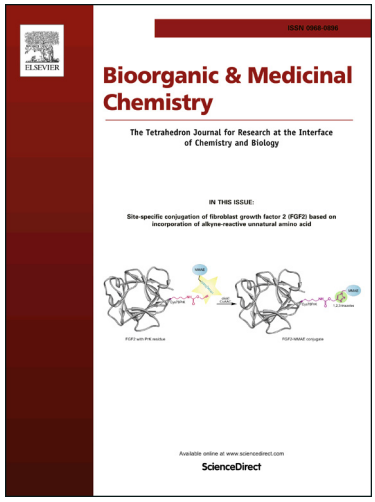
Accepted Manuscript



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Identification of highly selective and potent orexin receptor 1 antagonists derived from a dual orexin receptor 1/2 antagonist based on the structural framework of pyrazoylethylbenzamide

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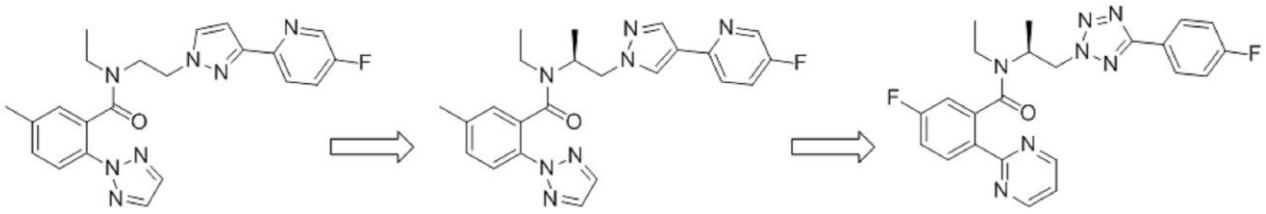


1
hOX_1R IC_{50} = 3.22 nM
hOX_2R IC_{50} = 2.81 nM
SI = 0.87

9b
hOX_1R IC_{50} = 0.767 nM
hOX_2R IC_{50} = 5.87 nM
SI = 7.7

24
hOX_1R IC_{50} = 2.01 nM
hOX_2R IC_{50} = 532 nM
SI = 265

Abstract

The design, synthesis, and structure activity relationships of the novel class of pyrazolylethylbenzamide orexin receptor 1-selective antagonists are described. Further derivatization of the prototype dual orexin receptor 1/2 antagonist lead (**1**) by installing a (*S*)-methyl group into the ethyl linker moiety between the pyrazole ring and benzamide resulted in an increase of the antagonist potency against orexin receptor 1/2 receptors. Optimization of the benzamide and pyrazole parts of compounds **2** and **9b** led to the identification of *N*-ethyl-5-fluoro-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-yl}-2-(pyrimidin-2-yl)benzamide (**24**), which exhibited excellent antagonistic activity against orexin receptor 1 with an IC_{50} of 2.01 nM and a 265-fold selectivity for orexin receptor 1 over orexin receptor 2.

Keywords:

Selective orexin receptor antagonist (SORA), Dual orexin receptor antagonist (DORA), Orexin 1

Abbreviations

OX_1R, orexin 1 receptor; OX_2R, orexin 2 receptor; DORA, dual orexin receptor antagonist; SORA, selective orexin receptor antagonist; SI, selectivity index; BBB, blood-brain barrier; CNS, central nervous system; SAR, structure-activity relationship; Boc, *tert*-butoxycarbonyl; DIPEA, *N*,*N*-diisopropylethylamine; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; T3P®, propylphosphonic acid anhydride; Ms, methanesulfonyl; RHS, right hand side; CHO, Chinese hamster ovary.

1. Introduction

Orexins, consisting of orexin-A and -B (also known as hypocretin-1 and -2), are hypothalamic neuropeptides that have been reported to originate from the common precursor protein prepro-orexin peptide[1,2]. They constitute the endogenous ligands for two subtypes of orexin receptors, orexin receptor 1 (OX_1R) and orexin receptor 2 (OX_2R), which are seven-transmembrane G-protein-coupled receptors. OX_1R and OX_2R are expressed differentially, although widely, in various regions of the brain, suggesting that each receptor subtype might have distinct physiological functions. The orexin system has been shown to play roles in a variety of important neurobiological processes, including the control of reward, sleep-wake regulation, feeding, and energy homeostasis. [3]

Research on the orexin system has mainly been directed toward the development of new hypnotic medications. Both dual orexin receptor antagonists (DORAs) and selective orexin 2 receptor antagonists (2-SORAs) have been developed for the treatment of sleep disorders. Proof-of-concept for treating human insomnia has been achieved with several DORAs, including almorexant[4], SB-649868[5] and suvorexant[6], and suvorexant was the first compound to be successfully launched in the market in both the United States and Japan in 2014 (Figure 1). Phase I trials have been completed for MK-1064[7] and JNJ-42847922[8], both 2-SORAs, and MK-1064 has been shown to induce

dose-dependent increases in the sleep efficacy and total sleep time, including rapid eye movement (REM) and non-rapid eye movement (NREM) sleep in healthy volunteers.[9] Although a number of DORAs and 2-SORAs have been developed, few selective OX_1R antagonist (1-SORA) tool compounds have been reported.[10,11] The first selective OX_1R antagonist to be reported was SB-334867, which is a diarylurea derivative.[12] Most *in vivo* and *in vitro* research conducted to investigate OX_1R has utilized this compound. However, SB-334867 has been demonstrated to also show affinity for the adenosine 2A (Ki = 0.67 μM) and 5-HT2c (Ki = 1.2 μM) receptors.[13] SB-334867, which has moderate OX_1R selectivity over OX_2R, with a selectivity index (SI = IC_{50} in OX_2R/IC_{50} in OX_1R) of 50, allowed the initial elucidation of the functions of OX_1R.[14] Subsequently, Actelion reported ACT-335827 as a structurally distinct selective OX_1R antagonist.[15] ACT-335827 (SI: 70) was reported as an orally available and BBB-penetrant 1-SORA. The OX_1R has been reported to play a much less significant role in the sleep-wake cycle than OX_2R based on studies carried out on orexin receptor knockout mice.[16,17] Consistent with previous reports from studies conducted using selective OX_1R antagonists, ACT-335827 did not promote sleep in telemeterized rats.[15]

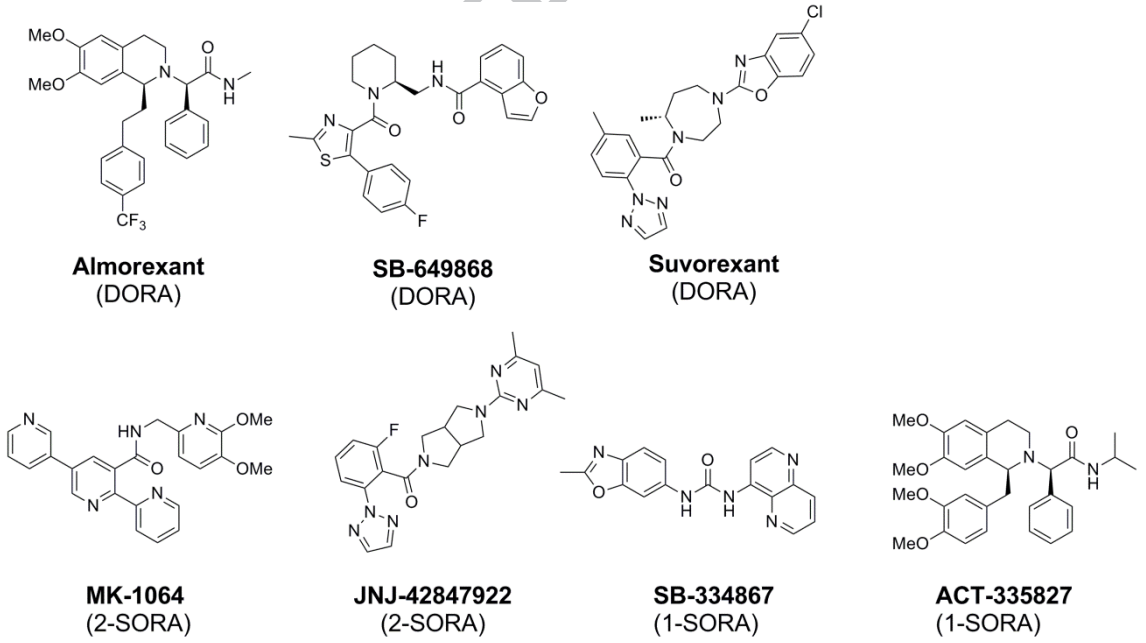


Figure 1. Representative DORAs, 2-SORAs, and 1-SORAs

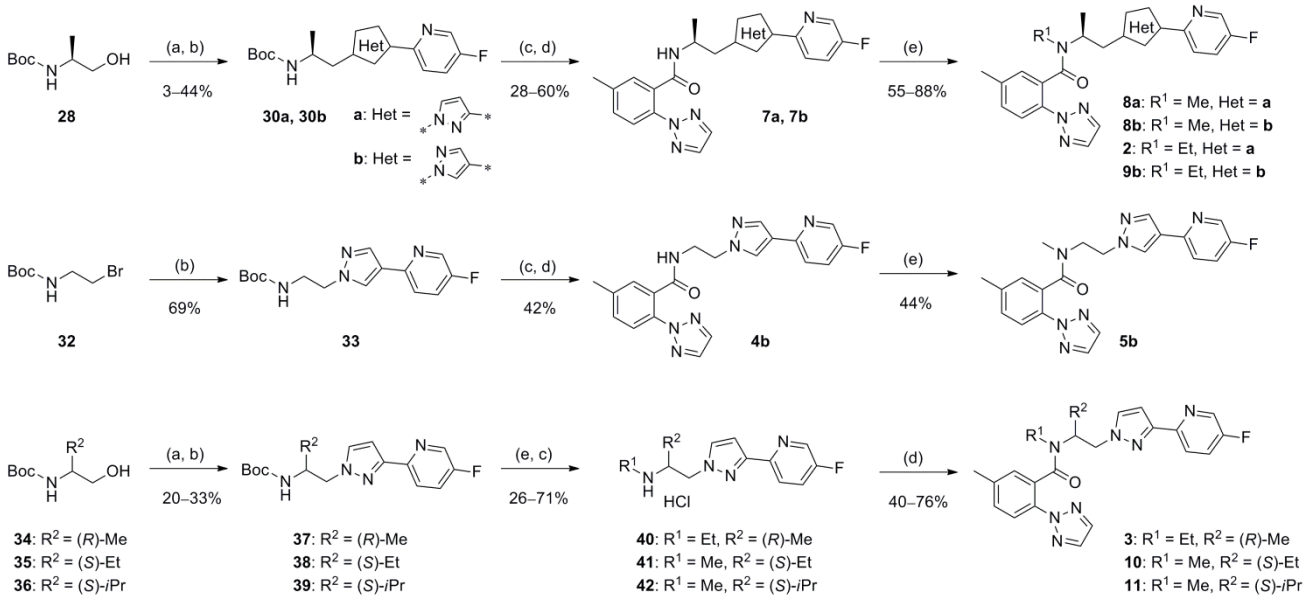
As the biology related to the orexin receptors emerges, selective compounds from multiple chemotypes will help

to further understand the role and biology of each receptor in the onset of CNS disorders. For the treatment of psychiatric disorders involving the OX_1R, one might see benefit in compounds which are selective for OX_1R over OX_2R, so that sedation is minimized.[15]

To move research on the pharmacology of orexin receptors forward and to clarify the functions of OX_1R, we explored highly potent and selective 1-SORAs, starting from DORA with a pyrazoylethylbenzamide structure[18], which was previously discovered as a new series of DORAs. Herein, we report our findings of research on the SARs of OX_1R and OX_2R based on a structural framework of pyrazolylethylbenzamide and the identification of new class of potent SORAs with high selectivity for OX_1R over OX_2R.

