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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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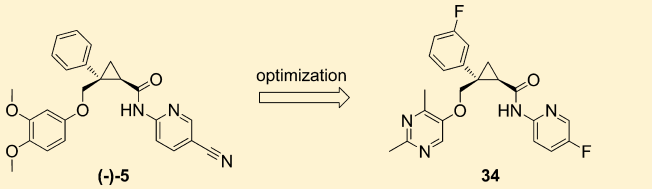
Supporting Information

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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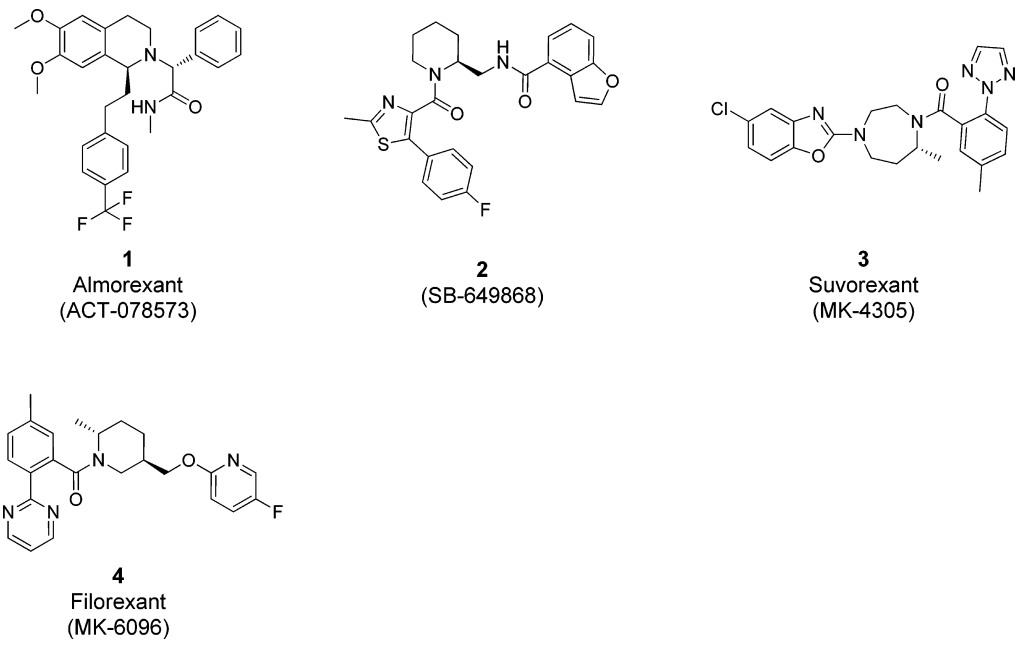


Figure 1. Orexin receptor dual antagonists that have advanced to clinical trials.

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]

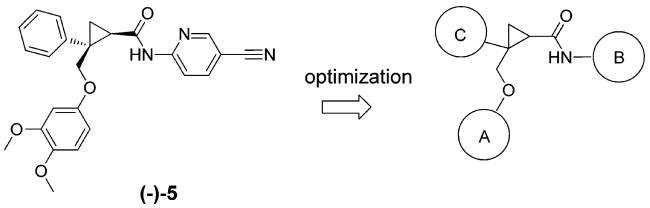
A dual OXR antagonist, almorexant, evaluated by Actelion Pharmaceuticals/GlaxoSmithKline was found to significantly decrease the behavioral indices of wakefulness in animals and has shown effectiveness in clinical trials.[11] Recently, a number of orally active selective OX_2R antagonists have been evaluated 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

