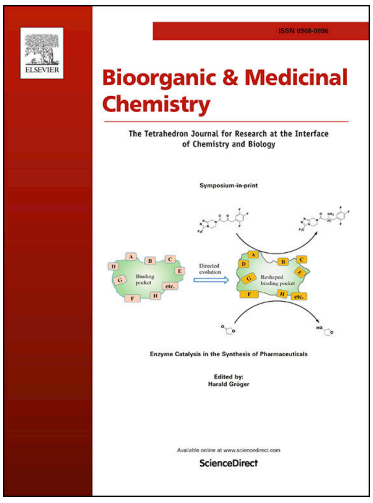
Journal Pre-proofs



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Aya Futamura, Ryo Suzuki, Yunoshin Tamura, Hiroshi Kawamoto, Mari Ohmichi, Noriko Hino, Yuichi Tokumaru, Sora Kirinuki, Tetsuaki Hiyoshi, Takeshi Aoki, Daiji Kambe, Dai Nozawa

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Discovery of ORN0829, a potent dual orexin 1/2 receptor antagonist for the treatment of insomnia

Aya Futamura[a], Ryo Suzuki[a], Yunoshin Tamura[a], Hiroshi Kawamoto[a], Mari Ohmichi[b], Noriko Hino[a], Yuichi Tokumaru[a], Sora Kirinuki[a], Tetsuaki Hiyoshi[a], Takeshi Aoki[a], Daiji Kambe[a] and Dai Nozawa[a,*]

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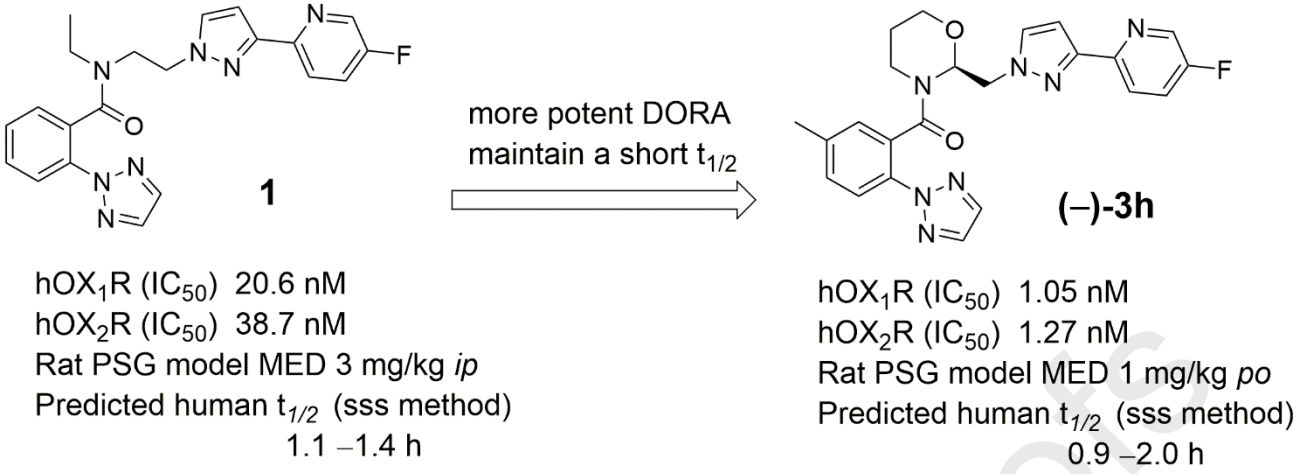
Abstract

Here, we present the design, synthesis, and SAR of dual orexin 1 and 2 receptor antagonists, which were optimized by balancing the antagonistic activity for orexin receptors and lipophilicity. Based on the prototype compound **1**, ring construction and the insertion of an additional heteroatom into the resulting ring led to the discovery of orexin 1 and 2 receptor antagonists, which were 3-benzoyl-1,3-oxazinane derivatives. Within these derivatives, **(−)-3h** enabled a high dual orexin receptor antagonistic activity and a low lipophilicity. Compound **(−)-3h** exhibited potent sleep-promoting effects at a *po* dose of 1 mg/kg in a rat polysomnogram study, and optimal PK properties with a rapid T_{max} and short half-lives in rats and dogs were observed, indicating a predicted human half-life of 0.9–2.0 h. Thus, **(−)-3h** (**ORN0829**; investigation code name, TS-142) was selected as a viable candidate and is currently in clinical development for the treatment of insomnia.



Abbreviations

OX_1R, orexin 1 receptor; OX_2R, orexin 2 receptor; DORA, dual orexin receptor antagonist; SORA, selective orexin receptor antagonist; PSG, polysomnography; SAR, structure-activity relationship; Cbz, benzyloxycarbonyl; Boc, *tert*-butoxycarbonyl; Ms, methanesulfonyl; IBX, 2-iodoxybenzoic acid; T3P®, propylphosphonic acid anhydride; DIPEA, *N*,*N*-diisopropylethylamine; CDI, 1,1'-carbonyldiimidazole; PPL, lipase from Porcine Pancreas; MTBE, methyl *tert*-butyl ether; REMD, replica-exchange molecular dynamics; CYP, cytochrome P450; CHO, Chinese hamster ovary; ER, efflux ratio; P-gp, P-glycoprotein; RHS, right hand side; PPB, plasma protein binding; CSF, cerebrospinal fluid; MED, minimum effective dose.

Introduction

Orexin neuropeptides (orexin-A and -B) were discovered in 1998 by two independent research groups[1–2] and were found to bind to seven-transmembrane G-protein-coupled receptors, orexin receptor 1 (OX_1R) and orexin receptor 2 (OX_2R).[1] Both orexin-A and -B are proteolytically derived from a common precursor protein, prepro-orexin peptide, produced in orexin neurons located in the lateral hypothalamus. OX_1R and OX_2R are widely distributed, including the basal forebrain, limbic structures, and brainstem regions, and have partially overlapping but distinct distributions throughout the brain, suggesting that each receptor subtype may have different physiological roles through different neuronal pathways.[3] The orexin system has been implicated in sleep-wake regulation, energy homeostasis, feeding,

and reward processing.[4] The contribution of OXRs to sleep-wake regulation has been studied using orexin receptor-knockout mice, and OX_2R reportedly plays a larger role than OX_1R.[5–6] Experiments using receptor-selective antagonists suggest that selective OX_2R antagonists (2-SORAs) induce sleep, whereas selective OX_1R antagonists (1-SORAs) did not influence sleep architectures.[7–8] However, emerging literature has reported that dual orexin receptor antagonists (DORAs) might be more effective for sleep promotion than 2-SORA,[9] and OX_1R antagonism has a subtle role in vigilance state gating, which is related to sleep onset, and influences stage transitions.[10] Additionally, OX_1R may have a role in the treatment of psychiatric disorders, such as stress and anxiety-related behaviors.[11–12]

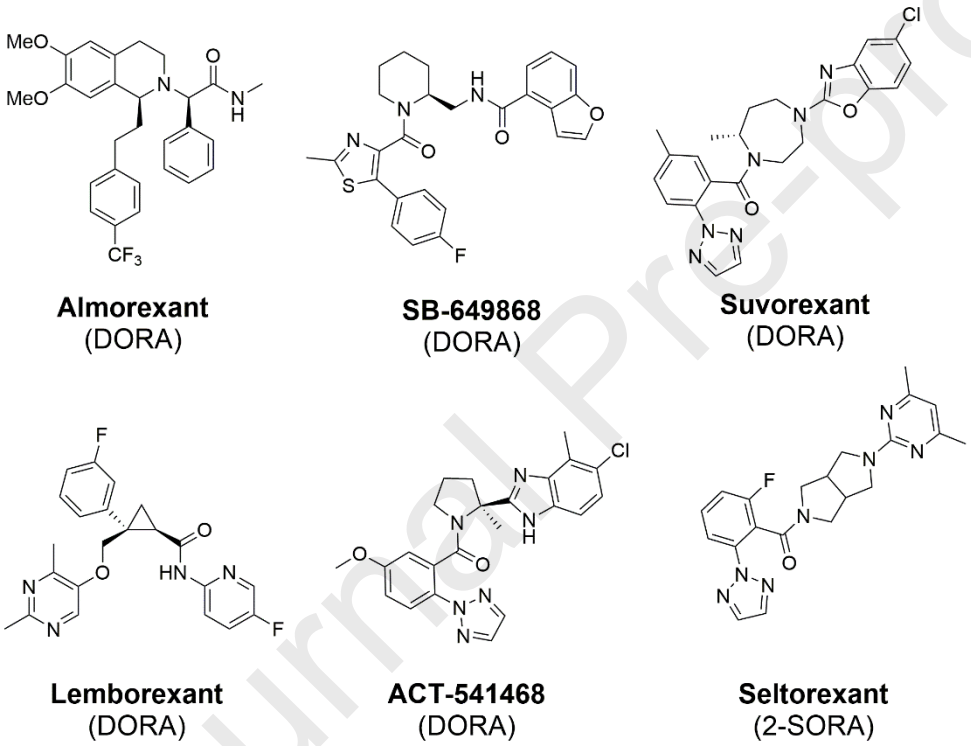


Figure 1. Representative DORAs and 2-SORA.

DORAs, as well as 2-SORAs, have been studied as new hypnotics (Figure 1). Almorexant[13], SB-649868[14], Suvorexant[15] and Lemborexant[16] are DORAs for which proof-of-concepts for the treatment of insomnia in humans have been completed. Moreover, Suvorexant and Lemborexant have received approval from the Food and Drug Administration (FDA). ACT-541468 is presently being used in ongoing clinical activities, and it reportedly has a short half-life in humans (approximately 6 h).[17] Seltorexant, a representative 2-SORA, reportedly displays favorable pharmacodynamic effects on sleep induction in addition to having a short half-life (approximately 2 h).[18]

In a regulatory review, the FDA has raised concerns about potential next-day residual effects associated with high doses of Suvorexant (a plasma half-life of approximately 12 h), and the FDA-approved maximum dose of Suvorexant is 20 mg once daily.[19] This prompted us to explore compounds with optimal pharmacokinetic (PK) profiles, such as rapid absorption and a favorable short half-life, to avoid the risks of long-acting sleep agents with next-day residual effects.

Previously, we reported a new chemical class of DORAs with a pyrazolylethylbenzamide structure (Figure 2).[20] These DORAs were identified through the optimization of pharmacophore A using a ligand-based drug design approach and retaining the characteristic U-shaped conformation.[21] Among this chemical class, compound **1** had modest *in vitro* antagonistic activities for OX_1R and OX_2R, while it was estimated to have an optimal PK profile with a rapid T_{max} and a short half-life (predicted human $T_{1/2}$: 1.2–1.4 h), which was a development target, based on animal PK data. In a rat sleep study using polysomnography (PSG), compound **1** showed a marginal *in vivo* efficacy, which was consistent with its moderate *in vitro* potency. On the other hand, compound **2** was identified as the most potent DORA among this class; unfortunately, it also had a slightly higher lipophilicity.

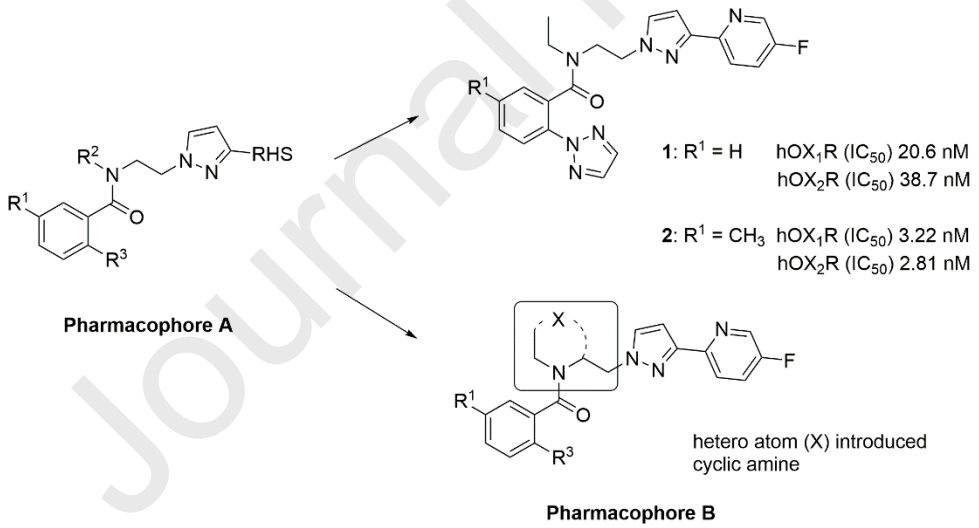


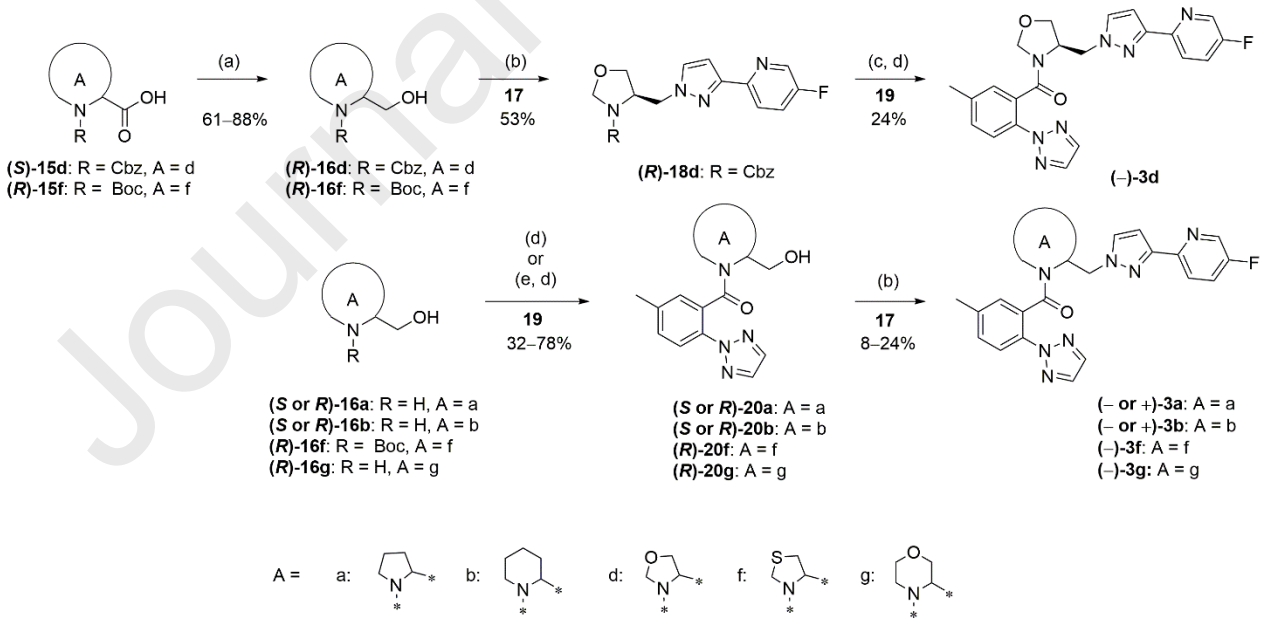
Figure 2. Design of pharmacophore **B**.