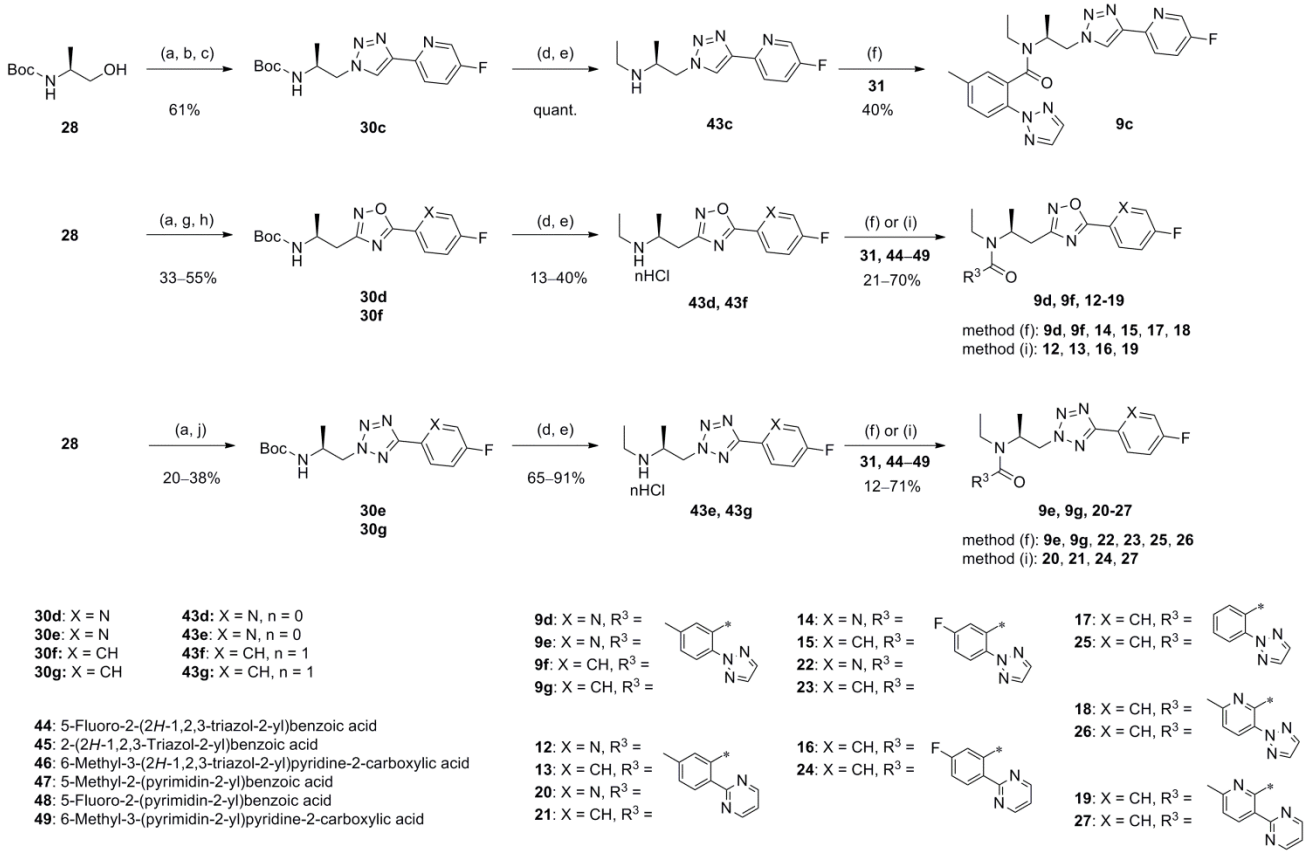
2. Chemistry

The pyrazole derivatives **2**, **3**, **4b**, **5b**, **7a**, **8a**, **7b**–**9b**, **10**, and **11** were synthesized as shown in Scheme 1. Compounds **2**, **7a**, **8a**, and **7b**–**9b** were prepared from the commercially available *N*-Boc-L-alaninol **28**, which was converted into the corresponding mesylate and successively alkylated with 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine (**29a**) or 5-fluoro-2-(1*H*-pyrazol-4-yl)pyridine (**29b**) to yield amine **30a** or **30b**, respectively. The resulting **30a** and **30b** were acidified to remove the Boc groups and then amidated with 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (**31**) in the presence of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) to yield **7a** and **7b**, which were reacted with the corresponding alkyl iodides to obtain **2**, **8a**, **8b** and **9b**. Compound **5b** was prepared by the same procedure as compound **8b**, except that *tert*-butyl *N*-(2-bromoethyl)carbamate (**32**) was used as the starting material. For the syntheses of compounds **3**, **10**, and **11**, compound **29a** was alkylated with mesylates which were converted from commercially available chiral alcohols **34**–**36** in the same manner as that to obtain **30a**, to obtain amines **37**–**39**, which were then ethylated and deprotected of the Boc group under acidic conditions to yield secondary amines **40**–**42**. The obtained compounds **40**–**42** were subsequently amidated with benzoic acid **31** to yield **3**, **10**, and **11**.



Scheme 1. Synthesis of **2**, **3**, **4b**, **5b**, **7a**–**8a**, **7b**–**9b**, **10**, **11**

Reagents and conditions: (a) MsCl, Et_3N, THF, 0 °C; (b) 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine (**29a**) or 5-fluoro-2-(1*H*-pyrazol-4-yl)pyridine (**29b**), Cs_2CO_3, DMF, 80 °C; (c) 4M HCl-EtOAc, EtOAc, rt; (d) HATU, DIPEA, DMF, 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (**31**); (e) NaH, DMF, then alkyliodide, 0–60 °C.



Scheme 2. Synthesis of **9c**–**9g**, **12**–**27**

Reagents and conditions: (a) MsCl, Et_3N, $CHCl_3$, 0 °C; (b) NaN_3, DMF, 80 °C; (c) 2-ethynyl-5-fluoropyridine, CuI,

L-ascorbic acid, 4-methylpiperidine, DMF, 80 °C; (d) NaH, DMF, then ethyliodide, 0–60 °C; (e) 4M HCl-EtOAc, EtOAc, rt; (f) HATU, DIPEA, DMF, **31**; (g) NaCN, DMF, 80 °C; (h) (1) NH_2OH aq., EtOH, 80 °C, (2) 5-fluoropyridine-2-carboxylic acid or 4-fluorobenzoic acid, 1,1'-carbonyldiimidazole, DMF, 40–80 °C; (i) HATU, DIPEA, DMF, **44**–**46** or T3P®, DIPEA, $CHCl_3$, **47**–**49**; (j) 5-fluoro-2-(1*H*-1,2,3,4-tetrazol-5-yl)pyridine (**29e**) or 5-(4-fluorophenyl)-1*H*-tetrazole, Cs_2CO_3, DMF, 80 °C.

The synthetic routes for triazole (**9c**), oxadiazole (**9d**, **9f**, **12**–**19**) and tetrazole derivatives (**9e**, **9g**, **20**–**27**) are depicted in Scheme 2. Heterocyclic intermediates **30c**–**g** were synthesized from alcohol **28**. For the synthesis of 1,2,3-triazole intermediate **30c** (for **9c**), alcohol **28** was mesylated and subsequently reacted with sodium azide (NaN_3) to yield an azide intermediate. 1,3-Dipolar cycloaddition of the azide intermediate to 2-ethynyl-5-fluoropyridine yielded **30c**. To synthesize **30d** and **30f** (for **9d**, **9f**, **12**–**19**), alcohol **28** was converted into its cyano derivative by mesylation and subsequent S_N2 reaction with sodium cyanide (NaCN). The resulting cyano derivative was then treated with hydroxylamine to obtain the amidoxime derivative. After *O*-acylation of the amidoxime with the corresponding acids by using 1,1'-carbonyldiimidazole, cyclization was achieved by heating to obtain the 1,2,4-oxadiazole intermediates **30d** and **30f**. Similarly, tetrazole intermediates **30e** and **30g** (for **9e**, **9g**, **20**–**27**) were prepared with 5-fluoro-2-(1*H*-1,2,3,4-tetrazol-5-yl)pyridine (**29e**) or 5-(4-fluorophenyl)-1*H*-tetrazole in the same manner as that to obtain **30a** in Scheme 1. The resulting compounds **30c**–**g** were subjected to *N*-ethylation followed by deprotection of the Boc group to obtain **43c**–**g**. Compounds **43c**–**g** were amidated with the corresponding benzoic acids using HATU or 1-propanephosphonic acid cyclic anhydride (T3P®) as a coupling agent to obtain **9c**–**9g** and **12**–**27**.

