



## Pharmacological characterization of RS 25259-197, a novel and selective 5-HT<sub>3</sub> receptor antagonist, *in vivo*

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**1** The pharmacological effects *in vivo*, of RS 25259-197, a selective 5-HT<sub>3</sub> receptor antagonist, have been investigated.

**2** In anaesthetized rats, RS 25259-197, administered by the intravenous, intraduodenal or transdermal route, dose-dependently inhibited the von Bezold-Jarisch reflex induced by 2-methyl 5-HT (ID<sub>50</sub> = 0.04 µg kg<sup>-1</sup>, i.v., 3.2 µg kg<sup>-1</sup>, i.d. and 32.8 µg per chamber, respectively). In this regard, when administered intraduodenally, RS 25259-197 was more potent and exhibited a longer duration of action than either ondansetron or granisetron.

**3** In conscious ferrets, RS 25259-197, administered intravenously or orally, dose-dependently inhibited emesis induced by cisplatin. The ID<sub>50</sub> estimates of RS 25259-197 were 1.1 µg kg<sup>-1</sup>, i.v. and 3.2 µg kg<sup>-1</sup>, p.o. In this respect, RS 25259-197 was more potent than ondansetron and equipotent with granisetron.

**4** In conscious dogs, RS 25259-197, administered intravenously or orally, dose-dependently inhibited emesis induced by cisplatin (ID<sub>50</sub> = 1.9 µg kg<sup>-1</sup>, i.v. and 8.5 µg kg<sup>-1</sup>, p.o.), dacarbazine (ID<sub>50</sub> = 4.1 µg kg<sup>-1</sup>, i.v. and 9.7 µg kg<sup>-1</sup>, p.o.), actinomycin D (ID<sub>50</sub> = 4.9 µg kg<sup>-1</sup>, i.v. and 2.5 µg kg<sup>-1</sup>, p.o.) and mechlorethamine (ID<sub>50</sub> = 4.4 µg kg<sup>-1</sup>, i.v. and 3.0 µg kg<sup>-1</sup>, p.o.). Against each of the emetogenic agents, RS 25259-197 was very much more potent than ondansetron. When tested at equal-effective intravenous doses against cisplatin-induced emesis in dogs, RS 25259-197 had a longer duration of anti-emetic activity (7 h) than ondansetron (4 h). At doses up to and including 1000 µg kg<sup>-1</sup>, p.o., neither RS 25259-197 nor ondansetron was capable of inhibiting apomorphine-induced emesis.

**5** At doses up to 1000 µg kg<sup>-1</sup>, i.v., RS 25259-197 produced no meaningful haemodynamic changes in anaesthetized dogs.

**6** In summary, RS 25259-197 is a novel, highly potent and orally active 5-HT<sub>3</sub> receptor antagonist *in vivo*. With respect to its anti-emetic activity, RS 25259-197 appears to be a significant improvement over ondansetron in terms of potency and duration of action.

**Keywords:** 5-HT<sub>3</sub> antagonist; RS 25259-197; RS 25259; ondansetron; emesis; cisplatin; cancer chemotherapy; anti-emetic; von Bezold-Jarisch reflex

### Introduction

It is now well established that the side-effects of nausea and emesis associated with anti-cancer therapy are due to activation of 5-HT<sub>3</sub> receptors at a peripheral and/or central locus (Kilpatrick *et al.*, 1990; Tyers, 1991). The vast majority of evidence favours a peripheral mechanism involving activation of 5-HT<sub>3</sub> receptors, localized on vagal afferents supplying the upper gastrointestinal tract, by 5-HT released from enterochromaffin cells as a consequence of exposure to cytotoxic drugs or X-ray irradiation (Costall & Naylor, 1992). The advent of selective 5-HT<sub>3</sub> receptor antagonists has shown that, in cancer patients, these drugs are highly effective in preventing chemotherapy-induced emesis and therefore improving the quality of life.

