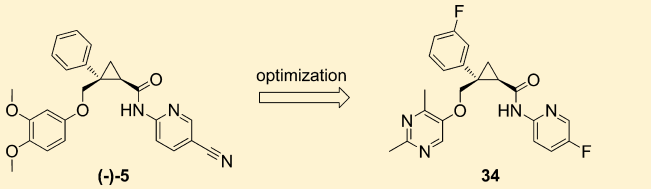
Supporting Information

ABSTRACT: The orexin/hypocretin receptors are a family of G protein-coupled receptors and consist of orexin-1 (OX_1) and orexin-2 (OX_2) receptor subtypes. Orexin receptors are expressed throughout the central nervous system and are involved in the regulation of the sleep/wake cycle. Because modulation of these receptors constitutes a promising target for novel treatments of disorders associated with the control of sleep and wakefulness, such as insomnia, the development of orexin receptor antagonists has emerged as an important focus in drug discovery research. Here, we report the design, synthesis, characterization, and structure−activity relationships (SARs) of novel orexin receptor antagonists. Various modifications made to the core structure of a previously developed compound (-)-**5**, the lead molecule, resulted in compounds with improved chemical and pharmacological profiles. The investigation afforded a potential therapeutic agent, (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)-*N*-(5-fluoropyridin-2-yl)-cyclopropanecarboxamide (E2006), an orally active, potent orexin antagonist. The efficacy was demonstrated in mice in an in vivo study by using sleep parameter measurements.



INTRODUCTION

Insomnia, a disorder in which the patient suffers from an inability to sleep or stay asleep for as long as desired, constitutes a widespread issue in today's society, as approximately 30% of adults in the United States experience some symptoms of insomnia and approximately 10% describe their insomnia as chronic and/or severe. Despite the prevalence of this disorder, research regarding medical treatments for insomnia is relatively limited.[1]

The effects of insomnia are dramatic, as it leads to a reduction in the quality of life, poor productivity, and a high risk of traffic and work-related safety incidents. Additionally, insomnia is a costly disorder, as it is estimated to cost over $63 billion per year in the United States.[2] Commonly prescribed medications for the treatment of insomnia include agents that positively modulate $GABA_A$ receptors, with zolpidem, a nonbenzodiazepine sleep drug, being the current market leader. Nonbenzodiazepine sleep aids are structurally distinct from benzodiazepines, as reflected in their naming, and bind to benzodiazepine sites with high specificity. Additionally, their pharmacological profiles are believed to be better in comparison with those of benzodiazepines owing to the less severe side effects. Despite the availability of various sleep-modifying drugs, the prevalence of insomnia has not decreased substantially because of remaining concerns about the overall safety and efficacy of treatments that target the GABA signaling pathway.[3,4] Notably, two new non-GABA-related sleep drugs have recently been approved for the treatment of insomnia. Ramelteon, a melatonin (MT) receptor MT_1/MT_2 agonist, was approved in 2005, and doxepin, a histamine H_1 receptor antagonist, received regulatory approval in 2010. In contrast to benzodiazepine and nonbenzodiazepine agents, these two sleep-aid drugs are nonscheduled drugs in the U.S.. However, questions remain about the effectiveness of ramelteon and doxepin, owing to the limited number of reports demonstrating their superiority to other sleep drugs, including those that positively modulate $GABA_A$.[5,6] The medical needs of patients with insomnia warrant the development of sleep medications with novel mechanisms of action.

Toward that end, antagonism of the orexin receptor may serve as a promising route. Orexin A and orexin B, also known as hypocretin 1 and hypocretin 2, are endogenous neuropeptide ligands for orexin/hypocretin receptors (OXRs), which are G protein-coupled receptors (GPCRs). Orexin-1 receptor (OX_1R) and orexin-2 receptor (OX_2R) mediate the action of these ligands by postsynaptic neuronal signal transduction. OX_1R and OX_2R are expressed throughout the central nervous

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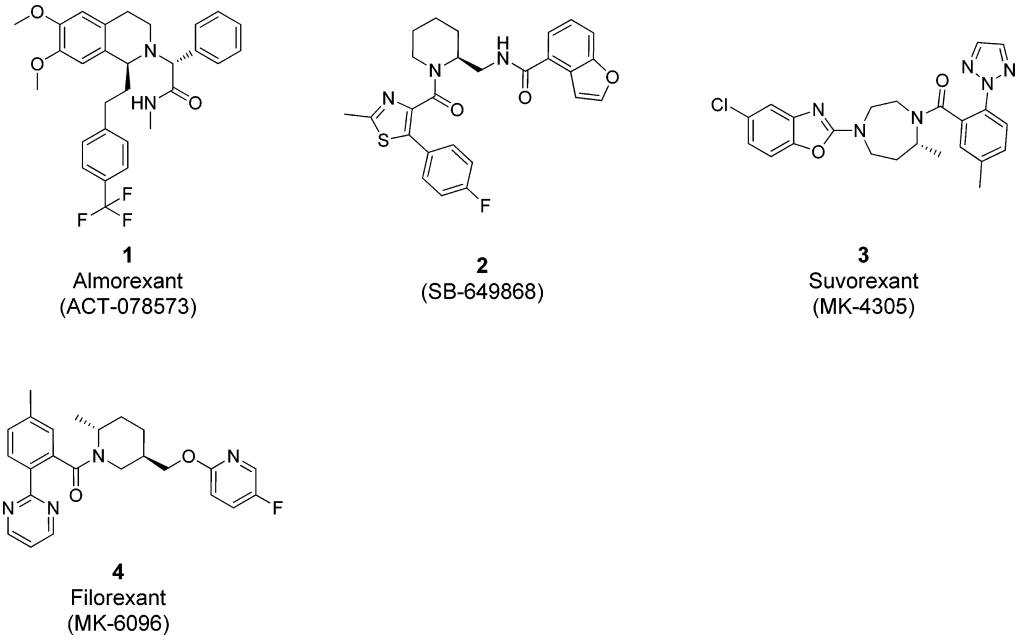


Figure 1. Orexin receptor dual antagonists that have advanced to clinical trials.

system and are involved in the regulation of the sleep/wake cycle.[7−9] To date, the specific contribution of OXR to the regulation of the sleep/wake cycle has recently starting emerged. A number of genetic and pharmacological studies have suggested that the orexin pathway plays an important role in regulating the sleep/wake cycle.[10−19] Studies performed in *orexin/ataxin-3* transgenic mice and rats, which lack orexin peptide-producing neurons, and OXR-deficient mice have shown that both receptors are involved in the regulation of the sleep/wake cycle because the lack of activation of both receptors results in a narcolepsy−cataplexy phenotype, while the lack of activation of either receptor alone elicits an attenuated sleep phenotype.[20−23] Furthermore, very low levels of orexin in cerebrospinal fluid (CSF), indicative of nearly complete or complete loss of orexinergic neurons, have been observed in humans suffering from narcolepsy−cataplexy syndrome.[24−26]

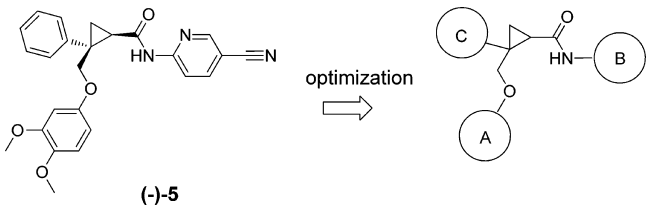
A dual OXR antagonist, almorexant, evaluated by Actelion Pharmaceuticals/GlaxoSmithKline was found to significantly decrease the behavioral indices of wakefulness in animals and has shown effectiveness in clinical trials.[11] Recently, a number of orally active selective OX_2R antagonists have been evaluated in animal studies.[27,28] However, whether a dual receptor antagonist[29] or an OX_2R-selective antagonist[30] will be better suited for use as a sleep aid is a topic of ongoing debate. We think that it is feasible to speculate that a sleep drug which promotes both non-REM and REM sleep would provide patients with a more natural sleep architecture, and we are providing animal data in this manuscript which suggest that a dual orexin antagonist is a potential candidate to fulfill this requirement.

To date, a number of dual OXR antagonists have been described. Almorexant (**1**) and was tested in the treatment of insomnia,[31] but a phase III clinical trial was discontinued. SB-649868 (**2**) from GlaxoSmithKline proceeded to a phase II clinical trial.[32] Suvorexant (**3**), developed by Merck, received approval from the Pharmaceutical and Medical Devices Agency in Japan and the US Food and Drug Administration (FDA) in 2014 and is marketed for the treatment of insomnia.[33,34] Merck has entered another dual OXR antagonist filorexant (**4**) into a phase II clinical trial for multiple indications, including the treatment of insomnia (Figure 1).[35]

In this paper, we report the synthesis, structure−activity relationships (SARs), optimization of drug-likeness parameters, and in vivo efficacy of a series of cyclopropane compounds reported previously.[36] Our efforts in this area led to the discovery of **34** (E2006) as a promising dual OXR antagonist that has advanced into clinical trials.

A novel series of compounds containing a cyclopropane core structure were identified as promising orally active orexin receptor antagonists, as exemplified by (−)-**5**[36] (Table 1). (−)-**5** exhibits low-nanomolar affinity toward human OX_2R, as measured by radio ligand-displacement binding assays. However, this compound exhibits a number of drawbacks in its drug-likeness parameters that need to be improved, such as

Table 1. Properties of Compound (−)-5



(-)-5

	in vitro binding affinity (K_i, nM)		solubility[a] (μM)		TDI of CYP3A[d]	metabolic stability[e]
compd	OX_2R	OX_1R	pH 7.4[b]	pH 1.2[c]	(% of control at 10 μM)	(residual ratio %)
(−)-**5**	5	106	15	25	49	80

[a]DMSO solution precipitation method.[36] [b]Dulbecco's phosphate-buffered saline (PBS). [c]Japanese Pharmacopoeia 1st fluid (aqueous HCl solution containing 34 mM NaCl). [d]Time-dependent inhibition evaluated using a cocktail of probe substrates with human liver microsomes. [e]Metabolism after incubation of compounds at 0.3 μM in 0.1% DMSO with human liver microsomes for 15 min.

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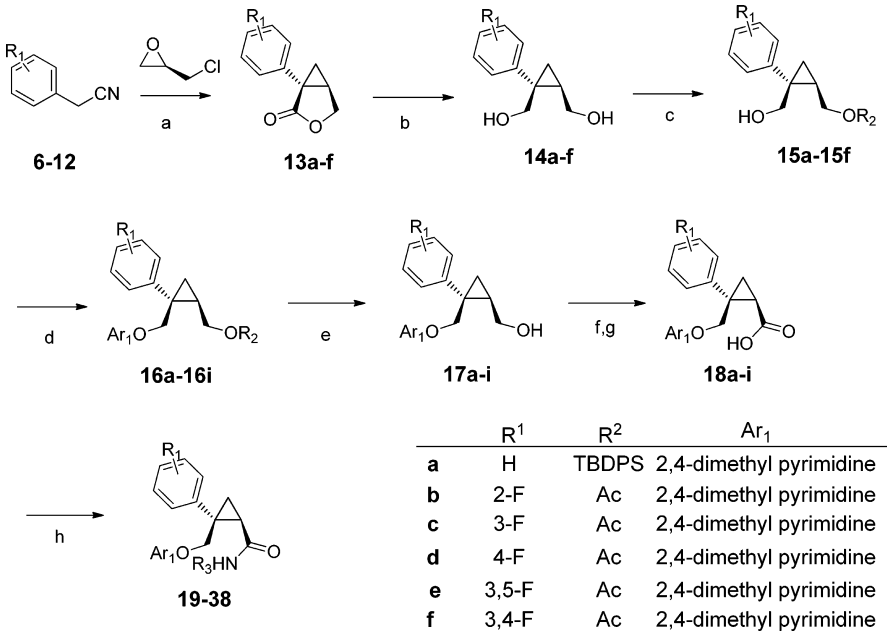
Scheme 1[a]



	R^1	R^2	Ar_1
a	H	TBDPS	2,4-dimethyl pyrimidine
b	2-F	Ac	2,4-dimethyl pyrimidine
c	3-F	Ac	2,4-dimethyl pyrimidine
d	4-F	Ac	2,4-dimethyl pyrimidine
e	3,5-F	Ac	2,4-dimethyl pyrimidine
f	3,4-F	Ac	2,4-dimethyl pyrimidine
g	H	TBDPS	4-ethyl-2-methyl pyrimidine
h	H	TBDPS	3,5-dimethyl pyrazine
i	H	TBDPS	4-methoxymethyl-2-methyl pyrimidine

[a]Reagents and conditions: (a) (1) NaHMDS, THF, 0 °C, 3 h, (2) KOH, EtOH, reflux, 8 h, (3) HCl, 0 °C−rt, 3 h; (b) $NaBH_4$, MeOH−THF, 0 °C−rt; (c) TBDPS-Cl, imidazole, DMF, −10 °C−rt, or vinyl acetate, lipase acrylic resin from *Candida antarctica*, rt; (d) Ar_1-OH, DIAD, PPh_3, 0 °C−rt, overnight or (1) MsCl, TEA, DCM, (2) Ar_1-OH, Cs_2CO_3, MeCN, 70 °C; (e) TBAF, THF, rt, 1 h or NaOH, EtOH−H_2O, rt, 1 h (2 steps); (f) $(COCl)_2$, DMSO, TEA, DCM, −78 °C−rt, 1 h; (g) 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, acetone−H_2O, rt, 2 h; (h) amine, HATU, DIPEA, DMF, 60 °C, overnight or $(COCl)_2$, cat. DMF, DCM, rt, 1 h, then amine, DIPEA, THF, 60 °C.

