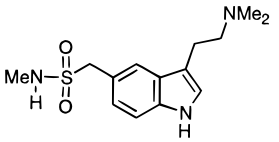
Isochroman-6-carboxamides as Highly Selective 5-HT$_{1D}$ Agonists: Potential New Treatment for Migraine without Cardiovascular Side Effects

Michael D. Ennis,*[,†] Nabil B. Ghazal,[†] Robert L. Hoffman,[†] Martin W. Smith,[‡] Siusaidh K. Schlachter,[‡] Charles F. Lawson,[‡] Wha Bin Im,[§] Jeffrey F. Pregenzer,[§] Kjell A. Svensson,[§] Richard A. Lewis,[§] Edward D. Hall,[§] Dorothy M. Sutter,[§] Lorri T. Harris,[||] and Robert B. McCall[||]

Pharmacia & Upjohn, Inc., Kalamazoo, Michigan 49001

Received March 4, 1998

Migraine headache is a debilitating, chronic disease affecting the lives of millions of people worldwide.[1,2] Migraine is most prevalent in working age populations (35−40 years old), and its economic impact in the United States alone has been estimated in the billions of dollars.[3,4] Currently, the primary treatment for migraine is Sumatriptan (**1**), a nonselective serotonergic agent which binds with high affinity to both the 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors (K_i of 3.4 and 7.7 nM respectively).[5,6] Recent theories regarding the etiology of migraine suggest that selective agonists at only the 5-HT$_{1D}$ site could be effective at ameliorating migraine symptoms.[7,8] Indeed, it has been proposed that the 5-HT$_{1B}$ properties of Sumatriptan are responsible for its vasoconstrictive properties and associated cardiovascular side effects.[9,10] Nearly all of the compounds currently in development for treating migraine are tryptamine derivatives which possess little or no selectivity between the 5-HT$_{1D}$ and 5-HT$_{1B}$ sites.[11] In this Communication, we describe the first class of highly selective, non-indole 5-HT$_{1D}$ agonists. One of these compounds, (*S*)-(−)-**10**, is significantly more potent than Sumatriptan in animal models of migraine yet devoid of vasoconstrictive properties.



1, sumatriptan

While exploring the structure−affinity relationships in a series of isochroman−arylpiperazines targeting the dopamine D4 receptor,[12] we prepared the 6-carboxamide analogue **7** shown in Scheme 1. Reaction of 4-bromophenethyl alcohol **2** with ethyl 3,3-diethoxypropionate in the presence of $TiCl_4$ provided the isochroman **3**. Hydrolysis of **3** to the carboxylic acid **4** and subsequent DECP coupling with 4-methoxyphenylpiperazine provided the amide **5**, which was reduced with borane to give **6**. Metal−halogen exchange of **6** with *t*-BuLi and subsequent quenching of the resulting aryl anion with *N*-trimethylsilylisocyanate afforded the 6-carboxamide **7**. When evaluated in a battery of CNS receptor binding assays (Table 1), **7** was found to possess poor affinity at the D_4 site (K_i 1054 nM), in sharp contrast to the unsubstituted isochroman analogue **8** (K_i 2.4 nM), a selective dopamine D_4 antagonist.[12] However, amide **7** displayed unexpected affinity for the 5-HT$_{1D}$ receptor (K_i 8.6 nM). Furthermore, **7** showed little affinity for the 5-HT$_{1B}$ site, exhibiting at least a 100-fold preference for 5-HT$_{1D}$. Thus, it appeared that isochroman-6-carboxamides such as **7** may represent a new, nontryptamine-based family of selective 5-HT$_{1D}$ agents.

