Substituted Azabicyclo[2.2.1]heptanes as Selective Orexin-1 Antagonists: Discovery of JNJ-54717793

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ABSTRACT: The orexin system consists of two neuropeptides (orexin-A and orexin-B) that exert their mode of action on two receptors (orexin-1 and orexin-2). While the role of the orexin-2 receptor is established as an important modulator of sleep/wake states, the role of the orexin-1 receptor is believed to play a role in addiction, panic, or anxiety. In this manuscript, we describe the optimization of a nonselective substituted azabicyclo[2.2.1]heptane dual orexin receptor antagonist (DORA) into orally bioavailable, brain penetrating, selective orexin-1 receptor (OX1R) antagonists. This resulted in the discovery of our first candidate for clinical development, JNJ-54717793.

KEYWORDS: Orexin, OX1, OX2, SORA-1, neuropeptides

JNJ-54717793
hOX1R K_i =16 nM
hOX2R K_i =700 nM
human extraction ratio = <0.3
ex vivo OX1 ARG
plasma EC_{50} =85 ng/mL

Orexin-A and -B are excitatory neuropeptides that originate in neurons situated in the hypothalamus and play important roles in the regulation of sleep/wake cycles, circadian rhythms, energy metabolism, reward-directed behavior, anxious arousal, stress responses, and monoaminergic neurotransmitter release via a relatively discrete network of neuroanatomical projections.^{1–5} The orexins stimulate two distinct G-protein coupled receptors, orexin-1 (OX1R) and orexin-2 (OX2R), that are collocated or selectively located in specific brain areas suggesting differentiated roles.^{6–12} An association of OX1R pathway activation with panic or anxiety states is consistent with this anatomical distribution.^{13–16}

Although the role of the OX1R in more complex emotional behavior such as panic, anxiety, and addictive disorders is emerging, most of the in vivo and in vitro biology reports interrogating the OX1R have utilized knockout animals, siRNA experiments, or the tool compound SB-334867 (**1**; Figure 1).¹⁷ However, the chemical stability, solubility, and overall selectivity profile of this molecule limit the ability to properly interpret many of the results.¹⁸ A variety of tool compounds with improved properties have been reported that can properly interrogate the receptor preclinically and its role in the CNS.^{10–12} For example, Actelion (now Idorsia) recently published the selective OX1R antagonist tetrahydroisoquinoline ACT-335827 (**2**) which also demonstrated efficacy in preclinical models of anxiety.¹⁹ Fluoropiperidines **3** and **4** have been disclosed by Merck as potent and selective OX1R antagonists that have good CNS exposure in rat after an oral dose.²⁰ Recently, Idorsia disclosed phase I clinical data for the selective OX1R antagonist ACT-539313.²¹

Figure 1. Representative selective orexin-1 receptor antagonists.

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Table 1. In Vitro Binding Affinity of Compounds 5–13 at the Human OX1R and OX2R

Compd	R ₁	R ₂	hOX1R ^{a,b} K _i (nM)	hOX2R ^b K _i (nM)	HLM ER ^d	RLM ER ^e	cLogP
(±)-5		F	10 ± 2 ^c	13 ± 3.2 ^c	>0.95	>0.92	4.8
(±)-6		F	10 ± 2.7	72 ± 24	0.91	0.90	4.0
(-)-6		F	3.2 ± 1	32 ± 1.5	0.93	>0.92	4.0
(±)-7		F	59 ± 23	200 ± 57	0.81	0.80	3.7
(±)-8		F	8.7 ± 0.3 ^c	40 ± 7 ^c	0.91	0.89	3.2
(±)-9		F	41 ± 23	160 ± 33	0.81	0.76	2.9
10		F	14 ± 1.7	110 ± 12	0.77	0.81	3.1
11		H	18 ± 1.5	430 ± 67	0.77	0.91	2.9
12		H	19 ± 0.7	230 ± 38	0.61	0.82	2.0
(±)-13		CF ₃	500 ± 110	420 ± 100	-	-	3.9

^aAll compounds were confirmed at the rat OX1R before obtaining human OX1R data. ^bK_i's ± s.d. are the mean of at least three experiments in triplicate, unless otherwise stated. ^cn = 2. ^dStability in human liver microsomes reported as extraction ratios. ^eStability in rat liver microsomes reported as extraction ratios.