3. Results and discussion

Among compounds of the pyrazolylethylbenzamide class reported previously, compound **1** was one of the representative DORAs, which exhibited comparable inhibitory activity against both OX_1R and OX_2R, with IC_{50} values of 3.22 and 2.81 nM, respectively. This compound was generated by the ligand-based drug design approach carrying the characteristic U-shaped conformation[19], with which potent OXRs ligands, such as suvorexant, are known to bind to the OXRs.[18] Therefore, we first investigated the effects of restriction of the U-shaped

conformation on the inhibitory potency and selectivity. As shown in Figure 2, conformational analysis of the derivatives which were introduced as substituents at the ethyl linker between the pyrazole ring and benzamide suggested that the distribution of the pyridine moiety of **1** spread widely, while that of the methyl substituted compounds **2** and **3** was limited; especially that of (*S*)-enantiomer **2** was localized to the bioactive U-shaped conformation (Figure 2).[20] Based on the above findings, we synthesized a number of compounds bearing an ethyl linker and (*S*)-alkyl-substituted ethyl linkers (Table 1) and compared their inhibitory activities on OX_1R and OX_2R. The inhibitory activities (IC_{50}) on OX_1R and OX_2R were assessed in functional assays by measuring the intracellular Ca^{2+} mobilization in CHO cells overexpressing the human receptors.

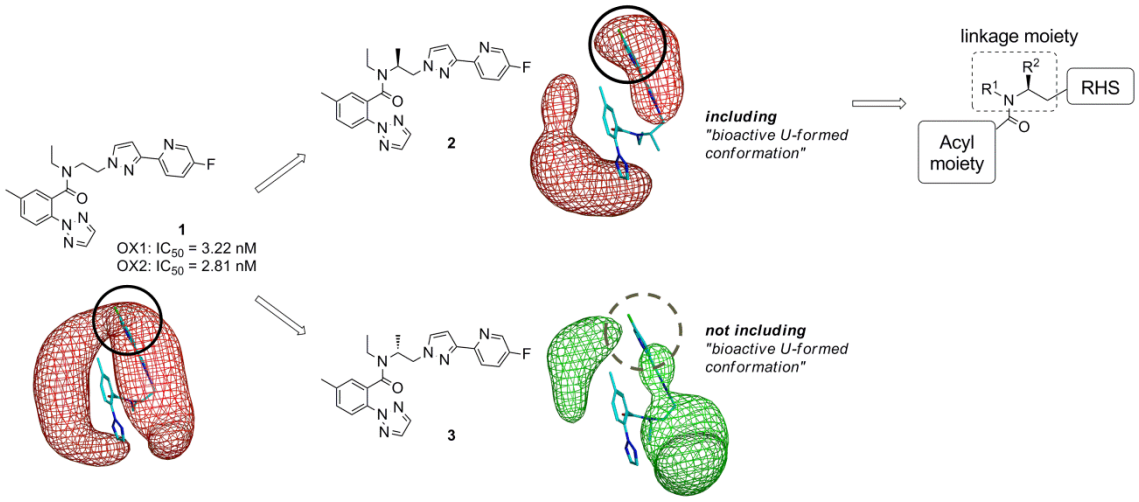


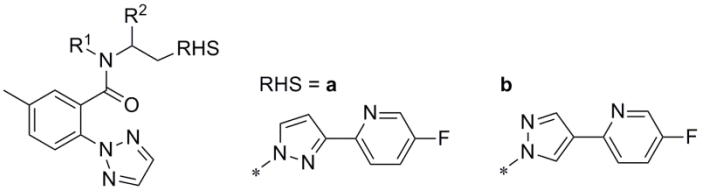
Figure 2. Conformational analysis of the derivatives

Red and green meshes show the distribution of the pyridine moiety under the alignment of the 5-tolyl triazole substructures. The conformations of the compounds were calculated using the replica-exchange molecular dynamics (REMD) simulation.[20]

All of the six (*S*)-methyl substituted derivatives (**7a**, **7b**, **8a**, **8b**, **2**, **9b**) exhibited more potent inhibitory activities as compared to the corresponding unsubstituted derivatives (**4a**, **4b**, **5a**, **5b**, **1**, **6b**) on both OX_1R and OX_2R. Comparison of the OX_1R inhibitory potency of (*S*)-methyl substituted compound **2** with that of (*R*)-methyl substituted derivative **3** suggested that placement of the (*S*)-methyl group into the ethyl linker played an important role in enhancing the inhibitory potency against both OX_1R and OX_2R, as expected. In addition, these (*S*)-methyl

substituted derivatives showed a trend towards higher OX_1R selectivity (SI) as compared to the corresponding unsubstituted derivatives. The (*S*)-ethyl-substituted derivative **10** and the (*S*)-isopropyl-substituted **11** showed higher OX_1R selectivity than the (*S*)-methyl-substituted **8a**, while the OX_1R inhibitory potencies of **10** and **11** were weaker than that of **8a**. Among the derivatives bearing a 3-(5-fluoropyridin-2-yl)pyrazole on the right hand side (RHS), compound **2** with an ethyl group at the amide nitrogen exerted the most potent activity on OX_1R (IC_{50}: 1.62 nM), and compound **9b** was the most potent derivative (IC_{50}: 0.767 nM) among the compounds of the 4-(5-fluoropyridin-2-yl)pyrazole class (Table 1). Interestingly, compound **2** did not show OX_1R selectivity (SI: 1.0), while compound **9b** showed slight selectivity (SI: 7.7).

Table 1. SAR of linkage moiety



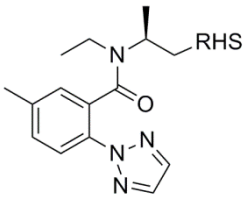
Compd	R^1	R^2	RHS	IC_{50} (nM)[a]		SI
				OX_1R	OX_2R	
4a	H	H	**a**	119	198	1.7
4b	H	H	**b**	908	1460	1.6
5a	Me	H	**a**	5.54	11.9	2.1
5b	Me	H	**b**	56.1	51.7	0.92
1	Et	H	**a**	3.22	2.81	0.87
6b	Et	H	**b**	9.06	14.2	1.6
7a	H	(*S*)-Me	**a**	10.1	58.4	5.8
7b	H	(*S*)-Me	**b**	245	840	3.4
8a	Me	(*S*)-Me	**a**	1.90	5.68	3.0
8b	Me	(*S*)-Me	**b**	4.39	21.2	4.8
2	Et	(*S*)-Me	**a**	1.62	1.61	1.0
9b	Et	(*S*)-Me	**b**	0.767	5.87	7.7
3	Et	(*R*)-Me	**a**	86.6	250	2.9
10	Me	(*S*)-Et	**a**	4.39	29.0	6.6
11	Me	(*S*)-*i*Pr	**a**	61.5	952	16

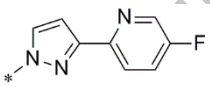
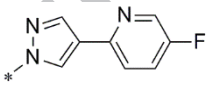
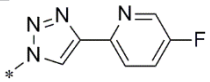
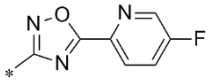
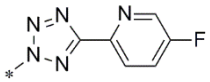
[a]The experiments were performed two to three times, and each test was performed in duplicate or triplicate.

The different selectivity (SI) of compounds **2** and **9b** suggested that the heteroaromatic motif on the RHS may have an influence on conferring selectivity for OX_1R over OX_2R. Therefore, we further synthesized five-membered

heteroaromatic derivatives and evaluated the inhibitory potencies on OX_1R and OX_2R, to examine the selectivity for OX_1R (Table 2). Indeed, the oxadiazole derivative **9d** and tetrazole derivative **9e** showed better selectivity for OX_1R over OX_2R (SI: **9d** = 9.0, **9e** = 16), without impairing the OX_1R antagonistic activity (IC_{50}: **9d** = 1.26 nM, **9e** = 1.10 nM). These results encouraged us to further modify compounds **9d** and **9e**, in which two substituents on the benzamide moiety (R^3) were replaced and/or the nitrogen on the 4-fluoropyridyl group on the RHS was substituted with a carbon atom to improve the selectivity and maintain the antagonist potency for OX_1R.

Table 2. SAR of RHS

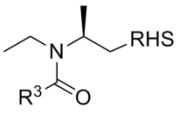


Compd	RHS	IC_{50} (nM)[a]		SI	*C*log*P*[b]
		OX_1R	OX_2R		
2		1.62	1.61	1.0	4.2
9b		0.767	5.87	7.7	4.0
9c		1.93	22.8	12	3.6
9d		1.26	11.3	9.0	2.8
9e		1.10	17.9	16	3.4

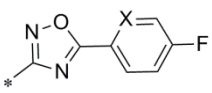
[a]The experiments were performed two to four times, and each test was performed in duplicate or triplicate. [b]The ClogP values were calculated using software from Daylight Chemical Information Systems, Inc.

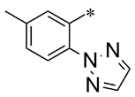
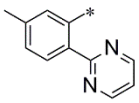
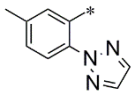
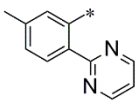
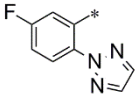
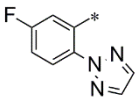
Table 3 shows the inhibitory potencies for OX_1R and OX_2R and the OX_1R selectivity (SI) of the oxadiazole derivatives (**9f**, **12**–**19**). These derivatives, except for **14**, exhibited single digit nano-molar potency for OX_1R. With respect to the OX_1R selectivity, it is worthy of note that the three derivatives **14**, **16** and **19** showed greater than 50-fold selectivity for OX_1R over OX_2R. As for the tetrazole derivatives (**9g**, **21**–**27**) (Table 4), two derivatives (**24** and **27**) exhibited single digit nano-molar potency for OX_1R and greater than 200-fold selectivity for OX_1R over OX_2R. Interestingly, 5-fluorobenzene and 2-methylpyridine as the R^3 moiety emerged as the common structures for enhancing the OX_1R selectivity, through modifications of **9d** and **9e**. We speculated on why these structures improved the selectivity for OX_1R over OX_2R. According to recent publications on the crystal structures of human OX_1R[21] and OX_2R[22] bound to suvorexant, OX_1R and OX_2R are highly similar, but there are two divergent residues between the subtypes at the orthosteric pocket (OX_1R to OX_2R: Ser103 to Thr111 and Ala127 to Thr135).