Table 1. CNS Binding Affinities (K_i, nM) for Sumatriptan (**1**), **8**, and Isochroman-6-carboxamides[a]

compd	5-HT$_{1D}$	5-HT$_{1B}$	5-HT$_{1A}$	5-HT$_{2A}$	D_2	D_4
1	2.0	6.4	341	376	>218	I[b]
7	8.6	5862	2463	140	348	1054
8	6.7	7242	899	76	1431	2.4
(*S*)-(−)-**7**	3.8	3023	967	248	325	I
(*R*)-(+)-**7**	238	26021	>3356	220	386	I
(*S*)-(−)-**10**	0.9	5775	1092	168	241	>3704
(*R*)-(+)-**10**	298	150300	>3356	586	158	I

[a] Values for 5-HT$_{1D}$ and 5-HT$_{1B}$ were determined using membranes prepared from cloned gorilla receptors expressed in cultured HEK 293 cells (see ref 13). Cloned human receptors were used for the 5-HT$_{1A}$, 5-HT$_{2A}$, D_2, and D_4 determinations (see Supporting Information). [b] I = inactive, defined as less than 50% inhibition of test ligand binding at 10^{-6} M.

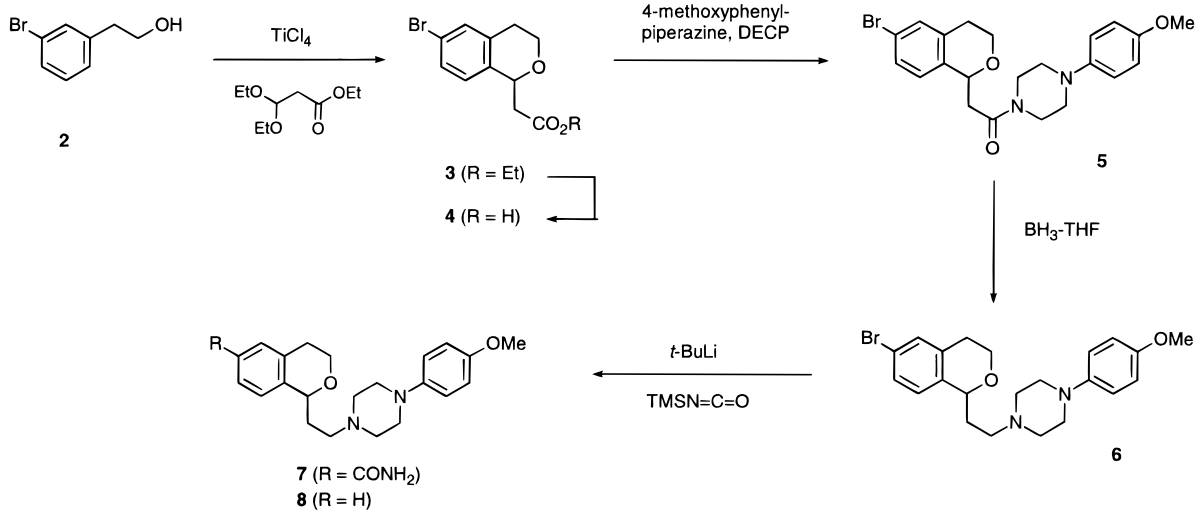
The serendipitous finding of 5-HT$_{1D}$ selectivity within the isochroman-6-carboxamide family prompted us to prepare the enantiomers of **7**. For this purpose, we employed an enzymatic-based resolution procedure similar to that previously described for the related D_4 isochroman compounds.[12] As illustrated in Scheme 2, treatment of racemic bromo ester **3** with Amano P-30 lipase results in a highly selective hydrolysis of the (*S*)-ester antipode to provide a readily separable mixture of the carboxylic acid (*S*)-(−)-**4** and the recovered ester (*R*)-(+)-**3** in yields of 86% and 91% and optical purities of 99% and 96% ee, respectively.[14] From these resolved products were prepared the corresponding enantiomers (*S*)-(−)-**7** and (*R*)-(+)-**7**. As can be seen from Table 1, binding affinity at the 5-HT$_{1D}$ receptor was exquisitely sensitive to the stereochemical configuration of the isochroman C-1 position, with the more potent isomer being the (*S*)-(−)-**7** compound. Interestingly, the other serotonin and dopamine receptors evaluated showed little dependence upon stereochemistry in their binding affinities for these compounds.