In the leading indications for an OX1R antagonist, for example, addiction, panic, anxiety, and major depressive disorders, 24 h coverage of the receptor is desired and presented an additional challenge in targeting selective OX1R antagonists as opposed to OX2R antagonists used for the treatment of insomnia.^{14–16,22–25} The goals of our research were to develop a selective OX1R antagonist that would provide at least 50% occupancy of the OX1 receptor for 24 h after q.d. dosing. While we have described the efficacy of JNJ-54717793 (23) this molecule in reversing a provocation by CO₂ or sodium lactate, the following will focus on the discovery, synthesis, and medicinal chemistry associated with this series of molecules ultimately leading to the identification of JNJ-54717793 (23).¹⁴

Our program began with the [2.2.1]-azabicyclo (±)-5, a compound prepared as part of an exploratory series in our selective OX2R program, Table 1. This was not pursued at the time since (±)-5 was a DORA. However, a related analog, (±)-6, provided a modest improvement in selectivity for the OX1 receptor. Resolution of the racemic material provided (–)-6 which was shown to be a potent OX1R antagonist with modest (10×) selectivity for OX1 over OX2. Based on this preliminary data we began to investigate this series with the

initial goal of improving OX1R selectivity to at least 50×. In addition, since we were targeting a compound that would be predicted to provide at least 50% OX1R occupancy in humans following a q.d. dose, we also focused on *in vitro* clearance.

Replacement of the 5-methyl substituent of (±)-6 with a fluorine ((±)-7) gave some improvements in microsomal stability but also resulted in a loss of affinity. The pyrimidine substituted benzamides (±)-8 and (±)-9 had similar potency, selectivity, and microsomal stability as the triazole counterparts, (±)-6 and (±)-7, respectively. In an effort to lower cLogP, the picolinic amides 10–13, were investigated where again, only modest improvements were observed. The trifluoromethyl substituted analog (±)-13 resulted in a decrease in affinity for both receptors. Shown in Table 2 are data obtained for compounds when the hydroxymethyl linker is replaced with a direct amino linkage.

It was determined that a 2-amino linked pyridine provided increased selectivity for the OX1R versus the OX2R. Although the data shown for 14–18 are obtained from racemic compounds, this early data set made it possible to uncover SAR which ultimately provided potent and selective molecules. For example, while the 5-Cl analog (±)-14 had only modest OX1R affinity, selectivity had increased to ~20×. The

Table 2. In Vitro Binding Affinity of Compounds 14–26 at the Human OX1R and OX2R

Compd	R ¹	R ²	hOX1R ^{a,b} K _i (nM)	hOX2R ^b K _i (nM)	HLM ER ^c	RLM ER ^d	cLogP
(±)-14			96 ± 19	2130 ± 230	0.88	>0.92	3.6
(±)-15			52 ± 6	1400 ± 130	0.69	0.87	3.9
(±)-16			75 ± 14	620 ± 60	0.93	>0.92	3.5
(±)-17			180 ± 15	3500 ± 170	0.94	>0.92	3.1
(±)-18			62 ± 24	1250 ± 150	0.36	0.55	3.1
18			30 ± 7.7	770 ± 160	<0.3	0.31	3.1
19			19 ± 3.8	520 ± 77	<0.3	0.38	2.3
20			25 ± 7	560 ± 33	-	-	2.3
21			65 ± 8.3	730 ± 120	-	-	2.5
22			61 ± 8.7	1700 ± 430	0.81	0.76	2.5
23			16 ± 3.8	700 ± 180	<0.3	0.31	2.5
24			9 ± 0.3	1100 ± 110	0.75	0.82	3.3
25			15 ± 5.3	1100 ± 170	0.49	0.8	3.4
26			14 ± 1.7	540 ± 27	0.61	0.83	4.0

