Discovery of Nonracemic Amisulpride to Maximize Benefit/Risk of 5-HT7 and D2 Receptor Antagonism for the Treatment of Mood Disorders

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In contrast to the dose-occupancy relationship in the treatment of schizophrenia, the minimal effective level of dopamine receptor 2 (D2R) blockade for antipsychotics in the treatment of bipolar depression is unknown. Lower doses aimed at reducing extrapyramidal side effects must be balanced against the need to retain the therapeutic benefit of D2R blockade on emergent cycling, mixed, manic, anxiety, and/or psychotic symptoms. Dose-reductions intended to lower D2R blockade, however, could also decrease concomitant serotonin receptor antagonism and its potential benefit on depressive symptoms. Here, we uncoupled the potential antidepressant activity in amisulpride, driven by 5-HT7 receptor (5-HT7R) antagonism, from the D2R-mediated antipsychotic activity by discovering that each enantiomer favors a different receptor. Aramisulpride was more potent at 5-HT7R relative to esamisulpride (*Ki* 47 vs. 1,900 nM, respectively), whereas esamisulpride was more potent at D2R (4.0 vs. 140 nM). We hypothesized that a nonracemic ratio might achieve greater 5-HT7R-mediated antidepressant effects at a lower level of D2R blockade. The dose-occupancy relationship of esamisulpride at D2R was determined by positron emission tomography (PET) imaging in human volunteers. Separately the dose-relationship of aramisulpride was established in humans using suppression of rapid eye movement (REM) sleep as a marker of 5-HT7R antagonism. These results led to the discovery of an 85:15 ratio of aramisulpride to esamisulpride (SEP-4199) that maximizes the potential for antidepressant benefit of aramisulpride via 5-HT7R and reduces esamisulpride to minimize D2R-related extrapyramidal side effects while still retaining D2R-mediated effects predicted to provide benefit in bipolar depression. The antidepressant efficacy of SEP-4199 was recently confirmed in a proof-of-concept trial for the treatment of bipolar depression (NCT03543410).

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The nonracemic form of amisulpride (SEP-4199) was a novel discovery whose development is reported here.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Is it possible to leverage the stereoselective binding affinities of the 2 enantiomers of amisulpride to enhance its 5-HT7 receptor-mediated antidepressant effects while retaining some D2 receptor antipsychotic benefits, but at a reduced level with improved safety and tolerability.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ We report the discovery of SEP-4199, a nonracemic 85:15 ratio of aramisulpride (potently targeting the 5-HT7 receptor) and esamisulpride (yielding D2 receptor occupancy < 50% at therapeutic doses). We present preclinical data demonstrating that aramisulpride has antidepressant-like activity and is associated with significant rapid eye movement (REM) suppression.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ SEP-4199 appears to represent a new class of antidepressant, a mixed D2/5-HT7 receptor antagonist with potential efficacy for the treatment of bipolar disorder and depression with mixed mood/psychotic states.

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The dose-occupancy relationship of antipsychotics in the treatment of schizophrenia suggests that relatively high D2 receptor (D2R) occupancies 65% to 90% are required for clinical efficacy.[1] However, also arising from increasing D2R blockade[2] are the dose-limiting extrapyramidal symptoms (EPS) of parkinsonism, dystonia, akathisia, and tardive dyskinesia. The balance of benefit/risk in the treatment of schizophrenia is shifted for the use of antipsychotics in mood disorders because the targeted level of D2R blockade for efficacy is unclear vs. an increased risk of EPS.[3,4] Currently, all antipsychotics approved for the treatment of bipolar disorder were initially approved for the treatment of schizophrenia. As a result, antipsychotics used in the treatment of bipolar disorder are approved over a wide dose-occupancy range that overlaps with the range used to treat schizophrenia. Systematic trials have not determined the minimal level of D2R blockade required for clinical benefit in bipolar disorder or to what extent varying levels of D2R occupancy are required during the different poles of the disorder, if at all.

The pharmacological activity of serotonin receptor antagonism improves the balance of benefit/risk for D2R blockade among antipsychotics. For example, serotonin 5-HT2A receptor (5-HT2AR) antagonism reduces the risk of EPS[5,6] and contributes antipsychotic benefits.[7,8] Atypical antipsychotics as a class have increased affinities for 5-HT2AR and produce relatively high occupancies at clinical doses.[9–11]

Antidepressant benefit among antipsychotics has been hypothesized to arise from serotonin 5-HT7 receptor (5HT7R) antagonism,[12] as it is present among several antipsychotics used successfully in the treatment of bipolar disorder.[12–14] Compounds with selective 5-HT7R antagonist activity have been shown to reduce rapid eye movement (REM) sleep and/or increase latency to onset of REM sleep; and to have antidepressant-like effects in mouse models.[15–23] The 5-HT7Rs are highly distributed in the suprachiasmatic nucleus, hippocampus, cortex, thalamus, and raphe nuclei, and are involved in central regulation of mood, cognition, pain, sleep, and circadian rhythms.[24–27] Among antipsychotics having 5-HT7R antagonism, their relative potencies between D2R and 5-HT7R imply that any dose-adjustments aimed at optimizing D2R benefit/risk (e.g., dose *decreases* to reduce EPS) will conflict with dose-adjustments taken to optimize 5-HT7R-mediated benefit/risk (e.g., dose *increases* to treat depression symptoms).