Numerous 5-HT<sub>3</sub> receptor antagonists have been synthesized to date. The most widely characterized compounds include ondansetron, granisetron, zacopride and tropisetron (Oxford *et al.*, 1992). Emerging evidence indicates that significant differences in potency, efficacy and duration of action exist between the compounds which may have an impact on their clinical usefulness (Marr *et al.*, 1991; Andrews *et al.*, 1992). For example, the anti-emetic effects have been reported to be dose-dependent for granisetron but not for ondansetron (Andrews *et al.*, 1992). Also, in the ferret,

granisetron was reported to be more potent as an anti-emetic when administered by the oral route than the intravenous route, whereas the reverse was true for ondansetron (Fitzpatrick *et al.*, 1990). Ondansetron, the most extensively studied 5-HT<sub>3</sub> receptor antagonist, is poorly effective in controlling mild and delayed emesis (Kris *et al.*, 1992; Levitt *et al.*, 1993). Furthermore, none of the currently available 5-HT<sub>3</sub> receptor antagonists is capable of completely blocking the incidence of nausea and emesis in cancer patients. Reports of side-effects, such as chest pain and extrapyramidal symptoms with ondansetron (Ballard *et al.*, 1992; Halpern & Murphy, 1992), and animal toxicity such as granisetron-induced hepatic carcinoma (Joss & Dott, 1993) may also limit the clinical utility of these compounds. Hence, there is clearly a need for the development of selective 5-HT<sub>3</sub> receptor antagonists which are more safe, potent and efficacious. RS 25259-197((3aS)-2-[(S)-1-azabicyclo[2.2.2]oct-3-yl]2,3,3a,4,5,6-hexahydro-1-oxo-1-*H*-benz[de]isoquinoline hydrochloride) is a novel selective 5-HT<sub>3</sub> receptor antagonist (Wong *et al.*, 1995) (Figure 1). Homogenate radioligand binding and functional pharmacological studies have established that RS 25259-197 exhibits a high affinity at 5-HT<sub>3</sub> receptors (pK<sub>i</sub> = 10.4 at 5-HT<sub>3</sub> receptors in rat cortex and pA<sub>2</sub> ~ 8.8 at 5-HT<sub>3</sub> receptors in guinea-pig ileum). In the present paper, we present our findings on the *in vivo* pharmacological effects of this compound using ondansetron and/or granisetron as reference compounds.

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Dr. Reddy's Laboratories, Ltd., et al.  
v.  
Helsinn Healthcare S.A., et al.  
U.S. Patent No. 8,729,094  
Reddy Exhibit 1011



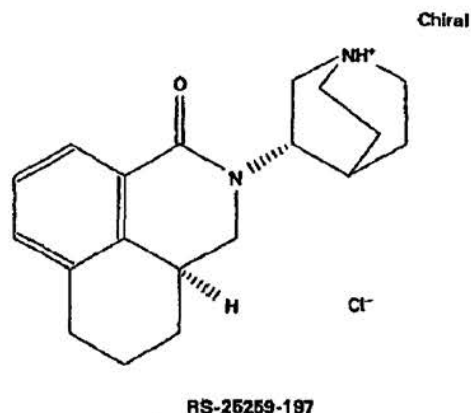


Figure 1 Chemical structure of RS 25259-197 ((3aS)-2-[(S)-1-aza-bicyclo[2.2.2]oct-3-yl]-2,3,3a,4,5,6-hexahydro-1-oxo-1H-benz[de]isoquinoline hydrochloride).

## Methods

### Studies on the von Bezold-Jarisch reflex in rats

Intravenously administered 5-HT or 2-methyl 5-HT elicits an immediate and short-lasting vagally mediated reflex bradycardia (referred to as the Von Bezold-Jarisch reflex) which is specifically mediated by 5-HT<sub>3</sub> receptors and is antagonized by selective 5-HT<sub>3</sub> receptor antagonists (Fozard, 1984). RS 25259-197 was evaluated for its ability to inhibit this reflex, using ondansetron and granisetron as reference compounds.

Male Sprague Dawley rats (Charles River, 250–380 g) were given food and water *ad libitum*, except those used for intraduodenal drug administration; these rats were deprived of food overnight. Rats were anaesthetized with urethane (1.5 g kg<sup>-1</sup>, i.p.) and placed on an aquatic K module heating pad to maintain body temperature at 37°C. The left jugular vein or duodenum, trachea and left femoral vein were cannulated for drug administration (i.v. or i.d.), facilitation of respiration and injection of 2-methyl 5-HT, respectively. Heart rate was derived from a limb lead II ECG monitored via subdermal platinum electrodes and was recorded with ECG/Biotach amplifiers connected to a Gould recorder (RS 3800). A dose-response curve to 2-methyl 5-HT (5–100 µg kg<sup>-1</sup>, i.v.) was constructed in each rat to establish a submaximal dose (usually 10 or 20 µg kg<sup>-1</sup>, i.v.) which would elicit a reproducible bradycardic response. Each rat then received a single dose of RS 25259-197, ondansetron or granisetron and was then challenged with 2-methyl 5-HT at 5, 15, 30, 60, 120, 180, 240, 300, 420 and 480 min post dosing. A separate group of rats receiving vehicle (saline for i.v., deionized water for i.d.) was similarly tested in each study. Preliminary experiments showed that there was no evidence of tachyphylaxis in the responses to 2-methyl 5-HT. Duration of action of the compounds was assessed by determining the period of time for which the inhibitory effects remained significantly different from vehicle controls.