^aAll compounds were confirmed at the rat OX1R before obtaining human OX1R data. ^bK_i's ± s.d. are the mean of at least three experiments in triplicate, unless otherwise stated. ^cStability in human liver microsomes reported as extraction ratios. ^dStability in rat liver microsomes reported as extraction ratios.

trifluoromethyl analog (±)-15 added another advantage in that a modest improvement in the human extraction ratio (ER) was observed. Since the picolinic analog (±)-16 resulted in slightly reduced selectivity, the strategic decision was made to keep the benzamide in place as opposed to the picolinamide and make changes to this portion of the molecule. In order to improve drug-like properties, additional heteroatoms were incorporated in the amino-heterocycle with the strategy being to improve metabolic stability by lowering cLogP. Hence, additional SAR on the heterocycle portion of the molecule included the pyrimidine (±)-17 and pyrazine (±)-18. While the pyrimidine did not improve the stability of the molecule when incubated with human liver microsomes, the pyrazine (±)-18 possessed a good balance of potency, selectivity, and an improvement in hER. Resolution of (±)-18 via SFC gave the single enantiomer

18. The more potent of the enantiomers was also the more stable in human liver microsomes which was encouraging. The remainder of the compounds in the table, 19–26, are single enantiomers. Replacing the triazole with a pyrimidine (19) maintained a favorable metabolic profile concurrent with a small gain in affinity for the OX1R. Analogs that explored substitution of the benzamide ring with fluorine were also synthesized. As shown for examples 20–23, the best balance of all properties resided with the 3-fluorobenzamide 23. The 5-trifluoromethylpyridine 24, 3-fluoro-5-trifluoromethylpyridine 25, and 3-chloro-5-trifluoromethylpyridine 26 were also investigated; however, these exhibited reduced microsomal stability.

Representative examples of alkoxy linked heterocycles as opposed to amino linked heterocycles are shown in Figure 2.

Figure 2. In vitro binding affinity of compounds 27–29 at the human OX1R and OX2R.

Generally, the compounds possessed good selectivity but were slightly less potent than their direct comparisons in the amino-linked examples. Illustrating this point, compounds 27–29 possessed weaker OX1R affinity than their amino linked counterparts, (±)-15, 23, and 24, respectively.

The ORTEP diagram of compound 23 is shown in Figure 3. The crystal structure of 23 shows an intramolecular π -stacking

Figure 3. Single X-ray crystal structure of 23.

between the benzamide phenyl and the trifluoromethyl pyrazine enforcing an overall shape of the molecule consistent with the U-shaped conformation of previously disclosed SORAs and DORAs.^{26,27} An observed hydrogen bond between the NH of the aminopyrazine and the pyrimidine nitrogen (distance of 2.1 Å) is thought to favor this conformation and also provides a possible explanation for the decreased affinity of compounds 27–29. When the hydrogen bond donating NH is replaced with an oxygen, a negative electrostatic interaction could result due to repulsion of the lone pair electrons. However, this appears to be a minor contribution since the oxygen linked analog (±)-29 possessed modest, albeit lower affinity for the OX1R. The absolute configuration was confirmed by measuring the Flack parameter.

Using *ex vivo* receptor binding autoradiography as a measure of target engagement, we screened selected compounds by measuring occupancy of the OX1R in the tenia tecta of rat brain following a 10 or 30 mg/kg po dose.^{14,15} Binding data for representative examples are shown in Table 4. As can be seen in the data, the hydroxymethyl analogs 6, 10, 11, and 22 showed only modest to poor occupancy after a 30 mg/kg po dose. The highest occupancy observed from this set was

Table 4. Ex-Vivo Brain OX1R Binding Autoradiography by Selected Compounds Measured in the Tenia Tecta

Cmpd	%OX1 Occ (0.5/2/6 h) ^a	[B]/[P] (@ 0.5 h) (ng/mL)	Dose mg/kg po
6	49/43/–	16/55	30
10	50/30/–	313/930	30
11	50/20/–	8/118	30
22	75/17/–	37/257	30
23	100/100/–	687/4964	30
23	70/41/17	188/1191	10
19	77/83/48	296/1483	10
20	51/43/29	654/1597	10
24	70/69/21	72/586	10
25	81/70/46	539/837	10