Amisulpride is a D2R/D3R antagonist with 5-HT7R activity.[12] At high doses (400–800 mg) amisulpride has demonstrated efficacy in treating acute psychosis.[28–30] At intermediate doses, amisulpride is effective in treating predominant negative symptoms,[31–33] whereas at a low dose (50 mg) it is used for the treatment of depression.[34,35] In multiple-treatments meta-analyses of schizophrenia, amisulpride ranked second after clozapine among all first and second generation antipsychotics in terms of efficacy, and lowest in all-cause discontinuations.[36,37] Amisulpride has remarkable tolerability in healthy volunteer studies[38,39] even at doses that result in high D2R blockade. The dose-occupancy relationship of amisulpride has demonstrated high 60% to 90% D2R occupancies for the 400–800 mg dose range[40–44] and well-below 50% at the 50 mg dose. The efficacy demonstrated for amisulpride at 100–300 mg/d in the treatment of predominant negative symptoms of schizophrenia is possibly attributable to a reduction in depressive symptoms.[45] It is unclear whether the efficacy of lower doses in depressive symptoms is driven by enhanced dopaminergic tone at presynaptic D2/D3Rs in the prefrontal cortex,[46] or by its 5-HT7R antagonism,[12] or a combination of both.

Amisulpride is a chiral compound, comprising a racemic (50:50) mixture of R- and S-enantiomers, whose receptor binding profile is characterized by potent antagonist activity at D2R, D3R, and 5-HT7R[12,47] and is relatively selective over the weaker affinities to other receptors, such as 5-HT2B, and adrenergic α_{2A} and α_{2C} receptors.[12,47] In amisulpride, D2R/D3R affinity predominates over 5-HT7R receptor affinity in a ratio of ~ 3:1.[12]

The pharmacology of amisulpride is enantiomer-specific[48,49] with antagonist activity at 5-HT7R residing in the R-enantiomer (aramisulpride), and antagonist activity at the D2R residing in the S-enantiomer (esamisulpride). This discovery enabled the development of SEP-4199, a nonracemic ratio of aramisulpride and esamisulpride developed to enhance the potential benefit of 5-HT7R-mediated antidepressant activity and minimize the risk of D2R blockade-related EPS while still retaining D2R-mediated benefit in bipolar depression. We describe a series of preclinical and clinical studies that led to the discovery of a nonracemic ratio of the two enantiomers comprising SEP-4199, a novel compound with hypothesized to have enhanced antidepressant efficacy for the treatment of bipolar disorder and depression with mixed mood/psychotic states.

MATERIALS AND METHODS

Drugs

Single enantiomers of amisulpride were synthesized from 4-Amino -5-(ethylsulfonyl)-2-methoxybenzoic acid and the single R- and S-enantiomers of (RS)-(1-ethylpyrrolidin-2-yl) methanamine to form esamisulpride as (S)-(-)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-(ethylsulfonyl)-2-methoxybenzamide and to form aramisulpride as (R)-(+)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-(ethylsulfonyl)-2-methoxybenzamide. Alternatively, single enantiomers of amisulpride were prepared by separation of racemic amisulpride using simulated moving bed chromatography. The products obtained from both routes was sufficient for clinical use (> 99.0% pure).

Radioligand binding

Binding affinities were measured by displacement of radioligand binding to membranes prepared from cells expressing human receptors, using [^{3}H]-spiperone for D2R (Eurofins Discovery #219700), and [^{3}H]-lysergic acid diethylamide for 5-HT7R (Eurofins Discovery #272320). Binding curves were generated from three independent determinations and fitted parameters (K_i expressed as mean ± SE).

Rodent tests

A series of rodent studies were conducted that were approved by the Institutional Animal Care and Use Committee of Sumitomo Dainippon Pharma Co., Ltd., Drug Research Division and were performed in accordance with the regulations for animal experiments of the division.

In vivo 5-HT7R-mediated activity of aramisulpride was evaluated in male Wistar rats in the forced swim test [16,50] and in a rat polysomnography (PSG) study[17]. The rat forced swim test consisted of two sessions. In the training session, each animal was gently placed into the plastic cylinder containing 5.8 L of water set at 25 ± 1°C. Fifteen minutes after the beginning of the training session, the animal was removed from the water and then dried and returned to its home cage. Twenty-four hours after the beginning of the training session, the swim test was performed for 5 minutes

in the same manner as the training session. The vehicle (0.1 M phosphoric acid + 0.1 M NaOH, pH6-7) or aramisulpride (0.15, 0.5, and 1.5 mg/kg) was intraperitoneally administered 3 times at 23.5 hours, 5 hours, and 1 hour prior to testing, and total animal immobility time was measured by an observer blinded to study treatment.

REM sleep time, non-rapid eye movement (NREM) sleep time, and WAKE time were measured using electroencephalogram (EEG) and electromyogram (EMG) recordings in rats. EEG electrodes were stereotaxically implanted at frontoparietal and parietal locations. EMG recordings were obtained at the dorsal neck muscle. Following 1 week recovery, EEG/EMG recordings were conducted for sleep stage analyses of WAKE, REM, and NREM. Aramisulpride was administered orally 10 minutes before the beginning of recording, during the light phase. EEG and EMG recordings were made for 6 hours starting at the beginning of the light phase.

Human studies

Three phase I studies in healthy volunteers were conducted following administration of single-doses of esamisulpride (study 1), aramisulpride (study 2), and SEP-4199 (study 3). In all three studies, written informed consent was obtained from each subject prior to initiation of any study procedures. Each study was approved by an Independent Ethics Committee before enrollment of any subject, and each study was conducted in accordance with International Council for Harmonization (ICH) Guidance for Industry. Study design and methods for the three studies are briefly summarized below. A more detailed summary is provided in the online Supplementary section.

In study 1, D2R occupancy was evaluated by ^{11}C-PHNO positron emission tomography (PET) scans in healthy subjects after administration of single oral doses of esamisulpride (Invicro, London, UK). PET scans were acquired at three timepoints (~ 3.5 hours, ~ 8.5 hours, and ~ 27 hours after dosing), to assess the onset of receptor occupancy. Only 27-hour PET scan results are reported here; the full time-course of pharmacokinetic and pharmacodynamic relationships will be reported separately.