In a separate series of experiments, RS 25259-197, when applied topically on the abdominal skin of rats, was evaluated for its ability to inhibit the reflex. An aqueous solution of the drug was absorbed onto a Hill Top chamber (25 mm in diameter) in a volume of 0.4 ml. A submaximal dose of 2-methyl 5-HT that could elicit a reproducible bradycardic response was first determined in each rat. RS 25259-197 (0.01–1000 µg per chamber) was then applied topically to the depilicated abdominal area and the rat was challenged with 2-methyl 5-HT at 5, 15, 30, 60, 120, 180, 240 and 300 min post dose.

### Anti-emetic studies in ferrets and dogs

The ferret (Florczyk *et al.*, 1982) and the dog (Gylys *et al.*, 1979) are well established animal models of emesis which respond to cancer chemotherapeutic agents in a manner similar to that observed in man.

Adult male ferrets (1–1.4 kg, Marshall farms) were randomly assigned to different treatment groups. Each animal was anaesthetized with metofane inhalant. A jugular vein was cannulated and exteriorized from the outside of the neck. Following recovery from anaesthesia, each animal was dosed with either RS 25259-197 (1–100 µg kg<sup>-1</sup>, i.v. or 0.3–100 µg kg<sup>-1</sup>, p.o.), ondansetron (30–1000 µg kg<sup>-1</sup>, p.o.) or vehicle (1 ml kg<sup>-1</sup>, i.v. or p.o.) 30 min prior to administration of cisplatin (10 mg kg<sup>-1</sup>, i.v.). The compounds were given orally in hard gelatin capsules. Each animal was counted for the number of emetic episodes for 5 h following cisplatin administration.

Adult male dogs (8–20 kg) (Hazleton Research Products) were randomly assigned to different treatment groups. Each animal was dosed with either RS 25259-197 (0.3–300 µg kg<sup>-1</sup>, i.v. or p.o.), vehicle control (0.1 ml kg<sup>-1</sup>, i.v. or p.o.), ondansetron (1–1000 µg kg<sup>-1</sup>, i.v. or p.o.) or haloperidol (5 mg kg<sup>-1</sup>, p.o.) at 30 min prior to the administration of apomorphine (0.1 mg kg<sup>-1</sup>, s.c.), or 120 min prior to the administration of either dacarbazine (30 mg kg<sup>-1</sup>, i.v.) or mechlorethamine (0.4 mg kg<sup>-1</sup>, i.v.) and actinomycin D (0.15 mg kg<sup>-1</sup>, i.v.) or at 60 min after the administration of cisplatin (3 mg kg<sup>-1</sup>, i.v.). The compounds were given intravenously via the cephalic vein or orally in hard gelatin capsules. Each animal was counted for the number of emetic episodes for 5 h following the administration of each emetic. An emetic episode was defined as the successful evacuation of stomach contents. In separate experiments designed to examine the duration of anti-emetic activity, dogs were pretreated with vehicle (0.1 ml kg<sup>-1</sup>, i.v.), RS 25259-197 (30 µg kg<sup>-1</sup>, i.v.) or ondansetron (300 µg kg<sup>-1</sup>, i.v.) at 24, 12, 8, 7, 6, 5, 4, 3, 2 or 1 h prior to the administration of cisplatin, after which each animal was observed for an additional 5 h.