^aPercentage of OX1R occupancy.

compound 22, which demonstrated 75% OX1R occupancy at 0.5 h but had diminished to 17% at 2 h. The amino linked analog 23 was shown to fully occupy the receptor following a 30 mg/kg po dose. Lowering the dose to 10 mg/kg and extending the time of the experiment to 6 h led to 70% occupancy over 2 h and 41% occupancy at 6 h. In general, the amino linked compounds proved to have good occupancy at the 0.5 h time point with sustained occupancy over the 6 h time course, at lower dose (10 mg/kg, po) as compared to the hydroxy methyl series with the added advantage of a lower dose as shown in the table for compounds 19, 20, 24, and 25. Based on this data, compounds 19, 23, and 25 were selected for further profiling and that data is shown in Table 5.

Table 5. In Vitro ADME Data for Compounds 19, 23, and 25^a

Parameter	19	23	25
ER h/r/d/ms ^a	<0.3/0.38	<0.3/0.34/<0.3/0.4	0.5/0.8
Plasma Protein Binding			
%f _{p,u} h/r/d ^b	15/21.4	20/23/21	5.6/6.4
%f _{b,u} ^c	6.7	9	2.7
MDCK			
(B-A/A-B) ^d	68/1.1	64.4/1.95	71.3/7.7
A-B + Inh.	46.2	13.1	8.6
PXR 3A4 induction			
% control @ 10 μ M ^e	18	29	39

^aStability in liver microsomes (human, rat, dog, and mouse) reported as extraction ratios. ^bReported as % free fraction (human, rat, and dog). ^cReported free fraction using rat brain tissue. ^dReported as [B–A ($\times 10^{-6}$) cm/s]/[A–B ($\times 10^{-6}$) cm/s] and A–B ($\times 10^{-6}$) cm/s in the presence of the Pgp inhibitor elacridar. ^eInduction of hPXR as an indirect measure of CYP3A4 induction. Reported as % of hPXR activation relative to control (rifampicin) at 10 μ M.

Plasma protein binding for 23 was 20–23% free across species. In addition, the brain tissue binding was determined to be 9% free. Permeability studies in LLC-PK1/MDR-1 cell lines indicated that 23 and 19 are strong PGP substrates consistent with the observation of low brain to plasma ratios. Although 25 is considered a substrate, the reduced MDCK ratio is consistent with the improved brain to plasma ratio of 0.6. This series of compounds were determined to be mild inducers of the 3A4 enzyme based on the PXR data shown in the table. Compound 25 had the greatest liability in this case followed by 23 and 19.

As shown in Figure 4, progressing compounds **23** and **25** into a dose response assay for receptor occupancy proved

Figure 4. Ex vivo OX1R occupancy with compound **23** and **25** in rat tectal: dose dependency after p.o. administration ($t = 60$ min).

informative and allowed us to further differentiate the two compounds. Although **23** has less than optimal brain penetration for a CNS drug, it was concluded from our *ex vivo* OX1R occupancy dose response experiment that compound **23** had a lower ED_{50} and EC_{50} than **25**. The corresponding EC_{50} 's are 85 and 250 ng/mL for **23** and **25**, respectively.

Pharmacokinetic study parameters for **23** in mouse, rat, nonhuman primate, and dog are shown in Table 6. Clearance

Table 6. Mouse, Rat, Dog, and Monkey Pharmacokinetics for **23**

Species	%F (po)	In vivo CL ^d	In vitro CL	C_{max} ^e	V_{ss} ^f
Mouse ^a	58	21	29	1497	1.1
Rat ^b	119	17	17	1369	1.4
Dog ^c	48	0.9	4.8	2237	0.6
Nonhuman primate	22	14	13	349	0.8

^aCompound was dosed as a solution in 20% HP- β -CD at 1.0 mg/kg i.v. and 5.0 mg/kg p.o. (3 animals per dose). ^bCompound was dosed as a solution in 20% HP- β -CD with 2 equiv of HCl at 1.0 mg/kg iv and 5.0 mg/kg po (3 animals per dose). ^cCompound was dosed as a solution in 20%HP- β -CD at 0.5 mg/kg i.v. and 2.5 mg/kg p.o. ^dClearance values are reported in mL/min/kg. ^e C_{max} is reported in ng/mL from the po arm. ^f V_{ss} is reported in L/kg.

was found to be low in dogs and moderate in rats, mice, and nonhuman primates. In addition, compound **23** was found to have good agreement between predicted and measured *in vivo* clearance providing an additional level of confidence in projecting a human clearance. The compound also exhibited good bioavailability and overall exposures following an oral dose as shown below in all species, except nonhuman primates in which case %F was low.

