CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-107

PHARMACOLOGY REVIEW(S)

NOV - 4 1999

PHARMACOLOGIST'S REVIEW OF NDA 21,107
AMENDMENT DATED OCTOBER 25, 1999

Sponsor & Address: GlaxoWellcome Inc.
Research Triangle Park, North Carolina

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Date of Submission: October 25, 1999

Date of HFD-180 Receipt:

Date of Review: November 4, 1999

DRUG: Alosetron Hydrochloride/GR 68755, Tablets

CATEGORY: 5-HT_3 receptor antagonist

Submission Contents: **(1)** Correspondence and **(2)** a cardiovascular study.

Background: The ischemic colitis was found in three patients treated with alosetron. Sponsor further investigated the effects of alosetron on the mesenteric artery tone isolated from guinea pigs and dogs. A report of this study along with the results of re-evaluation of the toxicity studies in mice, rats, and dogs was submitted in this submission.

Effect of Alosetron on Mesenteric Artery Tone in Guinea Pigs and Dogs

The inferior mesenteric arteries were removed from the guinea pig and dog. The ring preparations of the arteries were mounted in organ bath and the tension was recorded. Fourteen vessels from 5 guinea pigs and 13 vessels from 2 dogs were tested. The results indicated that addition of alosetron did not alter the resting tone of these vessels at concentrations up to 10^{-6} M (Figure 1). Alosetron did not affect the response to the nerve stimulation of these vessels at concentrations up to 10^{-6} M (Table 1). Figure 1 and Table 1 are attached below.

Lack of effect of alosetron on resting tone of the dog and guinea-pig inferior mesenteric artery

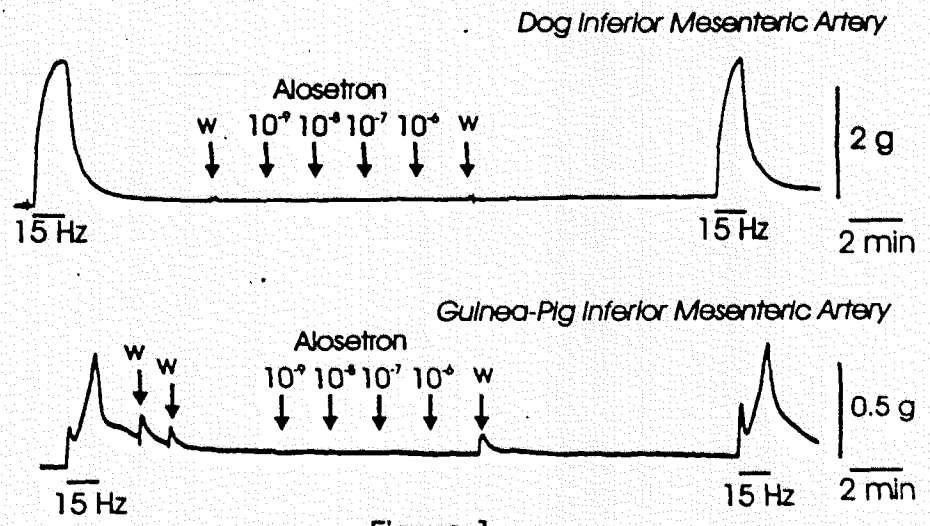


Figure 1

	Canine Mesenteric Artery		
	Date	*100%* (Control NS)	*%of control* (After cumulative treatment with Alosetron)
	10/11/99	100	98.8
	10/11/99	100	91.9
	10/11/99	100	124
	10/12/99	100	123
	10/13/99	100	96
	Mean	100.00	106.74
	SEM	0.00	6.93
	n	5	5
	Guinea-Pig Mesenteric Artery		
	Date	*100%* (Control NS)	*%of control* (After cumulative treatment with Alosetron)
	10/11/99	100	102
	10/12/99	100	93
	10/12/99	100	94
	10/12/99	100	92
	10/13/99	100	100
	Mean	100.00	96.20
	SEM	0.00	2.01
	n	5	5

Table 1

Review of Intestinal Changes in the Preclinical Studies with Alosetron

Following studies were reviewed: 1-month oral toxicity study in rats, 2-year oral carcinogenicity studies in mice and rats, 1-month, 6-month, and 12-month oral toxicity studies in dogs. The results indicated that no significant treatment related histopathological changes were found in the intestinal tract in these studies.

