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RS-100235: A HIGH AFFINITY 5-HT$_4$ RECEPTOR ANTAGONIST

R. D. Clark,*[a] A. Jahangir,[a] L. A. Flippin,[a] J. A. Langston,[a] E. Leung,[b]

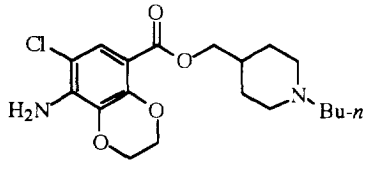
D. W. Bonhaus,[b] E. H. F. Wong,[b] L. G. Johnson [b] and R. M. Eglen [b]

[a]*Institutes of Organic Chemistry and* [b]*Pharmacology, Syntex Research, Palo Alto, CA 94304*

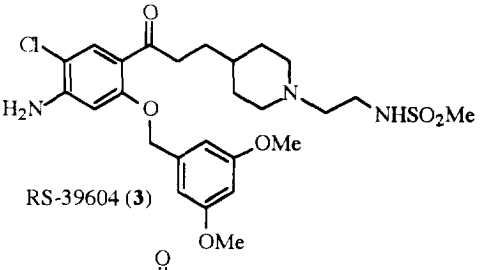
Abstract: The 1,4-benzodioxanyl ketone **14** (RS-100235) was found to be high affinity 5-HT$_4$ antagonist with potent *in vivo* activity and a sustained duration of action in the anesthetized pig.

The pharmacological relevance of 5-HT$_4$ receptors in various disease states is currently being elucidated through the agency of selective agonists and antagonists of this receptor.[1] Data from animal models suggest that activation of brain 5-HT$_4$ receptors enhances cognitive function, and in the periphery, 5-HT$_4$ receptors appear to play a modulatory role in the gastrointestinal tract: e.g., agonists have gastrointestinal pro-kinetic activity.[1] On the basis of several observations, including the ability of 5-HT$_4$ receptor antagonists to reverse 5-HT induced diarrhea in animal models, it has been proposed that 5-HT$_4$ receptor antagonists may have utility in the treatment of irritable bowel syndrome.[2]

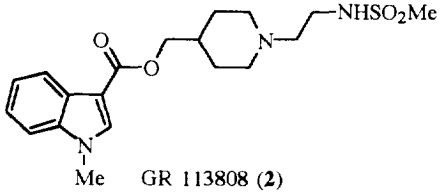
Potent and selective 5-HT$_4$ antagonists have recently been reported,[1] including the esters SB 204070 (**1**)[3] and GR 113808 (**2**)[4], and the ketone RS-39604 (**3**)[5]. Although the latter antagonist has lower affinity than **1** or **2**, the enhanced stability of this compound relative to the ester antagonists affords an advantage for *in vivo* studies. In this paper we report that ketones related to SB 204070 are also potent 5-HT$_4$ receptor antagonists and that, in particular, 1-(8-amino-7-chloro-1,4-benzodioxan-5-yl)-3-[[3-(3,4-dimethoxyphenyl)prop-1-yl] piperidin-4-yl]propan-1-one (RS-100235, **14**), is a selective 5-HT$_4$ receptor antagonist with potent *in vivo* activity.



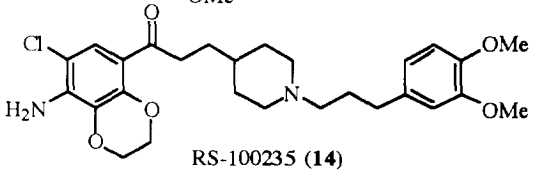
SB 204070 (**1**)



RS-39604 (**3**)



GR 113808 (**2**)