These studies provided a basis for a human dose prediction of 50–320 mg, dependent on the level of occupancy required for efficacy. For a dose of 50 mg once daily, plasma trough levels at steady state are expected to be maintained above the plasma EC_{50} , as determined in the *ex vivo* autoradiography experiments, of 85 ng/mL over 24 h. A dose of 320 mg is projected to provide 90% receptor occupancy over 12 h. The plasma concentration over time profile was simulated using predicted human clearance of 0.9 mL/min/kg, V_{ss} of 0.6 l/kg and assuming 50% bioavailability. The estimated steady state

C_{max} and AUC in humans for the dose of 50 mg once daily are 537 ng/mL and 6868 h·ng/mL, respectively.

Initial characterization of the metabolites of compound **23** was carried out *in vitro* using liver microsomes supplemented with NADPH as summarized in Figure 5. In addition to mono-

Figure 5. Metabolite identification for compound **23**.

oxidation metabolites related to **32**, the ketone **33** and aminopyrazine **34** were identified by comparison to authentic samples. *In vivo*, ketone **33** and an oxidative metabolite related to **32** were determined to be the major circulating metabolites from samples obtained following a 14-day toleration study. These products are consistent with hydroxylation of the methine carbon to give a hemiaminal which then fragments into **33** and **34**. Consistent with this mechanism, the deuterated compound **30** had a reduction in the amounts of ketone **33** produced *in vitro*. In contrast to rat, ketone **33** was only observed as a minor metabolite in dogs using *in vivo* plasma samples obtained from a pharmacokinetic study. In this case, the major circulating metabolite was a hydroxylated metabolite related to **32**. MS/MS spectral data localizes the site of hydroxylation to occur either at the azabicyclo or at the pyrazine. Based on *in vitro* liver microsomal metabolite i.d. studies using the trideuterated compound **31**, the deuterated methylene was confirmed not to be a site of metabolism as the resulting hydroxylation product was formed without the loss of deuterium.

The pK_a and $\log D_{7.4}$ of **23** were measured and determined to be <2 and 2.18, respectively. The low dissociation constant does not allow for salt formation. The solution and solid-state properties of **23** were measured by PXRD, TGA, DSC, moisture isotherm analysis, and HPLC. In addition to an amorphous form, two crystalline forms were discovered of the final compound with an endothermic transition onset temperature of 176.64 and 197.69 °C, respectively, for each form. Interconversion between the two forms was not observed. Formulations used to support *in vivo* studies with **23** were either a solution in 20% HP- β -CD or suspension dosing in 0.5% HPMC. There were no developability properties found that precluded the advancement of **23**.

In a 5-day repeat dose rat toxicology study, **23** was administered orally to male rats at 100, 250, or 1000 mg/kg. Evidence of enzyme induction in rats was apparent due to both the loss of exposure at day 5 and up regulation of several drug metabolizing enzyme genes such as CYP3A1 mRNAs. On day 5, a NOAEL was determined at 250 mg/kg with corresponding $C_{\max}$ and AUC values of 15,000 ng/mL and 101,000 h·ng/mL, respectively. Following the 5-day study in rats, a 14-day rat study only analyzing drug concentration was carried out in both rats and mice, Table 7. In this study **23** was dosed at 200

Table 7. Toxicokinetic of Compound **23 in Rats or Mice Dosed Orally at 200 mg/kg per Day for 14 Days**