SUMMARY AND EVALUATION:

The ischemic colitis was found in three patients treated with alosetron. Sponsor further investigated the effects of alosetron on the mesenteric artery tone isolated from guinea pigs and dogs. The results of this study indicated that alosetron did not alter the resting tone nor the response to the nerve stimulation of the isolated inferior mesenteric arteries from the guinea pig and dog at concentrations up to 10^{-6} M.

Sponsor re-evaluated the histopathological findings of the toxicity studies in animals including the 2-year oral carcinogenicity studies in mice and rats, 1-month oral toxicity study in rats, 1-month, 6-month, and 12-month oral toxicity studies in dogs. No significant treatment related histopathological changes in the intestinal tract were found.

RECOMMENDATION: None.

/S/ 11/4/99
Ke Zhang, Ph.D.

/S/ 11/4/99

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: J. Choudary 11/4/99

KZ/hw/11/4/99

NOV - 4 1999

NDA 21,107

REVIEW #1

Sponsor & Address: GlaxoWellcome, Inc.
Research Triangle Park,
North Carolina

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Date of Submission: Initial Submission - June 29, 1999
Amendment - August 25, 1999
Amendment - September 3, 1999
Amendment - September 17, 1999

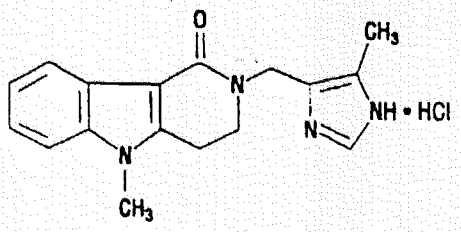
Date of HFD-180 Receipt: Initial Submission - July 1, 1999
Amendment - August 26, 1999
Amendment - September 3, 1999
Amendment - September 20, 1999

Date of Review: October 29, 1999

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUG: Alosetron Hydrochloride/Lotronex™/GR 68755, 1 mg, Tablets.

2,3,4,5-tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-1H-pyrido[4,3-b]indol-1-one, monohydrochloride



Molecular Formula: $C_{17}H_{18}N_4O \bullet HCl$ MW: 330.8

CATEGORY: Serotonin 5-HT_3 receptor antagonist.

Related INDs: IND [redacted]

Marketing Indications and Dose: Lotronex is indicated for the treatment of irritable bowel syndrome (IBS) in female patients with diarrhea predominance. The recommended adult oral dose is 1 mg twice a day for up to 12 weeks.

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study #	Lot #	Lab	Review Page #
Pharmacology				4-11
Absorption, Distribution, Metabolism And Excretion				12-29
Mice	MET862 BPW191			
Rats	MET541/579 MET703 BPW335 MET536/578 MET698 MET564/594 MET533/711 BPW198 AE-1585			
Rabbits	MET779			
Dogs	MET535/566			
Protein binding study (in vitro)	MET788			
Distribution in whole blood	MET787			
Bis-oxidized metabolite N-desmethyl metabolite	MET/594 WBP/91/002			
Interaction with cytochrome p-450	MET877 WBP/90/069 WD1998/00330/00			
GR 62202 in human plasma	WBP/91/099			
Acute Toxicity				29-30
Oral toxicity study in mice	M11909/M11910	C1281/83/1,C1017/133/1	1	
I.v. toxicity study in mice	M11903/M11904	C1281/83/1,C1017/133/1	1	
Oral toxicity study in rats	R11898/R11917	C1281/83/1,C1017/133/1	1	
I.v. toxicity study in rats	R11899/R11900	C1281/83/1,C1017/133/1	1	
Subacute/Subchronic/Chronic Toxicity				
1-month i.v. study in rats	R11655	C1281/16/1	1	30-32
34/35-day oral study in rats	R11832	DR11363	1	33-36
6-month oral study in rats	R11867	C1034/102/1	1	36-38
12-month oral study in rats	R12486	C1757/106/1	1	38-41
1-month i.v. study in dogs	D11654	C1281/58/60/2,C1281/58/1 C1281/54/1,C1297/52/2	1	42-44
35-day oral study in dogs	D11825	C1017/69/1,C1017/77/1	1	44-47
6-month oral study in dogs	D11865	C1034/104/1,C1034/98/1 C1017/189/1, C1034/102/1	1	48-51
12-month oral study in dogs	D12561	M91/025, M91/026, M91/027	2	51-54