Cyclic amine derivatives, such as SB-649868 (Figure 1), have been known to exhibit a high activity for OX_1R and OX_2R, however these derivatives have relatively high lipophilicity. Based on pharmacophore A, construction of a ring (as SB-649868) and insertion of an additional heteroatom into the resulting ring was designed, to maintain an

active conformation and to lower the lipophilicity of the molecule (Pharmacophore B in Figure 2). This effort led to the discovery of orexin 1 and 2 receptor antagonists, which were 3-benzoyl-1,3-oxazinane derivatives. Herein, we report the design, synthesis, and structure-activity relationship of compounds with unique heterocyclic core ring structures. We also provide the detailed PK profiles and results of PSG sleep studies for the clinical candidate **(−)-3h**.

Chemistry

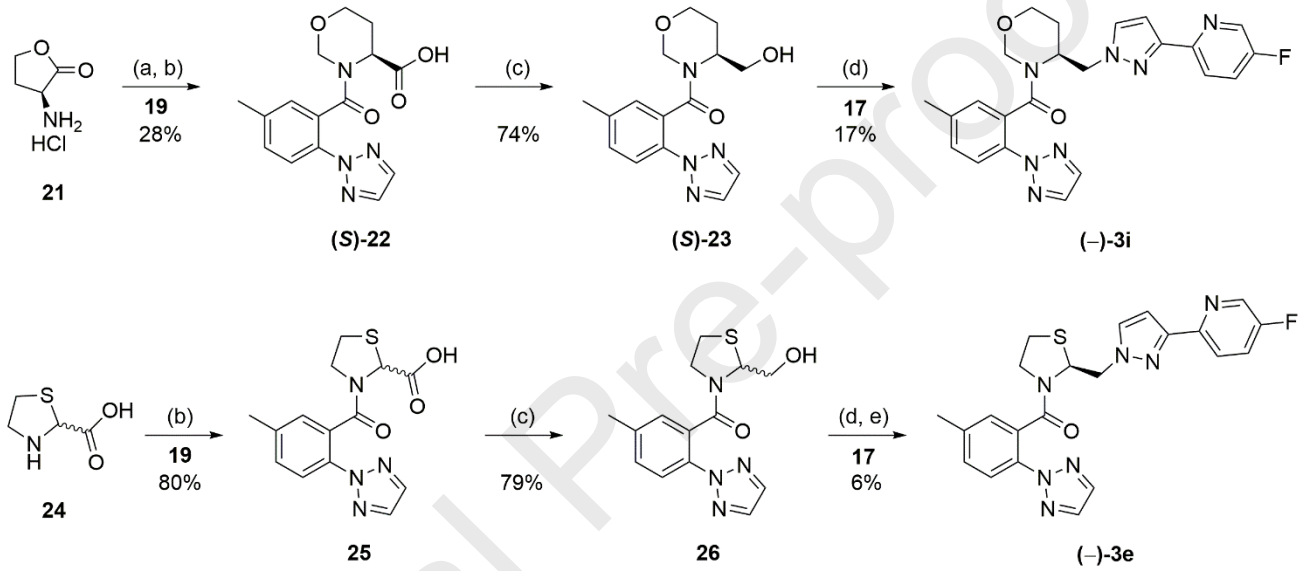
Scheme 1 summarizes the synthetic routes of **(− or +)-3a**, **(− or +)-3b**, **(−)-3d**, **(−)-3f**, and **(−)-3g**. Alcohol **(*R*)-16d**, obtained from the reduction of mixed anhydride (prepared *in situ* from commercially available carboxylic acid **(*S*)-15d** with $NaBH_4$, was converted into mesylate, which was subsequently alkylated with 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine **17** to yield **(*R*)-18d**. The deprotection of the Cbz group in **(*R*)-18d** followed by the amidation of the resulting secondary amine with 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl chloride **19** yielded **(−)-3d**. Compounds **(− or +)-3a**, **(− or +)-3b**, **(−)-3f**, and **(−)-3g** were prepared in a manner similar to that of **(−)-3d** but using **(*S* or *R*)-16a**, **(*S* or *R*)-16b**, **(*R*)-16f**, and **(*R*)-16g** as a starting material.



Scheme 1. Reagents and conditions: (a) isobutyl chloroformate, 4-methylmorpholine, then $NaBH_4$, THF-H_2O, -10 °C ; or BH_3-THF, THF, 0°C–rt; (b) (1) MsCl, Et_3N, $CHCl_3$, 0°C, (2) 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine **17**, *t*-

BuONa, DMSO, 80°C; or **17**, Cs_2CO_3, DMF, 80°C; (c) $Pd(OH)_2/C$, H_2, Et_3N, THF, rt; (d) 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl chloride **19**, Et_3N, $CHCl_3$, 0°C; (e) 4M HCl-EtOAc, EtOAc, rt.

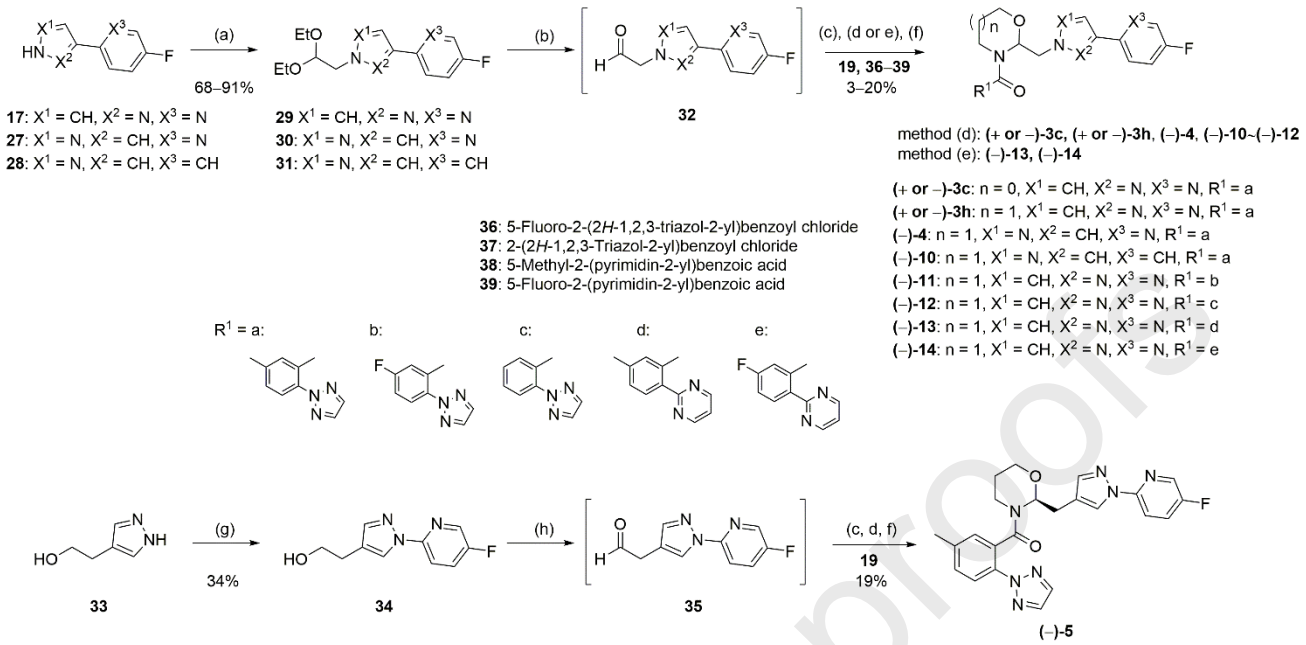
Compounds **(−)-3e** and **(−)-3i** were synthesized as shown in Scheme 2. Homoserine lactone hydrochloride **21** was treated with aqueous formaldehyde solution under an acidic condition to form (4*S*)-1,3-oxazinane-4-carboxylic acid, which was acylated with **19** to yield **(*S*)-22**. Compounds **(−)-3i** and **(−)-3e** were prepared from **(*S*)-22** and **25** in a similar manner as described in Scheme 1.



Scheme 2. Reagents and conditions: (a) formaldehyde (37% in water), 1M hydrochloric acid, H_2O, rt; (b) **19**, Et_3N, $CHCl_3$, 0°C; (c) isobutyl chloroformate, 4-methylmorpholine, then $NaBH_4$, THF-H_2O, -10°C; (d) (1) MsCl, Et_3N, $CHCl_3$, 0°C, (2) **17**, *t*-BuONa, DMSO, 80°C; (e) chiral separation using HPLC.

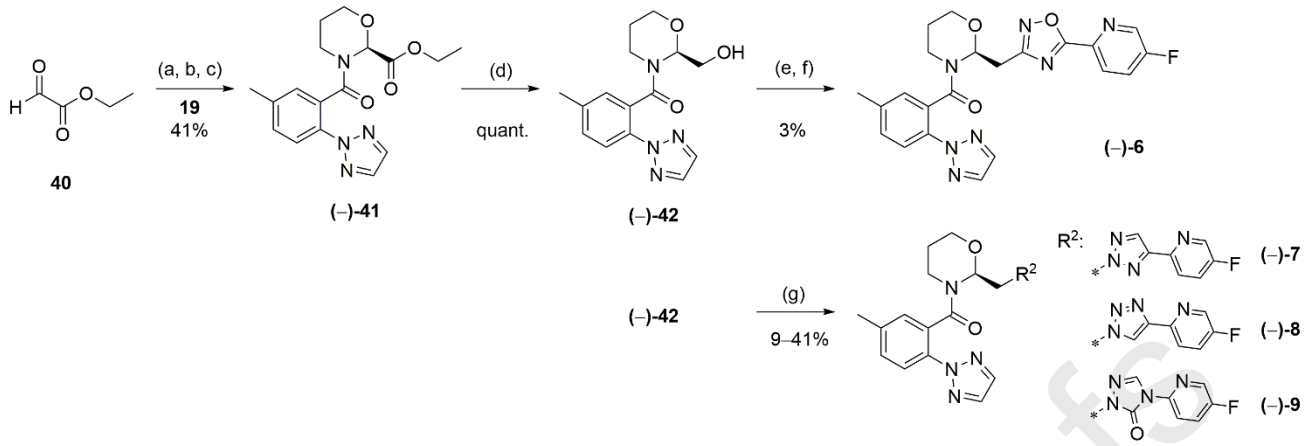
Oxazolidine and oxazinane derivatives with a pyrazole moiety [**(− or +)-3c**, **(− or +)-3h**, **(−)-4**, **(−)-5** and **(−)-10**–**(−)-14**] were synthesized as depicted in Scheme 3. The cyclic hemiaminal frameworks of these compounds were constructed by reacting **32** or **35** with the corresponding aminoalcohols in the presence of molecular sieves, and the subsequent amidation of the secondary amine moieties with the corresponding benzoic acids or benzoyl chlorides yielded the target compounds (**3c**, **3h**, **4**, **5** and **10**–**14**). Optical resolution of the racemates using chiral stationary phase HPLC yielded the corresponding chiral compounds. Compound **32** (for **3c**, **3h**, **4** and **10**–**14**) was prepared *via* the alkylation of **17**, **27**, and **28** with bromoacetaldehyde diethyl acetal followed by hydrolysis under acidic conditions. Compound **35** (for **5**) was synthesized starting from the *N*-arylation of **33** with 2,4-difluoropyridine, followed by

oxidation with 2-iodoxybenzoic acid (IBX).



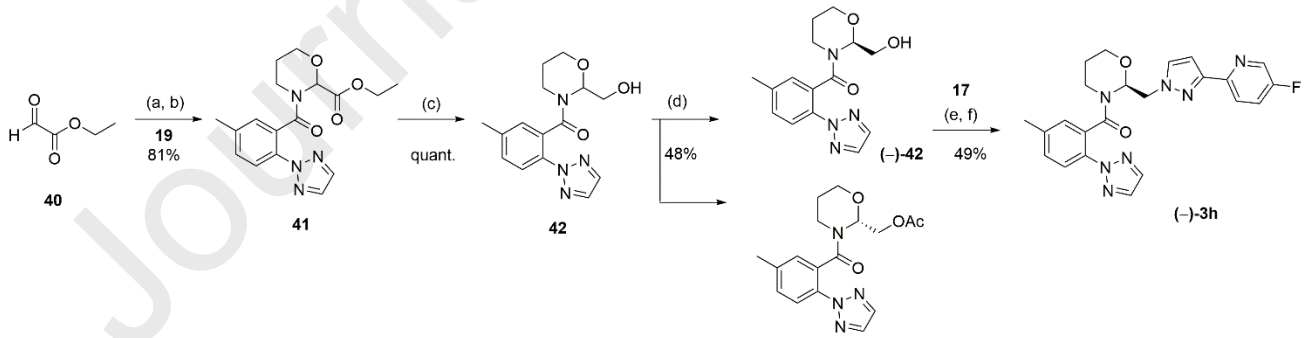
Scheme 3. Reagents and conditions: (a) bromoacetaldehyde diethylacetal, Cs_2CO_3, DMF, 80°C; (b) TFA, $CHCl_3$, rt; (c) 3-aminopropan-1-ol or 2-aminoethanol, molecular sieves 4A, $CHCl_3$, rt; (d) R^1-COCl (**19**, **36**, **37**), Et_3N, $CHCl_3$, 0 °C–rt; (e) R^1-CO_2H (**38**, **39**), T3P®, DIPEA, $CHCl_3$; (f) chiral separation using HPLC; (g) 2,5-difluoropyridine, Cs_2CO_3, MeCN, 80°C; (h) IBX, DMSO, rt.