### Haemodynamic studies in anaesthetized dogs

Mongrel dogs (4 male, 4 female, 11–16 kg, Hazleton Laboratories) were anaesthetized with pentobarbitone sodium (36 mg kg<sup>-1</sup>, i.v.). The dogs were then artificially ventilated with Harvard respirators. A catheter inserted into a carotid artery was used for measurement of arterial pressure and for blood sampling (pH/blood gas analysis with a Corning Model 168 analyser). The ipsilateral femoral vein was cannulated for the administration of test compound or vehicle and for the administration of a maintenance infusion of pentobarbitone sodium (3–8 mg kg<sup>-1</sup> h<sup>-1</sup>). A Millar 7F Mikro-Tip Catheter pressure transducer was inserted into the left ventricle for the measurement of left ventricular systolic pressure (LVP) and left ventricular end-diastolic pressure (LVEDP), and  $dp/dt_{max}$  was obtained by electronic differentiation of the LVP signal. The right external jugular vein was isolated and a 5F Swan-Ganz catheter (Spectramed SP5105H) placed in the pulmonary artery to permit determination of cardiac output (CO) by thermodilution using a Gould Cardiac Index Computer (Model 435). Heart rate (HR) was measured with a cardiometer triggered by the R-wave of a limb lead II ECG recorded from needle electrodes inserted subcutaneously. Mean arterial pressure (MAP) was calculated as 1/3 (systolic arterial pressure-diastolic arterial pressure) + diastolic arterial pressure. Directly monitored parameters as well as electronically derived  $dp/dt_{max}$  and HR were recorded on a polygraph (Gould RS 3800).

Following completion of instrumentation, each dog was allowed to equilibrate for 30–60 min before beginning an experiment. Each dog received vehicle (0.02 ml kg<sup>-1</sup>, i.v.) or



a single dose of RS 25259-197 (10, 100 and 1000  $\mu\text{g kg}^{-1}$ , i.v.). Recorded parameters were measured immediately prior to the first treatment dose and at 2, 5, 15, 30, 45 and 60 min after each dose.

#### Statistical analysis

Statistical analysis of the data was performed by a repeated measure analysis of variance (ANOVA) and, in some cases, was followed by pairwise comparisons against control at each time period using Fisher's LSD multiple comparison test. Statistical significance was defined as  $P < 0.05$ . In emetic studies, an  $\text{ID}_{50}$  (dose required to produce 50% of the maximal inhibition) was calculated wherever appropriate.  $\text{ID}_{50}$  was calculated with NONLIN84 software, a nonlinear modelling programme.

#### Materials

RS 25259-197, granisetron and ondansetron were synthesized at the Institute of Organic Chemistry, Syntex Research. 5-HT, 2-methyl 5-HT, actinomycin D, cisplatin, dacarbazine, mechlorethamine and apomorphine were obtained from Sigma Chemical Co (St Louis, MO, U.S.A.).

### Results

#### Studies on the von Bezold-Jurisch reflex

When administered intravenously, RS 25259-197 (0.01–10  $\mu\text{g kg}^{-1}$ ), ondansetron (1–300  $\mu\text{g kg}^{-1}$ ) and granisetron (0.03–300  $\mu\text{g kg}^{-1}$ ) produced dose-dependent inhibition of the 2-

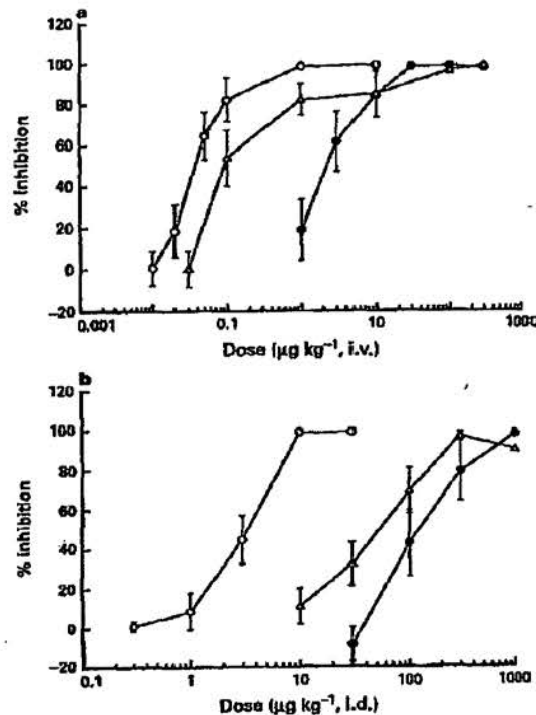


Figure 2 Inhibitory effects of RS 25259-197 (O), ondansetron (●) and granisetron (Δ), administered intravenously (a) and intraduodenally (b), on the 2-methyl 5-HT-induced bradycardia. Each point represents the mean  $\pm$  s.e.mean,  $n = 8-10$ .