1 = Sponsor's lab in Hertfordshire, UK
2 = Sponsor's lab in Verona, Italia

Type of Study	Study #	Lot #	Lab	Review Page #
Special Toxicity				
Skin irritation study in guinea-pigs	G12367	C1019/168/1	1	54
Eye irritation study in rabbits	L12445	C1019/168/1	1	54
Evaluation of contact sensitizing potential in guinea-pigs	G12434	C1019/168/1	1	55
Assessment of the effects on hearing in dog	D20192	C1819/87/1	1	55-56
Carcinogenicity				
13-week (drinking water) study in mice	M12419	C1026/120/1	1	56-58
Oral (drinking water) carcinogenicity study in mice	M12401	C1026/120/1	1	59-73
3-month oral (diet) study in rats	R12457	C1026/120/1	3	73-75
3-month oral (gavage) study in rats	R12569	C1026/120/1	3	75-77
Oral (diet) carcinogenicity study in rats	R12458	C1026/120/1 C1026/123/1 C1757/106/1	3	78-86
Reproductive Toxicity				
Segment I oral fertility and reproductive toxicity study in rats	R12036	C1028/98/1	1	86-90
Segment II oral teratology study in rats	R12151	C1034/96/1 C1017/133/1	1	91-94
Segment II oral teratology study in rabbits	L11998	C1017/133/1 C1028/98/1	1	94-97
Segment III oral pre- and postnatal reproductive toxicity study in rats	R20827	C1028/280/1	1	97-100
Mutagenicity				
Ames test	V12622	C1026/133/1	1	101-102
Ames test	V12468	C1017/321/1	1	106-108
In vitro chromosome aberration tests	V1192	C1017/133/1	1	102-103
Mouse lymphoma cell (L5178Y/TK$^{+/-}$) Forward Mutation assay at tk locus	V13036	C1026/133/1	1	104-105
Rat micronucleus test	115E/92	C1017/133/1	1	105-106
In Vivo unscheduled DNA synthesis in rat hepatocytes	R12689	R12689	1	103-104

1 = Sponsor's lab in Hertfordshire, UK
3 = [redacted]

Following studies were previously submitted to IND [redacted] **(1)** pharmacology, **(2)** ADME, **(3)** acute toxicity studies in mice and rats, **(4)** 34/35-day oral toxicity studies in rats and dogs, **(5)** 6-month oral toxicity studies in rats and dogs, **(6)** 12-month oral toxicity studies in rats and dogs, **(7)** 13-week oral (in drinking water) oral dose ranging study in mice, **(8)** 3-month oral (in diet) dose ranging study in rats, **(9)** 3-month oral (gavage) dose ranging study in rats, **(10)** 94/95-week oral (in drinking water) carcinogenicity study in B6C3F1 mice, **(11)** 104-week oral (in diet) carcinogenicity study in Wistar rats, **(12)** Segment I oral fertility and reproductive toxicity study in rats, **(13)** Segment II oral teratology studies in rats and rabbits, **(14)** Ames tests, **(15)** in vitro chromosome aberration tests in human lymphocytes, **(16)** mouse lymphoma cell (L5178Y/$TK^{+/-}$) forward mutation assays at tk locus, **(17)** unscheduled DNA synthesis in rat hepatocytes, and **(18)** rat micronucleus test. These studies were previously reviewed on April 16, 1996. The review has been reproduced in the appropriate portion of the present NDA review. In addition, new studies submitted in the present NDA have been reviewed below.