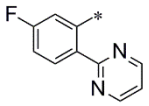
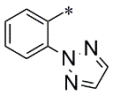
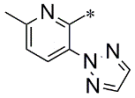
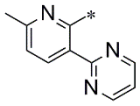
Table 3. SAR of acyl moiety (RHS = 4-fluoropyridyl oxadiazole ring **d**)



RHS = **d**

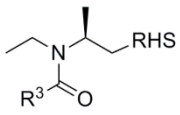


Compd	R^3	X	IC_{50} (nM)[a]		SI	*Clog*P[b]
			OX_1R	OX_2R		
9d		N	1.26	11.3	9.0	2.8
12		N	3.24	43.2	13	2.0
9f		CH	1.07	2.73	2.6	3.9
13		CH	1.40	8.85	6.3	3.1
14		N	14.7	887	60	2.5
15		CH	1.26	23.6	19	3.6

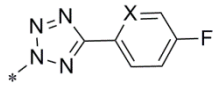
16		CH	1.58	80.1	51	2.8
17		CH	1.25	11.6	9.3	3.4
18		CH	1.82	40.5	22	3.0
19		CH	3.58	254	71	2.0

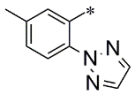
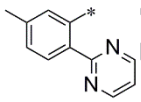
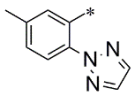
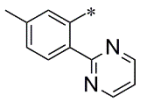
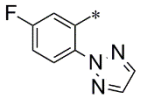
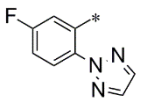
[a]The experiments were performed two to three times, and each test was performed in duplicate or triplicate. [b]The ClogP values were calculated using software from Daylight Chemical Information Systems, Inc.

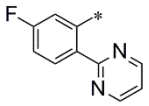
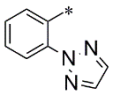
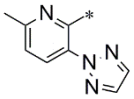
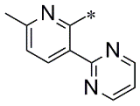
Table 4. SAR of acyl moiety (RHS = 4-fluoropyridyl tetrazole ring **e**)



RHS = **e**



Compd	R^3	X	IC_{50} (nM)[a]		SI	*Clog*P[b]
			OX_1R	OX_2R		
9e		N	1.10	17.9	16	3.4
20		N	10.6	129	12	2.6
9g		CH	0.820	2.57	3.1	4.6
21		CH	1.81	36.0	20	3.8
22		N	9.45	471	50	3.1
23		CH	3.06	139	45	4.3

24		CH	2.01	532	265	3.5
25		CH	2.45	61.0	25	4.1
26		CH	1.84	119	65	3.7
27		CH	6.15	1310	213	2.7

[a]The experiments were performed two to three times, and each test was performed in duplicate or triplicate. [b]The ClogP values were calculated using software from Daylight Chemical Information Systems, Inc.

To clarify the reason for the increased OX_1R selectivity conferred by replacement of the 5-tolyl substituent with 5-fluorobenzene, we calculated the binding modes of **9g** and **23** with OX_1R and OX_2R by molecular docking simulation using the crystal structures.[23] The binding modes of the compounds to OX_1R and OX_2R suggest that the 5-tolyl moiety in **9g** and 5-fluorobenzene moiety in **23** were directed toward the Ser103 in OX_1R or toward Thr111 in OX_2R, which represent one of the two divergent positions between the subtypes (Figure 3). Since the binding modes of **9g** and **23** to OX_2R are very similar, it might be considered that the antagonistic activity of fluorine-substituted **23** on OX_2R was attenuated by electrostatic factors, resulting in improved selectivity for OX_1R. More precisely, it appears that the electronegative fluorine atom on the benzamide moiety of **23** leads to repulsion in the location of the relatively negative electrostatic potential surface generated by Thr111 in OX_2R, resulting in improved selectivity for OX_1R. On the other hand, we remain unable to explain the reason for the increased OX_1R selectivity of compounds such as compound **27** obtained by the replacement of 5-tolyl with 6-methyl-2-pyridine.

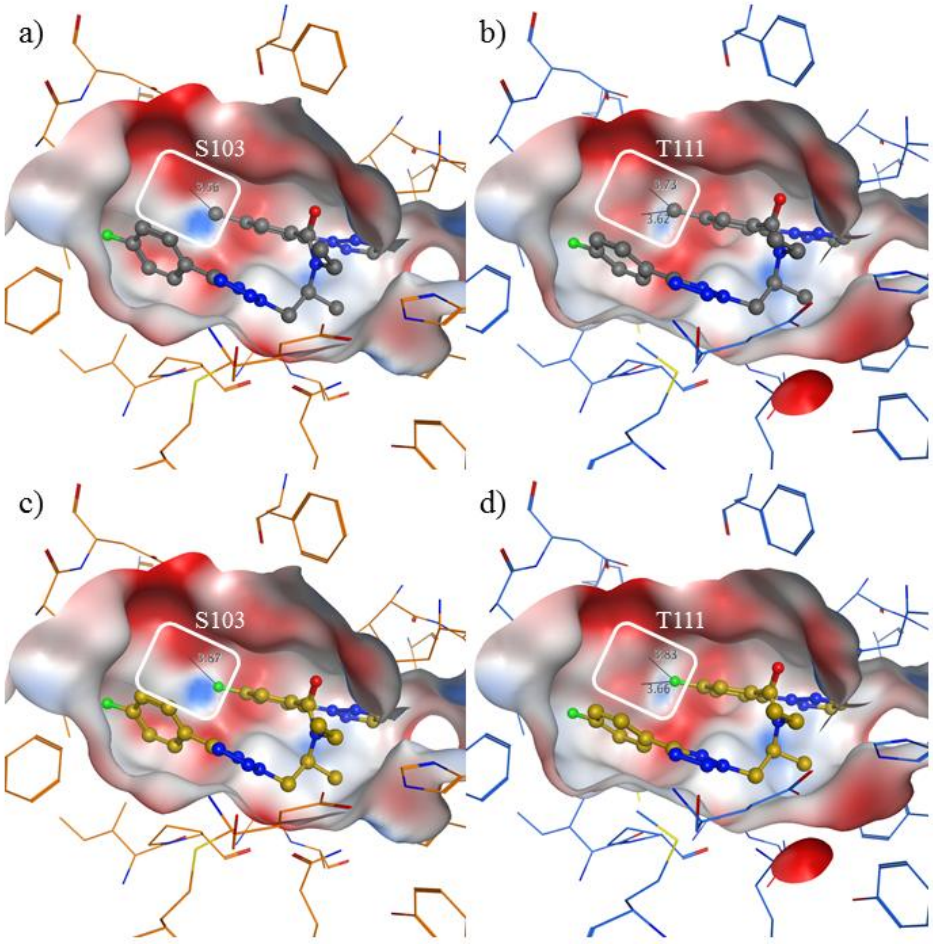


Figure 3. The predicted binding modes of compounds **9g** and **23** with OX_1R and OX_2R.

The surface areas of OX_1R and OX_2R of positive, neutral and negative electrostatic potential energy are shown in blue, white and red. Compounds, **9g** (gray carbon) and **23** (yellow carbon), are shown as balls and sticks. All residues within 5 angstroms from the compounds are shown as lines (OX_1R: orange carbon, OX_2R: blue carbon).

a,b) Interactions between **9g** and OX_1R or OX_2R. The distance between the carbon of methyl group in **9g** (Me_{9g}) and the oxygen of hydroxyl group of S103 (O_{S103}) in OX_1R was 3.56 angstroms. These between Me_{9g} and the oxygen of hydroxyl group of T111 (O_{T111}) or the carbon of methyl group of T111 (Me_{T111}) in OX_2R were 3.62 or 3.73 angstroms, respectively.

c,d) Interactions between **23** and OX_1R or OX_2R. The distance between fluorine in **23** (F_{23}) and O_{S103} was 3.87 angstroms. These between F_{23} and O_{T111} or Me_{T111} were 3.66 or 3.83 angstroms, respectively.

4. Conclusion

In conclusion, we investigated the SARs in detail from the structural framework of pyrazolylethylbenzamide **1**, which was previously revealed as a DORA, and identified the potent and highly selective OX_1R antagonist **24** with

an IC_{50} of 2.01 nM and 265-fold selectivity. The results of investigation of the SARs suggested that incorporation of a (*S*)-methyl group into the ethyl linker enhanced the inhibitory potencies on OX_1R and OX_2R with OX_1R preference, and the acyl moiety was relatively susceptible to OX_1R selectivity. Especially, replacement of the 5-tolyl moiety with 5-fluorobenzene or 2-methylpyridine enhanced the selectivity, and this feature might be associated with electrostatic factors at the divergent positions between the OX_1R and OX_2R subtypes by molecular docking simulations based on the X-ray structures of the orexin receptors in the case of replacement with 5-fluorobenzene.

The present SAR findings could provide helpful information to transform potent DORAs into 1-SORAs, which could serve as probes in research on the pharmacological functions of OX_1R. Existence of a link between stress and anxiety related behaviors[24,25] and activation of the OX_1R is highly likely, and future clinical studies will hopefully provide final confirmation about the efficacy, without inducing somnolence[15], with appropriate 1-SORA compounds such as compound **24**.

5. Experimental section

5.1. Biology

5.1.1. Antagonistic activity on human OX1 and OX2 receptors

The antagonistic activities of the test compounds were determined as described previously[18]. Chinese hamster ovary cells stably expressing recombinant human OX_1R or human OX_2R were seeded into a 96-well black/clear bottom plate at 2.4×10^4 cells/well one day before the experiment. The cells were incubated in loading buffer [Hank's balanced salt solution (HBSS) (pH 7.4) containing 20 mM HEPES, 0.1% BSA, 0.2 mg/ml amaranth, 2.5 mM probenecid, 0.02% pluronic F-127 and 0.15 μM of Fluo-4AM (Invitrogen)] at 37 °C for 1 h. Then, after removal of the loading buffer, the cells were incubated with assay buffer [HBSS (pH 7.4) containing 20 mM HEPES, 0.1% BSA, 0.2 mg/ml amaranth, 2.5 mM probenecid], with or without the test compounds at various concentrations, for 30 min at room temperature. The changes in the intracellular Ca^{2+} concentrations 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 or 1 nM for human OX_2R).

The concentration-response curves were fitted using nonlinear regression analyses; 50% inhibitory concentration (IC_{50}) values were calculated using the GraphPad Prism software (version 5.04; GraphPad Software Inc., San Diego, CA). The experiments were performed two to four times, and each test was performed in duplicate or triplicate.

5.2. Chemistry

5.2.1. General methods

All 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, acetonitrile + 0.1% CF_3CO_2H; 10% B up to 95% B in 12 min; Flow rate 40 mL/min. ^1H and ^{13}C NMR spectra were recorded on a JOEL 600 MHz, 500 MHz, and BRUKER 400 MHz NMR spectrometers, and all chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS, δ 0.00 ppm), used as the internal standard. High-resolution mass spectral data were acquired by a Shimadzu LCMS-IT-TOF equipped with an ESI/APCI dual ion source. Mass spectra (MS) were recorded on a Shimadzu LCMS-IT-TOF or Agilent LC-MS equipped with an ESI/APCI dual ion source. The LC-MS was performed under the following conditions: 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, acetonitrile + 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).