In study 2, the effect of aramisulpride on REM sleep suppression (as a proxy measure for 5-HT7R antagonist activity) was evaluated in a single-center, single-blind, placebo-controlled, 2-stage, 2-way, crossover PSG study conducted at a sleep center (Hammersmith Medicines Research, London, UK). In stage 1, a 600 mg dose of aramisulpride was administered to 13 subjects with clear evidence of REM sleep suppression (vs. placebo). Therefore, in stage 2, the dose was reduced and 340 mg of aramisulpride was administered to 20 subjects.

In study 3, D2R occupancy was evaluated by ^{11}C-PHNO PET scan in healthy subjects (N-11) after administration of single oral doses of compound SEP-4199 (in a ratio of 85% aramisulpride to 15% esamisulpride): 200 mg (N = 3 subjects), 300 mg (N = 2), 400 mg (N = 2), 600 mg (N = 2), and 700 mg (N = 2). PET scans were acquired up to 99 hours postdose, to assess the rate at which the occupancy washes out. Only the 27-hour PET scans are reported here. Pharmacokinetic and pharmacodynamic relationships will be reported separately in a pooled analysis.

PET analyses

D2R and D3R occupancies were calculated for each postdose PET scan via regional estimate of the binding potential relative to the nondisplaceable component (BP_{ND}). These estimates were derived using the simplified reference tissue model (SRTM). Brain regions of interest considered included D_2-rich regions such as caudate and putamen and D_3-rich regions, such as substantia nigra. All calculations/derivations were performed by Invicro LLC (London, UK).

Polysomnography/pharmacodynamic analyses

The primary PSG analyses were descriptive statistical summaries of each of the three co-primary REM measures (latency to REM sleep, total REM time, and REM as a percent of total sleep time) analyzed by treatment group for each stage, and by treatment sequence at each night for each stage. An exploratory linear mixed model analysis was performed for the three co-primary REM end points that evaluated least square (LS) mean and 90% confidence interval (CI) for differences between active treatment and placebo by each stage.

RESULTS

In vitro pharmacology of aramisulpride and esamisulpride

Amisulpride enantiomers demonstrated stereoselective affinities to D2R and 5-HT7R (**Figure 1**). Aramisulpride was the more potent (Ki ± SEM) enantiomer for 5-HT7R (47 ± 4 nM) relative to esamisulpride (1,860 ± 260 nM). In contrast, for D2R, esamisulpride was the more-potent enantiomer (4.43 ± 0.70 nM) relative to aramisulpride (140 ± 31 nM). Increasing aramisulpride content in nonracemic ratios increased 5-HT7R affinity and reduced D2R affinity (**Figure 1b**). The 85:15 ratio of aramisulpride:esamisulpride (SEP-4199) resulted in significant differences in relative potencies for the two distinct receptors in comparison to the racemate, favoring 5-HT7R activity relative activity at D2R (**Figure 1b**).

The stereoselectivity of esamisulpride vs. aramisulpride was also evident in binding to D3R (Ki values of 0.72 vs. 13.9 nM). Binding to adrenergic α receptors also favored esamisulpride over aramisulpride: affinities (K*i*s) for α_{2A} receptors were 290 vs. 590 nM, respectively, and for α_{2c} receptors were 170 vs. 750 nM. Binding to other serotonin receptor subtypes was substantially weaker than to 5-HT7R and did not demonstrate any stereoselectivity between esamisulpride and aramisulpride: affinities (K*i*s) for 5-HT2A receptors were 430 and 380 nM for esamisulpride and aramisulpride, respectively, and for 5-HT2B receptors were 270 and 240 nM.

Effects of aramisulpride in rodent models

The potential contribution of the aramisulpride to antidepressant-like effects in animal was evaluated in the rat forced swim test. Aramisulpride demonstrated antidepressant like activity in rats with a dose-dependent effect similar in magnitude to the positive control compound imipramine (**Figure 2**), suggesting that aramisulpride alone is sufficient for antidepressant-like effect under these testing conditions. Selective 5-HT7R antagonists are known to modulate REM sleep in rodents and humans.[18] Therefore, the effect of aramisulpride on sleep architecture was evaluated in freely moving rats in the light phase. As shown in **Figure 2**, oral doses of aramisulpride reduced REM sleep duration in a dose-dependent manner. There was no effect of aramisulpride on NREM sleep time and wake time.

Esamisulpride and D2R occupancy: PET study in healthy volunteers

The specific aim of study 1 was to discover the dose-range of esamisulpride that targeted a level of D2R occupancy in the low-to-middle range (30% to 50%) in healthy subjects (N = 6; **Table 1**) using PET radiotracer ^{11}C-PHNO. D2R occupancy around 30–50% was chosen (1) to be below the level targeted in schizophrenia to treat psychosis, (2) to provide a range that would minimize the adverse effects of dopamine blockade (e.g., parkinsonism and akathisia), (3) to be at a low level to retain or not interfere with