methyl 5-HT induced bradycardic response, yielding  $\text{ID}_{50}$  estimates (95% confidence interval) of 0.04(0.02–0.06), 2.2(0.5–3.96) and 0.1(0.01–0.19)  $\mu\text{g kg}^{-1}$ , respectively (Figure 2a). When administered intraduodenally, RS 25259-197, ondansetron and granisetron produced dose-dependent inhibition of the bradycardic response (Figure 2b). By the i.d. route, RS 25259-197 was much more potent ( $\text{ID}_{50}$  (95% confidence interval): [3.2(2.7–3.7)  $\mu\text{g kg}^{-1}$ ] than ondansetron [144(53.2–234.2)  $\mu\text{g kg}^{-1}$ ] and granisetron [49.1(21.2–76.9)  $\mu\text{g kg}^{-1}$ ]. The duration of inhibitory effects was dose-dependent for all three compounds (Figure 3a–c). At equi-effective doses (lowest dose required to produce 100% inhibition) the duration of the inhibitory effects for RS 25259-197 (10  $\mu\text{g kg}^{-1}$ , i.d., 420 min) was greater than that of ondansetron (1000  $\mu\text{g kg}^{-1}$ , i.d., 300 min) and granisetron (300  $\mu\text{g kg}^{-1}$ , i.d., 300 min).

Transdermal administration of RS 25259-197 (0.01–1000  $\mu\text{g/chamber}$ ) produced a dose-dependent inhibition of the bradycardia induced by 2-methyl 5-HT (Figure 4a) yielding an  $\text{ID}_{50}$  (95% confidence interval) of 32.8 (12.8–52.7)  $\mu\text{g}$  per chamber. The onset of the inhibitory effects was inversely proportional to the concentration of the drug in the chamber (Figure 4b). At doses of 1–100  $\mu\text{g}$  per chamber, the inhibitory effects of the drug lasted for greater than 300 min.

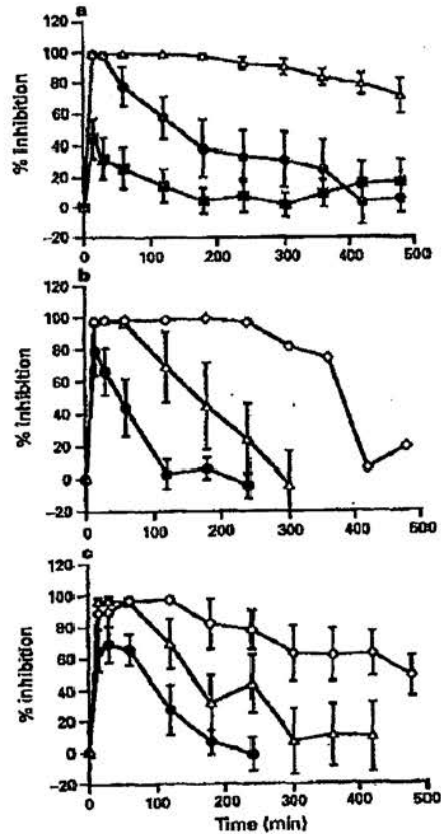


Figure 3 Duration of the inhibitory effects of RS 25259-197 (a), ondansetron (b) and granisetron (c) on the 2-methyl 5-HT-induced bradycardia. (a) RS 25259-197, (■) 3  $\mu\text{g kg}^{-1}$ , i.d., (●) 10  $\mu\text{g kg}^{-1}$ , i.d., (Δ) 30  $\mu\text{g kg}^{-1}$ , i.d., (b) Ondansetron, (●) 300  $\mu\text{g kg}^{-1}$ , i.d., (Δ) 1000  $\mu\text{g kg}^{-1}$ , i.d., (○) 3000  $\mu\text{g kg}^{-1}$ , i.d. (c) Granisetron, (●) 100  $\mu\text{g kg}^{-1}$ , i.d., (Δ) 300  $\mu\text{g kg}^{-1}$ , i.d., (○) 1000  $\mu\text{g kg}^{-1}$ , i.d. Each point represents the mean  $\pm$  s.e.mean,  $n = 8-10$ .