The *N*-alkylated benzamide 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 ^1H and ^{13}C NMR spectra of compounds **2**, **3**, **5b**, **8a**, **8b**, **9b**–**9g**, and **10**–**27** consisted of broad and complicated multiplets, analysis in detail of the coupling constants of which was difficult. Thus, the NMR resonances of these compounds are not listed in numerical format. Instead, we have included pictures of the ^1H and ^{13}C NMR spectra at 25 °C of these compounds in the Supplementary data. **1**, **4a**, **5a**, **6b**, **29a**, and **29b** were prepared according to procedures reported in the literature.[21]

5.2.2. *tert*-Butyl {(2*S*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}carbamate (30a)

To a solution of **28** (10.0 g, 57.1 mmol) in THF (114 mL) was added Et_3N (11.9 mL, 85.6 mmol) and methanesulfonyl chloride (4.70 mL, 59.9 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and then filtered through a pad of KC flock®. The filtrate was concentrated under reduced pressure to obtain (2*S*)-2-[(*tert*-butoxycarbonyl)amino]propyl methanesulfonate as a colorless oil. To a solution of the methanesulfonate in DMF (100 mL) was added 5-fluoro-2-(1*H*-pyrazol-3-yl)pyridine **29a** (8.40 g, 51.4 mmol) and Cs_2CO_3 (37.2 g, 0.110 mol) at room temperature. After the mixture was stirred at 80 °C for 3 h, water was added, and the reaction 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 solid was washed with EtOAc to obtain the title compound **30a** as a colorless solid (0.50 g, 3% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 2.81 Hz, 1H), 7.95 (dd, J = 4.46, 8.74 Hz, 1H), 7.39–7.47 (m, 2H), 6.84 (d, J = 2.20 Hz, 1H), 4.86 (br s, 1H), 4.18–4.33 (m, 2H), 4.01–4.14 (m, 1H), 1.42 (s, 9H), 1.16 (d, J = 6.72 Hz, 3H); MS (ESI/APCI dual): *m/z* 321 $[M+H]^+$.

5.2.3. *tert*-Butyl {(2*S*)-1-[4-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}carbamate (30b)

The title compound was prepared from 5-fluoro-2-(1*H*-pyrazol-4-yl)pyridine **29b** according to the procedure as described for compound **30a** (44% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 2.69 Hz, 1H), 7.93 (s, 1H), 7.89 (s, 1H), 7.34–7.48 (m, 2H), 4.96 (br s, 1H), 4.14–4.35 (m, 2H), 3.98–4.13 (m, 1H), 1.43 (s, 9H), 1.14 (d, J = 6.72 Hz, 3H); MS (ESI/APCI dual): *m/z* 321 $[M+H]^+$.

5.2.4.

***N*-{(2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (7a)**

To a solution of **30a** (0.50 g, 1.6 mmol) in EtOAc (4.0 mL) was added hydrogen chloride in EtOAc (4 mol/L, 3.9 mL, 0.016 mol) at room temperature, and the resulting mixture was stirred for 15 h. The organic solvent was removed under reduced pressure to obtain the primary amine as a colorless amorphous.

A mixture of the primary amine, 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid **31** (0.33 g, 1.6 mmol),

N,*N*-diisopropylethylamine (DIPEA, 2.5 mL, 14.7 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU, 0.91 g, 2.4 mmol) in DMF (3.2 mL) 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. The resulting residue was purified by preparative HPLC to obtain the title compound **7a** as a colorless solid (0.18 g, 28% over 2 steps). HRMS (ESI/APCI dual) for $C_{21}H_{21}FN_7O$ $[M+H]^+$, calcd: 406.1786, found: 406.1785; LC-MS t = 0.78 min, $[M+H]^+$ = 406; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, J = 2.68 Hz, 1H), 7.87 (dd, J = 4.59, 8.79 Hz, 1H), 7.74 (s, 2H), 7.64 (d, J = 8.03 Hz, 1H), 7.50 (d, J = 2.29 Hz, 1H), 7.37–7.45 (m, 2H), 7.32–7.36 (m, 1H), 6.83 (d, J = 2.68 Hz, 1H), 6.44 (br d, J = 7.64 Hz, 1H), 4.45–4.54 (m, 1H), 4.30 (dd, J = 5.35, 13.38 Hz, 1H), 4.21–4.27 (m, 1H), 2.37 (s, 3H), 1.12 (d, J = 6.88 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 159.8, 157.8, 151.3, 148.5, 148.5, 139.0, 137.4, 137.2, 135.5, 134.8, 132.2, 131.3, 131.0, 129.5, 124.3, 123.5, 123.3, 121.0, 121.0, 104.3, 55.8, 46.2, 20.9, 17.4.

5.2.5.

***N*-{(2*S*)-1-[4-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (7b)**

The title compound was prepared from **30b** according to the procedure as described for compound **7a** (60% yield in 2 steps, colorless solid). HRMS (ESI/APCI dual) for $C_{21}H_{21}FN_7O$ $[M+H]^+$, calcd: 406.1786, found: 406.1767; LC-MS t = 0.74 min, $[M+H]^+$ = 406; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (d, J = 3.06 Hz, 1H), 7.96 (s, 1H), 7.89 (s, 1H), 7.76 (s, 2H), 7.65 (d, J = 8.03 Hz, 1H), 7.32–7.45 (m, 4H), 6.44 (br d, J = 7.64 Hz, 1H), 4.43–4.52 (m, 1H), 4.31 (dd, J = 5.00, 13.40 Hz, 1H), 4.25 (dd, J = 5.00, 13.80 Hz, 1H), 2.41 (s, 3H), 1.12 (d, J = 6.88 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.1, 158.9, 156.9, 148.2, 148.1, 139.0, 137.7, 137.5, 135.6, 134.8, 131.3, 130.9, 129.5, 129.1, 124.2, 123.6, 123.4, 122.7, 120.2, 120.2, 55.8, 46.2, 21.0, 17.3.

5.2.6.

***N*-{(2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-*N*,5-dimethyl-2-(2*H*-1,2,3-triazol-2-yl)benz**

amide (8a)

NaH (55% in mineral oil, 3.9 mg, 0.089 mmol) was added to a solution of **7a** (0.030 g, 0.074 mmol) in DMF (0.74 mL), and the mixture was stirred at 80 °C for 1 h. After cooling in an ice bath, MeI (5.1 μL, 0.081 mmol) was added dropwise, and the resulting mixture was stirred at room temperature 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 Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (20–100% EtOAc in hexanes) to obtain the title compound **8a** as a colorless amorphous (0.017 g, 55%). HRMS (ESI/APCI dual) for $C_{22}H_{23}FN_7O$ $[M+H]^+$, calcd: 420.1943, found: 420.1941; LC-MS t = 0.80–0.89 min, $[M+H]^+$ = 420. 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.

5.2.7. Preparation of Compounds 8b, 2, and 9b

These compounds were synthesized from their corresponding precursors according to the procedure as described for compound **8a**.

5.2.7.1.

***N*-{(2*S*)-1-[4-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-*N*,5-dimethyl-2-(2*H*-1,2,3-triazol-2-yl)benz amide (8b)**

The title compound was prepared from **7b** (55% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{22}H_{23}FN_7O$ $[M+H]^+$, calcd: 420.1943, found: 420.1941; LC-MS t = 0.75–0.83 min, $[M+H]^+$ = 420. 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.

5.2.7.2.

***N*-Ethyl-*N*-{(2*S*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl) benzamide (2)**

The title compound was prepared from **7a** (73% yield, colorless amorphous). HRMS (ESI/APCI dual) for

$C_{23}H_{25}FN_7O$ $[M+H]^+$, calcd: 434.2099, found: 434.2099; LC-MS t = 0.91 min, $[M+H]^+$ = 434. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.7.3.

***N*-Ethyl-*N*-{(2*S*)-1-[4-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl) benzamide (9b)**

The title compound was prepared from **7b** (88% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{23}H_{25}FN_7O$ $[M+H]^+$, calcd: 434.2099, found: 434.2096; LC-MS t = 0.90–0.95 min, $[M+H]^+$ = 434. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.8. *tert*-Butyl {2-[4-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]ethyl}carbamate (33)

Compound **32** (0.50 g, 2.2 mmol) and Cs_2CO_3 (1.5 g, 4.5 mmol) were added to a solution of **29b** (0.36 g, 2.2 mmol) in DMF (2 mL) at room temperature, and the resulting 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 by column chromatography (20–80% EtOAc in hexanes) to obtain the title compound **33** as a colorless solid (0.47 g, 69%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 2.69 Hz, 1H), 7.94 (s, 1H), 7.88 (s, 1H), 7.34–7.47 (m, 2H), 4.94 (br s, 1H), 4.27 (t, J = 5.40 Hz, 2H), 3.57–3.68 (m, 2H), 1.43 (s, 9H); MS (ESI/APCI dual): m/z 307 $[M+H]^+$.

5.2.9. *N*-{2-[4-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]ethyl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (4b)

The title compound was prepared from **33** according to the procedure as described for compound **7a** (42% yield in 2 steps, colorless solid). HRMS (ESI/APCI dual) for $C_{20}H_{19}FN_7O$ $[M+H]^+$, calcd: 392.1630, found: 392.1619; LC-MS t = 0.69 min, $[M+H]^+$ = 392; ^1H NMR (500 MHz, $CDCl_3$) δ 8.39 (d, J = 2.68 Hz, 1H), 7.95 (s, 1H), 7.89 (s, 1H), 7.75 (s, 2H), 7.63 (d, J = 8.03 Hz, 1H), 7.35–7.43 (m, 3H), 7.31–7.34 (m, 1H), 6.45 (br t, J = 5.35 Hz, 1H), 4.33 (t, J = 5.35 Hz, 2H), 3.83 (dd, J = 6.12, 11.47 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0,

158.9, 156.9, 148.1, 148.1, 138.9, 137.9, 137.7, 137.5, 135.6, 134.8, 131.4, 130.5, 129.5, 128.8, 124.2, 123.6, 123.5, 122.6, 120.2, 120.2, 51.0, 40.1, 20.9.

5.2.10.

***N*-{2-[4-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]ethyl}-*N*,5-dimethyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (5b)**

The title compound was prepared from **4b** according to the procedure as described for compound **8a** (44% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{21}H_{21}FN_7O$ $[M+H]^+$, calcd: 406.1786, found: 406.1784; LC-MS t = 0.80 min, $[M+H]^+$ = 406. 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.