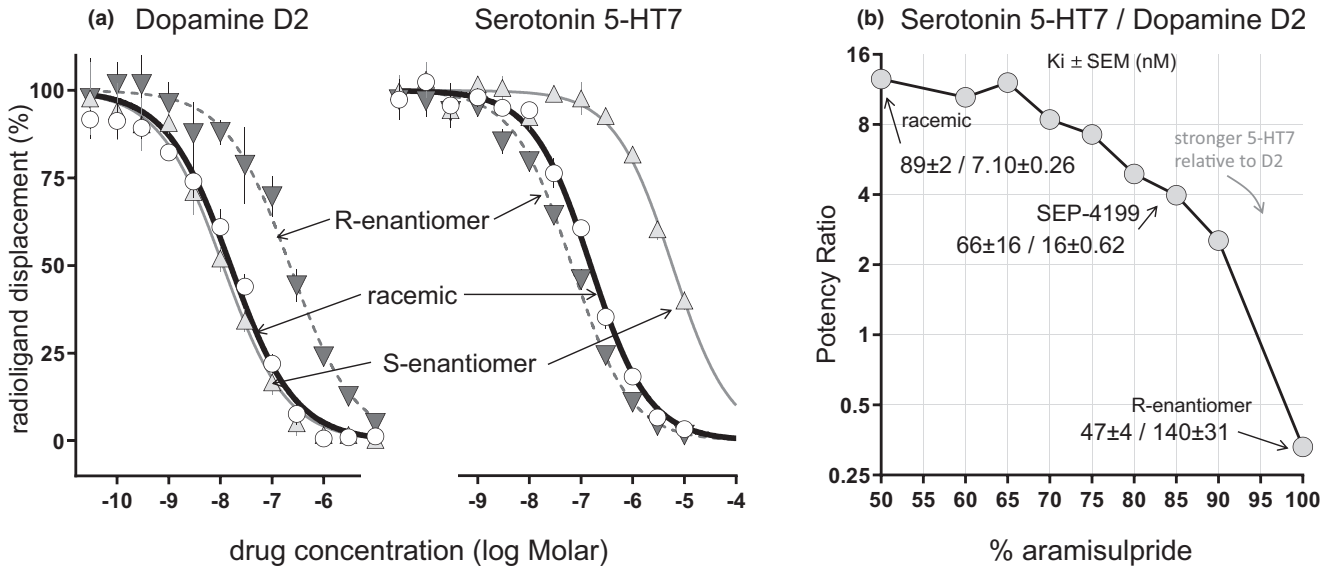

Figure 1 (**a**) Radioligand binding *in vitro* of amisulpride enantiomers to recombinantly expressed human dopamine receptor 2 (D2R) and 5-HT7 receptor (5HT7R) demonstrate stereoselectivity. Radioligand displacement was determined in triplicate as a function of increasing drug concentrations, where symbols represent the average and SD. (**b**) Relative potencies (Ki values) were determined in triplicate as a function of increasing aramisulpride content, where potency ratio is the Ki for 5-HT_7 divided by the Ki for D_2, and average Ki is noted ± SEM.

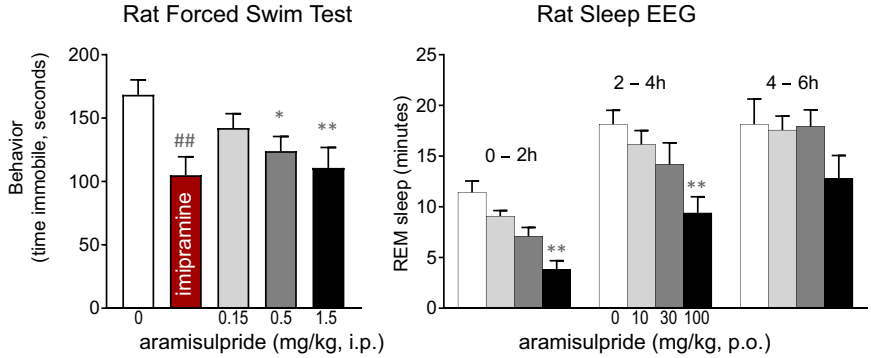

Figure 2 *Forced Swim Test:* rats were administered vehicle, imipramine, or aramisulpride at 23.5 hours, 5 hours and 1 hour prior to testing. Values are mean ± SEM, 18 animals per treatment group. ##P < 0.01 vs. vehicle (two-sided t-test), *P < 0.05 or **P < 0.01 vs. vehicle (parametric Dunnett's multiple comparison test, two-sided). *Rat Sleep* electroencephalogram (*EEG*)*:* rats were administered aramisulpride or vehicle 10 minutes before the beginning of the recording light phase. EEG and electromyogram (EMG) were recorded for 6 hours starting at the beginning of the light phase and rapid eye movement (REM) sleep time was measured. Data are expressed as mean ± SEM, n = 7 animals. *P < 0.05, **P < 0.01 (two-way analysis of variance (ANOVA) followed by *post hoc* parametric Dunnett multiple comparison test).

antidepressant effects overall, and (4) to still retain antimanic and antipsychotic effects via D2R. Following administration of doses between 25 mg and 200 mg esamisulpride in 6 healthy male subjects (mean age = 32.8 years, range = 26–38 years, body mass index (BMI) range = 21.0–28.0 kg/m^2), D2R occupancy for esamisulpride was found to be dose-dependent with an estimated RO_{50} of 91.7 mg (95% CI: 75.1–108.4 mg). The dose-occupancy relationship for esamisulpride is shown in **Figure 3a** (red symbols). These results suggested that single doses of esamisulpride below 100 mg would be required to achieve a D2R occupancy level that was < 50%. The occupancy of esamisulpride for D3R was similar to the occupancy measured for D2R, but its measurement exhibited much greater uncertainty. This is likely to be a result of several factors, including the fact that the substantia nigra is a relatively small region with weaker ^{11}C-PHNO signal than the D2R-rich regions used (dorsal caudate and dorsal putamen).

Vital signs and electrocardiograms (ECGs) were normal. Laboratory values remained normal, except for increases in prolactin levels. All doses of esamisulpride in study 1 were well-tolerated with no drug-related adverse events.