PHARMACOLOGY:

Alosetron hydrochloride (GR 68755) is indicated for the treatment of irritable bowel syndrome (IBS) with diarrhea predominance. IBS is a functional disorder of the gastrointestinal (GI) tract characterized by abdominal pain, discomfort, and changes of bowel function (constipation or diarrhea). The 5-HT_3 receptor is thought to play an important role in the IBS. Activation of 5-HT_3 receptor located in GI tract stimulates release of 5-HT which subsequently produces pain and increases motor activity of the gut. Therefore, by blocking 5-HT_3 receptor, Alosetron, a selective 5-HT_3 receptor antagonist, would be therapeutically useful for treatment of the IBS. The pharmacological activities of GR 68755 were demonstrated in a number of *in vitro* and *in vivo* animal models.

Primary Activity

1. In Vitro Studies:

The *in vitro* studies have demonstrated that alosetron is a selective 5-HT_3 receptor antagonist with pKi of 9.4 in rat entorhinal cortex, 9.8 in rat vagus nerve, and 7.7-7.8 in guinea pig ileum. It was also demonstrated that alosetron has a high

affinity for 5-HT_3 receptor with IC_{50} of 1.1 x 10^{-9} M and low to no affinity for other types of receptors. These results were summarized in Table 5.2 on page 120 in volume 1.1 and this table is attached below.

Table 5.2. Receptor Selectivity of Alosetron in Isolated Tissue Preparations

Receptor Type	Isolated Tissue (Brain Region or Organ)	Ligand	IC_{50} (M)
Adenosine A_1	Cortex	^{3}H-DPCPX	$>10^{-5}$
Adenosine A_2	Striatum	^{3}H-CGS-21680	$>10^{-5}$
Adrenergic α_1	Brain minus cerebellum	^{3}H-Prazosin	$>10^{-5}$
Adrenergic α_2	Cortex	^{3}H-Idazoxan	1.3×10^{-6}
Adrenergic β_1	Cortex	^{3}H-CGP-26505	$>10^{-5}$
Adrenergic β_2	Lung	^{3}H-ICYP	$>10^{-5}$
Dopamine D_1	Striatum	^{3}H-SCH-23390	$>10^{-5}$
Dopamine D_2	Striatum	^{3}H-YM-091512	$>10^{-5}$
$GABA_A$	Brain	^{3}H-Muscimol	$>10^{-6}$
$GABA_B$	Cerebellum	^{3}H-Baclofen	$>10^{-5}$
5-HT_1	Cortex	^{3}H-5-HT	$>10^{-5}$
5-HT_{2C} (previously called 5-HT_{1C})	Choroid plexus	^{3}H-Mesulergine	5.3×10^{-6}
5-HT_3	Cortex	^{3}H-GR65630	1.1×10^{-9}
Muscarinic M_1	Cortex	^{3}H-Pirenzepine	$>10^{-5}$
Muscarinic M_2	Heart	^{3}H-NMS	$>10^{-5}$
Muscarinic M_3	Pancreas	^{3}H-DAMP	$>10^{-5}$
Nicotinic	Cortex	^{3}H-NMCI	$>10^{-5}$
Histamine H_1	Cortex	^{3}H-Mepyramine	$>10^{-5}$
Histamine H_3	Cortex	^{3}H-N-α-methyl-histamine	1.4×10^{-4}
NMDA	Brain minus cerebellum	^{3}H-CGS-19755	$>10^{-6}$
Opiate μ	Brain minus cerebellum	^{3}H-CTOP	$>10^{-5}$
Opiate κ	Cortex	^{3}H-U-69593	$>10^{-5}$
Bradykinin	Ileum	^{3}H- Bradykinin	$>10^{-5}$
CCK_A	Pancreas	^{3}H-L-364,718	$>10^{-5}$
CCK_B	Brain minus cerebellum	^{3}H-L-365,260	$>10^{-5}$
Neurokinin 1	Brain minus cerebellum	^{3}H-Substance P	$>10^{-5}$
Neurokinin 2	Colon	^{3}H-Neurokinin A	$>10^{-5}$
Neurokinin 3	Cortex	^{3}H-Senktide	$>10^{-5}$
CGRP	Hypothalamus	^{125}1-CGRP	$>10^{-5}$
Neuropeptide Y	Hippocampus	^{125}1-Neuropeptide Y	$>10^{-5}$
Bombesin	Brain minus cerebellum	^{125}1-Bombesin	$>10^{-5}$
Endothelin	Heart	^{125}1-Endothelin	$>10^{-5}$
Somatostatin	Cortex	^{125}1-Somatostatin	$>10^{-5}$
Vasopressin 1	Liver	^{3}H-Vasopressin Arg	$>10^{-5}$
Vasopressin 2	Kidney	^{3}H-Vasopressin Arg	$>10^{-5}$
VIP	Cortex	^{125}1-VIP	$>10^{-6}$
Galanin	Brain minus cerebellum	^{125}1-Galanin	$>10^{-5}$
Calcium channel (Type T & L)	Cortex	^{3}H-Nitrendipine	$>10^{-5}$
Calcium channel (Type N)	Anterior brain	^{125}1-ω Conotoxin	$>10^{-5}$
Benzodiazepine	Cortex	^{3}H-Flunitrazepam	$>10^{-5}$
Glycine (Strychnine insensitive)	Cortex	^{3}H-5,7,DCKA	$>10^{-5}$