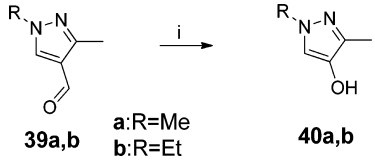
its time-dependent inhibition (TDI) of cytochrome P450 3A (CYP3A) and low aqueous solubility under both acidic and neutral conditions (pH 1.2 and pH 7.4). (−)-**5** also demonstrated moderate reversible inhibition of CYPs (IC_{50} values of 8 and 8.6 μM for CYP2C8 and CYP2C19, respectively, and >10 μM for CYP1A2, CYP2C9, and CYP2D6). In an effort to find a clinical candidate within the chemical series, the A, B, and C rings in the molecule were optimized through chemical modifications.

RESULTS AND DISCUSSION

Chemistry. The general synthetic route used to produce the cyclopropane compounds is presented in Scheme 1. Chiral cyclopropane ring formation was carried out by the reaction of corresponding aryl acetonitriles **6**−**12** with (*R*)-epichlorohydrin to yield the desired lactones **13a**−**f**.[37] The reported racemic compounds[36] can be obtained using racemic epichlorohydrin instead of (*R*)-epichlorohydrin by following a procedure similar to the one described in Scheme 1. Reduction of the lactone with sodium borohydride afforded diol products **14a**−**f** in excellent yields. Protection of the hydroxy group of **14a** with TBDPS-Cl gave the desired monoprotected compound **15a**. In the selective monoacylation of diols **14b**−**f**, enzymatic acylation with lipase acrylic resin from *Candida antarctica* was successfully applied in the presence of vinyl acetate to obtain compounds **15b**−**f** in good yields. The Mitsunobu reaction of the corresponding alcohols with various phenols led to the production of **16a**−**i**. Deprotection of **16a** and **16g**−**i** using TBAF, or hydrolysis of the acyl group of **16b**−**f** with sodium hydroxide, followed by Swern oxidation and Pinnick oxidation, afforded carboxylic acid intermediates **18a**−**i**. Subsequent amidation of **18a**−**i** with various amines yielded the desired products **19**−**38**.

Components of the heteroaromatic A ring were synthesized as outlined in Schemes 2 and 3. Pyrazole derivatives **40a** and **40b** were synthesized from commercially available aldehydes **39a** and **39b** via Baeyer−Villiger oxidation (Scheme 2). The Pd-catalyzed coupling of commercially available 2,4-dichloro-5-methoxy pyrimidine **41** with trimethylaluminum afforded **42** in a good yield. An analogous transformation yielded **45** from chloro intermediate **44**, which was accessed by iron-catalyzed ethylation of **41** with ethyl magnesium chloride (Scheme 3). Deprotection of the methoxy group of **42** and **45** in the presence of BBr_3 produced hydroxypyrimidines **43** and **46**. Compound **49** was synthesized from **43**. Protection of the hydroxyl group with benzyl bromide followed by selective bromination of the methyl group in the 4 position yielded the crude bromomethyl-containing compound. Subsequent substitution of the alkyl bromide in the presence of sodium

Scheme 2[a]



[a]Reagents and conditions: (i) *m*-CPBA, $CHCl_3$, rt, overnight.

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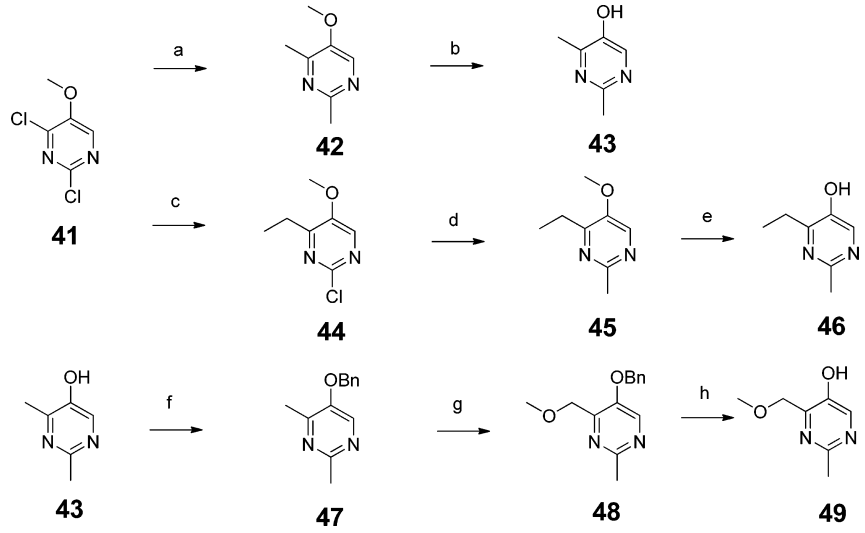
Scheme 3[a]



[a]Reagents and conditions: (a) Me_3Al, $Pd(PPh_3)_4$, THF, 75 °C, overnight; (b) BBr_3, DCM, rt, 4 d; (c) EtMgCl, $Fe(acac)_3$, THF, rt, overnight; (d) Me_3Al, $Pd(PPh_3)_4$, THF, 70 °C, 2 d; (e) BBr_3, DCM, rt, 4 d; (f) BnBr, NaH, THF, 0 °C to rt, overnight; (g) Br_2, $CHCl_3$, 0 °C to rt, overnight, then NaOMe, MeOH, 90 °C, 12 h; (h) Pd-C, H_2 gas, EtOAc, rt, 1 h.

methoxide afforded methoxymethyl product **48**. Deprotection of the benzyl group gave desired product **49**.

Pharmacology. Our efforts were primarily aimed at reducing the TDI effect on CYP3A and improving the aqueous solubility. The TDI effect was believed to be related to the demethylation of the 4-OCH_3 or 3-OCH_3 groups on the A-ring, where a second oxidative metabolism step may produce quinone intermediates that can react with nucleophiles.[38] Therefore, to resolve the TDI and solubility issues, we tried to introduce a substituted hetero Ar into the A-ring position instead of 3,4-di-OMe-Ph. According to our previous work, the methoxy groups are critical pharmacophores; the orientations of the small lipophilic group and lone pair were shown to strongly influence in vitro binding affinity.[36] On the basis of the information regarding 3,4-di-OMe-Ph in the Cambridge Structural Database (CSD), the Me groups face opposite directions (Figure 2). Therefore, two types of dimethylpyridines (**50** and **51**) were designed with the hope of imitating the pharmacophores of 3,4-di-OMe-Ph, the lone pair and small lipophilic group (Table 2).

Experimentally, dramatically different in vitro binding affinities were observed between **50** and **51**, as 3-pyridine-containing **50** exhibited a moderate affinity, whereas introduction of the 4-pyridine-containing **51** resulted in reduced in vitro binding affinity toward both OX_1R and OX_2R. From these results, computational simulations of superposition using Molecular Operating Environment (MOE, Ryoka System, Inc., Tokyo, Japan) were conducted with (−)-**5** and **50** or **51**, with a particular focus on the position of the three aromatic rings and the di-OMe substituents (Figure 2). Accordingly, we considered the superposition of the aromatic rings of **50** and **51** with (−)-**5**, together with the overall molecular shape including directionality of the ether linker, the angle of the upper-left aromatic ring (C-ring), and the direction of the NH of amide bond. On the basis of these observations, it is reasonable to exhibit better in vitro binding affinity for **50** than **51**. We successfully converted 3,4-di-OMe-Ph to a hetero Ar group with reasonable activity, and **50** showed improved solubility. This finding encouraged us to

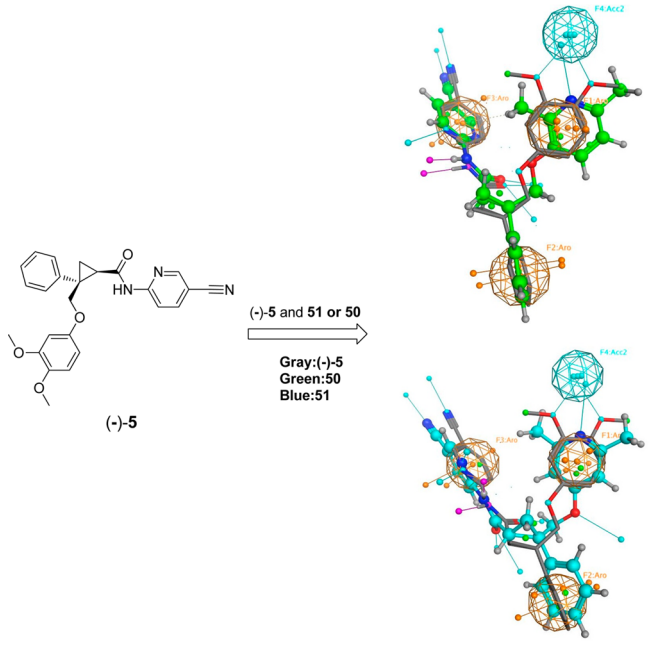


Figure 2. Overlay of (−)-**5** and **50** and **51**.

further investigate hetero Ar rings other than dimethylpyridine to improve the parameters, although the compound did not show an improvement in the TDI.

Dimethyl pyrazole **52**, which was expected to meet the structural requirements, was synthesized and it showed an affinity similar to that shown by **50** as well as a significant improvement in the TDI. To further increase the affinity, various substituents were installed at the 1-position of pyrazole **52**, owing to the results of the computational simulations. Ethyl-substituted **53** was thought to have more favorable interactions with the small lipophilic site, and it exhibited an in vitro binding affinity comparable to that of (−)-**5**. This racemic mixture could be resolved by chiral high-performance liquid chromatography (HPLC) to provide (+)-**53** and (−)-**53**. The

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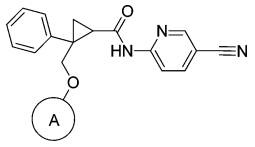
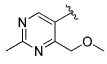
Table 2. A-Ring Modifications: Compounds 50−53 and 19−22



Compound	A-ring	*In vitro* binding affinity[a] (K_i, nM)		Solubility (μM)[b]	TDI of CYP3A[c] (% of control at 10 μM)	corrected P-gp FR[d] (MDR1 FR / PK1 FR)
		OX_2R	OX_1R	pH 7.4		
(-)-5		5	106	15	49	1.0
rac-50[e]		222	938	22	34	NT
rac-51[e]		>2000	>2000	NT	94	NT
rac-52[e]		204	>2000	85	85	NT
rac-53[e]		10	391	78	84	1.5
(+)-53		>2000	>2000	NT	NT	NT
(-)-53		8	171	64	93	0.9
19[f]		49	2491	80	96	2.7
20[f]		7	121	44	91	2.1
21[f]		21	73	47	97	1.3
22[f]		9	43	93	92	4.0

[a] K_i values are calculated from single experiments run in triplicate. [b] Measured in Dulbecco's PBS by DMSO solution precipitation method.[36] [c] Time-dependent inhibition using a cocktail of probe substrates with human liver microsomes. [d] P-Glycoprotein (P-gp) transport assay.[36] [e] Derived from *rac*-epichlorohydrin. [f] Derived from (*R*)-epichlorohydrin.

two stereoisomers exhibited dramatically different receptor affinities; the (−)-**53** isomer exhibited potent affinity toward both OX_2R and OX_1R, while the (+)-**53** isomer did not. Notably, it was found that (−)-**53** could be synthesized stereoselectively from (*R*)-epichlorohydrin using the procedure shown in Scheme 1,[36] suggesting that (−)-**53** had the chiral configuration of (1*R*,2*S*), which is the preferred configuration for OXR antagonist activity.

Another approach for enhancing the in vitro receptor binding affinity was attempted in diazine derivatives, as exemplified by pyrimidine- or pyrazine-containing compounds **19** and **21**. Specifically, this approach was carried out to modulate the pK_a of the ring because the di-OMe phenyl moiety of (−)-5 is nonbasic. As a result, a 5-fold increase in the in vitro binding affinity was achieved with compound **19**. Compound **20**, which contained an Et group instead of the Me group in **19**, was synthesized with the same expectations as we had for Et substituted pyrazole (−)-**53**; it exhibited increased in vitro binding affinity compared to that shown by **19** and exhibited an affinity comparable to that of (−)-**5**. Moreover, the pyrazole and pyrimidine derivatives, especially **19**, showed some improved properties compared to those of (−)-**5**, namely a reduced TDI (96% for **19** vs 49% for (−)-**5**) and aqueous solubility: 80 μM for **19** vs 15 μM for (−)-**5**. Pyrazine **21** exhibited a similar affinity to those of the pyrimidine derivatives but exhibited a concomitant deterioration in certain aspects of its physicochemical and drug likeness such as its aqueous solubility and reversible inhibition of CYPs (data not shown). Further reduction of the lipophilicity by the introduction of an OMe group in the 4-position of the dimethyl pyrimidine, as exemplified in **22**, resulted in a stronger binding affinity than that of **19**. However, **22** served as a substrate for P-glycoprotein (P-gp) with a corrected flux ratio of 4.0. Evaluation of the SARs of the A ring revealed that dimethyl pyrimidine-containing **19** possessed the optimal profile in terms of overall balance because it exhibited a low lipophilicity (ClogP of 3.3 for **19** vs 3.9 for (−)-**5**) with acceptable affinity toward OX_2R, improved TDI and solubility, and moderate liability in a P-gp transport assay. Therefore, compound **19** was selected for optimization of the B-ring moiety.