Aramisulpride suppresses REM sleep: PSG study in healthy volunteers

Based on results from two preclinical studies (forced swim test and PSG in rats) that demonstrated potential antidepressant and REM sleep suppression effects for aramisulpride, a PSG study in healthy subjects (N = 33; **Table 1**) was conducted to evaluate 5-HT7R-mediated effect of aramisulpride on REM sleep

Table 1 Demographic characteristics at baseline in human studies

	Study 1	Study 2		Study 3
	(*N* = 6)	Stage 1 (*N* = 13)	Stage 2 (*N* = 20)	(*N* = 11)
Male, *n* (%)	6 (100)	8 (61.5)	15 (75.0)	8 (72.7)
Age, years, mean (SD)	32.8 (4.4)	31.2 (8.3)	28.7 (7.1)	31.0 (4.6)
Race, *n* (%)				
White	6 (100)	13 (100)	12 (60.0)	6 (54.5)
Black/African American	0	0	5 (25.0)	2 (18.2)
Other	0	0	3 (15.0)	3 (27.3)
Body mass index, kg/m^2, mean (SD)	24.9 (2.5)	25.0 (3.2)	24.7 (2.7)	26.0 (3.6)

suppression. Aramisulpride suppressed REM parameters compared to placebo. Doses of 340 mg and 600 mg aramisulpride decreased time spent in REM sleep (LS mean ± SE) by 18 ± 4.9 and 31 ± 8.0 minutes, respectively **Figure 3b**) compared to placebo. Aramisulpride effects on REM sleep (LS mean ± SE) were also described by a latency to REM sleep that was increased by 20 ± 9.4 and 28 ± 9.8 minutes, and by the percent of time spent in REM sleep decreased by 4.2 ± 1.1 and 6.2 ± 1.5 percentage points, respectively, for doses of 340 and 600 mg compared to placebo. Effects of aramisulpride on other sleep parameters (LPS, TST, WASO, SE, NREM stages N1, N2, and N3) were characterized by overlapping 90% CIs compared with placebo. Vital signs were normal. Laboratory values remained normal, except for transient increases in prolactin levels. No subjects discontinued the study due to an adverse event. Aramisulpride was well-tolerated at the doses tested in this population of healthy adults.

In summary, single doses of aramisulpride (340 and 600 mg) were sufficient to produce clinically meaningful suppression of REM sleep parameters, indicating 5-HT7R-mediated effects at these doses in humans.

Nonracemic ratio SEP-4199

Based on the intervals of dose-effect demonstrated for each enantiomer of amisulpride in studies 1 and 2, a large gap was discovered for racemic amisulpride (**Figure 3c**), between doses of the S-enantiomer necessary to remain below 50% D2R occupancy demonstrated in study 1, and the doses of the R-enantiomer necessary to achieve 5-HT7R-mediated REM suppression above the thresholds for effect demonstrated in study 2. In contrast to racemic, the 85:15 ratio of aramisulpride to esamisulpride was discovered to provide significant overlap (**Figure 3c**) while remaining within the total dose range for the corresponding use of racemic amisulpride in schizophrenia (400–800 mg/day).

D2R occupancy of SEP-4199: PET study in healthy volunteers

The specific aim of study 3 was to determine, in a sample of healthy volunteers (*N* = 11; **Table 1**) the D2R occupancy of oral doses of SEP-4199, in the fixed 85:15 ratio of aramisulpride-to-esamisulpride, using PET and radiotracer ^{11}C-PHNO. The RO_{50} for D2R occupancy following doses of the 85:15 ratio (SEP-4199) was estimated to be 727.7 mg (95% CI, 586–869 mg). **Figure 3a** (blue symbols) illustrates the dose-occupancy relationship for the nonracemic ratio. Taken together, these results demonstrate that the esamisulpride

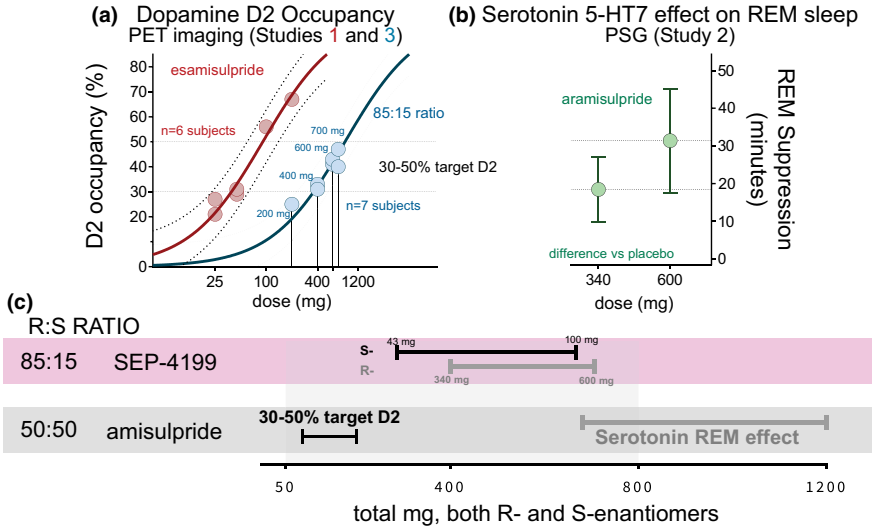

Figure 3 (**a**) Dopamine receptor 2 (D2R) occupancies for esamisulpride (red symbols) and SEP-4199 (blue symbols) are shown from studies 1 and 3. (**b**) Suppression of rapid eye movement (REM) sleep (green symbols) is shown for single doses of aramisulpride, difference vs. placebo, ±90% confidence interval (CI). (**c**) Horizontal bars indicate intervals of dose-effect for esamisulpride discovered in positron emission tomography (PET) imaging study 1 to occupy 30–50% D2R (horizontal black bars) and dose ranges of aramisulpride discovered in polysomnography (PSG) study 2 to produce serotonergic effects on REM suppression (horizontal grey bars). Intervals of dose-effect are expressed according to an x-axis of total amisulpride enantiomers (both aramisulpride and esamisulpride) RS, R- and S-enantiomers.

enantiomer contributes the great majority of D2R binding when dosed as the 85:15 ratio (15% of the overall dosage, 109 mg/727.7 mg). These results suggested SEP-4199 doses of 200 and 400 mg for further clinical study in the treatment of bipolar depression based on their D2R occupancy levels in the range of 25–40%.