Scheme 4 outlined the synthetic routes of 1,3-oxazinan-2-yl derivatives **(−)-6**–**(−)-9**, which were prepared from the chiral intermediate **(−)-42**. The 1,3-oxazinane ring was constructed by the cyclization of ethyl glyoxylate and 3-aminopropan-1-ol in the presence of molecular sieves, followed by the acylation of the amine moiety with **19** to give the racemate **41**. Chiral separation of the racemate using the chiral preparative HPLC method yielded **(−)-41**, which was reduced with $NaBH_4$ to produce the primary alcohol **(−)-42**. Conversion of the alcohol moiety in **(−)-42** into the 5-(5-fluoropydin-2-yl)-1,2,4-oxadiazol-3-ylmethyl group in **(−)-6** was conducted using the conventional 4-step reaction sequence *via* cyanation of the alcohol followed by the condensation of the hydroxyamidine with 5-fluoropyridine-2-carboxylic acid procedures. Compounds **(−)-7**–**(−)-9** were synthesized from **(−)-42** by alkylation with 1,2,3-trazole **43** and 1,2,4-triazol-4-one **44** in a similar procedure described above.



Scheme 4. Reagents and conditions: (a) 3-aminopropan-1-ol, molecular sieves 4A, $CHCl_3$, rt; (b) **19**, Et_3N, $CHCl_3$, 0 °C; (c) chiral separation using HPLC; (d) $NaBH_4$, MeOH, rt; (e) (1) MsCl, Et_3N, $CHCl_3$, 0 °C, (2) NaCN, DMF, 80°C; (f) (1) NH_2OH aq., EtOH, 80°C, (2) 5-fluoropyridine-2-carboxylic acid, 1,1'-carbonyldiimidazole (CDI), DMF, 40–80°C; (g) (1) MsCl, Et_3N, $CHCl_3$, 0°C, (2) R^2H (**43** or **44**), *t*-BuONa, DMSO, 80°C.

The synthetic procedures used to prepare **(−)-3h** in bulk for its preclinical studies are shown in Scheme 5. In the asymmetric synthesis of the key intermediate **(−)-42**, the enzymatically enantioselective acetylation of **(+)-42** using lipase from porcine pancreas (PPL) was accomplished in the presence of vinyl acetate to yield **(−)-42** in high enantiomeric excess (>99.5% *ee*). The following alkylation with **17** and EtOAc stallization in ethanol provided enantiomerically pure **(−)-3h**.



Scheme 5. Reagents and conditions: (a) 3-aminopropan-1-ol, molecular sieves 4A, $CHCl_3$, rt; (b) **19**, Et_3N, $CHCl_3$, 0°C; (c) $NaBH_4$, MeOH, rt; (d) PPL, vinyl acetate, MTBE; (e) (1) MsCl, Et_3N, $CHCl_3$, 0°C, (2) **17**, *t*-BuONa, DMSO, 80°C; (f) Recrystallization from EtOH, rt–1°C.

The absolute stereo-chemical structure of **(−)-3h** was determined using its X-ray crystallography, and the absolute

configurations of other compounds were tentatively assigned by comparing their optical rotations with that of **(−)-3h**. We found that the (−)-enantiomer was the active form by assessing the inhibitory activities of both enantiomers of **3a**–**3c**, **3h**, **4**, **10**, and **12**.

Results and Discussion

To explore the SARs of the compounds, the antagonist activities (IC_{50}s) against OX_1R and OX_2R were obtained using functional assays in which intracellular Ca^{2+} mobilization was measured in CHO-K1 cells overexpressing the human orexin receptors. In addition, the metabolic stability in human liver microsomes (hMS) and the efflux ratio (ER) of P-glycoprotein (P-gp) were evaluated for each compound. Also, the $LogD_{7.4}$ value of each compound was assessed using high-performance liquid chromatography ($LogD_{HPLC}$) as an indicator of lipophilicity.[22]

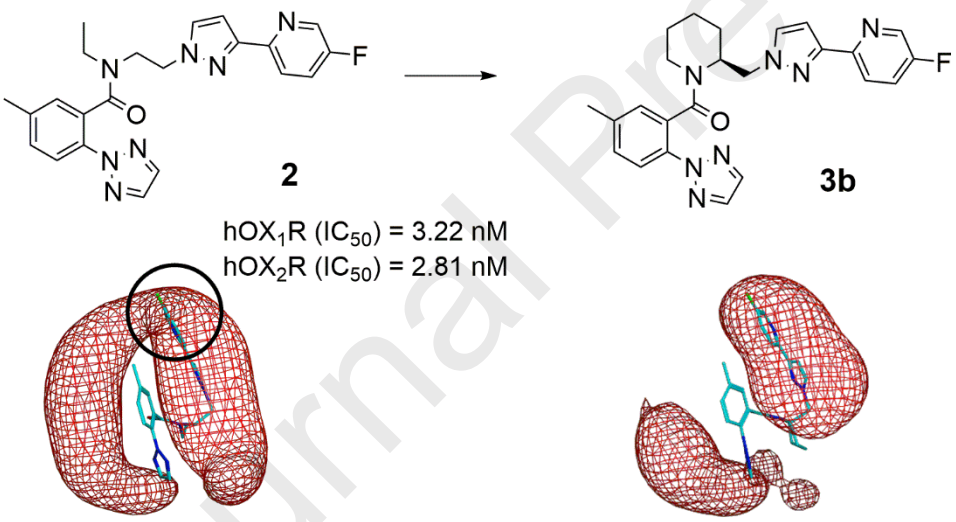


Figure 3. Conformational analysis of the derivatives.

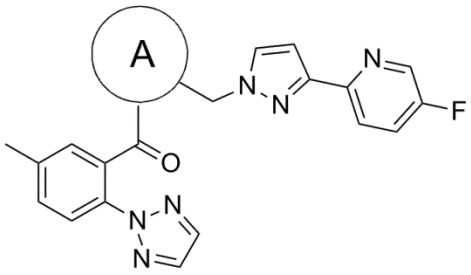
The red meshes show the distribution of the pyridine moiety under the alignment of the 5-tolyl triazole substructures. The black circles show the approximate positions of the pyridine moieties in the U-shape conformations. Conformations of the compounds were calculated using the replica-exchange molecular dynamics (REMD) simulation.[22]

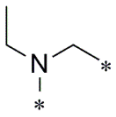
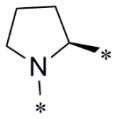
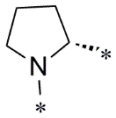
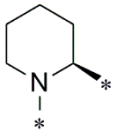
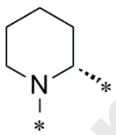
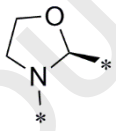
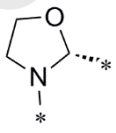
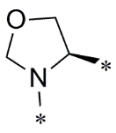
As stated in the Introduction, we have been focusing on the development of a potent and orally bioavailable DORA that possesses an optimal PK profile, including rapid absorption and a favorable short half-life. To achieve this goal,

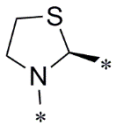
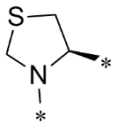
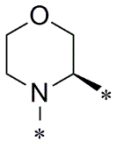
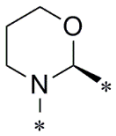
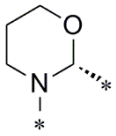
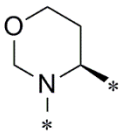
we aimed to enhance the antagonistic activity without increasing the lipophilicity of the linear compound (e.g., compound **1** and **2** in Figure 2), as a lower lipophilicity reportedly indicates a lower Vd, resulting in a short half-life.[20] At first, to maintain an active conformation, cyclization of the central core (as SB-649868 in Figure 1) was considered based on pharmacophore A. Conformational analysis revealed that the compound having a pyridyl-pyrazolyl-methyl side chain in its piperidine ring had a U-shape conformation, which is expected to contribute to the active conformation (**3b** in Figure 3). From these results, we initially built a simple cyclic amine motif, such as pyrrolidine or piperidine, and evaluated the functional activities for OX_1R and OX_2R of the resulting compounds [**(−)-3a, (+)-3a, (−)-3b, (+)-3b**] (Table 1). The enantiomers [**(−)-3a** and **(−)-3b**] with an (S)-configuration showed more potent inhibitory activities than those of the corresponding (R)-enantiomers [**(+)-3a** and **(+)-3b**]. The cyclic amine showed a potent antagonistic activity with single-digit, nano-molar potencies, as in **(−)-3b**, but also increased lipophilicity, with **(−)-3b** showing the most susceptible metabolic stability among these compounds (Table 1).

Next, a carbon atom on the pyrrolidine or piperidine core ring of **(−)-3a** and **(−)-3b** was replaced with a heteroatom, such as an oxygen or sulfur atom, to reduce lipophilicity.[23–24] Unfortunately, the 5-membered 1,3-oxazolidine rings [**(−)-3c** and **(−)-3d**] decreased the functional activities of both OX_1R and OX_2R, while they contributed to lower lipophilicity, to some extent, when compared with the pyrrolidine ring [**(−)-3a**]. In contrast, the 1,3-thiazolidine rings [**(−)-3e** and **(−)-3f**] did not show any virtues in either functional activity or lipophilicity. In the case of a 6-membered morpholine ring [**(−)-3g**], an approximately 10-fold reduction in the antagonist potency for OX_2R was observed, compared with that of piperidine **(−)-3b**. On the other hand, the 6-membered 1,3-oxazinane motifs [**(−)-3h** and **(−)-3i**] were well tolerated in terms of the antagonist activities for both OX_1R and OX_2R; in particular, **(−)-3h** was about 3-fold more potent than **(−)-3i** (Table 1).

Table 1. SAR of amino core A



Compound	A	IC_{50} (nM)[a]		$LogD_{HPLC}$[b]	hMS(%)[c]	P-gp (ER)
		OX_1R	OX_2R			
2		3.22	2.81	2.78	20.2	1.51
(−)-3a		3.30	3.15	3.08	37.2	1.96
(+)-3a		363	313	3.06	NT	NT
(−)-3b		2.27	1.64	2.96	51.9	1.63
(+)-3b		20.8	52.4	2.95	NT	NT
(−)-3c		8.08	12.1	2.67	15.7	1.21
(+)-3c		2690	2320	2.68	NT	NT
(−)-3d		149	133	2.52	18.0	1.65

(−)-3e		61.1	2300	3.51	53.0	1.34
(−)-3f		4.60	13.1	3.08	73.7	1.13
(−)-3g		3.50	15.0	2.33	24.5	3.56
(−)-3h		1.05	1.27	2.58	34.4	2.43
(+)-3h		344	274	2.56	NT	NT
(−)-3i		3.36	3.41	2.39	24.1	3.08

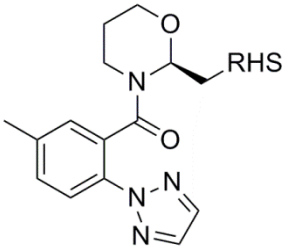
[a]The experiments were performed two to five times, and each test was performed in duplicate or triplicate. [b]LogD at pH 7.4. [c]Metabolized % after 15 min incubation in human liver microsomes (0.25 mg protein/mL, 1 μmol/L in DMSO).

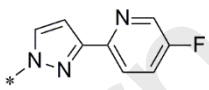
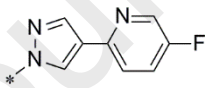
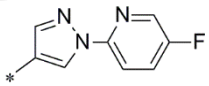
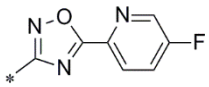
Interestingly, **(−)-3h** retained highly potent antagonistic activities despite a lower lipophilicity, compared with the parent piperidine **(−)-3b**. Moreover, **(−)-3h** had the lowest P-gp ER value (2.43) among the oxygen-inserted heterocyclic compounds [**(−)-3g**, **(−)-3h**, and **(−)-3i**]. While 1,3-oxazinane **(−)-3h** has a hemiaminal ether structure, fortunately there was no problem with its chemical stability under acidic conditions (JP 1st fluid (pH 1.2), 37°C, 24h). Since **(−)-3h** was one of the most potent DORAs in this class and had a relatively low lipophilicity and well-balanced metabolic stability (hMS), we were motivated to conduct further optimization (Table 1).

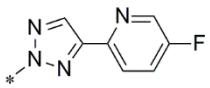
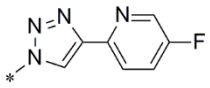
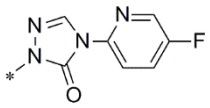
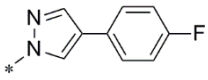
Optimization of the right hand side (RHS) of **(−)-3h** was performed, focusing on the internal 5-membered heterocycle between the 1,3-oxazinan-2-ylmethyl and 5-fluoropyridin-2-yl moieties, including pyrazole [**(−)-4**, **(−)-**

5; regioisomers of **(−)-3h**], oxadiazole [**(−)-6**], triazole [**(−)-7**, **(−)-8**] and triazolone [**(−)-9**] (Table 2). Compounds **(−)-4**–**(−)-7** were potent DORAs with single-digit, nano-molar potencies, while antagonistic activities of these compounds were lower than that of **(−)-3h**. Compound **(−)-4** had a lower lipophilicity ($LogD_{HPLC,}$ 2.38), but **(−)-4** had a higher P-gp ER value than **(−)-3h** [**(−)-4**: 3.54, **(−)-3h**: 2.43]. The replacement of the 4-fluoropyridine of **(−)-4** with a lipophilic 4-fluorobenzene led to a potent DORA [**(−)-10**] without any P-gp issues; however, further evaluations of **(−)-10** suggested concerns in terms of both the time-dependent inhibition of CYP 3A4 and its binding affinity to a hERG K^+ channel, which probably occurred because of a high lipophilicity.[25] From these results, we confirmed that 3-(5-fluoropyridin-2-yl)-1*H*-pyrazole was the most suitable RHS moiety.