### Anti-emetic activity in ferrets and dogs

Compared with vehicle control, RS 25259-197 (1–30  $\mu\text{g kg}^{-1}$ , i.v. and 1–30  $\mu\text{g kg}^{-1}$ , p.o.), ondansetron (30–1000  $\mu\text{g kg}^{-1}$ , p.o.) and granisetron (3–300  $\mu\text{g kg}^{-1}$ , p.o.) given 30 min prior to cisplatin produced significant and dose-dependent reduction in the number of emetic episodes in ferrets (Figure 5, Table 1). When administered orally, RS 25259-197 was 2 and 13 fold more potent than granisetron and ondansetron, respectively.

As shown in Figure 6 and Table 2, RS 25259-197 (0.3–100  $\mu\text{g kg}^{-1}$ , i.v. and 1–100  $\mu\text{g kg}^{-1}$ , p.o.) and ondansetron (3–300  $\mu\text{g kg}^{-1}$ , i.v. and 10–100  $\mu\text{g kg}^{-1}$ , p.o.) produced dose-dependent inhibition of the emesis induced by cisplatin, actinomycin D, dacarbazine and mechlorethamine in dogs. ID<sub>50</sub> estimates are shown in Table 3. When administered orally, RS 25259-197 was about 30 fold more potent than ondansetron as an anti-emetic against each of the emetogenic agents. In studies designed to examine the duration of anti-emetic activity, RS 25259-197 (30  $\mu\text{g kg}^{-1}$ , i.v.) was effective for 7 h in inhibiting cisplatin-induced emesis, whereas the anti-emetic activity of ondansetron (300  $\mu\text{g kg}^{-1}$ , i.v.) lasted for 4 h (Figure 7).

Neither RS 25259-197 (1–1000  $\mu\text{g kg}^{-1}$ , p.o.) nor ondansetron (1000  $\mu\text{g kg}^{-1}$ , p.o.) were effective in inhibiting apomorphine induced emesis in dogs (data not shown). In contrast, haloperidol (5  $\text{mg kg}^{-1}$ , p.o.) produced significant inhibition of apomorphine induced emesis (data not shown).

### Haemodynamic effects in anaesthetized dogs

Baseline values for the measured parameters did not differ significantly between the vehicle and RS 25259-197 treatment groups. Administration of vehicle or RS 25259-197 had no significant effects on MAP, CO,  $dP/dT$  and SVR (data not shown).

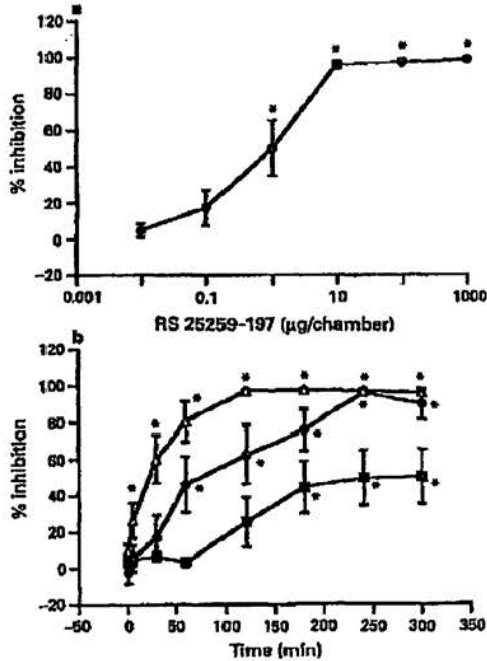


Figure 4 Inhibitory effects of RS 25259-197 (administered transdermally) on the 2-methyl 5-HT-induced bradycardia. (a) Dose-response curve to RS 25259-197. (b) Duration of the inhibitory effects of RS 25259-197, (■) 1  $\mu\text{g}$  per chamber, (●) 10  $\mu\text{g}$  per chamber, ( $\Delta$ ) 100  $\mu\text{g}$  per chamber. Each point represents the mean  $\pm$  s.e.mean,  $n = 10$ . \*Significantly different ( $P < 0.05$ ) from vehicle control.