First, 2-, 3-, and 4-pyridine were introduced to confirm the SAR regarding the position of nitrogen, as a comparison with our previous work (Table 3).[36] As a consequence, increased P-gp susceptibility was observed in 3- and 4-pyridine-containing

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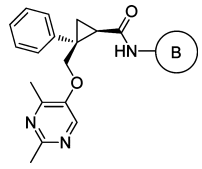
Table 3. Results of B-Ring Exploration: Compounds 23−32



Compound	B-ring	*In vitro* binding affinity[a] (K_i, nM) OX_2R	OX_1R	TDI of CYP3A[b] (% of control at 10 μM)	Corrected P-gp FR[c] (MDR1 FR / PK1 FR)	Reduction of wake time[d] (min/3 h; 30 mg/kg)
19		49	2491	96	2.7	
23		80	1016	99	1.9	
24		50	391	104	4.4	
25		163	1581	110	6.0	
26		7	137	87	1.7	72.3 min
27		56	138	94	1.4	
28		2	39	88	1.3	68.2 min
29		34	124	22	1.3	
30		29	224	90	1.5	
31		4	6	89	1.4	67.1 min
32		6	3	86	1.6	

[a] K_i values are calculated from single experiments run in triplicate. [b] Time-dependent inhibition using a cocktail of probe substrates with human liver microsomes. [c] P-gp transport assay.[36] [d] Sleep measurements conducted using an established method.[36]

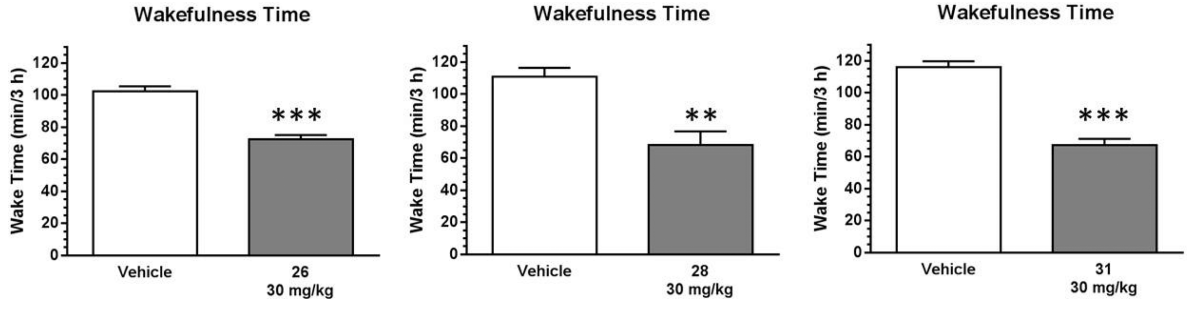


Figure 3. Wakefulness time measured after oral administration of **26**, **28**, and **31**. The vehicle (10% Cremophor EL, 5% DMSO in saline; $n = 4$ for **26**, $n = 7$ for **28** and **31**), **26** ($n = 4$), **28** ($n = 5$), and **31** ($n = 6$) at 30 mg/kg doses were administered immediately prior to the beginning of the dark cycle; cumulative wakefulness times were measured during the first 3 h after administration. Values are mean ± SEM.* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus vehicle control (unpaired parametric two-tailed t test).

derivatives (**24** and **25**, respectively), with a close resemblance to previously identified SARs involving the derivatization of the di-OMe phenyl series.[36] Subsequently, we evaluated the SARs of 2-pyridine-containing moieties in the B-ring, with the aim of enhancing the in vitro binding affinity and in vivo efficacy without adversely affecting other parameters. Introduction of substituents in the 5-position, such as in compounds **26** and **28**, resulted in a greater in vitro binding affinity than that of **23**. In contrast, the introduction of an Me substituent in the 4- or 5-position (**29**, **30**) also resulted in increased in vitro binding affinity relative to that of **23** although the magnitude of the observed increase was smaller with an Me group than with a halogen. These results suggested that the basicity of the B-ring may influence the in vitro binding affinity. Accordingly, incorporation of fluorine in the 5-position and an Me substituent in the 4-position (**31**) resulted in increased in vitro binding affinity, which was more potent than that of compound **30**. However, **31** exhibited decreased solubility compared to that of **26** (**31**, 51 μM, vs **26**, 92 μM, at pH 7.4).

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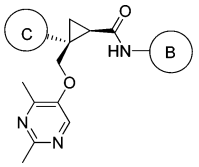
Table 4. Exploration of C-Ring with Different Combinations of B and C-Rings in Compounds 33−38



	C-ring	B-ring	*In vitro* binding affinity[a] (K_i, nM) OX_2R	*In vitro* binding affinity[a] (K_i, nM) OX_1R	TDI of CYP3A[b] (% of control at 10 μM)	Corrected P-gp FR[c] (MDR1 FR / PK1 FR)	hERG[d] (IC_{50}, μM)
26			7	137	87	1.7	7.7
33			17	81	77	1.4	5.9
34			3	6	76	1.6	6.1
35			8	39	85	1.3	2.2
36			3	7	62	1.5	4.8
37			4	14	75	1.5	2.2
38			4	2	83	1.4	>10

[a] K_i values are calculated from single experiments run in triplicate. [b] Time-dependent inhibition using a cocktail of probe substrates with human liver microsomes. [c] P-gp transport assay.[36] [d] Exploratory screening of inhibition of the human ether-a-go-go related gene (hERG) potassium channel.[39]

Introduction of an OMe group in the 4-position (**32**) also elicited a strong increase in the in vitro binding affinity.

With highly potent compounds **26**, **28**, and **31** in hand, in vivo characterizations were performed. The effects of these three compounds on wakefulness were evaluated in sleep experiments with mice via electroencephalogram/electromyogram (EEG/EMG) recordings during the first 3 h of the dark phase, as shown in Figure 3.[36] All compounds elicited a significant reduction in the time of wakefulness following an oral dose of 30 mg/kg (Figure 3). Because of its well-balanced in vitro profiles taking into account the in vitro binding affinity, solubility, and other parameters, compound **26** was selected for optimization of the C ring.

Previous synthetic efforts regarding the cyclopropane series were focused on modifying the A- and B-rings, while modifications of the C-ring were not explored. To investigate the SARs owing to changes in this component, a fluorine scan was conducted to explore favorable positions for substitutions (Table 4). Substitution at the 3-position (**34**) slightly increased the in vitro binding affinity relative to that of **26** without altering other parameters, while 2- or 4-F derivatives (**33**, **35**) exhibited 2−3-fold decreases in the in vitro binding affinity or increased hERG inhibition. 3,5-Difluoro-containing derivative **36** and 3,4-difluoro-containing derivative **37** exhibited strong in vitro binding affinities, but **37** also exhibited increased hERG inhibitory activity. Compound **38** was derived from **31**, which exhibited strong in vivo efficacy and relatively weak hERG inhibitory activity (**31**: hERG IC_{50} > 10 μM). Compound **38** exhibited an enhanced in vitro binding affinity compared to that of **26**, with reduced hERG inhibition, as expected. However, **38** exhibited decreased solubility (**34**, 74 μM, vs **38**, 46 μM at pH 7.4).

On the basis of the SAR studies, compound **34** showed the most promising balance of in vitro properties and was selected for further profiling (Table 5). Compound **34** exhibited very weak reversible inhibition of CYP with IC_{50} values of >20 μM for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. The aqueous solubility of **34** at room temperature was 74 μM at pH 7.4 and >100 μM at pH 1.2. Compound **34** showed weak TDI effects on CYP3A, measured as 76% of the control at 10 μM. Further evaluations of the possible risk of drug−drug interactions caused by CYP3A TDI are warranted, but the potential risk is expected to be marginal when the in vitro binding affinity and TDI screening data are considered.

Table 5. In Vitro Properties of Selected Compound 34

assay	**34**
hOX2R RBA K_i (nM)	3
hOX2R cell-based functional assay[a] K_i (nM)[b]	0.44
hOXIR RBA K_i (nM)	6
hOXIR cell-based functional assay[a] K_i (nM)[b]	5.7
corrected P-gp FR[c] (MDR1 flux ratio/PK1 flux ratio)	1.6
TDI[d] (CYP3A) (%)	76
solubility (pM)[e]	
pH 1.2[f]	>100
pH 7.4[g]	74
metabolic stability (residual ratio %)[h]	
human	90

[a] See cell-based functional assay section in Experimental section. [b] K_i values are calculated from single experiments run; [c] P-gp transport assay;[36] [d] Time-dependent inhibition using cocktail of probe substrates with human liver microsomes; [e] DMSO solution precipitation method;[36] [f] Japanese Pharmacopoeia 1st fluid; [g] Dulbecco's PBS; [h] Metabolism after incubation at 0.1 μM in 0.1% DMSO with human liver microsomes for 15 min.

The pharmacokinetic properties of **34** were evaluated in male mice after intravenous and oral administration at 3 mg/kg iv

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Table 6. Pharmacokinetic Parameters for 34 (iv and po) in Mice

	iv (1 mg/kg)		po (10 mg/kg)		po (30 mg/kg)	
PK parameter[a]	mean	SD	mean	SD	mean	SD
CL (mL/h/kg)	1060	375				
V_{ss} (mL/kg)	2710	1380				
$AUC_{(0-inf)}$ (ng·h/mL)	1030	368	1910	403	4130	555
$t_{1/2}$ (h)	2.16	0.289	2.20	1.00	1.94	0.585
t_{max} (h)			0.25	(0.25−0.50)	1.00	(0.50−1.00)
C_{max} (ng/mL)			488	25.2	961	127
F (%)[b]			18.5		13.4	

[a]See the Experimental section [b]F (%) = ((mean $AUC_{(0-inf)}$, po/$dose_{po}$)/(mean $AUC_{(0-inf)}$, iv/$dose_{iv}$)) × 100.

and 10 mg/kg po doses (Table 6). The results at 10 mg/kg po dose exhibited a plasma clearance of 1060 mL/h/kg, a half-life of 2.16 h for iv and 2.20 h for po administration, a T_{max} of 0.25 h, and an oral bioavailability of 18.5%. A C_{max} exhibited 488 ng/mL for 10 mg/kg and 961 ng/mL for 30 mg/kg po administration. In addition, brain penetration of **34** was evaluated in mice after 1 and 3 h following a single oral administration of 10 mg/kg. After 1 h postdose, the concentration of **34** reached 32.8 nmol/L in CSF and 633.3 nmol/L in plasma and reached 7.6 nmol/L in CSF and 157.6 nmol/L in plasma at 3 h, with 85.2% of the compound bound to plasma protein, indicating that **34** had sufficient brain penetrability and exposure was clearly above K_i on the target (Tables 5 and 7). Therefore, **34** was subjected to efficacy evaluations through measurements of its effects on sleep in mice.

Table 7. Mouse Brain Penetration Data of 34

34 (10 mg/kg)			
CSF/plasma[a] (nmol/L)	1 h	plasma	633.3 ± 67.8
		CSF	32.8 ± 22.1
	3 h	plasma	157.6 ± 22.3
		CSF	7.6 ± 2.3
PPB (%)[b]	C57BL/6N mice		85.2

[a]See the Experimental section. [b]See the Experimental section.

Compound **34** was administered orally at 10 and 30 mg/kg in mice, and the effect on sleep was evaluated (Figure 4).[36] Accordingly, **34** resulted in a significant decrease in time of wakefulness during the first 3 h following administration of 10 and 30 mg/kg doses (77.1 and 75.3 min, respectively, for **34** vs 116.7 min for vehicle, $P < 0.001$, Figure 4D). Both non-REM and REM sleep were increased following oral administration of 10 and 30 mg/kg doses (99.3 min at 10 mg/kg and 95.5 min at 30 mg/kg for **34** vs 62.6 min for vehicle, $P < 0.01$, Figure 4E; 3.6 min at 10 mg/kg and 9.3 min at 30 mg/kg for **34** vs 0.6 min for vehicle, not significant and $P < 0.001$, respectively, Figure 4F). Non-REM sleep-promoting effect in this sleep experiment was thought to be saturated at 10 and 30 mg/kg dose by taking of the PK results into consideration (Table 6). These data suggested that the compound **34** possessed robust sleep-promoting effects[36] and potential as a drug to treat sleep disorders such as insomnia.

Interestingly, the effects on sleep architecture were different between lead compound (−)-**5**[36] and lead optimized compound **34** (Figure 4). Especially, **34** caused a dose-dependent increase of REM sleep time, while (−)-**5** did not provoke REM-sleep even up to highest dose (100 mg/kg po).[36] The difference of the effects on REM sleep by these compounds probably can be explained by the difference of OXRs selectivity profiles, (−)-**5** is more OX_2R selective antagonist than **34**, which is nearly dual antagonist (about 3-fold selective for OX_2R). Our results strongly indicate that REM sleep increase is caused via OX_1R antagonism. This result is in alignment with orexin receptor KO mouse studies,[40,41] which demonstrate that both receptors are involved in sleep/wake regulation, with OX_2R more regulating non-REM sleep and OX_1R more regulating REM sleep. In addition, another sleep study, where orexin receptors were functionally inabled by pharmacological intervention by applying OX_2R selective or dual orexin receptor antagonists, has indicated similar results regarding the roles of OX_1R and OX_2R for sleep architecture.[42] Thus, in our humble opinion, the selectivity ratio for OX_1R and OX_2R can clearly explain the sleep architecture induced by both compounds. When clinical data using a SORA will become available in the future, it will be important for deepening our understanding of the predictability of rodent's sleep architecture data for humans. Nonetheless, we believe that both receptors should be targeted to promote physiological sleep by increasing both non-REM and REM sleep. Therefore, compound **34** is thought to induce a more favorable sleep architecture than (−)-**5**.