An *in vitro* study using cloned human 5-HT_3 receptors expressed in Chinese Hamster Ovary cells indicated that one of the major metabolites, GR 96105 (O-glucuronidated 6-hydroxy alosetron), had a pKi of 9.55 ± 0.12 similar to that of alosetron. However, other metabolites including GR 169307 (pKi=8.39±0.02), GR 163860 (pKi=7.43±0.03), GR 168355 (pKi=6.95±0.09), GR 153732 (pKi<6) had an affinity much lower than that of alosetron.

2. In Vivo Studies:

One of the major symptoms of IBS is pain or discomfort. To produce this symptom in animal model, rectal distension was induced by a step-wise inflation of a latex balloon inserted into the rectum of the anesthetized rats. Rectal distension subsequently causes a reduction of blood pressure. This hypotensive response is considered to be a result of pain. Alosetron inhibited the hypotensive response in a dose dependent manner with ID_{50} of 3 µg/kg (i.v. dose).

Alosetron inhibited small intestinal propulsion in rats. Egg albumin can cause wide-spread perturbation of small intestine motility and an increase in small intestinal propulsion. Intraperitoneal (i.p.) administration of alosetron at 0.3 mg/kg completely inhibited the egg albumin-induced increase in small intestinal propulsion (96%±4%).

Response to visceral (colorectal distension) stimulation were recorded in the convergent dorsal horn neurons in anesthetized rats. The response was then sensitized by rectal administration of the bile salt (deoxycholic acid) to ~174% and 202% at 40 and 90 minutes after the treatment. Intravenous administration of alosetron at 0.1 mg/kg 15 minutes prior to the bile salt significantly inhibited the sensitization of response to the bile (104% and 126%). However, alosetron did not affect the sensitization of the response to cutaneous stimulation, suggesting that alosetron selectively inhibits the sensitized response to visceral input. The repeated noxious colorectal distension induces Fos-like protein expression in the spinal cord of the anesthetized rat. The numbers of Fos-like immuno-reactive nuclei were significantly increased from 49 ± 25 to 1206 ± 86 following repeated noxious colorectal distension. Pretreatment with i.v. dose of alosetron at 0.1 mg/kg markedly reduced the number of the Fos-like immunoreactive nuclei to 591 ± 141. The above results suggest that 5-HT_3 receptor may play a role in the modulation of visceral nociceptive neurotransmission.