The optically pure primary amide (*S*)-(−)-**7** served as a useful starting point for the preparation of a number of secondary and tertiary amide derivatives. Treatment of (*S*)-(−)-**7** with di-*tert*-butyl dicarbonate generates the bis-BOC derivative (*S*)-(−)-**9** (Scheme 3), which can be reacted with a variety of primary and secondary amines.[15] Use of methylamine in this reaction provides the *N*-methyl analogue (*S*)-(−)-**10** (PNU-109291), which proved to be among the most potent and selective 5-HT$_{1D}$ compounds prepared to date, displaying a 5-HT$_{1D}$ K_i of 0.9 nM and an B/D ratio of over 5000. As

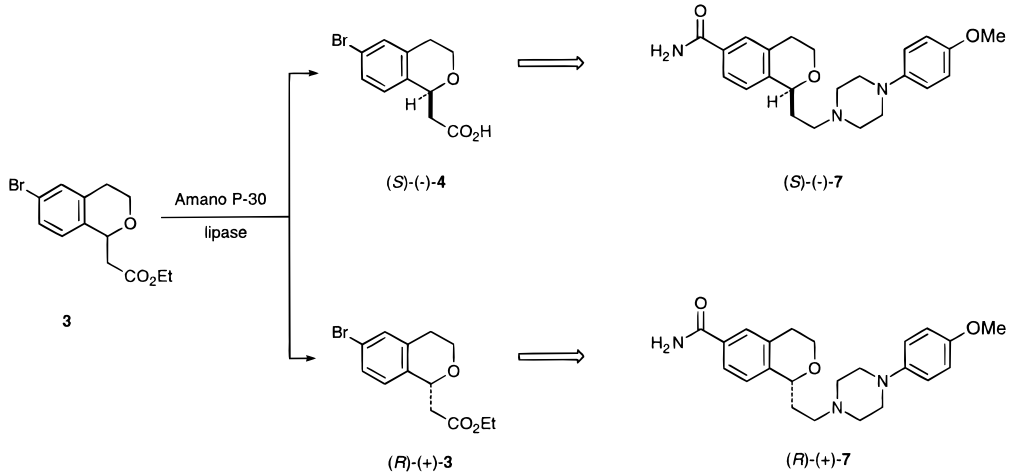
[†] Structural, Analytical and Medicinal Chemistry.
[‡] Discovery Technologies
[§] CNS Diseases Research
[||] Cardiovascular Pharmacology

S0022-2623(98)00137-X CCC: $15.00
Published on Web 06/03/1998

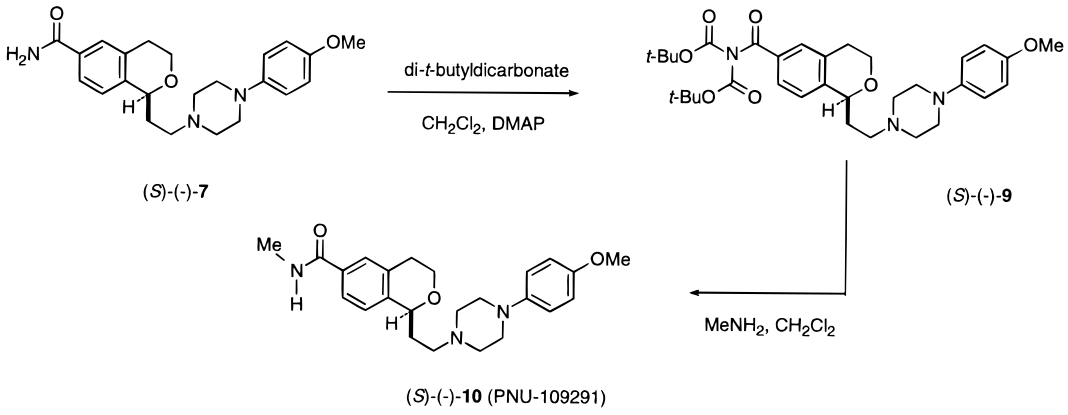
Scheme 1



Scheme 2



Scheme 3



with the primary amide **7**, the (*S*)-(−)-**10** isomer possessed far superior affinity for the 5-HT_{1D} site relative to the (*R*)-(+)-**10** enantiomer. In addition to its affinity at the 5-HT_{1D} site, (*S*)-(−)-**10** also possesses weak affinity for the 5-HT_{2A} (168 nM) and dopamine D_2 (241 nM) sites. Finally, (*S*)-(−)-**10** was found inactive (<50% inhibition of test ligand at 10^{-6} M) in a broad panel of CNS receptors, including the 5-HT_{2C}, 5-HT_3, 5-HT_4, and 5-HT uptake sites.