CONCLUSION

In conclusion, we optimized the structure of cyclopropane compounds based on (−)-**5** as a lead OXR antagonist compound. Modifications of the A, B, and C-ring, and appropriate combinations of these alterations, afforded several potentially valuable compounds. Compared to (−)-**5**, significant improvements in multiple aspects were achieved by our efforts. Specifically, **34** exhibited an overall improvement in other properties such as the TDI of CYP3A and aqueous solubility, which were previously recognized as the major drawbacks of (−)-**5**. Additionally, **34** exhibited high OXRs antagonist activity and sufficient brain penetration. Furthermore, **34** demonstrated high efficacy in preclinical sleep experiments. On the basis of these findings, it has been selected as a candidate compound, E2006, for further clinical evaluations toward the treatment of sleep disorders. Additional preclinical and clinical studies regarding this series will be reported in due course.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were recorded on a Bruker Avance spectrometer (operating at 600 MHz) or Varian Mercury 400 spectrometer (operating at 400 MHz). ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (operating at 150 MHz). Chemical shifts were calculated in ppm (δ) from the residual $CHCl_3$ signal at (δH) 7.26 ppm or DMSO signal at (δH) 2.50 and (δC) 77.0

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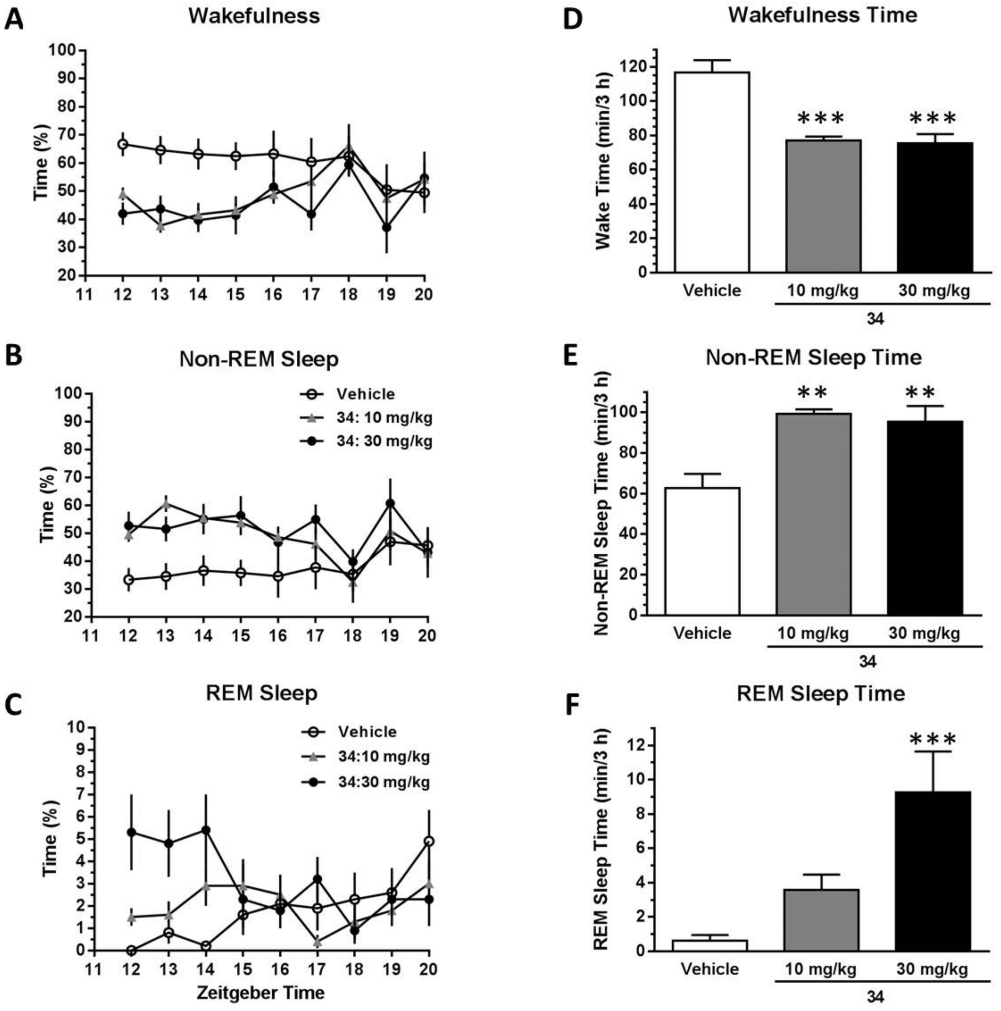

Figure 4. Wake and sleep time measurements after administration of **34**. (A–C) Vehicle (10% Cremophor EL, 5% DMSO in saline; $n = 7$) or **34** (10 or 30 mg/kg; $n = 6$) were orally administered immediately prior to the dark cycle (lights off), and (A) wakefulness, (B) non-REM sleep, and (C) REM sleep were measured hourly. (D–F) Cumulative amounts of (D) wakefulness, (E) non-REM sleep, and (F) REM sleep times measured during the first 3 h following administration of vehicle or **34** (derived from the time courses shown in A–C). Values are mean ± SEM * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle control (one-way ANOVA followed by Dunnett's multiple comparison test).

ppm in $CDCl_3$. Then 600 MHz ^1H or ^{13}C NMR data were processed using ACD Spectrus processor from ACD Laboratories. High-resolution mass spectra (HRMS) were recorded on a ThermoFisher-Scientific LTQ-Orbitrap XL spectrometer (using electrospray ionization).

The purity of the tested biological compounds was determined by an analytical LC–MS or UPLC method and was found to be greater than or equal to 95%. LC–MS analyses were performed using a Shimadzu LCMS-2010 EV and UPLC analyses were performed using a ACQUITY UPLC. The tested biological compounds were not PAINS compound. Optical rotation(±) was measured by Shimadzu HPLC SCL-10A system with a corresponding chiral column and JASCO OR-2090 optical rotation detector. Column chromatography was carried out using a Hi-Flash column (40 μm, silica gel and NH-silica gel, Yamazen Corporation). Chemicals and solvents were purchased from commercial sources.

*(1S,5R)-1-Phenyl-3-oxabicyclo[3.1.0]hexan-2-one (**13a**).* NaHMDS (85 mL, 2.0 M) was added dropwise to a solution of 2-phenylacetonitrile (7.96g) in THF (300 mL) under cooling in an ice–salt bath. The mixture was stirred for 2 h, then *R*-(−)-epichlorohydrin (7.548 g) was added dropwise (3 h, 0 °C). Stirring was continued for 2 h at 0 °C then overnight at room temperature. The reaction solution was then cooled on ice, and a small amount of water was added. The resulting mixture was concentrated under reduced pressure, then ethanol (100 mL) and 1.5 N/KOH aqueous solution (100 mL) were added to the residue. The obtained mixture was heated to reflux for 8 h. The reaction solution was allowed to cool to room temperature, and concentrated hydrochloric acid was added to adjust the pH to <2. This mixture was stirred at 0 °C for 2 h then was concentrated under reduced pressure. Ethyl acetate and water were added to the concentrated solution to carry out liquid separation. The organic layer was washed successively with a saturated sodium bicarbonate aqueous solution and a saturated sodium chloride aqueous solution. The organic layer was dried over magnesium sulfate, the drying agent was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, giving the title compound (9.0 g). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.37 (t, $J = 4.8$ Hz, 1H), 1.65 (dd, $J = 7.8$, 4.4 Hz, 1H), 2.54–2.58 (m, 1H), 4.30 (d, $J = 9.2$ Hz, 1H), 4.47 (dd, $J = 9.4$, 4.4 Hz, 1H), 7.25–7.45 (m, 5H).

*(1S,5R)-1-(2-Fluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one (**13b**).* The title compound was synthesized adapting the procedure described for compound **13a** (6.25 g, 67.5% yield) as a white solid. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.38 (t, $J = 4.9$ Hz, 1H), 1.73 (dd, $J = 7.9$, 4.9 Hz, 1H), 2.43–2.52 (m, 1H), 4.34 (d, $J = 9.4$ Hz, 1H), 4.57 (dd, $J = 9.1$, 4.5 Hz, 1H), 7.09 (t, $J = 9.1$ Hz, 1H), 7.14–7.18 (m, 1H), 7.31–7.42 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 17.2, 24.6, 27.9, 68.4, 115.6, 121.6, 124.3, 130.2, 131.6, 162.2, 175.5. HRMS (ESI(+)) calcd for $C_{11}H_{10}FO_2$ $[M + H]^+$, 193.0659; found, 193.0660.

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*(1S,5R)-1-(3-Fluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one (**13c**).* The title compound was synthesized adapting the procedure described for compound **13a** (9.96 g, 49.8% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41 (t, J = 5.2 Hz, 1H), 1.64 (dd, J = 8.0, 5.2 Hz, 1H), 2.56−2.63 (m, 1H), 4.30 (d, J = 9.2 Hz, 1H), 4.47 (dd, J = 9.2, 4.8 Hz, 1H), 6.96−7.02 (m, 1H), 7.16−7.21 (m, 2H), 7.28−7.35 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 20.7, 25.4, 31.2, 68.0, 114.7, 115.3, 123.6, 130.2, 136.6, 162.8, 175.3. HRMS (ESI(+)) calcd for $C_{11}H_{10}FO_2$ $[M + H]^+$, 193.0659; found, 193.0659.

*(1S,5R)-1-(4-Fluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one (**13d**).* The title compound was synthesized adapting the procedure described for compound **13a** (8.73g, 43.7% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.39 (t, J = 4.7 Hz, 1H), 1.62 (dd, J = 7.9, 4.9 Hz, 1H), 2.54−2.58 (m, 1H), 4.31 (d, J = 9.1 Hz, 1H), 4.49 (dd, J = 9.4, 4.5 Hz, 1H), 7.06 (m, 2H), 7.39−7.43 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 20.2, 25.0, 31.2, 68.1, 115.6, 129.9, 130.2, 162.3, 175.8. HRMS (ESI(+)) calcd for $C_{11}H_{10}FO_2$ $[M + H]^+$, 193.0659; found, 193.0658.

*(1S,5R)-1-(3,5-Difluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one (**13e**).* The title compound was synthesized adapting the procedure described for compound **13a** (6.9 g, 50.3% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.46 (t, J = 4.9 Hz, 1H), 1.64 (dd, J = 7.7, 5.1 Hz, 1H), 2.62 (dt, J = 8.1, 4.6 Hz, 1H), 4.32 (d, J = 9.4 Hz, 1H), 4.47 (dd, J = 9.4, 4.5 Hz, 1H), 6.76 (tt, J = 8.9, 2.3 Hz, 1H), 7.01 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 21.3, 25.7, 31.0, 67.8, 103.2, 110.9, 138.0, 162.2, 163.9, 174.6. HRMS (ESI(+)) calcd for $C_{11}H_9F_2O_2$ $[M + H]^+$, 211.0565; found, 211.0561.

*(1S,5R)-1-(3,4-Difluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one (**13f**).* The title compound was synthesized adapting the procedure described for compound **13a** (7.1 g, 51.7% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.42 (t, J = 4.9 Hz, 1H), 1.61 (dd, J = 7.7, 5.1 Hz, 1H), 2.57−2.61 (m, 1H), 4.31 (d, J = 9.4 Hz, 1H), 4.48 (dd, J = 9.3, 4.7 Hz, 1H), 7.12−7.19 (m, 2H), 7.32 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 20.7, 25.2, 30.9, 68.0, 117.5, 117.6, 124.2, 131.1, 149.2, 150.9, 175.2. HRMS (ESI(+)) calcd for $C_{11}H_9F_2O_2$ $[M + H]^+$, 211.0565; found, 211.0562.

*(1S,2R)-1-Phenylcyclopropan-1,2-dimethanol (**14a**).* The title compound was synthesized as a colorless oil (340 mg, 67.7%) from (1*S*,5*R*)-1-phenyl-3-oxabicyclo[3.1.0]hexan-2-one by adapting a previously described procedure.[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.78 (t, J = 5.2 Hz, 1H), 1.87 (dd, J = 8.6, 5.2, 1H), 1.60−1.76 (m, 1H), 3.42 (t, J = 11.6, 1H), 3.57 (dd, J = 9.4, 4.4 Hz, 1H), 4.14−4.28 (m, 2H) 7.22−7.44 (m, 5H).

*(1S,2R)-1-(2-Fluorophenyl)cyclopropan-1,2-dimethanol (**14b**).* The title compound was synthesized by adapting a previously described procedure[36] to give **14b** (745 mg, 33.3% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.88 (t, J = 5.5 Hz, 1H), 1.08 (dd, J = 8.7, 5.3 Hz, 1H), 1.67−1.75 (m, 1H), 2.50 (br s, 1H), 2.90 (br s, 1H), 3.46 (t, J = 11.3 Hz, 1H), 3.58 (d, J = 12.1 Hz, 1H), 4.15 (d, J = 12.1 Hz, 1H), 4.23 (dd, J = 11.3, 4.5 Hz, 1H), 7.04 (t, J = 9.3 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.22−7.27 (m, 1H), 7.42 (td, J = 7.6, 1.9 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.3, 24.8, 28.0, 63.4, 67.0, 115.6, 124.0, 128.8, 130.5, 132.7, 162.1. HRMS (ESI(+)) calcd for $C_{11}H_{14}FO_2$ $[M + H]^+$, 197.0972; found, 197.0972.

*(1S,2R)-1-(3-Fluorophenyl)cyclopropan-1,2-dimethanol (**14c**).* The title compound was synthesized as a colorless oil (10.3 g, quant) from (1*S*,5*R*)-1-(3-fluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one by adapting a previously described procedure.[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80 (t, J = 5.0 Hz, 1H), 1.10 (dd, J = 8.6, 5.0 Hz, 1H), 1.62−1.71 (m, 1H), 3.41 (t, J =11.4 Hz, 1H), 3.58 (d, J = 12.0 Hz, 1H), 4.12−4.25 (m, 2H), 6.90−6.96 (m, 1H), 7.08−7.14 (m, 1H), 7.16−7.21 (m, 1H) 7.24−7.32 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 17.1, 26.3, 32.1, 63.5, 67.3, 113.7, 116.0, 124.6, 129.8, 146.5, 162.8. HRMS (ESI(+)) calcd for $C_{11}H_{14}FO_2$ $[M + H]^+$, 197.0972; found, 197.0970.

*(1S,2R)-1-(4-Fluorophenyl)cyclopropan-1,2-dimethanol (**14d**).* The title compound was synthesized as a colorless oil (8.56g, 96.1% yield) from (1*S*,5*R*)-1-(4-fluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.79 (t, J = 5.3 Hz, 1H), 1.08 (dd, J =8.7, 5.3 Hz, 1H), 1.63−1.69 (m, 1H), 2.50 (br s, 1H), 2.86 (d, J = 6.0 Hz, 1H), 3.43 (t, J = 11.3 Hz, 1H), 3.59 (dd, J = 12.1, 2.3 Hz, 1H), 4.13 (dd, J = 11.9, 4.7 Hz, 1H), 4.18−4.25 (m, 1H), 6.99−7.05 (m, 2H), 7.35−7.43 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.7, 25.9, 31.8, 63.6, 67.7, 115.2, 131.0, 139.6, 161.7. HRMS (ESI(+)) calcd for $C_{11}H_{14}FO_2$ $[M + H]^+$, 197.0972; found, 197.0970.