Bezold-Jarisch reflex is characterized by decrease in heart rate and blood pressure following administration of certain chemicals or 5-HT agonist. This reflex includes the receptors on the sensory afferents in the left ventricle of the heart, the medulla (central component), and efferent limb (vagus nerve). It is believed that the receptor involved in the activation of this reflex is 5-HT_3 receptor. Intravenous administration of 5-HT_3 receptor agonist, 2-Methyl-5-HT, reduced the heart rate in a dose dependent manner in rats and cats. Intravenous administration of alosetron inhibited the 2-Methyl-5-HT-induced Bezold-Jarisch reflex at doses of 0.1 µg/kg or higher in cats. Intraduodenal administration of alosetron also blocked the 2-Methyl-5-HT-induced Bezold-Jarisch reflex at doses of 3 µg/kg or higher in cats. In rats, intravenous administration of alosetron caused a significant rightward shift of the dose-response curve (bradycardia response induced by 2-Methyl-5-HT) at 1 and 3 µg/kg.

Secondary Activity

1. Central Nervous System (CNS): In mice, GR 68755 had no observed effects at i.v. dose of 3 mg/kg but induced convulsion and death (all 5 treated mice) at i.v. dose of 10 mg/kg. In rats, GR 68755 had no effects on behavior or other observed effects at oral doses of 5 and 10 mg/kg and i.v. doses of 2.5 and 5 mg/kg but decreased the spontaneous activity at i.v. dose of 10 mg/kg. In dogs, GR 68755 had no observed effects at oral doses of 1.25-5 mg/kg and i.v. doses of 0.625-2.5 mg/kg.

GR 68755 had no effects on pentobarbitone-induced sleeping time at i.v. dose of 3 mg/kg in mice. Intragastrical administration of GR 68755 at 10 mg/kg had no significant effects on CNS function.

GR 68755 inhibited the cisplatin- and cyclophosphamide-induced emesis in ferrets at i.p. doses of 0.1 mg/kg or higher. At i.p. dose of 1 mg/kg it completely inhibited the cisplatin- and cyclophosphamide-induced emesis.

2. Cardiovascular System: GR 68755 had no effects on the contraction of the isolated rat aorta at concentrations up to 100 µM but decreased the noradrenaline-induced contraction by ~35% at 100 µM. GR 68755 had no effects on the contractile force and frequency of the isolated guinea pig atria at concentration of 1 µM. However, it increased the contractile force by 21-25% at 10 and 100 µM and decreased the contractile

frequency by 29% at 100 μM in this preparation. GR 68755 decreased the isoproterenol induced contraction at 100 μM (no effects on 1 and 10 μM) but did not affect the contractile frequency in the presence of isoproterenol.

Intravenous administration of GR 68755 at 0.1, 0.3, and 1 mg/kg had no clear effects on the heart rate, arterial blood pressure, tracheal inflation pressure, and ECG as compared pre-test values in an anesthetized cat. The i.v. dose (0.1 μg/kg) that blocked Bezold-Jarisch reflex is much lower that the high dose (1 mg/kg) tested in this cardiovascular study in the cat. GR 68755 was given intravenously to one monkey at 0.01, 0.03, 0.1, 0.3, and 1 mg/kg and a second monkey received control. Seven days later, the control monkey was given the treatment and the treated monkey received control. There were no clear treatment related effects on the heart rate and arterial blood pressure at doses up to 1 mg/kg. GR 68755 had no effects on PR and QT intervals at doses up to 0.3 mg/kg but prolonged QT interval (~12%) at 1 mg/ kg in one monkey. This monkey also had a single transient dysrhythmia. Intravenous administration of GR 68755 did not have any effects on heart rate, arterial blood pressure, ECG, and tracheal inflation pressure at cumulative doses up to 1 mg/ kg in anesthetized guinea pigs. The cardiovascular effects of GR 68755 were further investigated in dogs. GR 68755 had no marked effects on the action potential of the isolated canine Purkinje fibers at concentrations up to 100 ng/ml. The results were presented in Table 2 on page 92 in Amendment dated September 17, 1999 and this table is attached below.