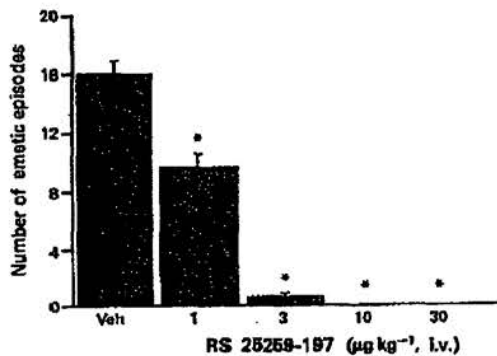


Figure 5 Inhibitory effects of intravenously administered RS 25259-197 on cisplatin-induced emesis in ferrets. Each column represents the mean  $\pm$  s.e.mean,  $n = 6$ . \*Significantly different ( $P < 0.05$ ) from vehicle control (Veh).

Table 1 ID<sub>50</sub> estimates of RS 25259-197, ondansetron and granisetron against cisplatin-induced emesis in ferrets

	ID <sub>50</sub> ( $\mu\text{g kg}^{-1}$ (95% confidence intervals))	
	<i>i.v.</i>	<i>p.o.</i>
RS 25259-197	1.1 (1.0–1.2)	3.2 (1.6–4.8)
Ondansetron	ND	43 (18.0–100)
Granisetron	ND	6.9 (1.0–40)

ND = not determined.

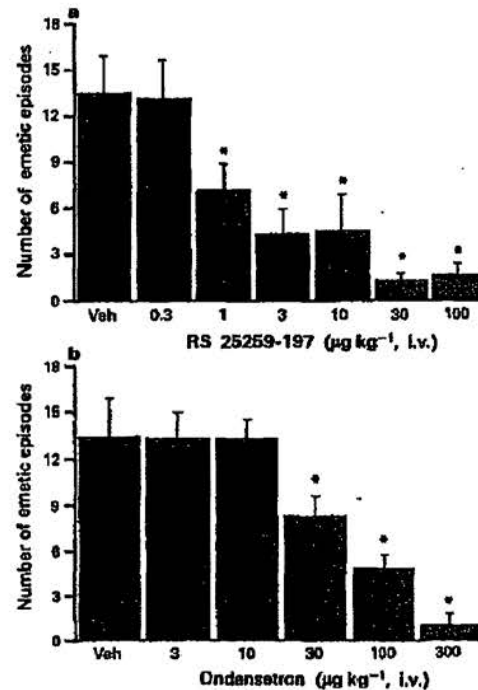


Figure 6 Inhibitory effects of intravenously administered RS 25259-197 (a) and ondansetron (b) on cisplatin-induced emesis in dogs. Each point represents the mean  $\pm$  s.e.mean,  $n = 6$ . \*Significantly different ( $P < 0.05$ ) from vehicle control (Veh).



shown). RS 25259-197 produced a significant decrease in HR at 1000 µg kg<sup>-1</sup>, i.v. but only at 45 min post-drug (Figure 8).

### Discussion

The data obtained in the present study show that RS 25259-197, a novel 5-HT<sub>3</sub> receptor antagonist, displays several pharmacodynamic and pharmacokinetic differences from the 5-HT<sub>3</sub> receptor antagonists, ondansetron and granisetron.

### Studies on the Von Bezold-Jarisch reflex

Inhibition of the 2-methyl 5-HT induced bradycardia (Von Bezold-Jarisch reflex) is a useful test for the evaluation of 5-HT<sub>3</sub> receptor antagonists *in vivo*. In this assay, RS 25259-197 administered either intravenously, intraduodenally or transdermally, was effective in inhibiting the responses to 2-methyl 5-HT. By the intravenous route, RS 25259-197 was approximately 3 and 55 fold more potent than granisetron and ondansetron, respectively. The affinity of RS 25259-197

Table 2 Anti-emetic activity of RS 25259-197 in dogs

Drug	Dose	Number of emetic episodes			
		Dacarbazine (30 mg kg <sup>-1</sup> , i.v.)	Actinomycin D (0.15 mg kg <sup>-1</sup> , i.v.)	Mechlorethamine (0.4 mg kg <sup>-1</sup> , i.v.)	Cisplatin (3 mg kg <sup>-1</sup> , i.v.)
Vehicle (ml kg <sup>-1</sup> , p.o.)	0.1	14.2 ± 1.6	14.5 ± 2.3	9.5 ± 2.9	14.2 ± 3.3
RS 25259-197 (µg kg <sup>-1</sup> , p.o.)	1	NT	NT	NT	12.8 ± 1.9
	3.2	12.8 ± 2.7	5.8 ± 0.6*	4.5 ± 1.4	16.2 ± 2.1
	10	6.8 ± 0.2*	6.7 ± 1.6*	2.8 ± 1.9*	4.0 ± 1.6*
	31.6	0.3 ± 0.2*	3.3 ± 1.9*	0.3 ± 0.3*	2.7 ± 0.7*
Ondansetron (µg kg <sup>-1</sup> , p.o.)	10	NT	NT	NT	12.7 ± 1.2
	31.6	10.8 ± 1.1	9.8 ± 3.1	5.7 ± 1.0	18.3 ± 3.4
	100	6.2 ± 2.2*	10.3 ± 1.9	8.5 ± 1.3	10.5 ± 1.3
	316	3.0 ± 1.2*	4.2 ± 0.8*	2.8 ± 1.5*	3.2 ± 0.5*
	1000	0.2 ± 0.2*	1.5 ± 0.6*	0 ± 0*	2.0 ± 0.9*