*(1S,2R)-1-(3,4-Difluorophenyl)cyclopropan-1,2-dimethanol (**14f**).* The title compound was synthesized as a colorless oil (7.1 g, 98% yield) from (1*S*,5*R*)-1-(3,4-difluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one **13f** by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.81 (t, J = 5.5 Hz, 1H), 1.10 (dd, J = 8.7, 5.3 Hz, 1H), 1.60−1.68 (m, 1H), 2.59−2.67 (m, 1H), 2.77 (d, J = 5.3 Hz, 1H), 3.43 (t, J = 11.3 Hz, 1H), 3.59 (dd, J = 11.9, 2.8 Hz, 1H), 4.13 (dd, J = 12.1, 6.4 Hz, 1H), 4.19−4.27 (m, 1H), 7.06−7.16 (m, 2H), 7.24 (ddd, J = 11.4, 7.8, 1.9 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.9, 26.3, 31.7, 63.4, 67.4, 117.0, 118.2, 125.0, 141.0, 149.3, 150.0. HRMS (ESI(+)) calcd for $C_{11}H_{13}F_2O_2$ $[M + H]^+$, 215.0878; found, 215.0875.

*(1S,2R)-1-(3,5-Difluorophenyl)cyclopropan-1,2-dimethanol (**14e**).* The title compound was synthesized as a colorless oil (3.2 g, 72.9% yield) from (1*S*,5*R*)-1-(3,5-difluorophenyl)-3-oxabicyclo[3.1.0]hexan-2-one **13e** by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.84 (t, J = 5.5 Hz, 1H), 1.14 (dd, J = 8.7, 5.3 Hz, 1H), 1.61−1.72 (m, 1H), 2.70 (br s, 1H), 2.74 (br s, 1H), 3.44 (t, J = 11.5 Hz, 1H), 3.61 (d, J = 12.1 Hz, 1H), 4.16−4.31 (m, 2H), 6.65−6.73 (tt, J = 8.9, 2.3 Hz, 1H), 6.92−6.97 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 17.3, 26.7, 31.9, 63.4, 67.0, 102.2, 111.7, 148.0, 162.9. HRMS (ESI(+)) calcd for $C_{11}H_{13}F_2O_2$ $[M + H]^+$, 215.0878; found, 215.0875.

*(1S,2R)-2-(tert-Butyldiphenylsilyloxymethyl)-1-phenylcyclopropylmethanol (**15a**).* The title compound was synthesized as a colorless oil (340 mg, 67.7% yield)from (1*S*,2*R*)-1-phenylcyclopropan-1,2-dimethanol **14a** by adapting a previously described procedure.[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.71 (t, J = 5.6 Hz, 1H), 1.04 (dd, J = 9.6, 5.2 Hz, 1H), 1.10 (s, 9H), 1.50−1.58 (m, 1H), 3.50 (dd, J = 12.4, 1.6 Hz, 1H), 3.53 (dd, J = 11.6, 1.6 Hz, 1H), 3.71 (dd, J = 12.4, 1.6 Hz, 1H), 4.10 (t, J = 12.0 Hz, 1H), 4.20 (dd, J = 12.0, 5.6 Hz, 1H), 7.21−7.46 (m, 10H). 7.7−7.76 (m, 5H).

*[(1R,2S)-2-(2-Fluorophenyl)-2-(hydroxymethyl)cyclopropyl]methyl Acetate (**15b**).* Lipase acrylic resin from *Candida antarctica* was added to a THF (50 mL)−vinyl acetate (1.51 g, 17.6 mmol) solution of (1*S*,2*R*)-1-(2-fluorophenyl)cyclopropan-1,2-dimethanol (2.28 g, 11.7 mmol) under cooling on ice. This mixture was stirred at room temperature for 17 h, then was filtered, and filtrate was concentrated, so as to give the title compound (2.1 g, 82.6% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.87 (t, J = 5.7 Hz, 1H), 1.15 (dd, J = 8.7, 5.3 Hz, 1H), 1.64 (ddd, J = 9.4, 5.7, 3.4 Hz, 1H), 2.08 (dd, J = 8.5, 3.6 Hz, 1H), 2.17 (s, 3H), 3.70 (dd, J = 12.1, 3.4 Hz, 1H), 3.96 (dd, J = 12.1, 8.7 Hz, 1H), 4.06 (dd, J = 11.9, 10.0 Hz, 1H), 4.65 (dd, J = 12.1, 5.7 Hz, 1H), 6.99−7.06 (m, 1H), 7.11 (td, J = 7.5, 0.9 Hz, 1H), 7.22−7.27 (m, 1H), 7.30 (td, J = 7.5, 1.7 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 15.1, 21.1, 22.1, 28.6, 64.5, 66.2, 115.6, 124.0, 128.9, 130.0, 132.1, 162.2, 171.1. HRMS (ESI(+)) calcd for $C_{13}H_{16}FO_3$ $[M + H]^+$, 239.1078; found, 239.1075.

*[(1R,2S)-2-(3-Fluorophenyl)-2-(hydroxymethyl)cyclopropyl]methyl Acetate (**15c**).* The title compound was synthesized as a light-yellow oil (1.21 g, quant) from (1*S*,2*R*)-1-(3-fluorophenyl)-cyclopropan-1,2-dimethanol **14c** by adapting the procedure described for compound **15b**. HRMS (ESI(+)) calcd for $C_{13}H_{16}FO_3$ $[M + H]^+$, 239.1078; found, 239.1074.

*[(1R,2S)-2-(4-Fluorophenyl)-2-(hydroxymethyl)cyclopropyl]methyl Acetate (**15d**).* The title compound was synthesized as a colorless oil (2.8 g, 92.3% yield) from (1*S*,2*R*)-1-(4-fluorophenyl)-cyclopropan-1,2-dimethanol by adapting the procedure described for compound **15b**. HRMS (ESI(+)) calcd for $C_{13}H_{16}FO_3$ $[M + H]^+$, 239.1078; found, 239.1072.

*[(1R,2S)-2-(3,5-Difluorophenyl)-2-(hydroxymethyl)cyclopropyl]methyl Acetate (**15e**).* The title compound was synthesized as a reddish oil (41.2 g, 99.2% yield) from (1*S*,2*R*)-1-(3,5-difluorophenyl)-cyclopropan-1,2-dimethanol by adapting the procedure described for

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compound **15b**. HRMS (ESI(+)) calcd for $C_{13}H_{15}F_2O_3$ [M + H]$^+$, 257.0984; found, 257.0979.

*[(1R,2S)-2-(3,4-Difluorophenyl)-2-(hydroxymethyl)cyclopropyl]-methyl Acetate (**15f**).* The title compound was synthesized as a colorless oil (2.9 g, 81% yield) from (1*S*,2*R*)-1-(3,4-difluorophenyl)-cyclopropan-1,2-dimethanolby adapting the procedure described for compound **15b**. HRMS (ESI(+)) calcd for $C_{13}H_{15}F_2O_3$ [M + H]$^+$, 257.0984; found, 257.0979.

*5-(((1S,2R)-2-(((tert-Butyldiphenylsilyl)oxy)methyl)-1-phenylcyclopropyl)methoxy)-2,4-dimethylpyrimidine (**16a**).* The title compound was synthesized as a colorless oil (340 mg, 67.7% yield) from ((1*S*,2*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-1-phenylcyclopropyl)methanol (**15a**) and 2,4-dimethyl-5-hydroxypyrimidine by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.95 (t, *J* = 5.7 Hz, 1H), 1.09 (s, 9H), 1.24 (dd, *J* = 8.7, 5.3 Hz, 1H), 1.64−1.70 (m, 1H), 2.30 (s, 3H), 2.61 (s, 3H), 3.80 (dd, *J* = 11.3, 8.3 Hz, 1H), 4.06 (dd, *J* = 11.3, 5.7 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.24 (d, *J* = 9.8 Hz, 1H), 7.24−7.28 (m, 1H), 7.31−7.39 (m, 6H), 7.41−7.45 (m, 4H), 7.66−7.70 (m, 4H), 7.89 (s, 1H). HRMS (ESI(+)) calcd for $C_{33}H_{39}N_2O_2Si$ [M + H]$^+$, 523.2775; found, 523.2783.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(2-fluorophenylcyclopropyl)methyl Acetate (**16b**).* Diisopropyl azodicarboxylate (310 μL, 1.57 mmol) was added dropwise to a THF solution (10 mL) of the compound **15b** (300 mg, 1.26 mmol), triphenylphosphine (413 mg, 1.57 mmol), and 2,4-dimethyl-pyrimidin-5-ol **43** (195 mg, 1.57 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 30 min, then allowed to warm to ambient temperatureand stirred overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using *n*-heptane/ethyl acetate (4/1 to 0/1, v/v) to give **16b** (154 mg, 35.5% yield) as a colorless oil. HRMS (ESI(+)) calcd for $C_{19}H_{22}FN_2O_3$ [M + H]$^+$, 345.1609; found, 345.1607.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(4-fluorophenylcyclopropyl)methyl Acetate (**16d**).* The title compound was synthesized as a colorless oil (276 mg, 63.6% yield) from [(1*R*,2*S*)-2-(4-fluorophenyl)-2-(hydroxymethyl)cyclopropyl]methyl acetate **15d** by adapting the procedure described for compound **16b**. HRMS (ESI(+)) calcd for $C_{19}H_{22}FN_2O_3$ [M + H]$^+$, 345.1609; found, 345.1606.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,4-difluorophenylcyclopropyl)methyl Acetate (**16f**).* The title compound was synthesized as a colorless oil (87 mg, 20.5% yield) from [(1*R*,2*S*)-2-(3,4-difluorophenyl)-2-(hydroxymethyl)cyclopropyl]-methyl acetate **15f** by adapting the procedure described for compound **16b**. HRMS (ESI(+)) calcd for $C_{19}H_{21}F_2N_2O_3$ [M + H]$^+$, 363.1515; found, 363.1511.

*((1R,2S)-2-(((2,4-Dimethylpyrimidin-5-yl)oxy)methyl)-2-phenylcyclopropyl)methanol (**17a**).* The title compound was synthesized as a white solid (155 mg, 83.9% yield) from 5-(((1*S*,2*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-1-phenylcyclopropyl)methoxy)-2,4-dimethylpyrimidine by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.98 (t, *J* = 5.7 Hz, 1H), 1.29 (dd, *J* = 8.7, 5.3 Hz, 1H), 1.85 (ddd, *J* = 9.4, 5.6, 3.6 Hz, 1H), 2.25 (br s, 1H), 2.39 (s, 3H), 2.61 (s, 3H), 3.60 (t, *J* = 11.0 Hz, 1H), 4.06−4.15 (m, 2H), 4.46 (d, *J* = 10.2 Hz, 1H), 7.25−7.27 (m, 1H), 7.34 (m, 2H), 7.45 (m, 2H), 7.99 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.4, 19.0, 25.0, 26.9, 30.0, 63.2, 74.4, 127.1, 128.5, 129.4, 139.1, 143.0, 149.1, 157.1, 160.3. HRMS (ESI(+)) calcd for $C_{17}H_{21}N_2O_2$ [M + H]$^+$, 285.1598; found, 285.1599.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(2-fluorophenylcyclopropyl)methanol (**17b**).* The acetate **16b** was dissolved in EtOH−1N sodium hydroxide aqueous solution (5−0.34 mL). The reaction mixture was stirred for 1 h then was concentrated under reduced pressure. The residue was purified by NH−silica gel column chromatography using *n*-heptane/ethyl acetate (9/1 to 0/1, v/v) to give **17b** (84.5 mg, 62.5% yield) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.05 (t, *J* = 5.9 Hz, 1H), 1.26 (dd, *J* = 8.9, 5.5 Hz, 1H), 1.82−1.88 (m, 1H), 2.21 (dd, *J* = 9.3, 2.8 Hz, 1H), 2.35 (s, 3H), 2.60 (s, 3H), 3.60 (ddd, *J* = 12.2, 9.9, 2.8 Hz, 1H), 4.09−4.16 (m, 2H), 4.42 (d, *J* = 9.8 Hz, 1H), 7.05 (t, *J* =9.3 Hz, 1H), 7.13 (td, *J* = 7.6, 1.1 Hz, 1H), 7.25−7.29 (m, 1H), 7.46 (td, *J* = 7.6, 1.5 Hz, 1H), 7.98 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 15.9, 18.8, 25.0, 25.3, 25.9, 62.9, 73.3, 115.7, 124.1, 129.3, 129.7, 132.4, 139.0, 149.1, 157.2, 160.2, 162.1. HRMS (ESI(+)) calcd for $C_{17}H_{20}FN_2O_2$ [M + H]$^+$, 303.1503; found, 303.1500.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenylcyclopropyl)methanol (**17c**).* Diisopropyl azodicarboxylate (45.8 mL, 217 mmol) was added dropwise to a THF solution (400 mL) of the compound **15c** (43.1 g, 181 mmol), triphenylphosphine (57 g, 217 mmol), and 2,4-dimethyl-pyrimidin-5-ol **43** (24.7 g, 199 mmol) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and then was stirred overnight. The reaction mixture was partitioned between sat. $NaHCO_3$ aq and EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, filtered to remove the drying agent, then concentrated under reduced pressure. The residue was dissolved in EtOH−1N NaOH (200−200 mL), and this solution was stirred for 1 h at room temperature. Additional 5 N NaOH (100 mL) was added to the reaction mixture, which was then concentrated under reduced pressure. The residue was extracted with EtOAc. The organic layer was washed with brine and dried over with $MgSO_4$, filtered to remove the drying agent, and then concentrated under reduced pressure. The residue was purified first by silica gel column chromatography using *n*-heptane/ethyl acetate/methanol (1/4/0 to 0/1/1, v/v) and thereafter by using a NH−silica gel pad with EtOAc as the eluent to give **17c** (39.3 g, 71.8% yield) as a light-red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.00 (t, *J* = 5.2 Hz, 1H), 1.24−1.30 (m, 1H), 1.79−1.85 (m, 1H), 2.39 (s, 3H), 2.60 (s, 3H), 3.55−3.61 (m, 1H), 4.03−4.13 (m, 1H), 4.12 (d, *J* = 9.6 Hz, 1H), 4.43 (d, *J* = 9.6 Hz, 1H), 6.92−6.98 (m, 1H), 7.11−7.15 (m, 1H), 7.19−7.22 (m, 1H), 7.25−7.31 (m, 1H), 8.00 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.6, 19.0, 25.0, 27.2, 29.7, 63.0, 73.9, 114.1, 116.3, 124.8, 129.9, 139.1, 145.6, 149.0, 157.1, 160.4, 162.7. HRMS (ESI(+)) calcd for $C_{17}H_{20}FN_2O_2$ [M + H]$^+$, 303.1503; found, 303.1502.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(4-fluorophenylcyclopropyl)methanol (**17d**).* The title compound was synthesized as a colorless oil (222 mg, 93% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(4-fluorophenylcyclopropyl)methyl acetate by adapting the procedure described for compound **17b**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.98 (t, *J* = 5.5 Hz, 1H), 1.25 (dd, *J* = 8.7, 5.3 Hz, 1H), 1.76−1.83 (m, 1H), 2.22 (dd, *J* = 9.3, 2.8 Hz, 1H), 2.38 (s, 3H), 2.61 (s, 3H), 3.56−3.62 (m, 1H), 4.05−4.13 (m, 1H), 4.10 (d, 9.8 Hz, 1H), 4.39 (d, *J* = 9.8 Hz, 1H), 7.02 (m, 2H), 7.39−7.44 (m, 2H), 7.99 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.3, 19.0, 25.0, 26.9, 29.5, 63.0, 74.3, 115.3, 131.1, 138.8, 139.1, 149.0, 157.1, 160.4, 161.8. HRMS (ESI(+)) calcd for $C_{17}H_{20}FN_2O_2$ [M + H]$^+$, 303.1503; found, 303.1503.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,5-difluorophenylcyclopropyl)methanol (**17e**).* To a solution of acetate **15e** (597 mg, 2.33 mmol) was added triethyl amine (357 μL, 2.56 mmol) and methanesulfonyl chloride (180 μL, 2.33 mmol) at 0 °C. The reaction mixture was stirred for 1 h at this temperature, then water was added and the mixture was extracted with dichloromethane. The organic layer was dried over $MgSO_4$, filtered to remove the drying agent, and concentrated under reduced pressure. The residue was dissolved in acetonitrile (20 mL), and to the solution were added 2,4-dimethylpyrimidin-5-ol **43** (347 mg, 2.8 mmol) and cesium carbonate (1.52 g, 4.66 mmol). The reaction mixture was stirred for 4 h at 70 °C, then was filtered through a glass filter. The filtrate was concentrated under reduced pressure. The residue was dissolved in EtOH−1N NaOH (2.45−2.45 mL), after which it was stirred for 1 h at 70 °C. After cooling the reaction mixture, it was extracted with EtOAc, washed with brine, and concentrated undwer reduced pressure. The residue was purified by silica gel column chromatography using *n*-heptane/AcOEt/MeOH (4/1/0 to 0/1/0 to 0/1/1, v/v) to give **17e** (520 mg, 69.7% yield) as a reddish oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.03 (t, *J* = 5.67 Hz, 1H), 1.31 (dd, *J* = 8.9, 5.5 Hz, 1H), 1.77−