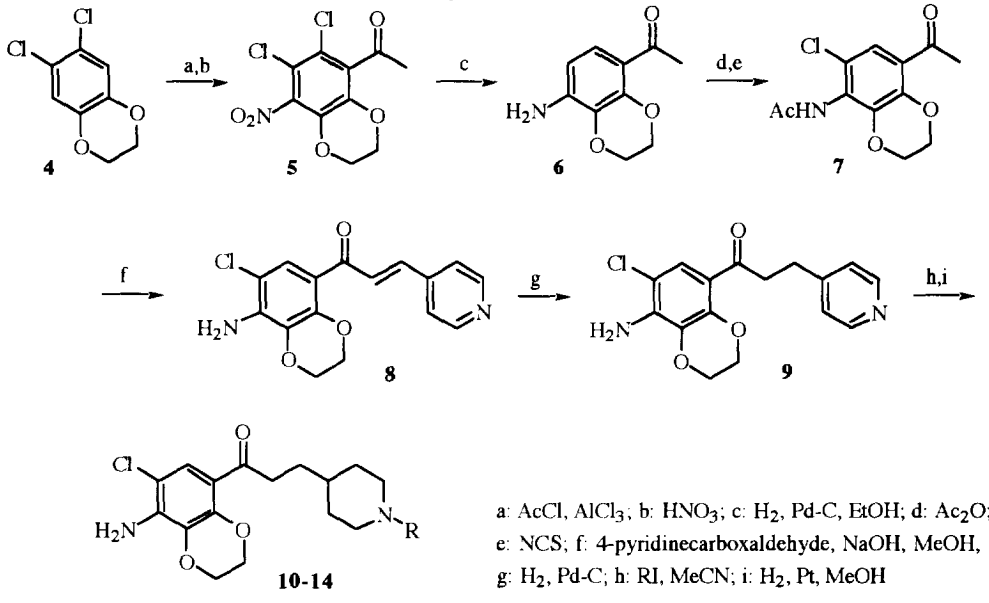


RS-100235 (**14**)

*Address correspondence to author at Roche Bioscience, 3401 Hillview Avenue, Palo Alto, CA 94304

The *N*-substituted 1-(1,4-benzodioxan-5-yl)-3-(piperidin-4-yl)-1-propanones **10-14** were prepared from 6,7-dichloro-1,4-benzodioxan (**4**)[6] by the multi-step sequence shown in Scheme I. Sequential acylation and nitration of **4** afforded the highly substituted benzodioxan **5**. Dechlorination of **5** with concomitant reduction of the nitro group was effected by catalytic hydrogenation to give aniline **6**. Acetylation of **6** followed by chlorination gave **7** which was condensed with 4-pyridinecarboxaldehyde under basic conditions to afford intermediate **8**. Partial hydrogenation of **8** furnished pyridine **9** which was quaternized and reduced by catalytic hydrogenation to give target compounds **10-14**. For reasons that are not readily apparent, catalytic hydrogenation of quaternary derivatives of **8** furnished mixtures of products that underwent decompostion upon attempted isolation. Hence it was necessary to introduce the additional step of conversion of **8** to **9**.

Scheme I



a: AcCl, $AlCl_3$; b: HNO_3; c: H_2, Pd-C, EtOH; d: Ac_2O; e: NCS; f: 4-pyridinecarboxaldehyde, NaOH, MeOH, g: H_2, Pd-C; h: RI, MeCN; i: H_2, Pt, MeOH

Compounds **10-14** and standards **1-3** were tested for functional 5-HT_4 receptor antagonism in the rat carbachol contracted esophagus[7] (Table 1). A slight reduction in antagonist activity was observed for the *n*-butyl derivative **10** relative to the directly related ester SB 204070 (**1**). The (methanesulfonamido)ethyl derivative **11** was equipotent to the butyl analog **10**, whereas the phenylpropyl analog **12** was slightly less active. However, introduction of one and two methoxy groups in the aromatic ring of **12** led to a progressive increase in antagonist activity (compounds **13** and **14**). The high pK_b value of 11.2 for **14** should be regarded as an estimate on the basis that this compound was an unsurmountable antagonist in this preparation, i.e., the maximum response to 5-HT was depressed in the presence of the antagonist. Similarly, we observed that SB 204070 was also an unsurmountable antagonist in the rat esophagus, in accord with previously reported unsurmountable antagonism in the guinea pig distal colon.[3,8]

TABLE 1. 5-HT_4 Receptor Antagonist Activity for Compounds **10-14**

compd.	R	Antagonist pK_b[a]
10	Bu-*n*	9.9 ± 0.1
11	$CH_2CH_2NHSO_2Me$	9.9 ± 0.1
12	$CH_2CH_2CH_2Ph$	9.6 ± 0.3
13	$CH_2CH_2CH_2C_6H_4OMe$-4	10.6 ± 0.1
14	$CH_2CH_2CH_2C_6H_3(OMe)_2$-3,4	11.2 ± 0.1
SB 204070 (**1**)		10.3 ± 0.1
GR 113808 (**2**)		9.0 ± 0.1
RS-39604 (**3**)		9.1 ± 0.1

[a]Antagonism of 5-HT mediated relaxation of rat carbachol contracted esophageal muscularis mucosae (± SEM).