5.2.11. Preparation of Compounds 37, 38, and 39

These compounds were from their corresponding precursors according to the procedure as described for compound **30a**.

5.2.11.1. *tert*-Butyl {(2*R*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}carbamate (37)

The title compound was prepared from **34** (20% yield in 2steps, colorless solid). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47 (d, J = 2.89 Hz, 1H), 7.95 (dd, J = 4.54, 8.67 Hz, 1H), 7.41–7.46 (m, 2H), 6.84 (d, J = 2.06 Hz, 1H), 4.87 (br s, 1H), 4.19–4.32 (m, 2H), 4.02–4.10 (m, 1H), 1.42 (s, 9H), 1.16 (d, J = 7.02 Hz, 3H).

5.2.11.2. *tert*-Butyl {(2*S*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]butan-2-yl}carbamate (38)

The title compound was prepared from **35** (33% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 2.69 Hz, 1H), 7.95 (dd, J = 4.52, 8.80 Hz, 1H), 7.39–7.47 (m, 2H), 6.83 (d, J = 2.32 Hz, 1H), 4.84 (br d, J = 5.75 Hz, 1H), 4.22–4.33 (m, 2H), 3.77–3.92 (m, 1H), 1.62–1.71 (m, 1H), 1.47–1.58 (m, 1H), 1.42 (s, 9H), 0.97 (t, J = 7.40 Hz, 3H); MS (ESI/APCI dual): *m/z* 335 $[M+H]^+$.

5.2.11.3. {(2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]-3-methylbutan-2-yl}carbamate (39)

The title compound was prepared from **36** (22% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 2.81 Hz, 1H), 7.94 (dd, J = 4.46, 8.74 Hz, 1H), 7.34–7.51 (m, 2H), 6.82 (d, J = 2.20 Hz, 1H), 4.82 (br d, J = 9.54 Hz, 1H), 4.29 (d, J = 5.40 Hz, 2H), 3.69–3.93 (m, 1H), 1.65–1.87 (m, 1H), 1.38 (s, 9H), 1.02 (d, J = 6.72 Hz, 3H), 0.95 (d, J = 6.85 Hz, 3H); MS (ESI/APCI dual): *m/z* 349 $[M+H]^+$.

5.2.12. (2*R*)-*N*-Ethyl-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-amine hydrochloride (40)

NaH (60% in mineral oil, 0.056 g, 1.4 mmol) was added to a solution of **37** (0.30 g, 0.94 mmol) in DMF (5.0 mL), and the mixture was stirred at room temperature for 30 min. EtI (90 μL, 1.1 mmol) was added dropwise to the reaction mixture under an ice bath and the mixture was stirred at room temperature for 1 day. 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 by column chromatography (20–100% EtOAc in hexanes) to obtain *tert*-butyl {(2*R*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}carbamate as a colorless oil (0.26 g, 80%).

To the solution of *tert*-butyl {(2*R*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}carbamate (0.26 g, 0.75 mmol) in MeOH (5.0 mL) was added hydrogen chloride in EtOAc (4 mol/L, 5.0 mL, 20 mmol) at room temperature, and the resulting mixture was stirred for 18 h. The precipitate was then collected by filtration to obtain the title compound **40** as a colorless solid (0.17 g, 71% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (br s, 2H), 8.59 (d, J = 2.93 Hz, 1H), 8.01 (dd, J = 4.58, 8.86 Hz, 1H), 7.96 (d, J = 2.32 Hz, 1H), 7.81 (dt, J = 2.93, 8.80 Hz, 1H), 6.85 (d, J = 2.20 Hz, 1H), 4.60 (dd, J = 5.01, 14.18 Hz, 1H), 4.44 (dd, J = 6.60, 14.18 Hz, 1H), 3.63–3.78 (m, 1H), 2.89–3.12 (m, 2H), 1.25 (t, J = 7.15 Hz, 3H), 1.19 (d, J = 6.60 Hz, 3H); MS (ESI/APCI dual): *m/z* 249 $[M+H]^+$.

5.2.13. Preparation of Compounds 41 and 42

These compounds were prepared from their corresponding precursors according to the procedure as described for compound **40**.

5.2.13.1. (2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]-*N*-methylbutan-2-amine hydrochloride (41)

The title compound was prepared from **38** (28% yield in 2 steps, colorless oil). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (br s, 2H), 8.58 (d, J = 2.93 Hz, 1H), 8.06 (dd, J = 4.46, 8.86 Hz, 1H), 7.98 (d, J = 2.32 Hz, 1H), 7.80 (dt, J = 2.93, 8.74 Hz, 1H), 6.84 (d, J = 2.32 Hz, 1H), 4.47–4.64 (m, 2H), 3.41–3.64 (m, J = 5.10, 8.00 Hz, 1H), 2.60 (t, J = 5.26 Hz, 3H), 1.61–1.74 (m, 1H), 1.49 (quind, J = 7.45, 14.69 Hz, 1H), 0.97 (t, J = 7.52 Hz, 3H); MS (ESI/APCI dual): *m/z* 249 $[M+H]^+$.

5.2.13.2. (2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]-*N*,3-dimethylbutan-2-amine hydrochloride (42)

The title compound was prepared from **39** (26% yield in 2 steps, colorless oil). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (br s, 1H), 9.10 (br s, 1H), 8.59 (d, J = 2.20 Hz, 1H), 7.98–8.13 (m, 2H), 7.73–7.92 (m, 1H), 6.80–6.93 (m, 1H), 4.45–4.56 (m, 2H), 3.45–3.61 (m, 1H), 2.45 (br t, J = 5.01 Hz, 3H), 1.99–2.23 (m, 1H), 1.00 (dd, J = 5.20, 6.91 Hz, 6H); MS (ESI/APCI dual): *m/z* 263 $[M+H]^+$.

5.2.14. Preparation of Compounds 3, 10, and 11

These compounds were prepared from their corresponding precursors according to the procedure as described for compound **7a**.

5.2.14.1.

***N*-Ethyl-*N*-{(2*R*)-1-[3-(5-fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl) benzamide (3)**

The title compound was prepared from **40** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (40% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{23}H_{25}FN_7O$ $[M+H]^+$, calcd: 434.2099, found: 434.2100; LC-MS t = 0.92 min, $[M+H]^+$ = 434. 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.

5.2.14.2.

***N*-{(2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]butan-2-yl}-*N*,5-dimethyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (10)**

The title compound was prepared from **41** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (60% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{23}H_{25}FN_7O$ $[M+H]^+$, calcd: 434.2099, found: 434.2096; LC-MS t = 0.87–0.95 min, $[M+H]^+$ = 434. 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.

5.2.14.3.

***N*-{(2*S*)-1-[3-(5-Fluoropyridin-2-yl)-1*H*-pyrazol-1-yl]-3-methylbutan-2-yl}-*N*,5-dimethyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (11)**

The title compound was prepared from **42** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (76% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{24}H_{27}FN_7O$ $[M+H]^+$, calcd: 448.2256, found: 448.2265; LC-MS t = 1.00 min, $[M+H]^+$ = 448. 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.

5.2.15. *tert*-Butyl {(2*S*)-1-[4-(5-fluoropyridin-2-yl)-1*H*-1,2,3-triazol-1-yl]propan-2-yl}carbamate (30c)

To a solution of **28** (19.9 g, 0.110 mol) in $CHCl_3$ (100 mL) were added Et_3N (31.4 mL, 0.230 mol) and methanesulfonyl chloride (13.2 mL, 0.170 mol), and the 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 concentrated to obtain (2*S*)-2-[(*tert*-butoxycarbonyl)amino]propyl methanesulfonate as a colorless oil.

To a solution of the methanesulfonate in DMF (100 mL) was added NaN_3 (8.10 g, 0.120 mol), and the resulting mixture was stirred at 80 °C for 3 h. The reaction was quenched by the addition of water, 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 by column chromatography (20–100% EtOAc in hexanes) to obtain *tert*-butyl *N*-[(2*S*)-1-azidopropan-2-yl]carbamate as a colorless oil (18.8 g, 83% yield).

tert-Butyl *N*-[(2*S*)-1-azidopropan-2-yl]carbamate (0.30 g, 2.5 mmol) and 2-ethynyl-5-fluoropyridine (0.50 g, 2.5 mmol) were suspended in a mixture of 4-methylpiperidine (2.0 mL) and DMF (8.0 mL). CuI (0.024 g, 0.12 mmol) and L-ascorbic acid (0.087 g, 0.50 mmol) were added to the mixture, and the mixture was stirred at 80 °C for 2 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 by column chromatography (20–100% EtOAc in hexanes) to obtain the title compound **30c** as a colorless solid (0.58 g, 73% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (d, J = 2.89 Hz, 1H), 8.18 (dd, J = 4.33, 8.46 Hz, 1H), 8.10 (s, 1H), 7.50 (dt, J = 2.89, 8.46 Hz, 1H), 4.67 (br s, 1H), 4.42–4.61 (m, 2H), 4.05–4.19 (m, 1H), 1.42 (s, 9H), 1.18 (d, J = 6.61 Hz, 3H); MS (ESI/APCI dual): *m/z* 322 $[M+H]^+$.

5.2.16. *tert*-Butyl {(2*S*)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}carbamate (30f)

To a solution of **28** (5.0 g, 29 mmol) in THF (50 mL) were added Et_3N (7.0 mL, 50 mmol) and methanesulfonyl chloride (2.1 mL, 27 mmol), in an ice bath. After this mixture was stirred at 0 °C for 15 min, the mixture was filtered through a pad of KC flock®. The filtrate was concentrated under reduced pressure to obtain (2*S*)-2-[(*tert*-butoxycarbonyl)amino]propyl methanesulfonate as a colorless oil.

The mixture of sodium cyanide (1.6 g, 32 mmol) in DMF (29 mL) was stirred at 35 °C for 30 min. To the mixture was added tetrabuthylammonium bromide (6.9 mL, 25 mmol). After this mixture was stirred for 2 h, a solution of the methanesulfonate in DMF (2.5 mL) was added to the mixture. The resulting mixture was stirred at 50 °C for 5 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 Na_2SO_4, filtered, and concentrated under reduced pressure to obtain *tert*-butyl *N*-[(1*S*)-2-cyano-1-methyl-ethyl]carbamate (3.2 g, 60% yield over 2 steps) as a colorless solid.