Table 2. SAR of RHS



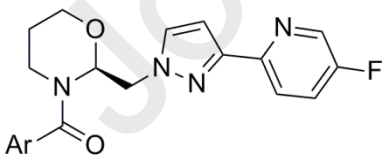
Compound	RHS	IC_{50} (nM)[a]		$LogD_{HPLC}$[b]	hMS(%)[c]	P-gp (ER)
		OX_1R	OX_2R			
(−)-3h		1.05	1.27	2.58	34.4	2.43
(−)-4		5.39	2.21	2.38	15.8	3.54
(−)-5		3.98	2.03	3.03	29.7	1.56
(−)-6		4.54	3.60	2.53	19.5	3.06

(−)-7		2.90	1.88	2.80	16.4	1.38
(−)-8		12.1	3.07	2.38	4.3	4.97
(−)-9		85.5	5.40	2.52	15.6	2.88
(−)-10		2.21	0.784	3.15	24.8	1.35

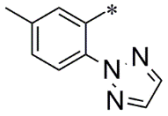
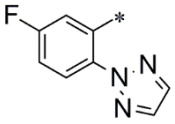
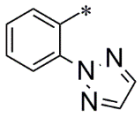
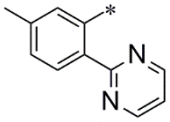
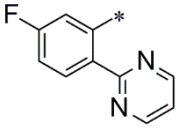
[a]The experiments were performed two to five times, and each test was performed in duplicate or triplicate. [b]LogD at pH 7.4. [c]Metabolized % after 15 min incubation in human liver microsomes (0.25 mg protein/mL, 1 μmol/L in DMSO).

Finally, we optimized the acyl moiety of **(−)-3h** as described in Table 3. Replacement of the 5-methyl-2-triazolylbenzamide in **(−)-3h** with 5-fluoro-2-triazolylbenzamide [**(−)-11**], 2-triazolylbenzamide [**(−)-12**], 5-fluoro-2-pyrimidinylbenzamide [**(−)-13**], or 5-methyl-2-pyrimidinylbenzamide [**(−)-14**] was well tolerated in terms of the OX_1R and OX_2R potency, while these replacements led to an increase in the P-gp susceptibility. Taking these results into consideration, we selected compound **(−)-3h** for further evaluation.

Table 3. SAR of acyl moiety



Compound	Ar	IC_{50} (nM)[a]		$LogD_{HPLC}$[b]	hMS(%)[c]	P-gp (ER)
		OX_1R	OX_2R			

(−)-3h		1.05	1.27	2.58	34.4	2.43
(−)-11		3.95	1.96	2.46	43.2	2.64
(−)-12		3.76	2.62	2.30	14.6	3.25
(−)-13		3.29	3.18	2.36	26.3	6.74
(−)-14		3.22	4.58	2.30	28.9	5.36

[a]The experiments were performed two to five times, and each test was performed in duplicate or triplicate. [b]LogD at pH 7.4. [c]Metabolized % after 15 min incubation in human liver microsome (0.25 mg protein/mL, 1 μmol/L in DMSO).

Pharmacokinetic parameters of **(−)-3h** in male rats and dogs are shown in Table 4. The intravenous administration (*iv*) of **(−)-3h** exhibited a plasma clearance of 2370 and 159 mL/h/kg and V_d values of 853 and 294 mL/Kg (rats and dogs), which were consistent with both microsomal stability results (rat: 51.0%, dog: 10.5%)[26] and the plasma protein binding (PPB) ratios. The plasma half-lives were as short as 0.238 h for rats and 1.16 h for dogs. For oral administration, a rapid T_{max} of 0.333 h and 0.583 h with oral bioavailabilities (*F*) of 5.5% and 61.6% were observed in rats and dogs, respectively. Regarding cerebral exposure, we evaluated the brain penetration (B/P: 13.5%) and cerebrospinal fluid penetration (CSF/P: 3.1%) after oral administration to rats (3 mg/kg). The concentration of **(−)-3h** reached 2.23 nM in CSF at 1 h, suggesting that **(−)-3h** had an acceptable brain penetrability enabling exposure above the IC_{50} to be attained. Based on these PK profiles, the efficacy of **(−)-3h** was next evaluated.

Table 4. Pharmacokinetic parameters of **(−)-3h** in rats and dogs

species	*iv*				*po*					
	dose (mg/kg)	V_d (mL/Kg)	CL (mL/h/kg)	$T_{1/2}$ (h)	dose (mg/kg)	T_{max} (h)	B/P (%)[a]	CSF/P (%)[b]	*F* (%)[c]	PPB (%)[d]
rat	1	853	2370	0.238	3	0.333	13.5	3.1	5.5	88.0
dog	0.5	294	159	1.16	3	0.583	-	-	61.6	96.4

The pharmacokinetic study was conducted in fasted Sprague-Dawley rats (n = 3) and dogs (n = 3). [a]B/P (%) was defined as the brain/plasma ratio. [b]CSF/P (%) was defined as the cerebrospinal fluid/plasma ratio. [c]*F* (%) was compared between intravenous administration and oral administration. [d]PPB (%) was defined as plasma protein binding, conducted at 2μg/mL.

Given the results of appropriate brain exposure and half-life in rats, the sleep-promoting effect of compound **(−)-3h** was evaluated using PSG (Figure 4). The vehicle or compound **(−)-3h** at a dose of 1, 3, or 10 mg/kg was orally administered to rats prior to turning the light off (start of the active phase). By measuring the sleep latency until sleep lasting 120 seconds or more, a dosing of **(−)-3h** at 1–10 mg showed a fast onset of action. Furthermore, a dose-dependent increase in the percentage of total sleep was observed. These data suggested that compound **(−)-3h** possessed promising sleep-inducing and sleep-promoting effects.

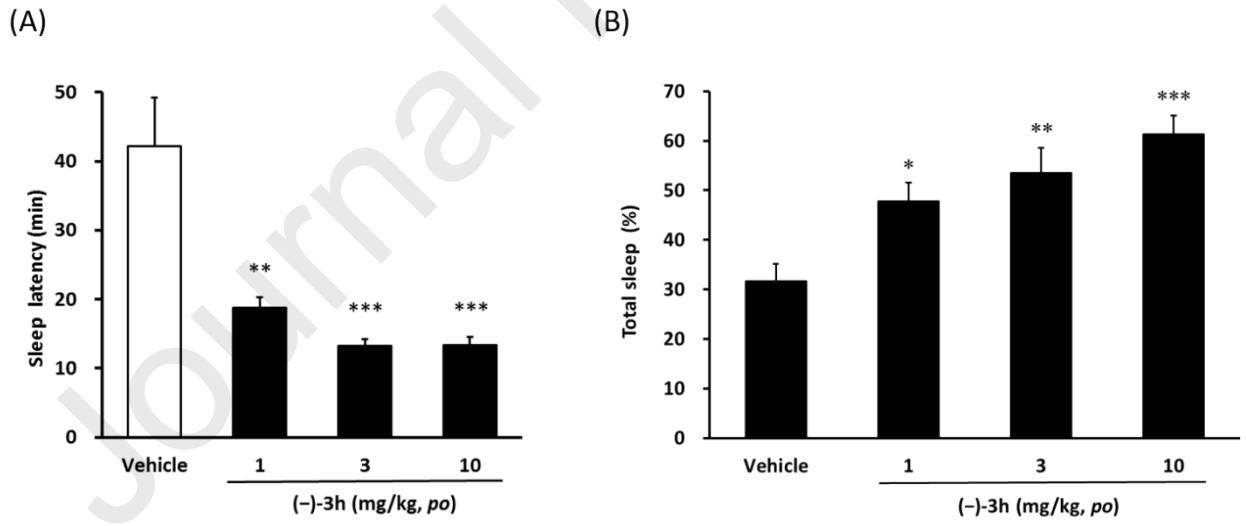
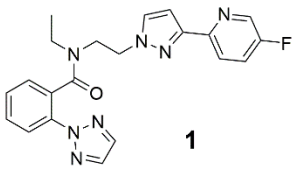
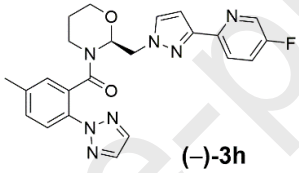


Figure 4. Effects of compound **(−)-3h** on sleep parameters in a rat PSG study.
The sleep-promoting effects on the sleep latency (A) and the percentage of total sleep (B) for the 2 h period after the oral administration of the vehicle or compound **(−)-3h** are shown. Values are shown as the mean and SEM (n = 10 animals/group). **P* <0.05, ***P* <0.01, ****P* <0.001 versus the vehicle-treated group, based on the Bartlett's test followed by the Dunnett multiple comparison post-hoc test.

Table 5 summarizes the profiles of prototype **1** and **(−)-3h**. During the course of the generation of **(−)-3h**, the 1,3-oxazinane ring in the central core played critical roles in both the high antagonistic activities for the orexin receptors and the low lipophilicity. These findings demonstrated that **(−)-3h** had both a strong pharmacological activity in animal models and a short plasma half-life because of its low Vd. Thus, **(−)-3h** could be a useful hypnotic with a low risk of next-day drowsiness. For this reason, **(−)-3h** was selected as the candidate compound ORN0829 (investigation code name, TS-142) and was used for further clinical evaluations.

Table 5. Profiles of compounds **1** and **(−)-3h**

Compound	 **1**	 **(−)-3h**
hOX_1R (IC_{50}, nM)	20.6	1.05
hOX_2R (IC_{50}, nM)	38.7	1.27
rOX_1R (Kb, nM)[a]	5.08	0.44
rOX_2R (Kb, nM)[a]	6.06	0.80
$LogD_{HPLC}$ (pH 7.4)	2.53	2.58
	rat: dog:	rat: dog:
Vd (mL/kg)[b]	1710 745	853 294
CL (mL/h/Kg)[b]	3960 598	2370 159
$T_{1/2}$ (h)[b]	0.412 1.07	0.238 1.16
Predicted human[c]	from rat: from dog:	from rat: from dog:
$T_{1/2}$ (h)	1.1 1.4	0.9 2.0
Rat PSG model MED	3 mg/kg *ip*	1 mg/kg *po*

[a]The K_b values are the mean of multiple results (at least three independent determinations performed in duplicate) with the standard error of the means. [b]The pharmacokinetic study was conducted in fasted Sprague-Dawley rats (n = 3) and dogs (n = 3) dosed at 2 mg/kg and 0.5 mg/kg for intravenous dosing. [C]sss method.

Conclusion

In conclusion, we identified 3-benzoyl-1,3-oxazinane **(−)-3h** as a highly potent DORA through iterative design and compound optimization based on the structural framework of pyrazolylethylbenzamide **1**, keeping the high antagonist activity, low lipophilicity and short half-life PK profile in mind. The unique structure of **(−)-3h**, an 1,3-oxazinane moiety, may play key roles in the formation of its bioactive U-shaped conformation and in lowering the lipophilicity. Compound **(−)-3h** exhibited potent sleep-promoting effects at a *po* dose from 1 mg/kg in a rat polysomnogram study, and optimal PK properties with rapid T_{max} and short half-lives were observed in rats and dogs, indicating a predicted human half-life of 0.9–2.0 h. Given the preclinical profiles as a useful hypnotic with a low risk of next-day drowsiness, **(−)-3h** (**ORN0829**; investigation code name, TS-142) was selected as a candidate compound and is currently in clinical development toward the treatment of insomnia.

Experimental section

Biology

Antagonistic activity against human and rat OX_1R and OX_2R

The antagonistic activities of the test compounds were determined as described previously.[20] Chinese hamster ovary (CHO)-K1 cells stably expressing recombinant human OX_1R, human OX_2R, rat OX_1R or rat OX_2R were seeded into a 96-well black/clear bottom plate at a density of 2.4×10^4 cells/well one day before the experiment. The cells were incubated with loading buffer (Hank's balanced salt solution [HBSS] [pH7.4] containing 20 mM HEPES, 0.1% BSA, 0.2 mg/ml amaranth, 2.5 mM probenecid, 0.01% pluronic F-127 and 0.5 μM of Fluo-4AM [Invitrogen]) at 37°C for 1 h. After the loading buffer was removed, the cells were incubated with assay buffer (HBSS [pH7.4] containing 20 mM HEPES, 0.1% BSA, 0.2 mg/ml amaranth, and 2.5 mM probenecid) with or without various concentrations of the test compounds for 30 min at room temperature. The changes in the intracellular Ca^{2+} concentration were determined by monitoring the changes in fluorescence using a Functional Drug Screening System (Hamamatsu

Photonics, Shizuoka, Japan) after the application of [Ala$^{6,\ 12}$]orexin-A (Peptide Institute, Osaka, Japan) (final concentration of 0.5 nM for human OX_1R, 1 nM for human OX_2R, 10 nM for rat OX_1R or 30 nM for rat OX_2R).

The concentration-response curves were fit using nonlinear regression analyses; the 50% inhibitory concentration (IC_{50}) was calculated using GraphPad Prism software (version 5.04; GraphPad Software Inc., San Diego, CA). The equilibrium dissociation constant (Kb) values were calculated according to the equation "Kb = IC_{50}/{1 + ([concentration of Ala-OXA used in the determination of IC_{50} value]/[50% effective concentration of Ala-OXA])}" using SAS (version 9.2; SAS Institute Japan Ltd., Tokyo, Japan). The experiments were performed two to five times, and each test was performed in duplicate or triplicate.

Effect on sleep-wake states during a dark period in rats

The effect of **(−)-3h** on sleep-wake states was evaluated during a dark period in rats. Male Wistar rats (10 rats/group, 10-12 weeks of age, Charles River Laboratories, Japan) that had been surgically implanted with electrodes for electroencephalogram (EEG) and electromyogram (EMG) studies were used. The rats were individually transferred to an acrylic chamber placed within an electrically shielded sound-proof box (light on: 5:00-17:00) 7-8 hours before the administration of **(−)-3h** to allow them to acclimate to the measurement environment. Rats were treated with **(−)-3h** at doses of 1, 3 and 10 mg/kg orally as a free base within 10 minutes prior to the onset of the dark period. The EEG and EMG recordings were continued for 6 hours after the administration. The sleep–wake states were classified every 20 seconds as wakefulness or sleep based on the EEG and EMG patterns. The rate of the sleep-wake state was calculated for 2 hours after administration. Sleep onset latency was defined as latency until the first sleep persisting for 120 seconds or more after administration. **(−)-3h** was suspended in 0.5% methyl cellulose 400 solution.