The discovery of the isochroman-6-carboxamide (*S*)-(−)-**10** as the first truly selective 5-HT_{1D} ligand has provided a valuable tool for the elucidation of the pharmacological role played by the 5-HT_{1D} receptor. We have been able to demonstrate in vivo activity with (*S*)-(−)-**10** which we believe reflects the 5-HT_{1D} agonist properties of this unique compound and suggests efficacy for the treatment of migraine headache.[16] In addition, we have determined that (*S*)-(−)-**10** does not

Table 2. Guinea Pig Hypothermia

compd	dose (μmol/kg, sc)	hypothermia[a] Δ °C at 30 min	Δ °C at 60 min
vehicle		0.06 ± 0.16	0.08 ± 0.19
sumatriptan (**1**)	25		−0.14 ± 0.13
(*S*)-(−)-**7**	50	−1.42 ± 0.14[b]	−1.14 ± 0.18[b]
(*S*)-(−)-**10**	25	−0.80 ± 0.11[b]	−0.54 ± 0.33
(*S*)-(−)-**10**	50	−1.84 ± 0.26[b]	−2.34 ± 0.53[c]
(*S*)-(−)-**10**	100	−1.38 ± 0.09[b]	−1.52 ± 0.07[b]

[a] The hypothermic response is presented as the difference in °C from control (vehicle) and is expressed as mean ± SEM (n = 4−5). [b] $P < 0.01$ vs vehicle. [c] $P < 0.05$ vs vehicle.

possess the vasoconstrictive activity which can be demonstrated with Sumatriptan.

The guinea pig hypothermia assay has been described as a simple in vivo assay for 5-HT_{1D} agonists.[17] The hypothermic response is believed to reflect agonist action within the CNS, since compounds with poor brain penetration (e.g., Sumatriptan) are essentially inactive after systemic administration. Given in Table 2 are the results from this assay for Sumatriptan, (*S*)-(−)-**7**, and (*S*)-(−)-**10**. Both of the isochroman amides exhibited good hypothermic effects, indicating that these compounds are agonists that readily cross the blood−brain barrier. The excellent binding selectivity of both (*S*)-(−)-**7** and (*S*)-(−)-**10** strongly suggests that this response is being mediated by 5-HT_{1D} receptors.[18]

The data presented in Tables 1 and 2 clearly indicate that the isochroman-6-carboxamide (*S*)-(−)-**10** is a selective and potent 5-HT_{1D} agonist with good CNS penetration. Recent reports regarding the involvement of 5-HT_{1D} receptors in meningeal pain prompted us to examine the potential antimigraine activity of (*S*)-(−)-**10**. Moskowitz has described a neurogenic inflammation model for migraine in which unilateral stimulation of the trigeminal ganglia results in the colateral release of permeability-enhancing autacoids (such as substance P and CGRP) from the trigeminal nerve terminals innervating meningeal blood vessels.[19] The release of these autacoids causes extravasation of plasma protein from these dural vessels. Pretreatment of guinea pigs with radiolabeled plasma protein (^{125}I bovine serum albumin) allows for quantification of the plasma extravasation in the stimulated and unstimulated halves of the dura and provides a means to measure the efficacy with which compounds inhibit this neurogenic inflammation. Moskowitz has shown that nonselective 5-$HT_{1D/1B}$ agonists such as Sumatriptan and dihydroergotamine are effective at blocking plasma extravasation in this model.[20,21] As shown in Figure 1, we have demonstrated that (*S*)-(−)-**10**, in addition to being 5-HT_{1D} selective, is also *significantly more potent than Sumatriptan in blocking neurogenic inflammation in the guinea pig model of migraine.* Nearly complete inhibition of plasma extravasation can be achieved in this model using 1.0 μg/kg iv of (*S*)-(−)-**10**, whereas a dose of 300 μg/kg iv of Sumatriptan is required for comparable activity.[22] The difference in potency between (*S*)-(−)-**10** and Sumatriptan in the neurogenic inflammation assay is not predicted by their nearly equivalent 5-HT_{1D} binding affinities (0.9 and 2.0 nM, respectively). One possible explanation for this difference recognizes the superior brain penetration of (*S*)-(−)-**10**, allowing for