Table 2
Effect of GR68755C or Vehicle on Action Potential Parameters (1 Hz)

Action Potential Parameter	GR68755C Baseline	GR68755C 1 ng/mL	GR68755C 10 ng/mL	GR68755C 100 ng/mL
DMP (mV)	-87.4 ± 2.2	-84.7 ± 2.8	-89.2 ± 3.0	-84.7 ± 1.2
UA (mV)	122.9 ± 1.0	121.3 ± 1.4	122.8 ± 2.0	122.0 ± 1.2
MRD (V/s)	738.1 ± 43.0	712.0 ± 31.7	733.4 ± 20.6	696.4 ± 17.4
APD_{30} (ms)	68.7 ± 17.3	65.5 ± 18.7	65.8 ± 17.7	65.7 ± 19.2
APD_{60} (ms)	254.8 ± 25.3	248.1 ± 33.2	262.2 ± 28.9	261.8 ± 30.3
APD_{90} (ms)	302.8 ± 24.2	302.9 ± 29.0	310.2 ± 29.6	313.4 ± 28.7

Action Potential Parameter	Saline Baseline	Saline 0.0009%	Saline 0.0009%	Saline 0.0009%
DMP (mV)	-87.8 ± 1.8	-87.0 ± 2.3	-87.8 ± 3.8	-86.0 ± 2.7
UA (mV)	120.2 ± 0.8	119.5 ± 0.9	119.9 ± 1.9	119.1 ± 1.8
MRD (V/s)	536.5 ± 70.5	549.2 ± 81.1	621.5 ± 44.5	626.8 ± 39.6
APD_{30} (ms)	47.4 ± 13.7	44.8 ± 10.7	52.9 ± 10.5	47.7 ± 9.2
APD_{60} (ms)	207.6 ± 11.1	216.4 ± 20.2	219.0 ± 19.0	217.1 ± 19.2
APD_{90} (ms)	265.0 ± 15.4	270.9 ± 20.5	271.8 ± 18.4	268.9 ± 19.9

Data are given as mean ± s.e. mean for n = 4 preparations per group, except for the MRD where n = 3 in the vehicle matched group for 10 and 100 ngGR68755C/mL. For abbreviations see text.

In contrast, dl-sotalol (30 μM) significantly prolonged action potential duration (~44-48%). GR 68755 had no significant effects on the delayed rectifying potassium currents (I_{kr}) and I_{ks} at concentrations ranging from 10^{-10} to 10^{-6} M in the isolated guinea pig cardiac myocytes using whole-cell voltage clamp technique. Intravenous administration of GR 68755 did not have any effects on heart rate, arterial blood pressure, and ECG at cumulative doses up to 1 mg/kg in dogs.

3. Learning/Memory Studies: GR 68755 is abbreviated GR.

GR showed activity in the following paradigms:

1) Mouse Habituation Test

GR active at 0.01 ng/kg i.p. b.i.d. (only dose used); this dose also attenuated the disruption of habituation induced by scopolamine.

2) Spontaneous Alternation in Rats:

GR at 1 ng-100 ug/kg i.p. b.i.d. partially reversed scopolamine-induced performance deficit; all GR doses tested in this range produced quantitatively similar effect.

3) Morris Water Maze Test in Rats:

GR at 1 ng-10 ug/kg i.p. antagonized scopolamine-induced performance deficit; all GR doses tested in this range produced quantitatively similar effect.

4) Wisconsin General Test Apparatus (Marmosets):

GR at 0.01 and 10 ng/kg s.c. b.i.d. reduced number of trials needed to reach criterion; however a higher dose (1 ug/kg) had no effect.

4. Anti-Anxiety Studies:

1) Social Interaction in Rats:

GR increased social interaction (without effect on locomotor activity); the effective dosage range was said to be 0.1-5000 ug/kg p.o. in one place and 0.01-10,000 ng/kg p.o. in another. In a separate study, GR was active at 0.1 and 10 (but not 0.001) ug/kg i.p. The magnitude of the effect was not dose-related. The effect of a single dose of 1 ug/kg p.o. lasted for 6 hours. Activity did not decrease with subacute treatment. Diazepam was effective in this paradigm; however, unlike GS, it produced a rebound decrease in social interaction after subacute treatment.