Chemistry

General methods

All the solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. The preparative HPLC purification conditions were as follows: Gilson preparative HPLC system; column Waters ODS sunfire, 50 mm × 30 mm; eluent A, water + 0.1% CF_3CO_2H; eluent B, CH_3CN + 0.1% CF_3CO_2H; 10% B up to 95% B in 12 min; flow rate 40 mL/min. Silica gel column chromatography was performed

using a Kanto 60 N silica gel or an appropriately sized pre-packed silica cartridge (Biotage® SNAP Ultra or SNAP KP-NH) on a Biotage system. Preparative supercritical fluid chromatography (SFC) was performed using a Waters SFC30 instrument. Preparative HPLC for the chiral compounds was performed using a Gilson preparative HPLC system. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL 600 MHz and 500 MHz NMR spectrometer, and all the chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS, δ 0.00 ppm) as an internal standard. High resolution mass spectral data were acquired using a Shimadzu LCMS–IT–TOF equipped with an ESI/APCI dual ion source. The final compounds exhibited ≥95% purity, as determined using HPLC and LC–MS on an Agilent instrument using electrospray ionization. The HPLC conditions were as follows: Shimadzu LC–20A; column Shim–Pack XR–ODS 2.2 μm, 3.0 mm × 75 mm; eluent A, water + 0.1% phosphoric acid; eluent B, CH_3CN; 1) 10% B in 4.0 min, 10%–90% B in 5.0 min, 90% B in 2.0 min, 2) 20% B in 4.0 min, 20%–90% B in 5.0 min, 90% B in 2.0 min or 3) 40% B in 4.0 min, 40%–90% B in 5.0 min, 90% B in 2.0 min; flow rate 0.8 mL/min; UV detection (λ = 210 nm). The LC–MS conditions were as follows: Agilent 1290 infinity and Agilent 6150; column Waters Acquity CSH C18, 1.7 μm, 2.1 mm × 50 mm; eluent A, water + 0.1% formic acid; eluent B, CH_3CN + 0.1% formic acid; 20%–99% B in 1.2 min, 99% B in 0.2 min; flow rate 0.8 ml/min; UV detection (λ = 254 nm). Optical rotations were measured using a Rudolph Research Analytical AUTOPOL V. Infrared spectroscopy (IR) was performed using a Shimadzu IRAffinity-1. A single crystal X-ray structure analysis was performed using Rigaku R-AXIS RAPID Ⅱ. The orexin receptor antagonists described in this paper exist in multiple conformations as a result of hindered rotations that were slow on the NMR timescale. The ^{1}H and ^{13}C NMR spectra of some compounds consisted of broad and complicated multiplets for which a detailed coupling constant was difficult to analyze. Thus, the NMR resonances of these compounds were not listed in numerical format. Instead, we included pictures of the ^{1}H and ^{13}C NMR spectra for these compounds at 25°C in the Supplementary Data. For compound **(−)-3h** only, the ^{1}H and ^{13}C chemical shifts were assigned based on an analysis of the COSY, HSQC, and HMBC spectra acquired for the sample dissolved in $CDCl_3$ at 25°C. Compounds **17**, **27**, and **28** were prepared according to previously reported procedures.[20]

Benzyl (4*R*)-4-(hydroxymethyl)-1,3-oxazolidine-3-carboxylate ((*R*)-16d)

4-Methylmorpholine (1.0 mL, 9.2 mmol) and isobutyl chloroformate (1.4 mL, 11 mmol) were successively added to a solution of (4*S*)-3-[(benzyloxy)carbonyl]-1,3-oxazolidine-4-carboxylic acid **(*S*)-15d** (2.1 g, 8.4 mmol) in 1,2-

dimethoxyethane (20 mL) at −15°C. The mixture was stirred at room temperature for 1 h and then filtered to remove a colorless solid. To the filtrate, a solution of $NaBH_4$ (0.47 g, 13 mmol) in water (10 mL) was added at −15°C within 30 min. The reaction mixture was stirred at −10°C for 1 h. The reaction was quenched by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with saturated NH_4Cl aqueous solution, saturated $NaHCO_3$ aqueous solution, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 20%–80% EtOAc in hexanes) to yield the title compound **(*R*)-16d** as a colorless oil (1.2 g, 61% yield). HRMS calcd for $C_{12}H_{15}NO_4$ $[M+H]^+$ 238.1074 found 238.1045; LC–MS t = 0.67 min, $[M+H]^+$ = 238. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.43 (m, 5H), 5.12–5.21 (m, 2H), 5.00 (br s, 1H), 4.81 (d, J = 4.12 Hz, 1H), 4.01–4.10 (m, 2H), 3.75–3.83 (m, 1H), 3.63–3.74 (m, 2H), 3.32–3.54 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.9, 128.6, 128.4, 128.1, 79.3, 69.4, 67.6, 63.8, 58.0.

***tert*-Butyl (4*R*)-4-(hydroxymethyl)-1,3-thiazolidine-3-carboxylate ((*R*)-16f)**

A borane-tetrahydrofuran complex (ca. 0.9 mol/L; 5.0 mL, 4.5 mmol) was added to a solution of (4*R*)-3-*tert*-butoxycarbonylthiazolidine-4-carboxylic acid **(*R*)-15f** (1.0 g, 4.3 mmol) in THF (6.1 mL) at 0°C. After stirring at 0°C for 5 min, the reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction was quenched by the addition of water, and the mixture was extracted using EtOAc. The combined organic layer was dried over Na_2SO_4 and then concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 30%–80% EtOAc in hexanes) to yield the title compound **(*R*)-16f** as a colorless oil (0.83 g, 88% yield). HRMS calcd for $C_9H_{17}NO_3S$ $[M+Na]^+$ 242.0821 found 242.0814; ^{1}H NMR (500 MHz, $CDCl_3$, 50 °C) δ 4.63 (d, J = 9.60 Hz, 1H), 4.31–4.39 (m, 1H), 4.26 (d, J = 9.61 Hz, 1H), 3.71 (t, J = 5.83 Hz, 2H), 3.14 (dd, J = 6.52, 11.66 Hz, 1H), 2.92 (br d, J = 9.61 Hz, 1H), 1.49 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 50 °C) δ 154.7, 81.5, 64.5, 61.9, 49.2, 33.2, 28.6; MS (ESI/APCI dual): *m/z* 220 $[M+H]^+$.

Benzyl (4*R*)-4-{[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazolidine-3-carboxylate ((*R*)-18d)

Et_3N (0.84 mL, 6.0 mmol) and methanesulfonyl chloride (0.37 mL, 4.8 mmol) were added to a solution of **(*R*)-16d**

(0.95 g, 4.0 mmol) in THF (10 mL) at 0°C. The mixture was stirred at room temperature for 1 h and then filtered through a pad of KC flock® (cellulose powder; Nippon paper group). The filtrate was concentrated under reduced pressure to yield benzyl (4*S*)-4-{[(methanesulfonyl)oxy]methyl}-1,3-oxazolidine-3-carboxylate. To a solution of the methanesulfonate in DMSO (10 mL) were added 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine **17** (0.85 g, 5.2 mmol) and *t*-BuONa (0.58 g, 6.0 mmol) at room temperature. The mixture was stirred at 90°C for 1 h. The reaction was quenched by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (KP-NH, 20%–80% EtOAc in hexanes) to yield the title compound **(*R*)-18d** as a pale yellow oil (0.81 g, 53% yield in 2 steps). HRMS calcd for $C_{20}H_{19}FN_4O_3$ $[M+H]^+$ 383.1514 found 383.1491; LC–MS t = 0.98 min, $[M+H]^+$ = 383. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(4*R*)-4-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3d)

Et_3N (0.55 mL, 3.9 mmol) and 20% $Pd(OH)_2$ on carbon (0.10 g) were added to a mixture of **(*R*)-18d** (0.58 g, 1.5 mmol) in THF (5.0 mL). The mixture was charged with H_2 and stirred at room temperature for 3 h. The reaction mixture was filtered through a pad of Celite® to yield a colorless solution of 5-fluoro-2-(1-{[(4*R*)-1,3-oxazolidin-4-yl]methyl}-1*H*-pyrazol-3-yl)pyridine. Thionyl chloride (0.095 mL, 1.3 mmol) and DMF (3 drops) were added to a mixture of 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (0.24 g, 1.2 mmol) in toluene (5.0 mL) at room temperature, and the mixture was stirred at 80°C for 1 h. The reaction mixture was cooled to room temperature, and the volatiles were distilled under reduced pressure to yield 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl chloride **19**. A solution of **19** in toluene (5.0 mL) was added dropwise at 0°C to a mixture of Et_3N (0.41 mL, 3.0 mmol) and the colorless solution of 5-fluoro-2-(1-{[(4*R*)-1,3-oxazolidin-4-yl]methyl}-1*H*-pyrazol-3-yl)pyridine obtained in the previous reaction. The mixture was allowed to warm to room temperature and then stirred for 1 h. The reaction was quenched by the addition of saturated $NaHCO_3$ aqueous solution, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The

resulting residue was purified by column chromatography (KP-NH, 10%–80% EtOAc in hexanes) to yield a mixture of the title compound **(−)-3d** and its region-isomer (0.17 g, 33% yield, **(−)-3d** : isomer = 17 : 1). The mixture (0.050 g, 0.12 mmol) was separated using preparative SFC (CHIRALPAK® AD-H column 2 cm * 25 cm, 40% MeOH/CO_2, flow rate = 30 mL/min), providing **(−)-3d** as a colorless amorphous substance (0.037 g, 74% yield). HRMS calcd for $C_{22}H_{20}FN_7O_2$ $[M+H]^+$ 434.1735 found 434.1723; LC–MS t = 0.91 min, $[M+H]^+$ = 434; $[\alpha]_D^{20}$ –65.6 (c 0.970, $CHCl_3$); IR (KBr, cm^{-1}) 825, 1111, 1226, 1427, 1495, 1651, 2877, 3118. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-(Hydroxymethyl)pyrrolidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*S*)-20a)

A solution of **19** (0.554 g, 2.46 mmol) and Et_3N (0.686 mL, 4.92 mmol) in $CHCl_3$ (5.00 mL) was added in a dropwise manner to a solution of L-prolinol **(*S*)-16a** (0.299 g, 2.95 mmol) in $CHCl_3$ (3.00 mL) at 0°C and within 15 min. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of saturated $NaHCO_3$ aqueous solution, and the mixture was extracted with $CHCl_3$. The organic layer was allowed to pass through an ISOLUTE® phase separator, and the filtrate was concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 20%–100% EtOAc in hexanes) to yield the title compound **(*S*)-20a** as a colorless oil (0.465 g, 66% yield). HRMS calcd for $C_{15}H_{18}N_4O_2$ $[M+H]^+$ 287.1503 found 287.1497; LC–MS t = 0.71 min, $[M+H]^+$ = 287; $[\alpha]_D^{26}$ –76.9 (c 0.775, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

Preparation of compounds (*R*)-20a, (*S* or *R*)-20b, (*R*)-20f, and (*R*)-20g

These compounds were synthesized from their corresponding precursors according to the procedure described for compound **(*S*)-20a**.

[(2*R*)-2-(Hydroxymethyl)piperidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*R*)-20a)

The title compound was prepared from D-prolinol **(*R*)-16a** (78% yield, colorless oil). $C_{15}H_{18}N_4O_2$ $[M+H]^+$ 287.1503 found 287.1486; LC–MS t = 0.71 min, $[M+H]^+$ = 287; $[\alpha]_D^{20}$ +70.5 (c 1.02, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

[(2*S*)-2-(Hydroxymethyl)piperidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*S*)-20b)

The title compound was prepared from [(2*S*)-piperidin-2-yl]methanol **(*S*)-16b** (32% yield, colorless solid). HRMS calcd for $C_{16}H_{20}N_4O_2$ $[M+H]^+$ 301.1659 found 301.1630; LC–MS t = 0.73 min, $[M+H]^+$ = 301. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

[(2*R*)-2-(Hydroxymethyl)piperidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*R*)-20b)

The title compound was prepared from [(2*R*)-piperidin-2-yl]methanol **(*R*)-16b** (41% yield, colorless solid). HRMS calcd for $C_{16}H_{20}N_4O_2$ $[M+H]^+$ 301.1659 found 301.1629; LC–MS t = 0.73 min, $[M+H]^+$ = 301. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

[(4*R*)-4-(Hydroxymethyl)-1,3-thiazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*R*)-20f)

Hydrogen chloride in EtOAc (4.0 mol/L, 7.33 mL, 29.3 mmol) was added to a solution of **(*R*)-16f** (0.643 g, 2.93 mmol) in EtOAc (7.00 mL) at 0°C, and the reaction mixture was stirred at room temperature for 2 h. The organic solvent was removed under reduced pressure to yield [(4*S*)-1,3-thiazolidin-4-yl]methanol hydrochloride as a colorless amorphous substance (0.345 g, 76% yield). The title compound was prepared from [(4*S*)-1,3-thiazolidin-4-yl]methanol hydrochloride according to the procedure described for compound **(*S*)-20a** (79% yield, colorless oil). HRMS calcd for $C_{14}H_{16}N_4O_2S$ $[M+H]^+$ 305.1067 found 305.1060; LC–MS t = 0.70 min, $[M+H]^+$ = 305; $[\alpha]_D^{20}$ −144 (*c* 1.15, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(3*R*)-3-(Hydroxymethyl)morpholin-4-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*R*)-20g)

The title compound was prepared from [(3*R*)-morpholin-3-yl]methanol hydrochloride **(*R*)-16g** (40% yield, colorless oil). HRMS calcd for $C_{15}H_{18}N_4O_3$ $[M+H]^+$ 303.1452 found 303.1431. LC–MS t = 0.57 min, $[M+H]^+$ = 303. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2S)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}pyrrolidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3a)

Et_3N (0.20 mL, 1.4 mmol) and methanesulfonyl chloride (0.082 mL, 1.1 mmol) were added to a solution of **(*S*)-20a** (0.27 g, 0.96 mmol) in THF (3.2 mL) at 0°C. The reaction mixture was stirred at 0°C for 30 min and then filtered through a pad of KC flock®. The filtrate was concentrated under reduced pressure to yield {(2*S*)-1-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]pyrrolidin-2-yl}methyl methanesulfonate as a colorless oil. 5-Fluoro-2-(1*H*-pyrazol-3-yl)pyridine **17** (0.16 g, 0.96 mmol) and Cs_2CO_3 (0.62 g, 1.9 mmol) were then added to a solution of the methanesulfonate in DMF (0.96 mL) at room temperature. The reaction mixture was stirred at 80°C for 2 h. The reaction was quenched by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 20%–80% EtOAc in hexanes) to yield a mixture of the title compound **(−)-3a** and its regioisomer (0.22 g, 63% yield in 2 steps, **(−)-3a** : regioisomer = 8 : 1). The mixture (0.22 g) was then separated using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 40% *i*PrOH in hexanes and a flow rate of 10 mL/min to yield the title compound **(−)-3a** as a colorless oil (0.093 g, 42% yield). HRMS calcd for $C_{23}H_{22}FN_7O$ $[M+H]^+$ 432.1943 found 432.1923; LC–MS t = 1.01 min, $[M+H]^+$ = 432; $[\alpha]_D^{26}$ –120 (c 1.05, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

Preparation of compounds (+)-3a, (− or +)-3b, (−)-3f, and (−)-3g

These compounds were synthesized from their corresponding precursors (**(*R*)-20a**, **(*S*)-20b**, **(*R*)-20b**, **(*R*)-20f**, and **(*R*)-20g**) according to the procedure as described for compound **(−)-3a**.