To a mixture of the above carbamate (1.5 g, 8.1 mmol) in EtOH (8.0 mL) was added hydroxylamine (50% in water, 0.96 mL, 16 mmol) at room temperature, and the mixture was stirred at 90 °C for 5 h. After cooling to room temperature, the mixture was concentrated under reduced pressure, water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine and concentrated under reduced pressure to obtain the crude

oil, which was used without further purification. To a mixture of the crude product and 4-fluorobenzoic acid (1.3 g, 9.0 mmol) in DMF (3.0 mL) was added 1,1'-carbonyldiimidazole (1.6 g, 9.8 mmol), and the resulting mixture was stirred at 90 °C for 13 h. After adding a saturated aqueous solution of $NaHCO_3$ to the mixture, the resulting precipitate was filtered to obtain the title compound **30f** as a colorless solid (1.4 g, 55% yield in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09–8.18 (m, 2H), 7.17–7.25 (m, 2H), 4.89 (br s, 1H), 4.12–4.28 (m, 1H), 2.93–3.01 (m, 2H), 1.43 (s, 9H), 1.23 (d, J = 6.72 Hz, 3H); MS (ESI/APCI dual): *m/z* 322 $[M+H]^+$.

5.2.17. *tert*-Butyl {(2*S*)-1-[5-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-3-yl]propan-2-yl}carbamate (30d)

The title compound was prepared from 5-fluoro-2-pyridinecarboxylic acid according to the procedure as described for compound **30f** (42% yield in 4 steps, colorless amorphous). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.67 (d, J = 2.89 Hz, 1H), 8.26 (dd, J = 4.13, 8.26 Hz, 1H), 7.61 (dt, J = 2.89, 8.26 Hz, 1H), 4.82 (br s, 1H), 4.15–4.29 (m, 1H), 2.88–3.13 (m, 2H), 1.42 (s, 9H), 1.25 (d, J = 6.61 Hz, 3H); MS (ESI/APCI dual): *m/z* 345 $[M+Na]^+$.

5.2.18. 5-Fluoro-2-(1*H*-1,2,3,4-tetrazol-5-yl)pyridine (29e)

To a solution of 2-cyano-5-fluoropyridine (2.0 g, 0.016 mol) in water (33 mL) were added NaN_3 (1.2 g, 0.019 mol) and $ZnBr_2$ (3.7 g, 0.016 mol), and the resulting mixture was stirred at 110 °C for 3 h. After cooling to room temperature, hydrochloric acid (1.2 mol/L, 66 mL, 0.079 mol) and EtOAc (70 mL) were added, and the mixture was stirred for 1 h. The mixture was extracted with EtOAc, and the organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain the title compound **29e** as a colorless solid (1.5 g, 56% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.67 (d, J = 2.93 Hz, 1H), 8.31 (dd, J = 4.22, 8.62 Hz, 1H), 7.85 (dt, J = 2.87, 8.53 Hz, 1H); MS (ESI/APCI dual): *m/z* 166 $[M+H]^+$.

5.2.19. Preparation of Compounds 30e and 30g

These compounds were prepared from the corresponding precursors according to the procedure as described for compound **30a**.

5.2.19.1. *tert*-Butyl {(2*S*)-1-[5-(5-fluoropyridin-2-yl)-2*H*-tetrazol-2-yl]propan-2-yl}carbamate (30e)

The title compound was prepared from **29e** (20% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 2.69 Hz, 1H), 8.27 (dd, J = 4.40, 8.68 Hz, 1H), 7.58 (dt, J = 2.87, 8.28 Hz, 1H), 4.64–4.88 (m, 3H), 4.22–4.40 (m, 1H), 1.40 (s, 9H), 1.23 (d, J = 6.85 Hz, 3H); MS (ESI/APCI dual): *m/z* 323 $[M+H]^+$.

5.2.19.2. *tert*-Butyl {(2*S*)-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-yl}carbamate (30g)

The title compound was prepared from 5-(4-fluorophenyl)-1*H*-tetrazole (38% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10–8.19 (m, 2H), 7.13–7.23 (m, 2H), 4.62–4.85 (m, 3H), 4.20–4.38 (m, 1H), 1.41 (s, 9H), 1.20 (d, J = 6.85 Hz, 3H); MS (ESI/APCI dual): *m/z* 322 $[M+H]^+$.

5.2.20. (2*S*)-*N*-Ethyl-1-[4-(5-fluoropyridin-2-yl)-1*H*-1,2,3-triazol-1-yl]propan-2-amine (43c)

To a solution of **30c** (0.27 g, 0.85 mmol) in DMF (10 mL) was added NaH (60% in mineral oil, 0.047 g, 1.2 mmol), and the resulting mixture was stirred at room temperature for 30 min. EtI (0.081 mL, 1.0 mmol) was added dropwise to the reaction solution and the mixture was stirred at room temperature 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 by column chromatography (20–100% EtOAc in hexanes) to obtain a crude oil (0.31 g, quant).

To the solution of the crude product (0.31 g, 0.88 mmol) in MeOH (5.0 mL) was added hydrogen chloride in dioxane (4 mol/L, 2.0 mL, 8.0 mmol) at room temperature, and the resulting mixture was stirred for 15 h. The reaction mixture was neutralized with a saturated aqueous solution of $NaHCO_3$, and the mixture was extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain the title compound **43c** as colorless oil (0.25 g, quant.). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (d, J = 2.89 Hz, 1H), 8.20 (dd, J = 4.54, 8.67 Hz, 1H), 8.16 (s, 1H), 7.50 (dt, J = 2.89, 8.46 Hz, 1H), 4.37 (dd, J = 1.86, 5.57 Hz, 2H), 3.18–3.26 (m, 1H), 2.70–2.77 (m, 1H), 2.66 (qd, J = 7.16, 11.15 Hz, 1H), 1.11 (d, J = 6.61 Hz, 3H), 1.09 (t, J = 8.67 Hz, 3H).

5.2.21. Preparation of Compounds 43d, 43e, 43f, and 43g

These compounds were prepared from the corresponding precursors according to the procedure as described for compound **43c**.

5.2.21.1. (2*S*)-*N*-Ethyl-1-[5-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-3-yl]propan-2-amine (43d)

The title compound was prepared from **30d** (13% yield in 2 steps, colorless solid). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.68 (d, J = 2.89 Hz, 1H), 8.25 (dd, J = 4.54, 8.67 Hz, 1H), 7.59–7.64 (m, 1H), 3.24–3.31 (m, 1H), 3.01 (dd, J = 5.99, 14.66 Hz, 1H), 2.87 (dd, J = 6.61, 14.45 Hz, 1H), 2.69–2.78 (m, 2H), 1.18 (d, J = 5.78 Hz, 3H), 1.13 (t, J = 7.22 Hz, 3H); MS (ESI/APCI dual): *m/z* 251 $[M+H]^+$.

5.2.21.2. (2*S*)-*N*-Ethyl-1-[5-(5-fluoropyridin-2-yl)-2*H*-tetrazol-2-yl]propan-2-amine (43e)

The title compound was prepared from **30e** (65% yield in 2 steps, colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 2.81 Hz, 1H), 8.29 (dd, J = 4.40, 8.68 Hz, 1H), 7.55–7.62 (m, 1H), 4.71 (dd, J = 6.60, 13.57 Hz, 1H), 4.63 (dd, J = 6.11, 14.31 Hz, 1H), 3.37–3.47 (m, 1H), 2.59–2.79 (m, 2H), 1.15 (d, J = 6.48 Hz, 3H), 1.09 (t, J = 7.09 Hz, 3H); MS (ESI/APCI dual): *m/z* 251 $[M+H]^+$.

5.2.21.3. (2*S*)-*N*-Ethyl-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-amine hydrochloride (43f)

The title compound was prepared from **30f** (40% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (br s, 1H), 8.07–8.21 (m, 2H), 7.14–7.25 (m, 2H), 3.72–3.89 (m, 1H), 3.60 (dd, J = 4.16, 15.04 Hz, 1H), 3.32 (dd, J = 9.41, 15.04 Hz, 1H), 3.10–3.27 (m, 2H), 1.52–1.62 (m, 6H); MS (ESI/APCI dual): *m/z* 250 $[M+H]^+$.

5.2.21.4. (2*S*)-*N*-Ethyl-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-amine hydrochloride (43g)

The title compound was prepared from **30g** (91% yield in 2 steps, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.10 (br s, 1H), 8.10–8.16 (m, 2H), 7.13–7.23 (m, 2H), 5.38 (dd, J = 4.71, 14.00 Hz, 1H), 5.11 (dd, J = 8.01, 14.00 Hz, 1H), 3.90–4.04 (m, 1H), 3.21–3.32 (m, 1H), 3.09–3.20 (m, 1H), 1.56 (t, J = 7.21 Hz, 3H), 1.51 (d, J = 6.60 Hz, 3H); MS (ESI/APCI dual): *m/z* 250 $[M+H]^+$.

5.2.22. Preparation of Compounds 9c–9g, 14, 15, 17, 18, 22, 23, 25, and 26

These compounds were prepared from the corresponding precursors according to the procedure as described for compound **7a**.

5.2.22.1.

***N*-Ethyl-*N*-{(2*S*)-1-[4-(5-fluoropyridin-2-yl)-1*H*-1,2,3-triazol-1-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (9c)**

The title compound was prepared from **43c** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (40% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{22}H_{24}FN_8O$ $[M+H]^+$, calcd: 435.2052, found: 435.2019; LC-MS t = 0.85 min, $[M+H]^+$ = 435. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.2.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (9d)**

The title compound was prepared from **43d** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (22% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{22}H_{23}FN_7O_2$ $[M+H]^+$, calcd: 436.1892, found: 436.1899; LC-MS t = 0.92 min, $[M+H]^+$ = 436. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.3.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(5-fluoropyridin-2-yl)-2*H*-tetrazol-2-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl) benzamide (9e)**

The title compound was prepared from **43e** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (71% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{21}H_{23}FN_9O$ $[M+H]^+$, calcd: 436.2004, found: 436.1990; LC-MS t =

0.89 min, $[M+H]^+$ = 436. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.4.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (9f)**

The title compound was prepared from **43f** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (21% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{23}H_{24}FN_6O_2$ $[M+H]^+$, calcd: 435.1939, found: 435.1940; LC-MS *t* = 1.06 min, $[M+H]^+$ = 435. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.5.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-yl}-5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzamide (9g)**

The title compound was prepared from **43g** and 5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (51% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{22}H_{24}FN_8O$ $[M+H]^+$, calcd: 435.2052, found: 435.2020; LC-MS *t* = 1.06 min, $[M+H]^+$ = 435. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.6.