All values are expressed as mean ± s.e.mean (n = 6 per group).

NT = not tested.

\*Statistically significant from vehicle control (P < 0.05).

Table 3 ID<sub>50</sub> (µg kg<sup>-1</sup>) estimates of RS 25259-197 and ondansetron against various emetogenic agents in dogs

Drug	Route of administration	Emetogenic agent			
		Cisplatin	Dacarbazine	Actinomycin D	Mechlorethamine
RS 25259-197	i.v.	1.9 (0.28-13.0)	4.1 (2.1-6.1)	4.9 (0.45-54)	4.4 (2.1-9.1)
	p.o.	8.5 (3.6-20)	9.7 (7.3-12.0)	2.5 <sup>a</sup>	3.0 (0.85-10.0)
Ondansetron	i.v.	46.0 (17.0-74.0)	80.0 <sup>b</sup>	200.0 (47.0-900)	36.0 <sup>b</sup>
	p.o.	160.0 (48.0-270)	83.0 <sup>b</sup>	160.0 <sup>b</sup>	280.0 <sup>b</sup>

<sup>a</sup>Meaningful confidence intervals could not be obtained because of lack of dose-dependency.

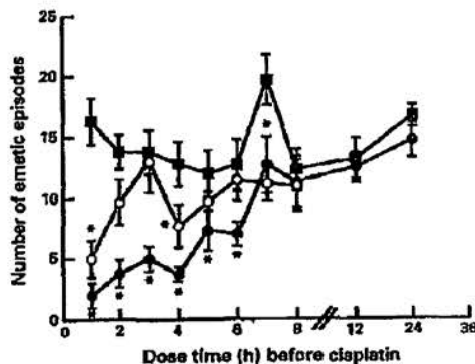


Figure 7 Duration of the inhibitory effects of vehicle (0.1 ml kg<sup>-1</sup>) (■), RS 25259-197 (30 µg kg<sup>-1</sup>, i.v.) (●) and ondansetron (300 µg kg<sup>-1</sup>, i.v.) (○) on cisplatin-induced emesis in dogs. Dogs were pre-treated with the drugs at 24, 12, 8, 7, 6, 5, 4, 3, 2 and 1 h prior to the administration of cisplatin. Each point represents the mean ± s.e.mean, n = 6. \*Significantly different (P < 0.05) from vehicle control.

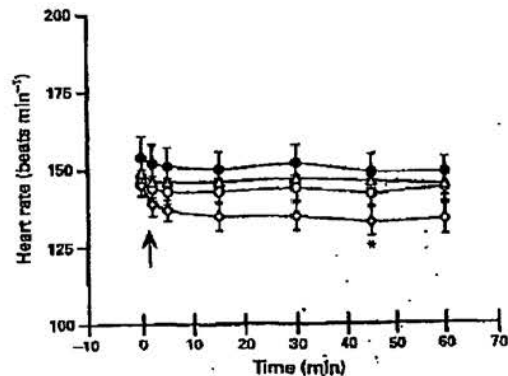


Figure 8 Effects of intravenously administered RS 25259-197 on heart rate (HR) in anaesthetized dogs. (○) Vehicle, (●) RS 25259-197 (10 µg kg<sup>-1</sup>, i.v.), (△) RS 25259-197 (10 µg kg<sup>-1</sup>, i.v.), (◇) RS 25259-197 (1000 µg kg<sup>-1</sup>, i.v.). Each point represents the mean ± s.e.mean, n = 6. \*Significantly different (P < 0.05) from vehicle control.

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With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

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Sync your system to PACER to automate legal marketing.