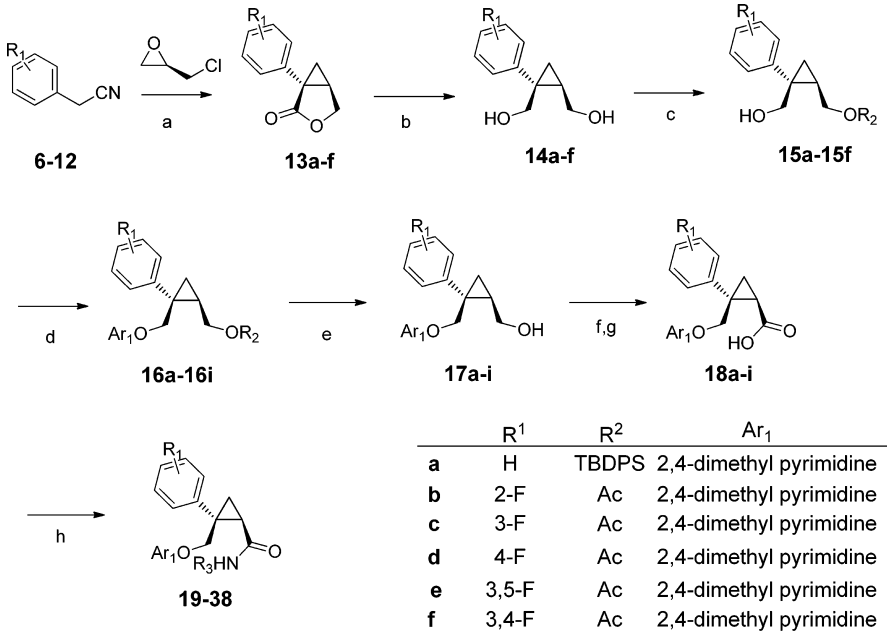


(-)-5

compd	in vitro binding affinity (K_i, nM) OX_2R	in vitro binding affinity (K_i, nM) OX_1R	solubility[a] (μM) pH 7.4[b]	solubility[a] (μM) pH 1.2[c]	TDI of CYP3A[d] (% of control at 10 μM)	metabolic stability[e] (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.

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.

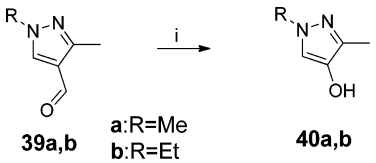
its time-dependent inhibition (TDI) of cytochrome P450 3A (CYP3A) and low aqueous solubility under both acidic and neutral conditions (pH 1.2 and pH 7.4). (−)-**5** also demonstrated moderate reversible inhibition of CYPs (IC_{50} values of 8 and 8.6 μM for CYP2C8 and CYP2C19, respectively, and >10 μM 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**.

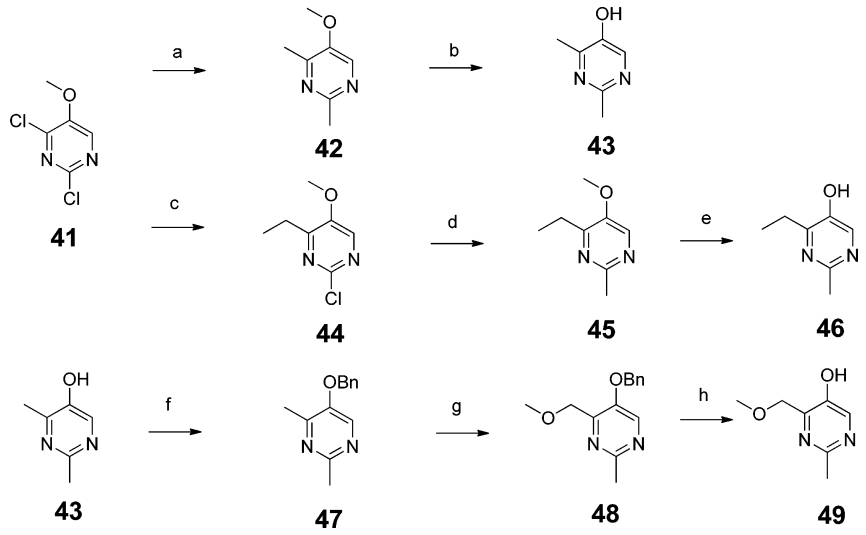
Components of the heteroaromatic A ring were synthesized as outlined in Schemes 2 and 3. Pyrazole derivatives **40a** and **40b** were synthesized from commercially available aldehydes **39a** and **39b** via Baeyer–Villiger oxidation (Scheme 2). The Pd-catalyzed coupling of commercially available 2,4-dichloro-5-methoxy 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.

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

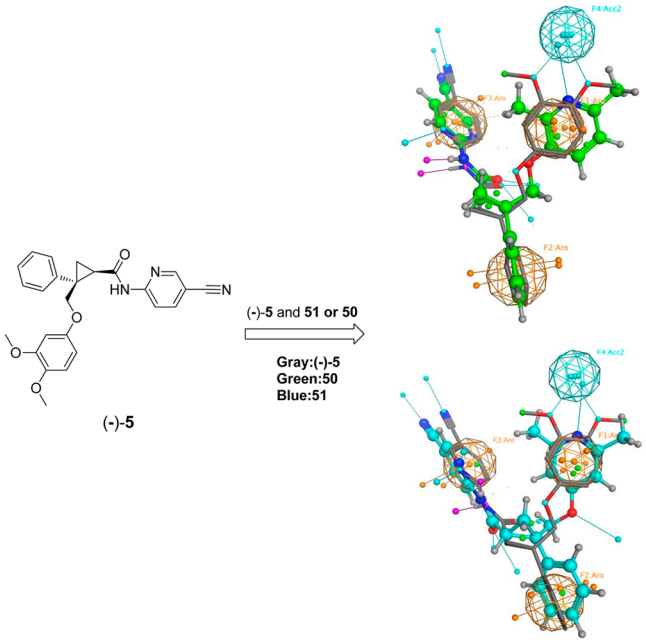
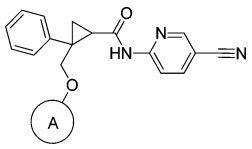


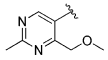
Figure 2. Overlay of (−)-**5** and **50** and **51**.

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

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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