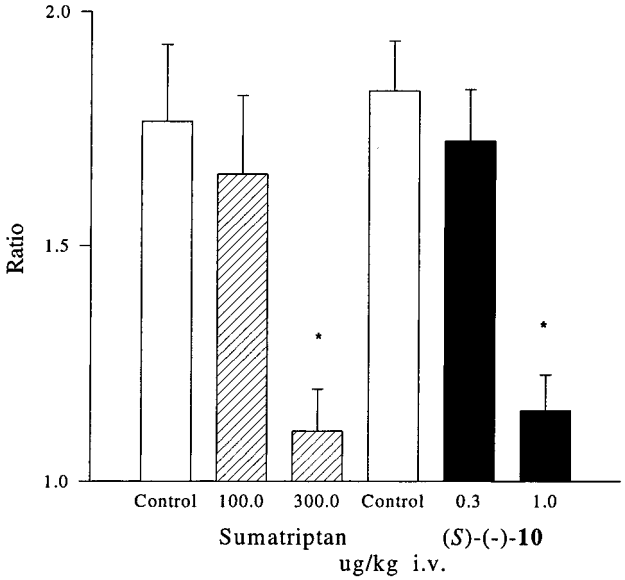


Figure 1. Inhibition of guinea pig dural plasma extravasation: comparison of Sumatriptan and (*S*)-(−)-**10**. Values = mean ratio of ^{125}I albumin extravasation in the stimulated side of the dura to that in the unstimulated side (± standard error) for n = 4−8 animals/group. $P < 0.05$ versus control.

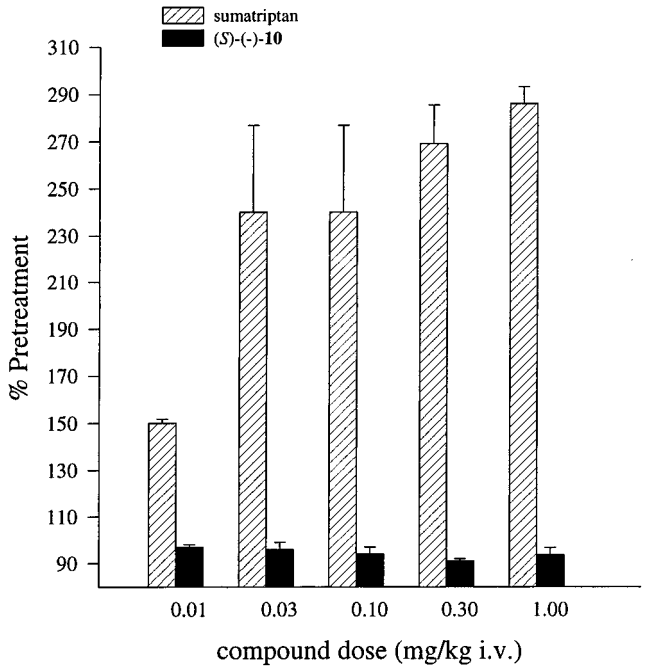


Figure 2. Comparison of Sumatriptan and (*S*)-(−)-**10** on carotid artery resistance in the cat. Values = mean increase ± standard error.

augmentation of the antiinflammatory effect by accessing centrally located 5-HT_{1D} receptors.