***N*-Ethyl-5-fluoro-*N*-{(2*S*)-1-[5-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-2-(2*H*-1,2,3-triazol-2-yl)benzamide (14)**

The title compound was prepared from **43d** and 5-fluoro-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (64% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{21}H_{20}F_2N_7O_2$ $[M+H]^+$, calcd: 440.1641, found: 440.1637; LC-MS *t* = 0.88 min, $[M+H]^+$ = 440. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.7.

***N*-Ethyl-5-fluoro-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-2-(2*H*-1,2,3-triazol-2-yl)benzamide (15)**

The title compound was prepared from **43f** and 5-fluoro-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (70% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{22}H_{21}F_2N_6O_2$ $[M+H]^+$, calcd: 439.1689, found: 439.1679; LC-MS *t* = 1.04 min, $[M+H]^+$ = 439. 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.

5.2.22.8.

***N*-Ethyl-*N*-{(2S)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-2-(2*H*-1,2,3-triazol-2-yl)benzamide (17)**

The title compound was prepared from **43f** and 2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (54% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{22}H_{22}FN_6O_2$ $[M+H]^+$, calcd: 421.1783, found: 421.1773; LC-MS t = 1.01 min, $[M+H]^+$ = 421. 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.

5.2.22.9.

***N*-Ethyl-*N*-{(2S)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-6-methyl-3-(2*H*-1,2,3-triazol-2-yl)pyridine-2-carboxamide (18)**

The title compound was prepared from **43f** and 6-methyl-3-(2*H*-1,2,3-triazol-2-yl)pyridine-2-carboxylic acid (69% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{22}H_{23}FN_7O_2$ $[M+H]^+$, calcd: 436.1892, found: 436.1880; LC-MS *t* = 0.96–1.02 min, $[M+H]^+$ = 436. 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.

5.2.22.10.

_N_-Ethyl-5-fluoro-_N_-{(2_S_)-1-[5-(5-fluoropyridin-2-yl)-2_H_-tetrazol-2-yl]propan-2-yl}-2-(2_H_-1,2,3-triazol-2-yl)benzamide (22)

The title compound was prepared from **43e** and 5-fluoro-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (45% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{20}H_{20}F_2N_9O$ $[M+H]^+$, calcd: 440.1753, found: 440.1731; LC-MS *t* = 0.89–0.91 min, $[M+H]^+$ = 440. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.11.

_N_-Ethyl-5-fluoro-_N_-{(2_S_)-1-[5-(4-fluorophenyl)-2_H_-tetrazol-2-yl]propan-2-yl}-2-(2_H_-1,2,3-triazol-2-yl)benzamide (23)

The title compound was prepared from **43g** and 5-fluoro-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (49% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{21}H_{21}F_2N_8O$ $[M+H]^+$, calcd: 439.1801, found: 439.1781; LC-MS *t* = 1.06–1.08 min, $[M+H]^+$ = 439. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.12.

_N_-Ethyl-_N_-{(2_S_)-1-[5-(4-fluorophenyl)-2_H_-tetrazol-2-yl]propan-2-yl}-2-(2_H_-1,2,3-triazol-2-yl)benzamide (25)

The title compound was prepared from **43g** and 2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (61% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{21}H_{22}FN_8O$ $[M+H]^+$, calcd: 421.1895, found: 421.1867; LC-MS *t* = 1.02 min, $[M+H]^+$ = 421. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.13.

_N_-Ethyl-_N_-{(2_S_)-1-[5-(4-fluorophenyl)-2_H_-tetrazol-2-yl]propan-2-yl}-6-methyl-3-(2_H_-1,2,3-triazol-2-yl)pyridine-2-carboxamide (26)

The title compound was prepared from **43g** and 6-methyl-3-(2*H*-1,2,3-triazol-2-yl)pyridine-2-carboxylic acid

(67% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{21}H_{23}FN_9O$ $[M+H]^+$, calcd: 436.2004, found: 436.1991; LC-MS t = 0.93–1.01 min, $[M+H]^+$ = 436. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.22.14.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-5-methyl-2-(pyrimidin-2-yl)benzamide (12)**

To a solution of 5-methyl-2-(pyrimidin-2-yl)benzoic acid (0.040 g, 0.19 mmol) in $CHCl_3$ (1.5 mL) were added DIPEA (0.092 ml, 0.53 mmol), **43d** (0.037 g, 0.15 mmol) and a solution of 1-propanephosphonic acid cyclic anhydride (T3P®, 1.7 mol/L in EtOAc, 0.30 ml, 0.52 mmol) at room temperature. After this mixture was stirred at 60 °C for 5 h, water was added, 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 by preparative HPLC to obtain the titled compound **12** as a colorless amorphous (0.024 g, 36% yield). HRMS (ESI/APCI dual) for $C_{24}H_{24}FN_6O_2$ $[M+H]^+$, calcd: 447.1939, found: 447.1923; LC-MS t = 0.87 min, $[M+H]^+$ = 447. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.23. Preparation of Compounds 13, 16, 19–21, 24, and 27

These compounds were prepared from the corresponding precursors according to the procedure as described for compound **12**.

5.2.23.1.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-5-methyl-2-(pyrimidin-2-yl)benzamide (13)**

The title compound was prepared from **43f** and 5-methyl-2-(pyrimidin-2-yl)benzoic acid (23% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{25}H_{25}FN_5O_2$ $[M+H]^+$, calcd: 446.1987, found: 446.1974; LC-MS t = 1.04 min, $[M+H]^+$ = 446. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR

spectra in $CDCl_3$ at 25 °C.

5.2.23.2.

***N*-Ethyl-5-fluoro-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-2-(pyrimidin-2-yl)benzamide (16)**

The title compound was prepared from **43f** and 5-fluoro-2-(pyrimidin-2-yl)benzoic acid (14% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{24}H_{22}F_2N_5O_2$ $[M+H]^+$, calcd: 450.1736, found: 450.1730; LC-MS *t* = 1.03 min, $[M+H]^+$ = 450. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.23.3.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl]propan-2-yl}-6-methyl-3-(pyrimidin-2-yl)pyridine-2-carboxamide (19)**

The title compound was prepared from **43f** and 6-methyl-3-(pyrimidin-2-yl)pyridine-2-carboxylic acid (23% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{24}H_{24}FN_6O_2$ $[M+H]^+$, calcd: 447.1939, found: 447.1931; LC-MS *t* = 0.92–0.99 min, $[M+H]^+$ = 447. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.23.4.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(5-fluoropyridin-2-yl)-2*H*-tetrazol-2-yl]propan-2-yl}-5-methyl-2-(pyrimidin-2-yl)benzamide (20)**

The title compound was prepared from **43e** and 5-methyl-2-(pyrimidin-2-yl)benzoic acid (20% yield, colorless amorphous). HRMS (ESI/APCI dual) for $C_{23}H_{24}FN_8O$ $[M+H]^+$, calcd: 447.2052, found: 447.2034; LC-MS *t* = 0.87 min, $[M+H]^+$ = 447. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.23.5.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-yl}-5-methyl-2-(pyrimidin-2-yl)benzamide (21)**

The title compound was prepared from **43g** and 5-methyl-2-(pyrimidin-2-yl)benzoic acid (15% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{24}H_{25}FN_7O$ $[M+H]^+$, calcd: 446.2099, found: 446.2083; LC-MS t = 1.06 min, $[M+H]^+$ = 446. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.23.6.

***N*-Ethyl-5-fluoro-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-yl}-2-(pyrimidin-2-yl)benzamide (24)**

The title compound was prepared from **43g** and 5-fluoro-2-(pyrimidin-2-yl)benzoic acid (12% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{23}H_{22}F_2N_7O$ $[M+H]^+$, calcd: 450.1848, found: 450.1834; LC-MS t = 1.04 min, $[M+H]^+$ = 450. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

5.2.23.7.

***N*-Ethyl-*N*-{(2*S*)-1-[5-(4-fluorophenyl)-2*H*-tetrazol-2-yl]propan-2-yl}-6-methyl-3-(pyrimidin-2-yl)pyridine-2-carboxamide (27)**

The title compound was prepared from **43g** and 6-methyl-3-(pyrimidin-2-yl)pyridine-2-carboxylic acid (22% yield, colorless solid). HRMS (ESI/APCI dual) for $C_{23}H_{24}FN_8O$ $[M+H]^+$, calcd: 447.2052, found: 447.2029; LC-MS t = 0.89–0.99 min, $[M+H]^+$ = 447. Please see the Supplementary data for pictures of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra in $CDCl_3$ at 25 °C.

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20. A) Conformations of compounds were calculated using the replica-exchange molecular dynamics (REMD) simulation implemented in GROMACS 5.0.4.[a] For each compound, 8 sets of 20 ns REMD simulation with 8 temperature replicas were carried out to sample sufficient conformational space. Temperatures of replicas were set to 310.0, 350.7, 396.0, 446.6, 503.0, 565.8, 635.9, and 714.0K. GAFF forcefield[b] and GBSA model[c] are applied to compounds and solvent, respectively. a) Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. *J. Chem. Theory Comput.* **2008**, *4*, 435. b) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004**, *25*, 1157. c) Onufriev, A.; Case, D. A.; Bashford, D. *J. Comput. Chem.* **2002**, *23*, 1297.

B) The probability density functions of pyridine positions under the alignment of toluene triazole substructures were calculated using the kernel density estimation method[d] of sampled conformations. The isosurface indicates the minimal space which 80% of conformations exist. d) R Core Team. R: A language and environment for statistical computing, R Foundation for Statistical Computing: Vienna, Austria, 2015.

C) Active U-shaped conformation was modeled by the crystal structure[22] of the human OX_2R bound to suvorexant.

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23. Molecular docking simulations were carried out by using the CDOCKER algorithm in Discovery Studio 2016[a]. The input coordinates for OX_1R and OX_2R were obtained from the PDB entries 4ZJ8 and 4S0V respectively. The "Input Site Sphere" parameter for CDOCKER was defined using the suvorexant molecule in the PDB entries. Hydrogen atoms were added and the ionization states were assigned using the Protonate-3D function of the Molecular Operating Environment program (MOE)[b], and then the positions of the hydrogen atoms were optimized by using the Amber10 forcefield implemented in MOE. The

electrostatic potential surfaces were calculated using the Poisson-Boltzmann equation solver in MOE. a) Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2016, San Diego: Dassault Systèmes, 2016. b) *Molecular Operating Environment (MOE)*, 2015.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2016**.

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