Day	$C_{\max}$ (ng/mL) rat	$C_{\max}$ (ng/mL) mouse	AUC _(0–24h) (h·ng/mL) rat	AUC _(0–24h) (h·ng/mL) mouse
0	30,900	32,905	386,000	225,157
6	8,800		62,000	
13	6,980	24,758	36,200	84,127

mg/kg/day in each species to determine if the level of induction observed in the 5-day study had reached equilibrium. After repeat administration of **23** to mice at a 200 mg/kg/day for 14 consecutive days, moderate auto induction was observed with the AUC reduced ~2-fold on day 13 compared to day 0. A more significant decrease in exposure (up to 11-fold) was observed in rats under similar conditions; however, the loss of exposure did appear to reach equilibrium by day 7. In a dog 6-day toxicology study, the NOAEL was determined to be 25 mg/kg with a $C_{\max}$ of 15,100 ng/mL. There were no consistent differences in exposures between males and females or between day 0 and day 5 of treatment.

The scale-up route utilized to synthesize gram quantities of **23** is shown in Scheme 1. Removal of the Cbz group from **35** with 10 wt % Pd/C under an atmosphere of hydrogen in EtOH gave the primary amine **36**. Formation of the amino pyrazine

37 using 2-chloro-5-trifluoromethylpyrazine in MeCN in the presence of TEA at 80 °C gave **37** in 84% yield. Boc removal provided the amine **38** quantitatively. Amide bond coupling using **38** and the carboxylic acid **39** mediated by T3P in 2-MeTHF in the presence of DIPEA gave **23** in 77% yield.

In summary, beginning with the azabicyclic DORA **5** as a template, sequential modification of the amide and the heterocyclic moieties established a robust SAR. Simultaneous improvements in selectivity, affinity, and druglike properties afforded a series of selective OX1R antagonists with pharmacokinetic properties predictive of q.d. dosing in humans. During the optimization process, changes to the amide and the heterocyclic portion of the molecule allowed for improvements in OX1R selectivity. Further improvements in selectivity and stability were achieved by manipulation of the linker between the azabicyclic and the pendant heterocycle. Eventually it was shown that an amino linked 5-trifluoromethylpyrazine was optimal for balancing these properties. However, this did come at the expense of relatively poor brain penetration. *Ex vivo* receptor occupancy studies allowed us to identify compound **23** (JNJ-54717793) which was shown to have good pharmacokinetics and evidence of target engagement and efficacy in a relevant and translatable disease model. The efficacy of this molecule in reversing a provocation by CO₂ or sodium lactate at doses that are consistent with occupancy of the OX1 receptors has been previously described.¹⁴ Based on these attributes, JNJ-54717793 was selected as a candidate for further development.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00085>.

A description of general methods, characterization of compounds **5–31**, and a comparison of the ¹H NMR of **30** and the protio compound **23** (PDF)

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Scheme 1. Synthesis of compound **23^a**

^aReagents and conditions: (a) H₂, 10 wt % Pd/C, EtOH, quantitative; (b) 2-chloro-5-trifluoromethylpyrazine, TEA, MeCN, reflux, 16.5 h, 84%; (c) 5.5 M HCl in *i*-PrOH, 60 °C, 99%; (d) 3-fluoro-2-(pyrimidin-2-yl)benzoic acid (**39**), T3P (50% in EtOAc), DIPEA, 2-MeTHF, 77%.

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ABBREVIATIONS

OX1R, orexin-1 receptor; OX2R, orexin-2 receptor; DORA, dual orexin receptor antagonist; SORA, selective orexin receptor antagonist; CNS, central nervous system; PK, pharmacokinetic; SAR, structure activity relationship; ADME, absorption, distribution, metabolism, and excretion; SFC, supercritical fluid chromatography; hER, human extraction ratio; rER, rat extraction ratio; PXR, pregnane X receptor; MDCK, Madin–Darby canine kidney cells; CL, clearance; V_{ss} , volume of distribution; PXRD, powder X-ray diffraction; TGA, thermogravimetric analysis; DSC, differential scanning calorimetry; TEA, triethylamine; T3P, 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide; DIPEA, diisopropylethylamine; CYP, cytochrome P450; NOAEL, no adverse event level; HPMC, hypromellose; PGP, P-glycoprotein.

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