It has been proposed that the 5-HT_{1B} affinity of Sumatriptan is responsible for its vasoconstrictive properties and that a selective 5-HT_{1D} agonist might be an effective treatment for migraine without cardiovascular side effects.[9,10] We have investigated the effects of Sumatriptan and (*S*)-(−)-**10** in a cat model of carotid resistance (Figure 2).[23] Sumatriptan (0.01−1.0 mg/kg, iv, n = 5) produced a dose-related increase in carotid resistance in the absence of a change in mean arterial blood pressure. The maximum increase in resistance was 286% of pretreatment. In contrast, (*S*)-(−)-**10**

(0.01−1.0 mg/kg, iv, $n = 5$) failed to alter either carotid resistance or mean arterial blood pressure. These data confirm numerous previous studies which demonstrate vasoconstrictor properties of Sumatriptan. The fact that (*S*)-(−)-**10** has no carotid constrictor effect supports the notion that the vasoconstrictor properties of Sumatriptan are mediated by its 5-HT_{1B} agonist properties. *Furthermore, these data suggest that (S)-(−)-**10** may be free of the cardiovascular complications observed with Sumatriptan.*

We have discovered a class of isochroman-6-carboxamides which possesses excellent affinity and selectivity for the 5-HT_{1D} receptor. One such compound, (*S*)-(−)-**10** (PNU-109291), is active as an agonist in the guinea pig hypothermia model and potently blocks neurogenic dural inflammation in the guinea pig model of migraine. Furthermore, we have demonstrated a lack of vasoconstrictive effects of (*S*)-(−)-**10** in a rabbit model of blood flow. When tested in the rat, (*S*)-(−)-**10** displayed excellent oral bioavailability (70 ± 20%, $n = 4$).[24] The debate whether the antimigraine efficacy of the current class of triptan drugs is due to neurogenic or vascular mechanisms remains unresolved. The discovery of truly selective 5-HT_{1D} agonists such as (*S*)-(−)-**10** should provide a valuable tool for probing therapeutic approaches to the treatment of migraine. We believe that the isochroman-6-carboxamides described herein represent a significant structural breakthrough in the search for safe, new therapies for migraine headache.

Supporting Information Available: Experimental details for the preparation of all compounds and detailed descriptions of the biological assays (22 pages). Ordering information is given on any current masthead page.

References

(1) Olesen, J. *Migraine and other headaches: the vascular mechanisms*; Raven Press: New York, 1991.

(2) Mushet, G. R.; Miller, D.; Clements, B.; Pait, G.; Gutterman, D. L. Impact of Sumatriptan on Workplace Productivity, Nonwork Activities, and Health-Related Quality of Life Among Hospital Employees With Migraine. *Headache* **1996**, *36*, 137−143.

(3) de Lissovoy, G.; Lazarus, S. S. The economic cost of migraine. *Neurology* **1994**, *44* (suppl. 4), S56−S62.

(4) Osterhaus, J. T.; Gutterman, D. L.; Plachetka, J. R. Healthcare resource and lost labour costs of migraine headache in the US. *Pharmacoeconomics* **1992**, *2*, 67−76.

(5) Weinshank, R. L.; Zgombic, J. M.; Macchi, M. J.; Branchek, T. A.; Hartig, P. R. Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3630−3634.

(6) The 5-HT_{1D} and 5-HT_{1B} receptors were formerly referred to as 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$, respectively. See: Hartig, P. R.; Hoyer, D.; Humphrey, P. P. A.; Martini, G. R. Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. *Trends Pharmacol. Sci.* **1996**, *17*, 103−105.

(7) Rebeck, G. W.; Maynard, K. I.; Hyman, B. T.; Moskowitz, M. A. Selective 5-$HT_{1D\alpha}$ serotonin receptor gene expression in trigeminal ganglia: Implications for antimigraine drug development. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3666−3669.

(8) Moskowitz, M. A.; Macfarlane, R. Neurovascular and molecular mechanisms in migraine headaches. *Cerebrovasc. Brain Metab. Rev.* **1993**, *5*, 159−177.

(9) Hamel, E. 5-HT_{1D} Receptors: pharmacology and therapeutic potential. *Serotonin* **1996**, *1*, 19−29.

(10) Hamel, E.; Gregoire, L.; Lau, B. 5-HT1 receptors mediating contraction in bovine cerebral arteries: a model for human cerebrovascular '5-$HT_{1D\beta}$' receptors. *Eur. J. Pharmacol.* **1993**, *242*, 75−82.