DISCUSSION

The pharmacology of amisulpride at its serotonin and dopamine receptor targets is split between its enantiomers. Here, we show that the R-enantiomer (aramisulpride) targets 5-HT7R, and the S-enantiomer (esamisulpride) targets D2R. Our results replicate and extend the observations reported on esamisulpride stereoselectivity for binding D2R and D3R by Castelli *et al*.,[51] on the racemate for binding 5-HT7R by Abbas *et al*.,[12] and aramisulpride stereoselectivity for binding 5-HT7R by Grattan *et al*.[48]

In broad panel screening, amisulpride also binds with weaker affinities to other members of the serotonin, adrenergic members of the G protein-coupled receptor (GPCR) family. Here, we showed that the affinities for adrenergic α_{2A} and α_{2B} receptors also favored esamisulpride, although to a much lesser extent, indicating a chirality in the adrenergic receptor pharmacophore may retain aspects of the pharmacophore of D2R that favored esamisulpride. In contrast, stereoselectivity was lost in the weaker binding to the other serotonin receptors 5-HT2A and 5-HT2B. Thus, it was unexpected that 5-HT7R binding would favor the R-enantiomer. Outside of the adrenergic, serotonin, and dopamine GPCRs, the 85:15 ratio of aramisulpride:esamisulpride did not show any significant activities (< 50% at 10 μM) on a broad panel of ~ 90 receptor, transporter, and enzyme targets.

Antidepressant-like effects of racemic amisulpride in preclinical studies are mediated via 5-HT7R antagonism as demonstrated using 5-HT7R knockout mice.[12] Other antipsychotic compounds with high affinity for 5-HT7R include lurasidone and aripiprazole. Antidepressant-like effects of lurasidone[14] and aripiprazole[13] in animal models are also mediated via 5-HT7R antagonism, suggesting that clinical antidepressant effects may be mediated via 5-HTR. Here, we found that administration of the single enantiomer, aramisulpride, in rodents is sufficient for antidepressant-like activity in the forced swim test. In addition, 5-HT7R antagonists are known to modulate REM sleep in rodents and humans.[18] In a PSG study in rats, we found that single doses of aramisulpride resulted in significant REM sleep suppression.

The next series of studies were conducted to leverage the stereoselective binding affinities of aramisulpride (5-HT7R) and esamisulpride (D2R) to discover a nonracemic ratio of enantiomers that enhanced the 5-HT7R-mediated antidepressant effects while retaining some D2R antipsychotic benefits, but at a reduced level that would provide improved safety and tolerability. The PSG study, using REM sleep suppression as proxy measure of 5-HT7R-mediated antidepressant effects, demonstrated that aramisulpride doses of 340 mg and 600 mg were consistent with marked pharmacodynamic effects on sleep. For esamisulpride, results of the PET study demonstrated that doses of 43–100 mg yielded D2R occupancies in the range of 30%–50%. The contribution of aramisulpride to occupancy of D2R was negligible (based on the results described here for pure esamisulpride and the 85:15 ratio) such that pure aramisulpride would not be expected to occupy D2R in the clinical dose range of 400–800 mg.

The stereoselective pharmacology of amisulpride enantiomers allowed the discovery of SEP-4199. Medicinal chemistry programs often set out to adjust the ratio of affinities for two different therapeutic targets, by synthesizing new molecules to optimize their relative binding affinities, but in this unique case of amisulpride, the ratio of affinities to two important therapeutic targets was optimized by changing the ratio of enantiomers for the same molecular entity. Here, we demonstrated, by dosing each enantiomer in human clinical studies, that the racemic form of amisulpride was insufficient to achieve a targeted balance of 5-HT7R-mediated effects together with the targeted level of D2R occupancy. The nonracemic ratio of 85:15 aramisulpride:esamisulpride was required to achieve the targeted levels of 5-HT7R antagonist-mediated antidepressant effects and the antimanic, anxiolytic, and antipsychotic benefits of low levels of D2R/D3R blockade. The doses of 200 and 400 mg were selected to improve the benefit/risk profile in bipolar disorder by enhancing aramisulpride for antidepressant benefit at 5-HT7R and reducing esamisulpride to minimize D2R-related extrapyramidal side effects while still retaining D2R-mediated efficacy against emergent cycling, mixed, manic, anxiety, and/or psychotic symptoms. The results described here supported utilizing doses of 200 and 400 mg for proof-of-concept testing in phase 2. The proof-of-concept trial (clinicaltrials.gov: NCT03543410) for the treatment of bipolar depression with SEP-4199 was recently completed. The double-blind trial provided initial confirmation of the efficacy of SEP-4199. On the primary depression measure, the MADRS, the LS mean difference in week 6 change scores for SEP-4199 vs. placebo was significant for both the 200 mg dose ($P = 0.025$; effect size, 0.34) and the 400 mg dose ($P = 0.025$; effect size, 0.31).[52]

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

S.C.H., S.W., T.J.C., H.N., K.N., A.L., and K.S.K. wrote the manuscript. S.C.H., A.L., and K.S.K. designed the research. S.W., T.J.C., H.N., and K.N. performed the research. S.C.H., H.N., A.L., and K.S.K. analyzed the data.