2) Light Aversion in Mice:

GR decreased light aversion at 0.1 ng/kg-10 mg/kg i.p. (No effect at 0.01 ng/kg; all doses tested in the active range produced quantitatively similar effects; 50% died at 10 mg/kg). Diazepam was active at 0.125 but not 0.063 mg/kg i.p. GR had a duration of action of 12-24 hours after a single dose of 1 ug/kg i.p. or p.o. Tolerance did not develop with subactue treatment with either GR or diazepam; a rebound effect (i.e. increased light aversion) was seen after cessation of treatment with diazepam only.

3) Elevated Plus Maze in Rats:

GR at 1 and 10 ug/kg i.p. increased the amount of time spent in the open arm section of the maze, which was interpreted as an anxiolytic effect.

5. Antipsychotic Activity:

A) GR68755 (hereafter abbreviated as GR) blocked the increased locomotor activity caused by amphetamine injection into the nucleus accumbens of rats. GR and ondansetron had similar effects at doses of 100 and 1 ng, respectively (injected into nucleus accumbens). GR was inactive at 1 and 10 ng.

B) GR partially blocked the increased locomotor activity caused by dopamine infused into the nucleus accumbens of rats. GR was active at doses of 1 ng/kg - 100 ug/kg given i.p. (inactive at 0.01 ng/kg); all doses tested within this active range produced a similar magnitude of effect.

C) Unilateral intrastriatal injection of GR (1 ug) in rats did not induce postural assymetries or circling behavior, either when given alone or when animals were subsequently challenged with systemic apomorphine. Fluphenazine, given at a higher dose (5 ug intra-striatal), did not have any effect when given alone but did cause assymetry in response to systemic apomorphine challenge.

D) GR at 0.5 - 5 mg/kg i.p. did not cause catalepsy in rats; haloperidol did so at 2 mg/kg i.p.

E) GR at 0.5 - 5 mg/kg i.p. did not antagonize apomorphine-induced stereotypy in rats; fluphenazine antagonized this at 0.1 mg/kg i.p.

F) GR partially antagonized the increased locomotor activity caused by the neurokinin receptor agonist DiMeC7 injected into the ventral tegmental area of rats. (It was stated that this procedure produces an increase in locomotor activity which is considered to be due to a selective increase in dopamine release in the mesolimbic and mesocortical dopaminergic systems, and can be abolished by haloperidol). GR was active at 1 - 1000 (but not 0.1) ug/kg s.c.; it was stated that these doses produced no effects on basal locomotor activity. (All doses within the active range produced a similar magnitude of effect). (Ondansetron had similar potency but lost activity at the highest dose). (This study was previously submitted to IND [redacted]

6. Tolerance and Withdrawal Studies: Alosetron (1 μg/kg, i.p.) reversed the inhibitory behavioral changes induced by cessation of repeated treatment with diazepam, nicotine, alcohol or cocaine in mice. The behavioral changes included decreased activity and duration in the light section of a chamber. The preventive effects of alosetron (1 μg/kg, i.p.) on the behavioral changes induced by cessation of repeated treatment with diazepam were also observed in rats. Alosetron at i.p. dose of 100 μg/kg b.i.d. inhibited the increased locomotor activity by cessation of repeated treatment with haloperidol (i.p., 0.3 mg/kg, b.i.d.) and dopamine (infusion into the nucleus accumbens) in rats. Alosetron did not result in any withdrawal behavior following an i.p. dose at 0.1 μg/kg, b.i.d. for 7 days or 1 μg/kg for 14 days in mice or following an oral dose at 0.02 mg/kg, b.i.d. for 21 days in rats.

No significant effects on the gastrointestinal, renal, and pulmonary systems were observed following intragastrical dose of 10 mg/kg.