(11) Branchek, T.; Audia, J. E. Recent Advances in Migraine Therapy. *Annu. Rep. Med. Chem.* **1997**, *32*, 1−10.

(12) TenBrink, R. E.; Bergh, C. L.; Duncan, J. N.; Harris, D. W.; Huff, R. M.; Lahti, R. A.; Lawson, C. F.; Lutzke, B. S.; Martin, I. J.; Rees, S. A.; Schlachter, S. K.; Sih, J. C.; Smith, M. W. (*S*)-(−)-4-[4-[2-(Isochroman-1-yl)ethyl]-piperazin-1-yl]benzenesulfonamide, a Selective Dopamine D4 Antagonist. *J. Med. Chem.* **1996**, *39*, 2435−2437.

(13) Pregenzer, J. F.; Alberts, G. L.; Bock, J. H., Slightom, J. L.; Im, W. B. Characterization of ligand binding properties of the 5-HT_{1D} receptors cloned from chimpanzee, gorilla, and rhesus monkey in comparison with those from the human and guinea pig receptors. *Neurosci. Lett.* **1997**, *235*, 117−120.

(14) The acid (*S*)-(−)-**4** was assayed for optical purity by reduction to the corresponding alcohol and analysis via HPLC (Chiracel OJ column; see Supporting Information for details). The ester (*R*)-(+)-**3** was hydrolyzed to the corresponding acid (*R*)-(+)-**4** and assayed in an identical fashion. A single recrystallization of this enriched chiral acid brought the optical purity to 99%.

(15) Davidsen, S. K.; May, P. D.; Summers, J. B. Di-tert-butyl N-acylimido dicarbonates as isolable acylating agents: Mild conversion of primary carboxamides to substituted amides. *J. Org. Chem.* **1991**, *56*, 5482−85.

(16) We have also been able to demonstrate agonist properties for (*S*)-(−)-**10** in vitro using the GTPγ^{35}S binding assay with cloned gorilla receptors. Unfortunately, similar evaluation in the more relevant guinea pig 5-HT_{1D} receptors fails because these receptors appear to be poorly coupled to intracellular G-proteins when expressed in HEK 293 cells (Wha Bin Im, manuscript in preparation).

(17) Skingle, M.; Higgins, G. A.; Feniuk, W. Stimulation of central 5-HT_{1D} receptors causes hypothermia in the guinea pig. *J. Psychopharmacol.* **1994**, *8*, 14−21.

(18) As observed with gorilla clones, (*S*)-(−)-**10** shows excellent selectivity for guinea pig 5-HT_{1D} receptors over 5-HT_{1B} (350−400-fold).

(19) Moskowitz, M. A.; Cutrer, F. M.; Lee, W. S.; Waeber, C.; Limmroth, V.; Yu, X. Drug Discovery in Migraine Circa 1995. In *Experimental Headache Models*; Olesen, J., Moskowitz, M. A., Eds.; Lippincott-Raven Publishers: Philadelphia, 1995; pp 63−73.

(20) Markowitz, S.; Saito, K.; Moskowitz, M. A. Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. *J. Neurosci.* **1991**, *7*, 4129−4136.

(21) Buzzi, M. G.; Moskowitz, M. A. The antimigraine drug sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. *Br. J. Pharmacol.* **1990**, *99*, 202−206.

(22) Similar results for (*S*)-(−)-**10** have been obtained independently: Cutrer, F. M.; Yu, X.-J.; Ayata, G.; Moskowitz, M. A.; Waeber, C. Effects of PNU-109291, a selective 5-HT_{1D} receptor agonist, on electrically induced dural plasma extravasation and capsaicin-evoked c-Fos immunoreactivity within trigeminal nucleus caudalis. *Neuropharmacology*, submitted.

(23) Carotid artery resistance was determined by dividing carotid blood flow (as measured by an implanted transit-time ultrasonic probe) by mean arterial blood pressure. Complete details are provided in the Supporting Information.

(24) We thank Ms. Grace Wilson, Endocrine Pharmacology and Metabolism, Pharmacia & Upjohn, for this determination.

JM980137O