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1. Kapur, S., Zipursky, R.B. & Remington, G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am. J. Psych.* **156**, 286–293 (1999).
2. Tauscher, J., Küfferle, B., Asenbaum, S., Tauscher-Wisniewski, S. & Kasper, S. Striatal dopamine-2 receptor occupancy as measured with [123I]iodobenzamide and SPECT predicted the occurrence of EPS in patients treated with atypical antipsychotics and haloperidol. *Psychopharmacology* **162**, 42–49 (2002).
3. Gao, K., Kemp, D.E., Ganocy, S.J., Gajwani, P., Xia, G. & Calabrese, J.R. Antipsychotic-induced extrapyramidal side effects in bipolar disorder and schizophrenia: a systematic review. *J. Clin. Psychopharmacol.* **28**, 203–209 (2008).
4. Ghaemi, S.N., Hsu, D.J., Rosenquist, K.J., Pardo, T.B. & Goodwin, F.K. Extrapyramidal side effects with atypical neuroleptics in bipolar disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **30**, 209–213 (2006).
5. Poyurovsky, M. & Weizman, A. Serotonergic agents in the treatment of acute neuroleptic-induced akathisia: open-label study of buspirone and mianserin. *Int. Clin. Psychopharmacol.* **12**, 263–268 (1997).
6. Miller, C.H., Fleischhacker, W.W., Ehrmann, H. & Kane, J.M. Treatment of neuroleptic induced akathisia with the 5-HT2 antagonist ritanserin. *Psychopharmacol. Bull.* **26**, 373–376 (1990).
7. Meltzer, H.Y., Matsubara, S. & Lee, J.C. The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs. *Psychopharmacol. Bull.* **25**, 390–392 (1989).
8. Meltzer, H.Y., Arvanitis, L., Bauer, D. & Rein, W., & Meta-Trial Study Group. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *Am. J. Psychiatry* **161**, 975–984 (2004).
9. Nordström, A.L., Farde, L. & Halldin, C. High 5-HT2 receptor occupancy in clozapine treated patients demonstrated by PET. *Psychopharmacology* **110**, 365–367 (1993).
10. Moresco, R.M. *et al*. Cerebral D2 and 5-HT2 receptor occupancy in Schizophrenic patients treated with olanzapine or clozapine. *J. Psychopharmacol.* **18**, 355–365 (2004).
11. Gefvert, O. *et al*. D(2) and 5HT(2A) receptor occupancy of different doses of quetiapine in schizophrenia: a PET study. *Eur. Neuropsychopharmacol.* **11**, 105–110 (2001).
12. Abbas, A.I., Hedlund, P.B., Huang, X.P., Tran, T.B., Meltzer, H.Y. & Roth, B.L. Amisulpride is a potent 5-HT7 antagonist: relevance for antidepressant actions in vivo. *Psychopharmacology* **205**, 119–128 (2009).
13. Sarkisyan, G., Roberts, A.J. & Hedlund, P.B. The 5-HT(7) receptor as a mediator and modulator of antidepressant-like behavior. *Behav. Brain Res.* **209**, 99–108 (2010).
14. Cates, L.N., Roberts, A.J., Huitron-Resendiz, S. & Hedlund, P.B. Effects of lurasidone in behavioral models of depression. Role of the 5-HT_7 receptor subtype. *Neuropharmacology* **70**, 211–217 (2013).
15. Thomas, D.R. *et al*. SB-656104-A, a novel selective 5-HT7 receptor antagonist, modulates REM sleep in rats. *Br. J. Pharmacol.* **139**, 705–714 (2003).
16. Hedlund, P.B., Huitron-Resendiz, S., Henriksen, S.J. & Sutcliffe, J.G. 5-HT7 receptor inhibition and inactivation induce antidepressant like behavior and sleep pattern. *Biol. Psychiatry* **58**, 831–837 (2005).
17. Bonaventure, P. *et al*. Selective blockade of 5-hydroxytryptamine (5-HT)7 receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. *J. Pharmacol. Exp. Ther.* **321**, 690–698 (2007).
18. Bonaventure, P. *et al*. Translational evaluation of JNJ-18038683, a 5-hydroxytryptamine type 7 receptor antagonist, on rapid eye movement sleep and in major depressive disorder. *J. Pharmacol. Exp. Ther.* **342**, 429–440 (2012).
19. Matthys, A., Haegeman, G., Van Craenenbroeck, K. & Vanhoenacker, P. Role of the 5-HT7 receptor in the central nervous system: from current status to future perspectives. *Mol. Neurobiol.* **43**, 228–253 (2011).
20. Monti, J.M., Leopoldo, M. & Jantos, H. The serotonin 5-HT7 receptor agonist LP-44 microinjected into the dorsal raphe nucleus suppresses REM sleep in the rat. *Behav. Brain Res.* **191**, 184–189 (2008).
21. Monti, J.M., Leopoldo, M., Jantos, H. & Lagos, P. Microinjection of the 5-HT7 receptor antagonist SB-269970 into the rat brainstem and basal forebrain: site-dependent effects on REM sleep. *Pharmacol. Biochem. Behav.* **102**, 373–380 (2012).
22. Monti, J.M. & Jantos, H. The role of serotonin 5-HT7 receptor in regulating sleep and wakefulness. *Rev. Neurosci.* **25**, 429–437 (2014).
23. Shelton, J., Bonaventure, P., Li, X., Yun, S., Lovenberg, T. & Dugovic, C. 5-HT7 receptor deletion enhances REM sleep suppression induced by selective serotonin reuptake inhibitors, but not by direct stimulation of 5-HT1A receptor. *Neuropharmacology* **56**, 448–454 (2009).
24. Meneses, A. 5-HT system and cognition. *Neurosci. Biobehav. Rev.* **23**, 1111–1125 (1999).
25. Hedlund, P.B. & Sutcliffe, J.G. Functional, molecular and pharmacological advances in 5-HT7 receptor research. *Trends Pharmacol. Sci.* **25**, 481–486 (2004).
26. Leopoldo, M., Lacivita, E., Berardi, F., Perrone, R. & Hedlund, P.B. Serotonin 5-HT7 receptor agents: Structure-activity relationships and potential therapeutic applications in central nervous system disorders. *Pharmacol. Ther.* **129**, 120–148 (2011).
27. Cortes-Altamirano, J.L. *et al*. Review: 5-HT1, 5-HT2, 5-HT3 and 5-HT7 receptors and their role in the modulation of pain response in the central nervous system. *Curr. Neuropharmacol.* **16**, 210–221 (2018).
28. Mota, N.E., Lima, M.S. & Soares, B.G. Amisulpride for schizophrenia. *Cochrane Database Syst. Rev.* **2002**, CD001357 (2002).
29. McKeage, K. & Plosker, G.L. Amisulpride: a review of its use in the management of schizophrenia. *CNS Drugs* **18**, 933–956 (2004).
30. Nuss, P., Hummer, M. & Tessier, C. The use of amisulpride in the treatment of acute psychosis. *Ther. Clin. Risk Manag.* **3**, 3–11 (2007).
31. Boyer, P., Lecrubier, Y., Puech, A.J., Dewailly, J. & Aubin, F. Treatment of negative symptoms in schizophrenia with amisulpride. *Br. J. Psychiatry* **166**, 68–72 (1995).
32. Danion, J.M., Rein, W. & Fleurot, O., & Amisulpride Study Group. Improvement of schizophrenic patients with primary negative symptoms treated with amisulpride. *Am. J. Psych.* **156**, 610–616 (1999).
33. Loo, H., Poirier-Littre, M.F., Theron, M., Rein, W. & Fleurot, O. Amisulpride versus placebo in the medium-term treatment of the negative symptoms of schizophrenia. *Br. J. Psych.* **170**, 18–22 (1997).
34. Lecrubier, Y., Boyer, P., Turjanski, S., Rein, W., & Amisulpride Study Group. Amisulpride versus imipramine and placebo in dysthymia and major depression. *J. Affect. Disord.* **43**, 95–103 (1997).
35. Boyer, P., Lecrubier, Y., Stalla-Bourdillon, A. & Fleurot, O. Amisulpride versus amineptine and placebo for the treatment of dysthymia. *Neuropsychobiology* **39**, 25–32 (1999).
36. Leucht, S. *et al*. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet* **382**, 951–962 (2013).
37. Huhn, M. *et al*. Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: a systematic review and network meta-analysis. *Lancet* **394**, 939–951 (2019).
38. King, D.J. & The BAP Consensus Group. Guidelines for the use of antipsychotic drug studies in healthy volunteers. *J. Psychopharmacol.* **11**, 201–209 (1997).
39. Ramaekers, J.G. *et al*. Psychomotor, cognitive, extrapyramidal, and affective functions of healthy volunteers during treatment with an atypical (amisulpride) and a classic (haloperidol) antipsychotic. *J. Clin. Psychopharmacol.* **19**, 209–221 (1999).
40. Martinot, J.L. *et al*. Central D2 receptor blockade and antipsychotic effects of neuroleptics. Preliminary study with positron emission tomography. *Psychiatr. Psychobiol.* **5**, 231–240 (1990).