[(2*R*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}pyrrolidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((+)-3a)

The title compound was prepared from **(*R*)-20a** (10% yield in 2 steps, colorless amorphous substance). HRMS calcd for $C_{23}H_{22}FN_7O$ $[M+H]^+$ 432.1943 found 432.1924; LC–MS t = 1.01 min, $[M+H]^+$ = 432; $[\alpha]_D^{20}$ +106 (c 0.410, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at

25°C.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}piperidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3b)

The title compound was prepared from **(*S*)-20b** (20% yield in 2 steps, colorless solid). HRMS calcd for $C_{24}H_{24}FN_7O$ $[M+H]^+$ 446.2099 found 446.2080; LC–MS t = 1.02 min, $[M+H]^+$ = 446; $[\alpha]_D^{20}$ –35.6 (c 1.29, $CHCl_3$); IR (KBr, cm^{-1}) 821, 1225, 1457, 1496, 1623, 2933, 3101. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*R*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}piperidin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((+)-3b)

The title compound was prepared from **(*R*)-20b** (8.4% yield in 2 steps, colorless solid). HRMS calcd for $C_{24}H_{24}FN_7O$ $[M+H]^+$ 446.2099 found 446.2086; LC–MS t = 0.99 min, $[M+H]^+$ = 446; $[\alpha]_D^{20}$ +34.0 (c 0.659, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(4*R*)-4-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-thiazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3f)

The title compound was prepared from **(*R*)-20f** (17% yield in 2 steps, colorless amorphous substance). HRMS calcd for $C_{22}H_{20}FN_7OS$ $[M+H]^+$ 450.1507 found 450.1500; LC–MS t = 0.99 min, $[M+H]^+$ = 450; $[\alpha]_D^{20}$ –74.1 (c 0.800, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(3*R*)-3-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}morpholin-4-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3g)

The title compound was prepared from **(*R*)-20g** (24% yield in 2 steps, colorless amorphous substance). HRMS calcd

for $C_{23}H_{22}FN_7O_2$ $[M+H]^+$ 448.1892 found 448.1871; LC–MS t = 0.90 min, $[M+H]^+$ = 448; $[\alpha]_D^{20}$ –47.9 (*c* 0.791, $CHCl_3$); IR (KBr, cm^{-1}) 825, 1114, 1228, 1458, 1496, 1642, 2863, 3117. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

(4*S*)-3-[5-Methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinane-4-carboxylic acid ((*S*)-22)

L-Homoserine lactone hydrochloride **21** (2.55 g, 18.5 mmol) was added to a mixture of formaldehyde (37% in water, 9.22 mL, 90.8 mmol) and hydrochloric acid solution (1.0 mol/L, 1.74 mL) in water (44.0 mL). The reaction mixture was stirred at room temperature for 17 h. The solvent was then distilled under reduced pressure to yield a white solid, which was then suspended in EtOH (100 mL). After stirring for 15 min, the insoluble matter was filtered off, and the filtrate was concentrated under reduced pressure to yield approximately 25 mL of crude residue. After the addition of EtOAc (25 mL), the crude residue was stored at −10°C overnight. The precipitate was then collected by filtration to yield (4*S*)-1,3-oxazinane-4-carboxylic acid hydrochloride as a colorless solid (2.70 g, 86% yield).

A solution of benzoyl chloride **19** (0.38 g, 1.7 mmol) and Et_3N (0.41 mL, 3.0 mmol) in $CHCl_3$ (11 mL) were added to a mixture of (4*S*)-1,3-oxazinane-4-carboxylic acid hydrochloride (0.40 g, 2.4 mmol) and Et_3N (0.83 mL, 5.9 mmol) in $CHCl_3$ (5.0 mL) in a dropwise manner at 0°C within 20 min. The solvent was then distilled under reduced pressure. The resulting residue was purified using preparative HPLC to yield the title compound **(*S*)-22** as a colorless solid (0.18 g, 33% yield). HRMS calcd for $C_{15}H_{16}N_4O_4$ $[M+H]^+$ 317.1244 found 317.1223; LC–MS t = 0.67 min, $[M+H]^+$ = 317. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in DMSO-d_6 at 25°C.

[(4*S*)-4-(Hydroxymethyl)-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((*S*)-23)

The title compound was prepared from **(*S*)-22** according to the procedure described for compound **16d** (74% yield, colorless solid). HRMS calcd for $C_{15}H_{18}N_4O_3$ $[M+H]^+$ 303.1452 found 303.1456. LC–MS t = 0.59 min, $[M+H]^+$ = 303. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(4*S*)-4-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3i)

The title compound was prepared from **(*S*)-23** according to the procedure described for compound **18d** (17% yield, colorless solid). HRMS calcd for $C_{23}H_{22}FN_7O_2$ $[M+H]^+$ 448.1892 found 448.1880; LC–MS t = 0.87 min, $[M+H]^+$ = 448; $[\alpha]_D^{20}$ –50.0 (c 0.730, $CHCl_3$); IR (KBr, cm^{-1}) 820, 1055, 1228, 1431, 1458, 1625, 2958, 3134. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[2-(Hydroxymethyl)-1,3-thiazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (26)

3-[5-Methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-thiazolidine-2-carboxylic acid **25** was prepared from 1,3-thiazolidine-2-carboxylic acid **24** according to the procedure described for compound **(*S*)-20a** (80% yield in 2 steps, colorless oil). LC–MS t = 0.73 min, $[M+H]^+$ = 319.

The title compound was prepared from **25** according to the procedure described for compound **(*R*)-16d** (79% yield, colorless oil). LC–MS t = 0.69 min, $[M+H]^+$ = 305. ^1H NMR (600 MHz, $CDCl_3$) δ 7.89 (d, J = 8.26 Hz, 1H), 7.81 (s, 2H), 7.37 (br d, J = 8.26 Hz, 1H), 7.31 (br s, 1H), 5.74 (s, 1H), 3.63 (br s, 1H), 3.23–3.52 (m, 1H), 3.10–3.21 (m, 1H), 2.81–2.90 (m, 1H), 2.44 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.8, 168.1, 138.8, 136.0, 136.0, 131.3, 128.5, 128.2, 128.2, 121.9, 59.6, 51.3, 30.6, 20.9.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-thiazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3e)

The title compound **3e** (racemate) was prepared from **26** according to the procedure described for compound **(−)-3a** (26% yield, colorless solid). The chiral resolution of racemate **3e** (41 mg, 0.091 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 50% *i*PrOH in hexanes with a flow rate of 10 mL/min to yield the title compound **(−)-3e** as a colorless solid (9.8 mg, 24% yield, tR = 23–26 min [(+)-form: 15–19 min]). HRMS calcd for $C_{22}H_{20}FN_7OS$ $[M+H]^+$ 450.1507 found 450.1495; LC–MS t = 1.08 min, $[M+Na]^+$ = 472; $[\alpha]_D^{20}$ –247 (c 0.490, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

2-[1-(2,2-Diethoxyethyl)-1*H*-pyrazol-3-yl]-5-fluoropyridine (29)

2-Bromo-1,1-diethoxyethane (6.0 mL, 40 mmol) was added to a stirred suspension of 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine **17** (5.0 g, 31 mmol) and Cs_2CO_3 (20 g, 61 mmol) in DMF (100 mL) in a dropwise manner within 5 min at room temperature. The reaction mixture was stirred at 80°C for 4 h. After cooling to room temperature, water was added, and the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 30%–80% EtOAc in hexanes) to yield the title compound **29** as a colorless solid (6.4 g, 75% yield). HRMS calcd for $C_{14}H_{18}FN_3O_2$ $[M+H]^+$ 280.1456 found 280.1450; LC–MS t = 0.91 min, $[M+H]^+$ = 280; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 8.48 (d, J = 2.74 Hz, 1H), 7.92 (dd, J = 4.12, 8.92 Hz, 1H), 7.52 (d, J = 2.40 Hz, 1H), 7.43 (dt, J = 3.10, 7.90 Hz, 1H), 6.79 (d, J = 2.40 Hz, 1H), 4.82 (t, J = 5.49 Hz, 1H), 4.26 (d, J = 5.49 Hz, 2H), 3.72 (qd, J = 7.09, 9.26 Hz, 2H), 3.44 (qd, J = 7.03, 9.43 Hz, 2H), 1.17 (t, J = 7.03 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 163.7, 161.7, 151.0, 132.3, 130.1, 130.0, 127.5, 127.4, 115.8, 115.6, 102.8, 101.8, 64.0, 55.5, 15.5.

Preparation of compounds 30 and 31

These compounds were synthesized from their corresponding precursors according to the procedure described for compound **29**.

2-[1-(2,2-Diethoxyethyl)-1*H*-pyrazol-4-yl]-5-fluoropyridine (30)

The title compound was prepared from 5-fluoro-2-(1*H*-pyrazol-4-yl)pyridine **27** (91% yield, colorless solid). HRMS calcd for $C_{14}H_{18}FN_3O_2$ $[M+H]^+$ 280.1456 found 280.1436; LC–MS t = 0.88 min, $[M+H]^+$ = 280; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.41 (d, J = 2.48 Hz, 1H), 7.94 (d, J = 3.72 Hz, 2H), 7.44 (dd, J = 4.13, 8.67 Hz, 1H), 7.38 (dt, J = 3.30, 8.67 Hz, 1H), 4.82 (t, J = 5.37 Hz, 1H), 4.24 (d, J = 5.37 Hz, 2H), 3.68–3.76 (m, 2H), 3.43–3.50 (m, 2H), 1.17 (t, J = 7.02 Hz, 6H).

1-(2,2-Diethoxyethyl)-4-(4-fluorophenyl)-1*H*-pyrazole (31)

The title compound was prepared from 4-(4-fluorophenyl)-1*H*-pyrazole **28** (68% yield, colorless oil). HRMS calcd for $C_{15}H_{19}FN_2O_2$ $[M+H]^+$ 279.1503 found 279.1488; LC–MS t = 1.04 min, $[M+H]^+$ = 279; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 7.72 (s, 1H), 7.66 (s, 1H), 7.38–7.46 (m, 2H), 7.00–7.10 (m, 2H), 4.80 (t, J = 5.54 Hz, 1H), 4.22 (d, J = 5.54 Hz, 2H), 3.72 (qd, J = 7.07, 9.36 Hz, 2H), 3.46 (qd, J = 7.07, 9.36 Hz, 2H), 1.17 (t, J = 7.07 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 162.5, 160.5, 136.9, 128.8, 128.8, 127.3, 127.0, 127.0, 115.8, 115.6, 101.4, 63.7, 54.2, 15.2.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3c)

[(2*R*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazolidin-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((+)-3c)

CF_3CO_2H (2.4 mL, 31 mmol) was added to a stirred suspension of **29** (1.4 g, 5.1 mmol) in $CHCl_3$ (51 mL) at room temperature. After stirring at room temperature for 5 h, trifluoroacetic acid (1.2 mL, 15 mmol) was added; the mixture was then stirred at room temperature for 17 h. The reaction mixture was added to water, neutralized to pH7 with saturated aqueous $NaHCO_3$ solution, and extracted with $CHCl_3$. The organic layer was allowed to pass through an ISOLUTE® phase separator and was concentrated under reduced pressure to yield **32** as a colorless oil (0.86 g, 81% yield).

2-Aminoethanol (0.15 g, 2.5 mmol) and molecular sieves 4A (2.5 g, powder type) were added to a stirred suspension of **32** (0.51 g, 2.5 mmol) in THF (8.3 mL) at room temperature. The reaction mixture was stirred at room temperature for 24 h, filtered, and the filtrate was then concentrated under reduced pressure to yield a colorless oil.

The resulting colorless oil in THF (8.3 mL) was added in a dropwise manner at a temperature of under 0°C to a solution of **19** and Et_3N (0.59 mL, 4.3 mmol) in toluene (11 mL). The mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 12%–88% EtOAc in hexanes) to yield racemate **3c** as a colorless solid (0.27 g, 36% yield over 2 steps). The chiral resolution of **3c** (0.061 g, 0.14 mmol) was performed using a

CHIRALPAK® AD-H column (2 cm * 25 cm) with 70% EtOH in hexanes with a flow rate of 10 mL/min, providing **(−)-3c** as a colorless oil (0.020 g, 32% yield, tR = 23–29 min) and **(+)-3c** as a colorless solid (0.014 g, 22% yield, tR = 44–60 min).