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1.84 (m, 1H), 2.12 (dd, J = 8.9, 2.8 Hz, 1H), 2.41 (s, 3H), 2.63 (s, 3H), 3.57−3.63 (m, 1H), 4.04−4.10 (m, 1H), 4.13 (d, J = 10.2 Hz, 1H), 4.42 (d, J = 10.2 Hz, 1H), 6.72 (tt, J = 8.9, 2.3 Hz, 1H), 6.93−6.99 (m, 2H), 8.03 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.7, 19.0, 25.0, 27.4, 29.7, 62.7, 73.4, 102.7, 112.0, 139.0, 146.9, 148.9, 157.1, 160.5, 162.9. HRMS (ESI(+)) calcd for $C_{17}H_{19}F_2N_2O_2$ $[M + H]^+$, 321.1409; found, 321.1407.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,4-difluorophenylcyclopropyl)methanol (**17f**).* The title compound was synthesized as a colorless oil (70 mg, 97% yield) from [(1*R*,2*S*)-2-(3,4-fluorophenyl)-2-(hydroxymethyl)cyclopropyl]methyl acetate by adapting the procedure described for compound **17b**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.00 (t, J = 5.7 Hz, 1H), 1.27 (dd, J = 8.9, 5.5 Hz, 1H), 1.76−1.82 (m, 1H), 2.13 (dd, J = 9.1, 3.0 Hz, 1H), 2.40 (s, 3H), 2.62 (s, 3H), 3.59 (ddd, J = 12.3, 9.6, 3.0 Hz, 1H), 4.05−4.10 (m, 1H), 4.11(d, J = 9.8 Hz, 1H), 4.38 (d, J = 9.8 Hz, 1H), 7.09−7.19 (m, 2H), 7.24−7.28 (m, 1H), 8.01 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 16.4, 19.0, 25.0, 27.1, 29.4, 62.8, 73.8, 117.2, 118.5, 125.3, 139.0, 140.1, 148.9, 149.4, 150.0, 157.1, 160.5. HRMS (ESI(+)) calcd for $C_{17}H_{19}F_2N_2O_2$ $[M + H]^+$. 321.1409; found, 321.1408.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-phenylcyclopropanecarboxylic Acid (**18a**).* The title compound was synthesized as a white solid (569 mg, 60.4% yield) from ((1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)methyl)-2-phenylcyclopropyl)-methanol (**17a**) by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.57 (dd, J = 8.3, 4.9 Hz, 1H), 1.77 (t, J = 5.5 Hz, 1H), 2.28 (dd, J = 8.3, 6.0 Hz, 1H), 2.34 (s, 3H), 2.58 (s, 3H), 4.50 (q, J = 9.4 Hz, 2H), 7.28−7.33 (m, 1H), 7.37 (m, 2H), 7.51 (m, 2H), 8.20 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.8, 19.4, 24.0, 25.5, 35.0, 71.9, 127.5, 128.5, 129.3, 138.4, 141.8, 149.6, 158.7, 158.7, 174.1. HRMS (ESI(+)) calcd for $C_{17}H_{19}N_2O_3$ $[M + H]^+$, 299.1390; found, 299.1388.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(2-fluorophenyl)cyclopropanecarboxylic Acid (**18b**).* The title compound was synthesized as a white solid by adapting the procedure previously described[36] for compound **18a** (47 mg, 73.8% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.49−1.55 (m, 1H), 1.74−1.81 (m, 1H), 2.21−2.30 (m, 4H), 2.53−2.60 (m, 3H), 4.47 (d, J = 9.4 Hz, 1H), 4.54 (d, J = 9.4 Hz, 1H), 7.02−7.18 (m, 2H), 7.28−7.33 (m, 1H), 7.45−7.54 (m, 1H), 8.20 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.6, 19.0, 24.0, 24.9, 30.0, 70.9, 115.7, 124.0, 128.6, 129.4, 132.3, 138.1, 149.6, 158.6, 158.7, 161.8, 174.3. HRMS (ESI(+)) calcd for $C_{17}H_{18}FN_2O_3$ $[M + H]^+$, 317.1296; found, 317.1293.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)cyclopropanecarboxylic Acid (**18c**).* The title compound was synthesized as a white solid (203 mg, 81.7% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenylcyclopropyl)methanol by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.58 (dd, J = 8.1, 5.1 Hz, 1H), 1.78 (t, J = 5.5 Hz, 1H), 2.28 (dd, J = 7.9, 6.0 Hz, 1H), 2.37 (s, 3H), 2.59 (s, 3H), 4.48−4.54 (m, 2H), 7.01 (td, J = 8.3, 2.3 Hz, 1H), 7.20−7.25 (m, 1H), 7.26−7.30 (m, 1H), 7.30−7.37 (m, 1H), 8.25 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.9, 19.4, 23.8, 25.7, 34.5, 71.7, 114.5, 116.4, 124.8, 130.0, 138.2, 144.2, 149.6, 158.7, 159.1, 162.7, 173.8. HRMS (ESI(+)) calcd for $C_{17}H_{18}FN_2O_3$ $[M + H]^+$, 317.1296; found, 317.1293.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(4-fluorophenyl)cyclopropanecarboxylic Acid (**18d**).* The title compound was synthesized as an oil (223 mg, 92.5% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(4-fluorophenylcyclopropyl)methanol by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.54 (dd, J = 8.3, 4.9 Hz, 1H), 1.76 (t, J = 5.5 Hz, 1H), 2.24 (dd, J = 8.3, 6.0 Hz, 1H), 2.34 (s, 3H), 2.58 (s, 3H), 4.44−4.50 (m, 2H), 7.06 (m, 2H), 7.46−7.51 (m, 2H), 8.22 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.8, 19.4, 24.0, 25.5, 34.3, 71.9, 115.3, 131.1, 137.6, 138.6, 149.6, 158.8, 158.9, 162.1, 173.9. HRMS (ESI(+)) calcd for $C_{17}H_{18}FN_2O_3$ $[M + H]^+$, 317.1296; found, 317.1292.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,5-difluorophenyl)cyclopropanecarboxylic Acid (**18e**).* The title compound was synthesized as a white solid (886 mg, 83.9% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,5-difluorophenylcyclopropyl)methanol by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.57 (dd, J = 8.3, 5.3 Hz, 1H), 1.79 (t, J = 5.7 Hz, 1H), 2.26 (dd, J = 8.3, 6.0 Hz, 1H), 2.37 (s, 3H), 2.58 (s, 3H), 4.50 (s, 2H), 6.77 (tt, J = 8.8, 2.1 Hz, 1H), 7.00−7.09 (m, 2H), 8.26 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.9, 19.4, 23.9, 25.8, 34.2, 71.3, 103.1, 112.1, 138.6, 145.5, 149.5, 159.0, 159.0, 162.9, 173.4. HRMS (ESI(+)) calcd for $C_{17}H_{17}F_2N_2O_3$ $[M + H]^+$, 335.1202; found, 335.1198.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,4-difluorophenyl)cyclopropanecarboxylic Acid (**18f**).* The title compound was synthesized as a white amorphous solid (662 mg, 63.1% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,4-difluorophenylcyclopropyl)methanol by adapting a previously described procedure.[36] ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.40 (br s, 1H), 1.65 (br s, 1H), 2.13 (br s, 1H), 2.28 (br s, 3H), 2.53 (s, 3H), 4.35−4.48 (m, 2H), 7.05−7.19 (m, 2H), 7.23−7.33 (m, 1H), 8.17 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.8, 19.2, 24.1, 26.6, 33.7, 71.5, 117.2, 118.4, 125.2, 138.7, 138.9, 149.4, 149.6, 150.0, 158.5, 159.1, 175.3. HRMS (ESI(+)) calcd for $C_{17}H_{17}F_2N_2O_3$ $[M + H]^+$, 335.1202; found, 335.1198.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(pyridin-2-yl)-2-phenylcyclopropanecarboxamide (**23**).* To a solution of carboxylic acid **18a** (40 mg, 0.13 mmol) in DMF (1 mL) were added 2-aminopyridine (37.8 mg, 0.40 mmol), DIPEA (35.4 μL, 0.27 mmol), and HATU (56 mg, 0.15 mmol). The reaction mixture was stirred at room temperature for 5 h, then was warmed up to 50 °C and stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified first by silica gel column chromatography using chloroform/methanol (1/0 to 19/1, v/v) and subsequently by using Prep-TLC (SiO_2, chloroform/methanol = 10/1 v/v) to give amide **23** (27.3 mg, 54.4% yield) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.63 (dd, J = 8.1, 5.1 Hz, 1H), 1.93 (t, J = 5.3 Hz, 1H), 2.13−2.19 (m, 1H), 2.22 (s, 3H), 2.56 (s, 3H), 4.43 (d, J = 9.4 Hz, 1H), 4.53 (d, J = 9.4 Hz, 1H), 7.02−7.06 (m, 1H), 7.30−7.34 (m, 1H), 7.38 (t, J = 7.4 Hz, 2H), 7.46−7.51 (m, 2H), 7.65−7.70 (m, 1H), 7.98 (s, 1H), 8.07 (d, J = 7.9 Hz, 1H), 8.27−8.30 (m, 1H), 8.37 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 15.7, 15.9, 22.2, 26.2, 32.6, 68.3, 111.0, 117.1, 124.8, 125.9, 126.1, 135.7, 136.1, 139.0, 145.1, 146.4, 148.4, 154.3, 156.8, 165.7. HRMS (ESI(+)) calcd for $C_{22}H_{23}N_4O_2$ $[M + H]^+$, 375.1816; found, 375.1810. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(pyridin-3-yl)-2-phenylcyclopropanecarboxamide (**24**).* The title compound was synthesized as a white amorphous solid (28.6 mg, 57% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)methyl)-2-phenylcyclopropanecarboxylic acid **18a** by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.66 (dd, J = 7.7, 5.1 Hz, 1H), 1.93 (t, J = 5.5 Hz, 1H), 2.13 (dd, J = 7.9, 5.7 Hz, 1H), 2.24 (s, 3H), 2.57 (s, 3H), 4.47−4.51 (m, 1H), 4.53−4.57 (m, 1H), 7.25−7.27 (m, 1H), 7.29−7.34 (m, 1H), 7.38 (m, 2H), 7.47 (m, 2H), 7.69 (br s, 1H), 8.01 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 8.36 (d, J = 4.2 Hz, 1H), 8.55 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.7, 18.9, 25.0, 29.0, 35.2, 71.0, 123.8, 127.1, 127.6, 128.6, 128.7, 134.6, 139.1, 140.8, 141.7, 145.5, 149.2, 156.9, 159.7, 168.7. HRMS (ESI(+)) calcd for $C_{22}H_{23}N_4O_2$ $[M + H]^+$, 375.1816; found, 375.1809. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(pyridin-4-yl)-2-phenylcyclopropanecarboxamide (**25**).* The title compound was synthesized as a white amorphous solid (37.4 mg, 74.5% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)methyl)-2-phenylcyclopropanecarboxylic acid **18a** by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.68 (dd, J = 8.1, 5.1 Hz, 1H), 1.94 (t, J = 5.5 Hz, 1H), 2.11 (dd, J = 7.9, 6.0 Hz, 1H), 2.22 (s, 3H), 2.57 (s, 3H), 4.46 (d, J = 9.8 Hz, 1H), 4.55 (d, J = 9.4 Hz, 1H), 7.29−7.34 (m, 1H), 7.38 (m, 2H), 7.42−7.47 (m, 4H), 7.78 (br s, 1H), 8.01 (s, 1H), 8.50 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.7, 19.1, 25.0, 29.2, 35.5, 70.9, 113.36, 127.7, 128.5, 128.7, 139.2, 141.5, 144.7, 149.2, 150.9, 156.9, 159.8, 168.9.