Antagonism of 5-HT induced tachycardia in the anesthetized pig[9,10] was used to determine the 5-HT_4 antagonist activity of lead compound **14** *in vivo* (Table 2). Intravenous (iv) and intraduodenal (id) routes of administration were used to assess potency and to obtain an indication of absorption,[11] and duration of action studies were (separately) carried out.[12] As predicted by its *in vitro* activity (Table 1), **14** was a potent 5-HT_4 antagonist in the pig with an ID_{50} value of 0.55 μg/kg iv. On the basis of id vs. iv potency, **14** appeared to have good absorption from the gut. The duration of action of **14** was >6 hours (essentially the duration of the study), which was significantly longer than the duration of the ester SB 204070. The antagonist activity of ketone **14** was significantly greater than that of the related ketone RS-39604 by both routes of administration.

TABLE 2. *In Vivo* 5-HT_4 Receptor Antagonist Activity[a]

compd.	Dose range[b] (μg/kg)	Route	ID_{50}[c] (μg/kg)	$T_{1/2}$ (min)
RS-100235 (**14**)	0.03 - 10	iv	0.55 (0.48-0.62)	>360[d]
	0.1 - 30	id	1.52 (1.24-1.87)	–
SB 204070 (**1**)	0.1 - 300	iv	8.0 (5.38-11.9)	<150[e]
	3 - 3000	id	no effect	–
RS-39604 (**3**)	1 -300	iv	4.68 (3.63-6.03)	315[e]
	30 - 3000	id	245 (190-323)	–

[a]Antagonism of 5-HT induced tachycardia in anesthetized, vagotomized Yucatan micropigs. [b]n = 4 for all determinations. [c]Mean (95% confidence interval) [d]Determined at an iv dose of 3 μg/kg. [e]Determined at an iv dose of 30 μg/kg.

A receptor binding profile of **14** indicated that this compound had at least a 1000-fold selectivity for the 5-HT_4 receptor (labeled with [^{3}H]GR 113808[13] in guinea-pig striata) over other serotonergic, adrenergic, dopaminergic, muscarinc, and angiotensin receptors (Table 3). Thus this compound would appear to be a useful agent for determining the potential therapeutic utility of a 5-HT_4 antagonist.

TABLE 3. Receptor Binding Profile of **14**

Receptor[a]	Binding pK_i[b]	Receptor[a]	Binding pK_i[b]
5-HT_{1A}	6.0 ± 0.01	Adrenergic α_{2B}	5.6 ± 0.03
5-HT_{2A}	6.3 ± 0.02	Dopamine D_1	<5
5-HT_{2C}	5.8 ± 0.09	Dopamine D_2	6.5 ± 0.35
5-HT_3	6.3 ± 0.09	Muscarinic M_1	5.8 ± 0.04
5-HT_4	10.0 ± 0.17	Muscarinic M_2	5.9 ± 0.06
Adrenergic α_{1B}	6.3 ± 0.04	Muscarinic M_3	5.7 ± 0.09
Adrenergic α_{1C}	6.5 ± 0.10	Angiotensin AT_1	<5
Adrenergic α_{2A}	7.0 ± 0.16	Angiotensin AT_2	<5

[a]Radioligand binding assays were performed as previously described.[14]
[b]mean pK_i ± SEM (n = 3)

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12. In duration of action studies, a single iv or id dose of test compound was administered. An ED_{50} dose of 5-HT was administered prior to test compound administration and subsequently at 15 min intervals. Inhibition of heart rate responses was then measured after each dose of 5-HT and the $T_{1/2}$ was determined. The $T_{1/2}$ was defined as the time for the 5-HT response to attain 50% of the original response. The study was performed for 6 h; the 5-HT response was near-maximally inhibited by **14** for the duration of the study.
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