(−)-3c: HRMS calcd for $C_{22}H_{20}FN_7O_2$ $[M+H]^+$ 434.1735 found 434.1727; LC–MS t = 0.92 min, $[M+H]^+$ = 434; $[\alpha]_D^{20}$ –73.6 (c 0.605, $CHCl_3$). IR (KBr, cm^{-1}) 825, 1110, 1224, 1439, 1494, 1656, 2875, 3121.

(+)-3c: HRMS calcd for $C_{22}H_{20}FN_7O_2$ $[M+H]^+$ 434.1735 found 434.1725; LC–MS t = 0.92 min, $[M+H]^+$ = 434; $[\alpha]_D^{20}$ +68.7 (c = 0.739, $CHCl_3$). IR (KBr, cm^{-1}) 825, 1110, 1223, 1439, 1494, 1656, 2875, 3121.

Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

Preparation of compounds (− or +)-3h, (−)-4, and (−)-10–(−)-12

These compounds were synthesized from their corresponding precursors according to the procedure described for compound **(− or +)-3c**.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3h)

[(2*R*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((+)-3h)

The racemate **3h** of the title compound was prepared from **29** (33% yield, colorless amorphous substance). The chiral resolution of **3h** (10 mg, 0.022 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 80% EtOH in hexanes and a flow rate of 10 mL/min, yielding **(−)-3h** as a colorless solid (4.8 mg, 48% yield, tR = 10–11 min) and **(+)-3h** as a colorless solid (4.8 mg, 48% yield, tR = 12–14 min). The absolute stereochemistry of **(−)-3h** was determined to be the (*S*)-form in a single crystal X-ray structural analysis of its monohydrochloride salt. The monohydrochloride salt was prepared with **(−)-3h** and hydrogen chloride in EtOAc, and then recrystallization from EtOAc.

(−)-3h: HRMS calcd for $C_{23}H_{22}FN_7O_2$ $[M+H]^+$ 448.1892 found 448.1874; LC–MS t = 0.94 min, $[M+H]^+$ = 448; $[\alpha]_D^{25}$ –33.2 (c 0.102, Ce_3); IR (KBr, cm^{-1}) 841, 1082, 1229, 1456, 1497, 1627, 2944, 3108. Compound **(−)-3h** exists

as a mixture of four rotamers in chemical exchange in $CDCl_3$ at room temperature (42:34:18:6). The ^1H and ^{13}C chemical shifts of the two most populated rotamers were assigned based on the results of the COSY, HSQC, and HMBC spectra analysis acquired for a sample dissolved in $CDCl_3$ at 25°C.

Rotamer 1 (42% of total): ^1H NMR (500 MHz, $CDCl_3$, 25 °C) δ 8.48 (1H), 8.04 (1H), 7.83 (1H), 7.79 (1H), 7.62 (1H), 7.44 (1H), 7.26 (1H), 7.03 (1H), 6.89 (1H), 6.02 (1H), 4.80 (1H), 4.64 (1H), 4.01 (1H), 3.66 (1H), 3.26 (1H), 2.89 (1H), 2.19 (1H), 1.49 (1H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 168.7, 159.0, 150.9, 149.0, 138.5, 137.6, 135.8, 133.9, 132.7, 130.8, 128.9, 128.7, 123.6, 122.1, 121.1, 104.9, 81.6, 62.2, 52.0, 39.9, 24.2, 20.9.

Rotamer 2 (34% of total): ^1H NMR (500 MHz, $CDCl_3$, 25 °C) δ 7.97 (1H), 7.91 (1H), 7.81 (1H), 7.66 (1H), 7.43 (1H), 7.36 (1H), 7.27 (1H), 6.24 (1H), 4.82 (1H), 4.62 (1H), 4.13 (1H), 3.78 (1H), 3.45 (1H), 3.08 (1H), 2.45 (1H), 1.88 (1H), 1.57 (1H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 168.6, 150.9, 149.0, 138.9, 137.5, 136.0, 134.3, 131.4, 131.2, 128.4, 128.2, 122.1, 121.3, 105.2, 80.3, 60.6, 48.5, 41.0, 24.7, 21.2.

(+)-3h: HRMS calcd for $C_{23}H_{22}FN_7O_2$ $[M+H]^+$ 448.1892 found 448.1872; LC–MS t = 0.94 min, $[M+H]^+$ = 448; $[\alpha]_D^{20}$ +34.7 (c 0.994, $CHCl_3$); IR (KBr, cm^{-1}) 822, 1077, 1226, 1457, 1505, 1629, 2964, 3101.

Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C, and X-ray crystal structure of compound **(−)-3h**.

[(2*S*)-2-{[4-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-4)

The racemate **4** of the title compound was prepared from **30** (52% yield, colorless amorphous substance). The chiral resolution of **4** (0.12 g, 0.27 mmol) was performed using a CHIRALPAK® IB column (2 cm * 25 cm) with 70% *i*PrOH in hexanes and a flow rate of 5 mL/min, providing **(−)-4** as a colorless amorphous substance (0.045 g, 38% yield, tR = 32 min [(+)-form: 38 min]). HRMS calcd for $C_{23}H_{22}FN_7O_2$ $[M+H]^+$ 448.1892 found 448.1886; LC–MS t = 0.90 min, $[M+H]^+$ = 448; $[\alpha]_D^{25}$ –31.8 (c 0.100, $CHCl_3$); IR (KBr, cm^{-1}) 826, 1081, 1227, 1431, 1488, 1639, 2960, 3114. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-{[4-(4-Fluorophenyl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-10)

The racemate **10** of the title compound was prepared from **31** (10% yield, colorless amorphous substance). The chiral resolution of **10** (0.22 g, 0.49 mmol) was performed using SFC (CHIRALPAK® IC column 2 cm * 25 cm, 30% MeOH/CO_2, flow rate: 30 mL/min), providing **(−)-10** as a colorless solid (0.065 g, 30% yield, tR = 4.7 min [(+)-form: 6.2 min]). HRMS calcd for $C_{24}H_{23}FN_6O_2$ $[M+H]^+$ 447.1939 found 447.1931; LC–MS t = 1.05 min, $[M+H]^+$ = 447; $[\alpha]_D^{20}$ –33.9 (c 0.743, $CHCl_3$); IR (KBr, cm^{-1}) 833, 1074, 1224, 1430, 1506, 1641, 2959, 3110. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-fluoro-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-11)

The racemate **11** of the title compound was prepared from **29** (45% yield, colorless amorphous substance). The chiral resolution of **11** (0.11 g, 0.25 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 80% EtOH in hexanes and a flow rate of 5 mL/min, providing **(−)-11** as a colorless amorphous substance (0.050 g, 45% yield, tR = 25–27 min [(+)-form: 30–39 min]). HRMS calcd for $C_{22}H_{19}F_2N_7O_2$ $[M+H]^+$ 452.1641 found 452.1625; LC–MS t = 0.93 min, $[M+H]^+$ = 452; $[\alpha]_D^{20}$ –36.3 (c 0.056, $CHCl_3$); IR (KBr, cm^{-1}) 827, 1085, 1227, 1439, 1505, 1646, 2960, 3120. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-12)

The racemate **12** of the title compound was prepared from **29** (39% yield, colorless oil). The chiral resolution of **12** (40.9 mg, 0.0944 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 80% EtOH in hexanes and a flow rate of 5 mL/min, providing **(−)-12** as a colorless oil (16.8 mg, 41% yield, tR = 25–31 min [(+)-form: 40–54 min]). HRMS calcd for $C_{22}H_{20}FN_7O_2$ $[M+H]^+$ 434.1735 found 434.1735; LC–MS t = 0.86 min, $[M+H]^+$

= 434; $[\alpha]_D^{20}$ –31.7 (*c* 0.734, $CHCl_3$); IR (KBr, cm^{-1}) 764, 1104, 1230, 1458, 1497, 1636, 2871, 3124. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(pyrimidin-2-yl)phenyl]methanone ((−)-13)

3-Aminopropan-1-ol (0.33 mL, 4.2 mmol) and molecular sieves 4A (4.0 g, powder type) were added to a stirred suspension of **32** (0.86 g, 4.2 mmol), synthesized according to the procedure described for **3c**, in $CHCl_3$ (17 mL). The reaction mixture was stirred at room temperature for 2 days, filtered, and the filtrate was then concentrated under reduced pressure to yield a colorless oil. 5-Methyl-2-(pyrimidin-2-yl)benzoic acid **38** (0.46 g, 2.1 mmol), T3P® (1.6 mol/L solution in DMF, 0.13 mL, 0.20 mmol), 2,6-lutidine (0.47 mL, 4.1 mmol), and trimethylacetyl chloride (0.38 mL, 3.1 mmol) were then added to a solution of the colorless oil in DMF (10 mL). The mixture was stirred at room temperature for 15 h. The reaction was quenched by the addition of water, and the mixture was extracted with $CHCl_3$. The organic layer was concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 20%–100% EtOAc in hexanes) to yield the racemate **13** as a colorless amorphous substance (0.10 g, 11% yield in 3 steps). The chiral resolution of **13** (0.10 g, 0.22 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 80% EtOH in hexanes with a flow rate of 5 mL/min, yielding **(−)-13** as a colorless oil (0.040 g, 40% yield, tR = 25–31 min [(+)-form: 40–54 min]). HRMS calcd for $C_{25}H_{23}FN_6O_2$ $[M+H]^+$ 459.1939 found 459.1929; LC–MS *t* = 0.88 min, $[M+H]^+$ = 459; $[\alpha]_D^{27}$ –32.5 (*c* 0.068, $CHCl_3$); IR (KBr, cm^{-1}) 839, 1077, 1228, 1416, 1494, 1637, 2972, 3110. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-fluoro-2-(pyrimidin-2-yl)phenyl]methanone ((−)-14)

The racemate **14** of the title compound was prepared from **29** and 5-fluoro-2-(pyrimidin-2-yl)benzoic acid **39** according to the procedure described for compound **13** (9% yield in 3 steps, colorless oil). The chiral resolution of **14** (65.2 mg, 0.141 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 80% EtOH in

hexanes with a flow rate of 5 mL/min, providing **(−)-14** as a colorless oil (29.8 mg, 46% yield, tR = 24–30 min [(+)-form: 45–65 min]). HRMS calcd for $C_{24}H_{20}F_2N_6O_2$ $[M+H]^+$ 463.1689 found 463.1674; LC–MS t = 0.87 min, $[M+H]^+$ = 463; $[\alpha]_D^{20}$ –33.5 (c 0.760, $CHCl_3$); IR (KBr, cm^{-1}) 808, 1090, 1226, 1417, 1496, 1638, 2963, 3054. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

2-[1-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-4-yl]ethan-1-ol (34)

Cs_2CO_3 (9.7 g, 18 mmol) and 2,5-difluoropyridine (0.89 mL, 9.8 mmol) were added to a stirred suspension of 2-(1*H*-pyrazol-4-yl)ethan-1-ol **33** (1.0 g, 8.9 mmol) in CH_3CN (45 mL). The reaction mixture was stirred at 80°C for 3 h. After cooling to room temperature, water was added, and the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 10%–70% EtOAc in hexanes) to yield the title compound **34** as a colorless solid (0.63 g, 34% yield). HRMS calcd for $C_{10}H_{10}FN_3O$ $[M+H]^+$ 208.0881 found 208.0871; LC–MS t = 0.64 min, $[M+H]^+$ = 208; ^1H NMR (500 MHz, $CDCl_3$, 25 °C) δ 8.35 (s, 1H), 8.22 (d, J = 3.09 Hz, 1H), 7.93 (dd, J = 3.94, 9.09 Hz, 1H), 7.60 (s, 1H), 7.49–7.55 (m, 1H), 3.85 (t, J = 6.52 Hz, 2H), 2.80 (t, J = 6.52 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 158.8, 156.8, 147.8, 142.3, 135.5, 135.3, 125.9, 125.7, 120.2, 113.2, 113.2, 62.9, 27.8.

[(2*S*)-2-{[1-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-4-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-5)

To a stirred suspension of **34** (0.314 g, 1.52 mmol) in DMSO (7.58 mL) was added 2-iodoxybenzoic acid (0.778 g, 1.67 mmol) at room temperature. The mixture was stirred at room temperature for 17 h. The reaction was quenched by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **35** as a colorless oil.

Molecular sieves 4A (2.5 g, powder type) and 3-aminopropan-1-ol (0.118 mL, 1.52 mmol) were added to a solution of **35** in $CHCl_3$ (6.08 mL) at room temperature. After stirring at room temperature for 17 h, the mixture was filtered through a pad of Celite®. The filtrate was concentrated under reduced pressure to yield a colorless oily residue.

The residue in $CHCl_3$ (4.00 mL) was added in a dropwise manner to a solution of **19** (0.202 g, 0.910 mmol) and

Et_3N (0.423 mL, 3.04 mmol) in $CHCl_3$ (10.0 mL) at 0°C within 15 min. The mixture was allowed to warm to room temperature and stirred for 17 h. The reaction was quenched by the addition of water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine and passed through an ISOLUTE® phase separator. The filtrate was concentrated under reduced pressure, and the resulting residue was purified using column chromatography (SiO_2, 12%–100% EtOAc in hexanes) to yield the racemate **5** as a colorless solid (0.189 g, 46% yield in 3 steps). The chiral resolution of **5** (31.8 mg, 0.0711 mmol) was performed using a CHIRALPAK® IB column (2 cm * 25 cm) with 20% EtOH in hexanes and a flow rate of 5 mL/min, providing **(−)-5** as a colorless oil (13.5 mg, 42% yield, less polar enantiomer). HRMS calcd for $C_{23}H_{22}FN_7O_2$ $[M+H]^+$ 448.1892 found 448.1878; LC–MS t = 0.99 min, $[M+H]^+$ = 448; $[\alpha]_D^{20}$ –17.1 (*c* 0.561, $CHCl_3$); IR (KBr, cm^{-1}) 828, 1075, 1234, 1434, 1486, 1639, 2869, 3119. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

Ethyl 3-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinane-2-carboxylate ((−)-41)

Molecular sieves 4A (5.0 g, powder type) and 3-aminopropan-1-ol (0.39 mL, 5.0 mmol) were added to a solution of ethyl glyoxalate (47% solution in toluene, polymer type, 1.1 mL, 5.0 mmol) in $CHCl_3$ (20 mL). The mixture was stirred at room temperature for 15 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to provide a colorless oily residue.