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HRMS (ESI(+)) calcd for $C_{22}H_{23}N_4O_2$ [M + H]$^+$, 375.1816; found, 375.1809. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-fluoropyridin-2-yl)-2-phenylcyclopropanecarboxamide (**26**).* The title compound was synthesized as a white amorphous solid (30.5 mg, 46.3% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)-methyl)-2-phenylcyclopropanecarboxylic acid **18a** by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.64 (dd, J = 8.1, 5.1 Hz, 1H), 1.92 (t, J = 5.3 Hz, 1H), 2.10−2.16 (m, 1H), 2.22 (s, 3H), 2.56 (s, 3H), 4.42 (d, J = 9.4 Hz, 1H), 4.52 (d, J = 9.4 Hz, 1H), 7.29−7.34 (m, 1H), 7.35−7.43 (m, 3H), 7.46−7.50 (m, 2H), 7.98 (s, 1H), 8.10 (dd, J = 9.1, 3.8 Hz, 1H), 8.14 (d, J = 3.0 Hz, 1H), 8.29 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.6, 18.7, 25.0, 28.9, 35.4, 71.1, 114.5, 125.3, 127.7, 128.7, 128.8, 135.4, 138.8, 141.7, 147.4, 149.2, 156.4, 157.0, 159.6, 168.3. HRMS (ESI(+)) calcd for $C_{22}H_{22}FN_4O_2$ [M + H]$^+$, 393.1721; found, 393.1716. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(6-fluoropyridin-2-yl)-2-phenylcyclopropanecarboxamide (**27**).* The title compound was synthesized as a white solid (8.8 mg, 16.7% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)methyl)-2-phenylcyclopropanecarboxylic acid **18a** by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.64 (dd, J = 7.9, 5.5 Hz, 1H), 1.92 (t, J = 5.5 Hz, 1H), 2.09−2.16 (m, 1H), 2.22 (s, 3H), 2.56 (s, 3H), 4.41 (d, J = 9.4 Hz, 1H), 4.50 (d, J = 9.4 Hz, 1H), 6.66 (dd, J = 7.9, 2.3 Hz, 1H), 7.29−7.34 (m, 1H), 7.38 (m, 2H), 7.47 (m, 2H), 7.75 (q, J = 8.2 Hz, 1H), 7.94 (br d, J = 7.6 Hz, 1H), 7.98 (s, 1H), 8.23 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.7, 24.9, 28.8, 35.7, 71.1, 104.3, 104.5, 110.1, 127.7, 128.7, 128.9, 138.8, 141.6, 143.4, 149.2, 149.3, 157.0, 159.6, 161.9, 168.6. HRMS (ESI(+)) calcd for $C_{22}H_{22}FN_4O_2$ [M + H]$^+$, 393.1721; found, 393.1717. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-chloropyridin-2-yl)-2-phenylcyclopropanecarboxamide (**28**).* The title compound was synthesized as a light-yellow solid (105.7 mg, 30.8% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)methyl)-2-phenylcyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.64 (dd, J = 7.9, 4.9 Hz, 1H), 1.92 (t, J = 5.5 Hz, 1H), 2.11−2.16 (m, 1H), 2.22 (s, 3H), 2.56 (s, 3H), 4.41 (d, J = 9.4 Hz, 1H), 4.52 (d, J = 9.4 Hz, 1H), 7.29−7.34 (m, 1H), 7.38 (t, J = 7.4 Hz, 2H), 7.45−7.50 (m, 2H), 7.63 (dd, J = 9.1, 2.6 Hz, 1H), 7.98 (s, 1H), 8.06 (d, J = 8.7 Hz, 1H), 8.24 (d, J = 2.6 Hz, 1H), 8.29 g(s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.7, 18.7, 25.0, 28.9, 35.6, 71.0, 114.4, 126.9, 127.7, 128.7, 128.8, 138.1, 138.9, 141.6, 146.5, 149.2, 149.4, 157.0, 159.6, 168.5. HRMS (ESI(+)) calcd for $C_{22}H_{22}ClN_4O_2$ [M + H]$^+$, 409.1426; found, 409.1422. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-methylpyridin-2-yl)-2-phenylcyclopropanecarboxamide (**29**).* The title compound was synthesized as a white amorphous solid (5.5 mg, 44.9% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)-methyl)-2-phenylcyclopropanecarboxylic acid **18a** by adapting the procedure described for compound **23**. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.60 (dd, J = 8.0, 5.2 Hz, 1H), 1.90 (t, J = 5.2 Hz, 1H), 2.11 (br, 1H), 2.20 (s, 3H), 2.28 (s, 3H), 2.54 (s, 3H), 4.40 (d, J = 9.4 Hz, 1H), 4.51 (d, J = 9.4 Hz,1H), 7.27−7.38 (m, 3H), 7.45−7.48 (m, 3H), 7.94 (br, 1H), 7.96(s,1H), 8.08 (q, J = 0.8 Hz, 1H), 8.27 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 17.8, 18.4, 18.7, 25.0, 28.9, 35.2, 71.1, 113.3, 127.6, 128.6, 128.9, 129.3, 138.8, 139.0, 141.9, 147.7, 149.0, 149.2, 157.1, 159.5, 168.3. HRMS (ESI(+)) calcd for $C_{23}H_{25}N_4O_2$ [M + H]$^+$, 389.1972; found, 389.1965. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(4-methylpyridin-2-yl)-2-phenylcyclopropanecarboxamide (**30**).* The title compound was synthesized as a white amorphous solid (6.5 mg, 53.0% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)-methyl)-2-phenylcyclopropanecarboxylic acid **18a** by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.58−1.62 (m, 1H), 1.91 (t, J = 5.5 Hz, 1H), 2.11−2.16 (m, 1H), 2.22 (s, 3H), 2.32 (s, 3H), 2.56 (s, 3H), 4.43 (d, J = 9.4 Hz, 1H), 4.53 (d, J = 9.4 Hz, 1H), 6.85 (d, J = 5.3 Hz, 1H), 7.29−7.33 (m, 1H), 7.37 (m, 2H), 7.45−7.51 (m, 2H), 7.90 (br s, 1H), 7.99 (s, 1H), 8.11 (d, J = 5.3 Hz, 1H), 8.44 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.5, 18.7, 21.4, 24.9, 29.0, 35.3, 71.0, 114.3, 121.1, 127.6, 128.6, 128.8, 138.8, 141.8, 147.4, 149.2, 150.0, 151.2, 157.1, 159.5, 168.5. HRMS (ESI(+)) calcd for $C_{23}H_{25}N_4O_2$ [M + H]$^+$, 389.1972; found, 389.1966. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-fluoro-4-methylpyridin-2-yl)-2-phenylcyclopropanecarboxamide (**31**).* The title compound was synthesized as a white amorphous solid (2.4 g, 61.7% yield) from (1*R*,2*S*)-2-(((2,4-dimethylpyrimidin-5-yl)oxy)-methyl)-2-phenylcyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.63 (dd, J = 8.1, 5.1 Hz, 1H), 1.91 (t, J = 5.5 Hz, 1H), 2.09−2.14 (m, 1H), 2.23 (s, 3H), 2.29 (s, 3H), 2.56 (s, 3H), 4.42 (d, J = 9.4 Hz, 1H), 4.52 (d, J = 9.4 Hz, 1H), 7.29−7.34 (m, 1H), 7.38 (m, 2H), 7.45−7.50 (m, 2H), 7.96 (d, J = 5.3 Hz, 1H), 7.98 (s, 1H), 8.02 (s, 1H), 8.23 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 14.7, 18.5, 18.7, 25.0, 28.9, 35.4, 71.0, 115.8, 127.6, 128.7, 128.8, 134.5, 136.6, 138.8, 141.7, 147.1, 149.2, 155.8, 157.0, 159.5, 168.2. HRMS (ESI(+)) calcd for $C_{23}H_{24}FN_4O_2$ [M + H]$^+$, 407.1878; found, 407.1871. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-fluoro-4-methoxypyridin-2-yl)-2-phenylcyclopropanecarboxamide (**32**).* To a solution of carboxylic acid **18a** (1 g, 3.35 mmol) was added oxalyl chloride (575 μL, 6.7 mmol). The reaction mixture was stirred for 2 h, then was concentrated under reduced pressure. The residue was dissolved in THF (15 mL), and 28% aqueous ammonia (4.08 mL, 67 mmol) was added at 0 °C. This mixture was stirred for 30 min, after which it was concentrated under reduced pressure to afford crude carboxamide. A mixture of this carboxamide (300 mg, 1.01 mmol), 2-chloro-5-fluoro-4-methoxypyridine (235 mg, 1.52 mmol), 4,5-bis-(diphenylphosphino)-9,9-dimethylxanthene (351 mg, 0.606 mmol), potassium triphosphate (429 mg, 2.02 mmol), and tris(dibenzylidene)-dipalladium(0) (185 mg, 0.202 mmol) in 1,4-dioxane (20 mL) was heated to 95 °C. and stirred for 15 h. The reaction solution was cooled to room temperature and filtered using Celite. The filtrate was concentrated under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine and dried with $MgSO_4$, filtered to remove the drying agent, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using *n*-heptane/ethyl acetate (7/3 to ethyl acetate, v/v) then by NH−silica gel chromatography using *n*-heptane/ethyl acetate (4:1 to 2:3, v/v). The crude product obtained was dissolved in chloroform, and *n*-hexane was added. The precipitated solid was colected by filtration and dried to give compound (304 mg, 71.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.63 (dd, J = 8.0, 5.6 Hz, 1H), 1.89 (t, J = 5.6 Hz, 1H), 2.11 (dd, J = 8.0, 5.6 Hz, 1H), 2.23 (s, 3H), 2.55 (s, 3H), 3.88 (s, 3H), 4.41 (d, J = 9.6 Hz, 1H), 4.51 (d, J = 9.6 Hz, 1H), 7.28−7.39 (m, 3H), 7.45−7.48 (m, 2H), 7.82 (d, J = 6.4 Hz, 1H), 7.97 (d, J = 2.8 Hz, 1H), 7.98 (s, 1H), 8.30 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.5, 18.7, 24.9, 29.0, 35.5, 56.1, 71.0, 98.4, 127.7, 128.7, 128.8, 134.7, 138.8, 141.6, 147.1, 148.6, 149.2, 155.2, 157.0, 159.6, 168.5. HRMS (ESI(+)) calcd for $C_{23}H_{24}FN_4O_3$ [M + H]$^+$, 423.1827; found, 423.1820. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(2-fluorophenyl)-N-(5-fluoropyridin-2-yl)cyclopropanecarboxamide (**33**).* The title compound was synthesized as a white solid (1.7 mg, 8.74% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(2-fluorophenyl)cyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.53 (dd, J = 8.3, 5.3 Hz, 1H), 1.94 (t, J = 5.5 Hz, 1H), 2.15−2.27 (m, 4H), 2.55 (s, 3H), 4.34 (d, J = 9.8 Hz, 1H), 4.43 (d, J = 9.4 Hz, 1H), 7.08−7.14 (m, 1H), 7.17 (t, J = 7.4 Hz, 1H), 7.29−7.35 (m, 1H), 7.39−7.49 (m, 2H), 7.93 (s, 1H), 8.10−8.19 (m, 2H), 8.35 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 17.6, 18.6, 24.9, 28.0, 30.3, 70.6, 114.5, 115.9, 124.4, 125.3, 128.5, 129.7, 131.9, 135.4, 138.5, 147.4, 149.2, 156.4, 157.1, 159.6, 161.9, 168.0. HRMS (ESI(+)) calcd for $C_{22}H_{21}F_2N_4O_2$ [M + H]$^+$, 411.1627; found, 411.1621. Purity: >95%.