41. Martinot, J.L., Paillère-Martinot, M.L., Poirier, M.F., Dao-Castellana, M.H., Loc'h, C. & Mazière, B. In vivo characteristics of dopamine D2 receptor occupancy by amisulpride in schizophrenia. *Psychopharmacology* **124**, 154–158 (1996).
42. Bressan, R.A., Erlandsson, K., Spencer, E.P., Ell, P.J. & Pilowsky, L.S. Prolactinemia is uncoupled from central D2/D3 dopamine receptor occupancy in amisulpride treated patients. *Psychopharmacology* **175**, 367–373 (2004).
43. Vernaleken, I. *et al*. High striatal occupancy of D2-like dopamine receptors by amisulpride in the brain of patients with schizophrenia. *Int. J. Neuropsychopharmacol.* **7**, 421–430 (2004).
44. la Fougère, C. *et al*. D2 receptor occupancy during high- and low-dose therapy with the atypical antipsychotic amisulpride: a 123I-iodobenzamide SPECT study. *J. Nucl. Med.* **46**, 1028–1033 (2005).
45. Krause, M. *et al*. Antipsychotic drugs for patients with schizophrenia and predominant or prominent negative symptoms: a systematic review and meta-analysis. *Eur. Arch. Psychiatry Clin. Neurosci.* **268**, 625–639 (2018).
46. Montgomery, S.A. Dopaminergic deficit and the role of amisulpride in the treatment of mood disorders. *Int. Clin. Psychopharmacol.* **17**(Suppl 4), S9–S15 (2002).
47. Sparshatt, A., Taylor, D., Patel, M.X. & Kapur, S. Amisulpride–--dose, plasma concentration, occupancy and response: implications for therapeutic drug monitoring. *Acta. Psychiatr. Scand.* **120**, 416–428 (2009).
48. Grattan, V., Vaino, A.R., Prensky, Z. & Hixon, M.S. Antipsychotic benzamides amisulpride and LB-102 display polypharmacy as racemates, S Enantiome.rs Engage Receptors D2 and D3, while R Enantiomers Engage 5-HT7. *ACS Omega*. **4**, 14151–14154.
49. Hopkins, S.C., Koblan, S., Snoonian, J.R. & Wilkinson, H.S. Nonracemic mixtures and uses thereof. *PCT Patent Application* WO2019113079 (2019).
50. Porsolt, R.D., Anton, G., Blavet, N. & Jalfre, M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* **47**, 379-391 (1978).
51. Casetlli, M.P., Mocci, I., Sanna, A.M., Gessa, G.L. & Pani, L. (-)S amisulpride binds with high affinity to cloned dopamine D(3) and D(2) receptors. *Eur. J. Pharmacol.* **432**, 143–147 (2001).
52. Loebel, A. *et al*. A randomized, double-blind, placebo-controlled study of SEP-4199 for the treatment of patients with bipolar depression. *Neuropsychopharmacol.* **45**, 95–96 (2020).