A mixture of the above residue and Et_3N (1.4 mL, 10 mmol) in $CHCl_3$ (5.0 mL) was added in a dropwise manner to a solution of **19** (0.55 g, 2.5 mmol) in $CHCl_3$ (20 mL) at 0°C within 10 min. The mixture was then stirred at room temperature for 17 h. The reaction was quenched by the addition of water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine and passed through an ISOLUTE® phase separator. The filtrate was concentrated under reduced pressure, and the resulting residue was purified using column chromatography (SiO_2, 12%–88% EtOAc in hexanes) to yield the racemate of the title compound **41** as a colorless oil (0.85 g, 99% yield in 2 steps). The chiral resolution of **41** (0.10 g, 0.29 mmol) was performed using a CHIRALPAK® AD-H column (2 cm * 25 cm) with 70% EtOH in hexanes with a flow rate of 5 mL/min, providing **(−)-41** as a colorless oil (0.041 g, 41% yield, tR = 22–26 min [(+)-form: 27–34 min]). HRMS calcd for $C_{17}H_{20}N_4O_4$ $[M+H]^+$ 345.1557 found 335.1543;

LC–MS t = 0.88 min, $[M+H]^+$ = 345; $[\alpha]_D^{20}$ –43.2 (c 0.789, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-(Hydroxymethyl)-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-42)

$NaBH_4$ (0.021 g, 0.55 mmol) was added to a solution of **(−)-41** (0.019 g, 0.055 mmol) in MeOH (0.30 mL) at 0°C. The mixture was stirred at 0°C for 0.5 h and then at room temperature for 1 h. $NaBH_4$ (0.021 g, 0.55 mmol) was then added to the mixture, and the resulting mixture was stirred at room temperature for 2 h. The solvent was distilled under reduced pressure. The reaction was quenched by the addition of water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine and passed through an ISOLUTE® phase separator, and the filtrate was concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 25%–100% EtOAc in hexanes) to yield the title compound **(−)-42** as a colorless amorphous substance (0.017 g, quant.). The optical purity of **(−)-42** was determined to be >99.9% *ee* using a chiral HPLC analysis (CHIRALPAK® IB column 0.5 cm * 25 cm, solvent: 10% EtOH in hexanes, flow rate: 1 mL/min, (−)-form: tR = 3.8 min, (+)-form: 4.4 min). HRMS calcd for $C_{15}H_{18}N_4O_3$ $[M+H]^+$ 303.1452 found 303.1428; LC–MS t = 0.60 min, $[M+H]^+$ = 303; $[\alpha]_D^{20}$ –38.7 (c 1.14, $CHCl_3$). Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

[(2*S*)-2-{[5-(5-Fluoropyridin-2-yl)-1,2,4-oxadiazol-3-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-6)

Et_3N (0.69 mL, 5.0 mmol) and methanesulfonyl chloride (0.28 mL, 3.6 mmol) were added to a solution of **(−)-42** (1.0 g, 3.3 mmol) in $CHCl_3$ (11 mL) at 0°C. The mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine and passed through an ISOLUTE® phase separator. The filtrate was concentrated under reduced pressure to yield [(2*S*)-2-(chloromethyl)-1,3-oxazinan-3-yl]-[5-methyl-2-(triazol-2-yl)phenyl]methanone as a colorless solid (0.47 g, 45% yield).

NaCN (0.038 g, 0.78 mmol) was added to a solution of the above compound (0.21 g, 0.64 mmol) in DMF (1.8 mL).

The mixture was stirred at 80°C for 3 h. After cooling to room temperature, water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 20%–80% EtOAc in hexanes) to yield {(2*S*)-3-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinan-2-yl}acetonitrile as a colorless oil (0.15 g, 78% yield).

Hydroxylamine (50% solution in water, 0.057 mL, 0.96 mmol) was added to a solution of {(2*S*)-3-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinan-2-yl}acetonitrile (0.10 g, 0.32 mmol) in EtOH (0.64 mL), and the mixture was stirred at 80°C for 4 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. Water and EtOAc were added to the residue, and the organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to provide *N*-hydroxy{(2*S*)-3-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinan-2-yl}ethanimidamide as a colorless oil.

5-Fluoropyridine-2-carboxylic acid (0.050 g, 0.35 mmol) and 1,1'-carbonyldiimidazole (0.063 g, 0.39 mmol) were added to a solution of the ethanimidamide in CH_3CN (0.90 mL) and DMF (0.090 mL), and the mixture was stirred at room temperature for 5 h and then at 70°C for 1 h. Water and EtOAc were added to the mixture. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using preparative HPLC to yield the title compound **(−)-6** as a colorless oil (0.0094 g, 7% yield in 2 steps). HRMS calcd for $C_{22}H_{20}FN_7O_3$ $[M+H]^+$ 450.1684 found 450.1686; LC–MS $t = 0.92$ min, $[M+H]^+ = 450$; $[\alpha]_D^{20}$ –32.2 (*c* 0.530, $CHCl_3$); IR (neat, cm^{-1}) 827, 1084, 1232, 1430, 1465, 1638, 2872, 2966. Please see the Supplementary Data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

Fluoro-2-(1*H*-1,2,3-triazol-4-yl)pyridine (43)

NaN_3 (0.64 g, 9.9 mmol) and NH_4Cl (1.3 g, 25 mmol) were added to a solution of 2-ethynyl-5-fluoropyridine (1.0 g, 8.3 mmol) in DMF (7.0 mL), and the mixture was stirred at 120°C for 6 h. The reaction was quenched by the addition of water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified using column chromatography (SiO_2, 2%–10% MeOH in $CHCl_3$) to yield the title compound **43** as a colorless solid (0.40 g, 29%

yield). LC–MS t = 0.46 min, $[M+H]^+$ = 165; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (d, J = 2.89 Hz, 1H), 8.27 (s, 1H), 8.03 (dd, J = 4.54, 8.67 Hz, 1H), 7.52 (dt, J = 2.89, 8.46 Hz, 1H).

[(2*S*)-2-{[4-(5-Fluoropyridin-2-yl)-2*H*-1,2,3-triazol-2-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-7)

[(2*S*)-2-{[4-(5-Fluoropyridin-2-yl)-1*H*-1,2,3-triazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-8)

To a solution of [(2*S*)-2-(chloromethyl)-1,3-oxazinan-3-yl]-[5-methyl-2-(triazol-2-yl)phenyl]methanone (0.18 g, 0.56 mmol) described in the synthesis of **(−)-6** in DMSO (0.70 mL) were added **43** (0.10 g, 0.62 mmol) and *t*-BuONa (0.076 g, 0.79 mmol) at room temperature. After the mixture was stirred at 80°C for 2 h, the reaction was quenched by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified using preparative HPLC to yield the title compound **(−)-7** (0.032 g, 13% yield, colorless oil) and **(−)-8** (0.022 g, 9% yield, colorless oil). **(−)-7**: HRMS calcd for $C_{22}H_{21}FN_8O_2$ $[M+H]^+$ 449.1844 found 449.1817; LC–MS t = 0.96 min, $[M+H]^+$ = 449; $[\alpha]_D^{20}$ –35.6 (*c* 0.971, $CHCl_3$); IR (KBr, cm^{-1}) 828, 1084, 1229, 1428, 1466, 1646, 2963, 3123.

(−)-8: HRMS calcd for $C_{22}H_{21}FN_8O_2$ $[M+H]^+$ 449.1844 found 449.1824; LC–MS t = 0.88 min, $[M+H]^+$ = 449; $[\alpha]_D^{21}$ –38.6 (*c* 0.954, $CHCl_3$); IR (KBr, cm^{-1}) 827, 1085, 1228, 1430, 1479, 1643, 2960, 3136.

Please see the Supplementary Data for pictures of 500 MHz ^{1}H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

4-(5-Fluoropyridin-2-yl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (44)

To a solution of 5-fluoropyridin-2-amine (1.00 g, 8.92 mmol) and pyridine (0.86 mL, 10.7 mmol) in THF (30.0 mL) was added phenyl carbonochloridate (1.54 mL, 9.81 mmol) at 0°C. The mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of water, and the mixture was stirred for 1 h. The precipitate was then collected by filtration to yield phenyl (5-fluoropyridin-2-yl)carbamate as a colorless solid (2.05 g, 99% yield).

Hydrazine monohydrate (0.419 mL, 8.61 mmol) was added to a solution of phenyl (5-fluoropyridin-2-yl)carbamate (1.00 g, 4.31 mmol) in 1,4-dioxane (15.0 mL). The mixture was stirred at 60°C for 2 h. The reaction was quenched

by the addition of water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The precipitate was then collected by filtration and washed with Et_2O to yield *N*-(5-fluoropyridin-2-yl)hydrazinecarboxamide as a colorless solid (0.686 g, 94% yield).

Formamidine acetate (0.840 g, 8.06 mmol) was added to a solution of *N*-(5-fluoropyridin-2-yl)hydrazinecarboxamide (0.686 g, 4.03 mmol) in DMF (10.0 mL). The mixture was stirred at room temperature for 30 min. Acetic acid (0.500 mL) was added to the mixture, and the mixture was stirred at 80°C for 1 h. Water was added to the reaction, and the mixture was stirred at room temperature for 30 min. The precipitate was then collected by filtration to yield the title compound **44** as a colorless solid (0.500 g, 69% yield). LC–MS t = 0.40 min, $[M+H]^+$ = 181; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.14 (br s, 1H), 8.52 (s, 1H), 8.51 (d, J = 2.89 Hz, 1H), 8.23 (dd, J = 4.13, 9.08 Hz, 1H), 7.97 (ddd, J = 2.89, 8.26, 9.08 Hz, 1H).

4-(5-Fluoropyridin-2-yl)-2-({(2*S*)-3-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinan-2-yl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one ((−)-9)

The title compound was prepared from **(−)-42** and **44** according to the procedure described for compound **(−)-7** (41% yield, colorless solid). HRMS calcd for $C_{22}H_{21}FN_8O_3$ $[M+H]^+$ 465.1793 found 465.1790; LC–MS t = 0.94 min, $[M+H]^+$ = 465; $[\alpha]_D^{20}$ –22.0 (c 0.522, $CHCl_3$); IR (KBr, cm^{-1}) 828, 1231, 1409, 1483, 1652, 1707, 2884, 3138. Please see the Supplementary Data for pictures of 500 MHz ^{1}H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25°C.

Compounds **(−)-42** and **(−)-3h** were alternatively synthesized using a different method, as follows.

[(2*S*)-2-(Hydroxymethyl)-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-42)

Porcine pancreas lipase (SIGMA, Lipase from Porcine Pancreas Type 2, 400 g) was added to a mixture of alcohol **42** (200 g, 0.66 mol) and vinyl acetate (1.6 L) in methyl *t*-butyl ether (6.4 L) under a N_2 atmosphere. The mixture was stirred at 25°C for 21 h. The resulting precipitates were filtrated through a pad of KC flock® and washed with EtOAc (2.0 L). The filtrate was concentrated under reduced pressure. The resulting residue was purified using column

chromatography (SiO_2, hexane/EtOAc/acetone = 50:50:0 → 0:100:0 → 0:0:100) to yield {(2*R*)-3-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,3-oxazinan-2-yl}methyl acetate as a brown solid (130 g, 58%, less polar) and **(−)-38** as a brown oil (97 g, 48%, polar). A chiral HPLC analysis showed that the optical purity of **(−)-42** was >99.5% *ee* (CHIRALPAK® IB column 0.5 cm * 25 cm, solvent: 10% EtOH in hexanes, flow rate: 1 mL/min, Rt = (−)-form: 3.8 min, (+)-form: 4.4 min).

[(2*S*)-2-{[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]methyl}-1,3-oxazinan-3-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone ((−)-3h)

Et_3N (100 g, 0.99 mol) in $CHCl_3$ (100 mL) and methanesulfonyl chloride (92 g, 0.80 mol) in $CHCl_3$ (100 mL) were added to a solution of **(−)-42** (200 g, 0.66 mol) in $CHCl_3$ (1.8 L) at a temperature of below 19°C. The mixture was allowed to warm to 23°C and was stirred for 5 h. The reaction was quenched by the addition of water (1 L), and the organic layer was separated. The aqueous phase was extracted with $CHCl_3$, and the combined organic layer was dried over Na_2SO_4 (100 g), filtered, and washed with $CHCl_3$ (500 mL). The filtrate was concentrated under reduced pressure. IPE (500 mL) and EtOAc (50 mL) were added to the residue, and the mixture was stirred for 12 h at room temperature. The resulting precipitate was then collected by filtration and washed with IPE (400 mL) to yield [(2*S*)-2-(chloromethyl)-1,3-oxazinan-3-yl]-[5-methyl-2-(triazol-2-yl)phenyl]methanone as a colorless solid (193 g, 91% yield).

To the mixture of *t*-BuONa (80 g, 0.84 mol) in DMSO (730 mL) was added **17** (125 g, 0.77 mol) in DMSO (730 mL) at 28°C, and the mixture was stirred at 82°C for 1 h. The alkyl chloride (244 g, 0.76 mol) obtained in the above reaction in DMSO (980 mL) was added to the mixture, and the mixture was stirred for 30 min. After cooling to 35°C, the reaction was quenched by the addition of water (2.4 L) and extracted with EtOAc. The organic layer was washed with brine (3.6 L) and concentrated under reduced pressure. The resulting residue was diluted with EtOH (2.0 L), and the mixture was stirred at 1°C. The precipitate was then collected by filtration and washed with EtOH (480 mL) to yield the title compound **(−)-3h** as a colorless solid (183 g, 54% yield).

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26. The values show %metabolized after 15 min incubation with rat or dog liver microsomes (0.25 mg protein/mL, 1 μmol/L in MeCN).