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*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)-N-(5-fluoropyridin-2-yl)cyclopropanecarboxamide (**34**).* The title compound was synthesized as a white solid (3.66 g, 56.4% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)cyclopropanecarboxylic acid **18c** by adapting the procedure described for compound **23**. ^{1}H NMR (400 MHz, DMSO-*d*) δ (ppm): 1.46−1.50 (m, 1H), 1.68 (t, *J* = 6.0 Hz, 1H), 2.01 (s, 3H), 2.36 (s, 3H), 2.59−2.63 (m, 1H), 4.27 (d, *J* = 10.4 Hz, 1H), 4.66 (d, *J* = 10.4 Hz, 1H), 7.06−7.11 (m, 1H), 7.37−7.44 (m, 3H), 7.60−7.65 (m, 1H), 7.85−7.89 (m, 1H), 8.11 (s, 1H), 8.30 (d, *J* = 3.2 Hz, 1H), 11.20 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.7, 18.7, 25.0, 29.0, 34.9, 70.7, 114.5, 114.7, 115.9, 124.2, 125.4, 130.2, 135.5, 138.9, 144.1, 147.3, 149.1, 156.4, 157.0, 159.8, 162.8, 167.9. HRMS (ESI(+)) calcd for $C_{22}H_{21}F_2N_4O_2$ $[M + H]^+$, 411.1627; found, 411.1622. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(4-fluorophenyl)-N-(5-fluoropyridin-2-yl)cyclopropanecarboxamide (**35**).* The title compound was synthesized as a white solid (102 mg, 39.3% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]-methyl}-2-(4-fluorophenyl)cyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (400 MHz, DMSO-*d*) δ (ppm): 1.43−1.45 (m, 1H), 1.66 (t, *J* = 4.4 Hz, 1H), 2.02 (s, 3H), 2.36 (s, 3H), 2.55−2.58 (m, 1H), 4.26 (d, *J* = 10.4 Hz, 1H), 4.59 (d, *J* = 10.4 Hz, 1H), 7.15−7.20 (m, 2H), 7.57−7.65 (m, 3H), 7.86−7.89 (m, 1H), 8.09 (s, 1H), 8.30 (d, *J* = 3.2 Hz, 1H), 11.18 (br s, 1H). ^{13}C NMR (150 MHz, CDCl3) δ (ppm): 18.6, 18.7, 24.9, 28.8, 34.8, 71.1, 114.5, 115.6, 125.4, 130.6, 135.4, 137.5, 138.9, 147.3, 149.1, 156.4, 157.0, 159.7, 162.1, 168.1. HRMS (ESI(+)) calcd for $C_{22}H_{21}F_2N_4O_2$ $[M + H]^+$, 411.1627; found, 411.1620. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,4-difluorophenyl)-N-(5-fluoropyridin-2-yl)cyclopropanecarboxamide (**37**).* The title compound was synthesized as a white amorphous solid (123 mg, 53.4% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,4-difluorophenyl)cyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.58−1.61 (m, 1H), 1.91 (t, *J* = 5.2 Hz, 1H), 2.07 (br t, *J* = 8.0 Hz, 1H), 2.21 (s, 3H), 2.56 (s, 3H), 4.39 (d, *J* = 9.6 Hz, 1H), 4.45 (d, *J* = 9.6 Hz, 1H), 7.12−7.41 (m, 4H), 7.97 (s, 1H), 8.04−8.08 (m, 1H), 8.12 (d, *J* = 2.4 Hz, 1H), 8.30 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.6, 18.7, 25.0, 28.9, 34.6, 70.7, 114.5, 117.5, 118.1, 124.8, 125.4, 135.5, 138.6, 138.9, 147.2, 149.0, 149.8, 150.1, 156.4, 156.87, 159.9, 167.7. HRMS (ESI(+)) calcd for $C_{22}H_{20}F_3N_4O_2$ $[M + H]^+$, 429.1533; found, 429.1527. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,5-difluorophenyl)-N-(5-fluoropyridin-2-yl)cyclopropanecarboxamide (**36**).* The title compound was synthesized as a white amorphous solid (140 mg, 60.7% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3,5-difluorophenyl)cyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.61−1.64 (m, 1H), 1.93 (t, *J* = 5.2 Hz, 1H), 2.09 (br t, *J* = 8.0 Hz, 1H), 2.22 (s, 3H), 2.56 (s, 3H), 4.40 (d, *J* = 9.6 Hz, 1H), 4.49 (d, *J* = 9.6 Hz, 1H), 6.74−6.79 (m, 1H), 6.98 (d, *J* = 6.0 Hz, 2H), 7.36−7.41 (m, 1H), 7.99 (s, 1H), 8.05 (dd, *J* = 3.6, 9.2 Hz, 1H), 8.11 (d, *J* = 2.8 Hz, 1H), 8.35 (br t, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 18.7, 18.7, 25.0, 29.1, 34.7, 70.3, 103.3, 111.7, 114.5, 125.4, 135.5, 138.9, 145.4, 147.2, 149.0, 156.5, 156.9, 160.0, 163.0, 167.4. HRMS (ESI(+)) calcd for $C_{22}H_{20}F_3N_4O_2$ $[M + H]^+$, 429.1533; found, 429.1526. Purity: >95%.

*(1R,2S)-2-{[(2,4-Dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-fluoro-4-methylpyridin-2-yl)-2-(3-fluorophenyl)cyclopropanecarboxamide (**38**).* The title compound was synthesized as a white amorphous solid (289 mg, 55.4% yield) from (1*R*,2*S*)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)cyclopropanecarboxylic acid by adapting the procedure described for compound **23**. ^{1}H NMR (400 MHz, $CDCl_3$) d (ppm): 1.55−1.65 (m, 1H), 1.91 (t, *J* = 5.6 Hz, 1H), 2.05−2.13 (m, 1H), 2.22 (s, 3H), 2.27 (s, 3H), 2.56 (s, 3H), 4.41 (d, *J* = 10.0 Hz, 1H), 4.50 (d, *J* = 9.2 Hz, 1H), 6.97−7.04 (m, 1H), 7.14−7.20 (m, 1H), 7.22−7.28 (m, 1H), 7.33 (td, *J* = 8.0, 5.8 Hz, 1H), 7.93 (d, *J* = 5.2 Hz, 1H), 7.99 (s, 1H), 8.00 (s, 1H), 8.24 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 14.7, 18.6, 18.7, 25.0, 29.0, 34.8, 70.6, 114.7,115.8, 115.8, 124.2, 130.2, 134.5, 136.7, 138.8, 144.1, 147.0, 149.1, 155.8, 157.0, 159.7, 162.8, 167.8. HRMS (ESI(+)) calcd for $C_{23}H_{23}F_2N_4O_2$ $[M + H]^+$, 425.1784; found, 425.1779. Purity: >95%.

Animals. All animal experiments were approved by the Animal Care and Use Committee of Eisai Co., Ltd. Male C57BL/6NCrlCrlj mice (Charles River, Yokohama, Japan) were maintained under a 12 h light−dark cycle with food and water available ad libitum.

In Vivo Sleep Experiments. *Recordings of Wake/Sleep Behavior.* The sleep assay was quantitated using polysomnography in mice implanted with electrodes for recording EEG and EMG activity as previously described.[36]

In male mice (C57BL/6NCrlCrlj; Charles River Laboratories, Japan), compounds **26**, **28**, **31**, and **34** were formulated in 10% Cremophor EL, 5% DMSO in saline. The compounds or vehicle were administered prior to switching the lights off at ZT (zeitgeber time) 12:00, and EEG/EMG data were recorded from ZT 12:00 until ZT 20:00.

After administration of compounds or vehicle, EEG/EMG activity recording results were analyzed by software (SleepSign 3.0, Kissei Comtech, Japan), followed up by visual confirmation by an experienced sleep researcher blinded as to treatment, to determine wakefulness, non-REM sleep, or REM sleep. The data were expressed as vigilance state time every hour (for time course) or for 3 h after lights out (for cumulative analysis).[36]

Data were expressed as mean ± SEM. Unpaired parametric two-tailed *t* test for **26**, **28**, and **31** or one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test for **34** were applied to test the null hypothesis. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 6.02 (GraphPad Software, Inc.).

Cell-Based Calcium Accumulation Functional Assay. HEK-293 cells expressing human OX_1R or OX_2R were cultured in Dulbecco's Minimum Essential Medium (DMEM)/10% fetal bovine serum and frozen in aliquots until use. On the day before the experiment, cells were thawed and seeded at 16000 cells/well into poly-D-lysine-coated 384-well plates (black walls with transparent bottom) in a culture medium and incubated overnight at 37 °C. On the day of the experiment, the cell supernatant medium was replaced with an assay buffer (115 mM NaCl, 5.4 nM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 13.8 mM D-glucose, 20 mM HEPES, 0.1% bovine serum albumin), containing Calcium 4 calcium-sensitive dye (FLIPR Calcium4 assay kit, Molecular Devices; Sunnyvale, CA, USA), and cells were subsequently incubated at 37 °C for 1 h, followed by 15 min at room temperature. After transfer to an FDSS6000 device (Hamamatsu Photonics; Hamamatsu, Japan), test compounds dissolved in the assay buffer were added to the cells and allowed to equilibrate for 30 min at room temperature. Baseline fluorescence was measured for approximately 10 s (9 measurements, 1.1 s each) before buffer alone or orexin-A in assay buffer were added in the 10th measurement, followed by 101 additional measurements (1.1 s each). All measurements were obtained using excitation and detection wavelengths of 480 and 540 nm, respectively. Calcium accumulation was expressed as the ratio of peak fluorescence intensity to fluorescence intensity at the baseline (9th measurement). In every experiment, the dose−response of orexin-A was measured to confirm the conditions of the cells and to calculate the half-maximal effective concentration (EC_{50}). Test compounds were challenged with orexin-A at a concentration (*L*) equivalent to EC_{80} on both receptors. The inhibition potential of the test compounds was expressed as the concentration necessary to reduce the orexin-A-elicited response by 50% (IC_{50}), which was then converted to the inhibition constant, K_i, by using the Cheng−Prusoff formula: $K_i = IC_{50}/[1 + (L/EC_{50})]$.

Evaluation of Pharmacokinetic Profile in Mice. Compound **34** was formulated for intravenous (1 mg/kg) and oral (10 mg/kg) administrations in 10% DMSO in water and 10% Cremophor EL, 5% DMSO in saline, respectively, and adeministered to male C57BL/6NCrlCrlj mice. After administration, blood was collected from the tail vein at the designated time points and centrifuged to obtain the plasma sample. Plasma samples were precipitated with 20 volumes of acetonitrile containing the internal standard (10 ng/mL imipramine). Following vortex mixing and centrifugation, the supernatant was

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filtered, and the resulting filtrate was injected into the LC−MS/MS system. Detection was performed by multiple reaction monitoring in positive ionization mode. The standard curve was obtained from the ratios of the peak area responses relative to the internal standard using linear least-squares regression with a $1/x^2$ weighting. On the basis of the plasma concentrations, pharmacokinetic parameters were calculated using a software of Phoenix WinNonlin Ver. 6.3 (Certara USA, Inc., Missouri, US) via noncompartment analysis.

Exploratory Determination of Plasma and CSF Concentrations in Mice. Compound **34** was formulated as a suspension in 0.5% aqueous methylcellulose and orally administered to male C57BL/6NCrlCrlj mice at a dose of 10 mg/kg. Subsequently, 1 or 3 h after administration, the mice were anesthetized with sodium pentobarbital and CSF was collected from the cisterna magna. Immediately after CSF sampling, a blood sample was collected from the abdominal aorta and centrifuged to obtain the plasma sample. The plasma and CSF samples were stored below −20 °C until analysis by liquid chromatography−tandem mass spectroscopy (LC−MS/MS).

Plasma samples were precipitated with 25 volumes of acetonitrile containing the internal standard (1 ng/mL deuterated **34**). Following vortex mixing and centrifugation, the supernatant was filtered and the resulting filtrate was injected into the LC−MS/MS system. CSF samples were mixed with 9 volumes of acetonitrile and 50 volumes of acetonitrile containing the internal standard. After vortex mixing, the mixture was injected onto the LC−MS/MS system. Plasma and CSF concentrations were determined by LC−MS/MS, and the values are reported as means ± SEM.

Exploratory Assessment of Plasma Protein Binding in Mice. The plasma protein binding of compound **34** in C57BL/6N mice was determined by equilibrium dialysis. Blood was collected from the abdominal vein under anesthesia, and the plasma was obtained via centrifugation. Plasma samples spiked with **34** (1000 ng/mL) were dialyzed against an equal volume of PBS over a cellulose membrane with a 14000 Da molecular weight cutoff for 24 h at 37 °C. Following the dialysis, plasma and PBS fractions were collected and mixed with 4 volumes of acetonitrile containing the internal standard (100 ng/mL propranolol). After vortex mixing, the mixture was injected into the LC−MS/MS system. Plasma protein binding was calculated based on the concentrations measured in the plasma and PBS fractions using the following equation:

$$\text{plasma protein binding (\%)} = \left(1 - \frac{\text{concentration in PBS fraction}}{\text{concentration in plasma fraction}}\right) \times 100\%$$

Metabolic Stability Screening in Liver Microsomes. Test compounds were incubated with human liver microsomes (0.2 mg/mL) in the presence or absence of an NADPH-generating system (60 mM $MgCl_2$ solution containing 3.6 mM β-$NADP^+$, 90 mM glucose-6-phosphate, and 1 unit/mL glucose-6-phosphate dehydrogenase, incubated for 5 min to generate NADPH) for 15 min at 37 °C. After the addition of an equal volume of acetonitrile/methanol (7:3) mixture containing 0.1 μM propranolol as the internal standard to the reaction mixture, the concentration of the test compound was measured using LC−MS. The residual ratio of the compound in the presence of NADPH relative to that in the absence of NADPH was evaluated.

Screening for Time-Dependent CYP Inhibition in Liver Microsomes. Test compounds were preincubated with human liver microsomes (0.2 mg/mL) in the presence or absence of the NADPH generating system for 30 min at 37 °C. After preincubation, a sample of the reaction solution was collected, diluted 10 times by mixing with a cocktail of CYP marker substrates (phenacetin for CYP1A2, rosiglitazone for CYP2C8, tolbutamide for CYP2C9, S-mephenytoin for CYP2C19, bufuralol for CYP2D6, and midazolam for CYP3A) and the NADPH generating system, and then incubated for 10 min at 37 °C. An equal volume of an acetonitrile/methanol (1:1) mixture containing 0.05 μM dextrorphan or 0.05 μM propranolol as an internal standard was added to the reaction mixtures, and the metabolites of the marker substrates were measured using LC−MS/MS. The ratio of residual activity in the presence of NADPH relative to that in the absence of NADPH was evaluated.[43]

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and analytical data of all compounds and intermediates not described in the Experimental Section. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00217.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

OX_1R, orexin 1 receptor; OX_2R, orexin 2 receptor; NaHMDS, sodium bis(trimethylsilyl)amide; TBDPS, *tert*-butyldiphenylsilyl; DIAD, diisopropyl azodicarboxylate; TEA, triethylamine; HATU, *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; *m*-CPBA, *m*-chloroperoxybenzoic acid; acac, acetylacetone; Bn, benzyl; EMG, electromyogram; ZT, zeitgeber time

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