N-[3-(2-Dimethylaminoethyl)-2-methyl-1*H*-indol-5-yl]-4-fluorobenzamide: A Potent, Selective, and Orally Active 5-HT$_{1F}$ Receptor Agonist Potentially Useful for Migraine Therapy

Yao-Chang Xu,*,† Kirk W. Johnson,† Lee A. Phebus,† Marlene Cohen,† David L. Nelson,† Kathy Schenck,† Clint D. Walker,† James E. Fritz,† Stephen W. Kaldor,† Michael E. LeTourneau,† Robert E. Murff,† John M. Zgombick,‡ David O. Calligaro,† James E. Audia,† and John M. Schaus†

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, and Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, New Jersey 07652

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Abstract: Recent studies have demonstrated that selective 5-HT$_{1F}$ receptor agonists inhibit neurogenic dural inflammation, a model of migraine headache, indicating that these compounds may be effective therapies for the treatment of migraine pain. This communication describes the synthesis and discovery of a novel compound, *N*-[3-(2-(dimethylamino)-ethyl)-2-methyl-1*H*-indol-5-yl]-4-fluorobenzamide (**4**), which possesses high binding affinity and selectivity at the 5-HT$_{1F}$ receptor relative to more than 40 other serotonergic and nonserotonergic receptors examined.

Serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter widely distributed in the brain and peripheral tissues, is involved in the regulation of various physiological functions such as mood, appetite, pain, sexual behavior, blood pressure, and body temperature.[1] Recent advances in the molecular cloning of seven serotonin receptor subfamilies (5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$) have provided a plethora of potentially important targets for drug discovery research. Among these 5-HT receptors, the 5-HT$_1$ receptor family appears to be the most complex and has been further subclassified into the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, and 5-HT$_{1F}$ subtypes.[2]

Sumatriptan (**1**) is the first 5-HT$_1$ receptor agonist approved for the clinical treatment of patients with migraine headaches.[3] Since the introduction of this and other structurally related triptans, their exact mechanism of action has been the subject of debate.[2f,4] For some time, 5-HT$_{1B/1D}$ receptor activation had been believed to be involved in mediating the therapeutic effects[4c] partly because triptans have high binding affinity for these receptor subtypes (Table 1). Phebus and colleagues have recently demonstrated that agonist potency to inhibit trigeminal-stimulated dural plasma extravasation in the guinea pig is highly correlated with their affinities for the 5-HT$_{1F}$ receptor.[5] This finding indicates that activation of the 5-HT$_{1F}$ receptor instead of the 5-HT$_{1B/1D}$ receptor could be the rational way for discovering effective migraine therapeutics. Sumatriptan has moderate 5-HT$_{1F}$ receptor agonist activity (K_i = 25.7 nM)[5] and is active in the neurogenic dural inflammation model (Figure 1). While the antimigraine effect of sumatriptan may be occurring via 5-HT$_{1F}$ receptor activation, the cardiovascular liabilities of this agent most likely result from coronary arterial vasoconstriction,[6] mediated via a receptor other than the 5-HT$_{1F}$ receptor.[4b] This finding has stimulated considerable interest recently in the identification of potent 5-HT$_{1F}$ receptor agonists without cross-reactivity at the 5-HT$_{1B}$ receptor or 5-HT$_{1D}$ receptor, which could ultimately lead to safer migraine therapy.[7,8]

Our effort in the search for more potent and selective 5-HT$_{1F}$ receptor agonists was directed toward the tryptamine core structure. On the basis of the structure features of compound **1** (LY334370),[8] which has an arylamido functional group at the C-5 position of the indole, we introduced the same substituent at a similar position of tryptamine, leading to compound **3** (Table 1). This analogue had a 5-HT$_{1F}$ affinity (K_i = 3.8 nM) higher than that of either serotonin (K_i = 10 nM) or sumatriptan (K_i = 25.7 nM).[5] Unfortunately, compound **3**, like sumatriptan, lacked selectivity versus other 5-HT$_1$ receptor subtypes.

A second modification was introduced by installation of a methyl group at the C-2 position of the indole nucleus, leading to a new analogue **4** (LY349950). A reduction in binding affinity at the 5-HT$_1$ receptor subtypes would be expected on the basis of a report[12] that 2-methyl-5-HT is more than 40-fold less potent in binding than 5-HT itself. To our surprise, **4** retained high affinity at the 5-HT$_{1F}$ receptor (K_i = 8.2 nM). As expected, the affinities for other 5-HT$_1$ receptor subtypes dropped significantly. The combination of these two modifications on the aryl-substituted tryptamine core led to the discovery of **4**, a potent, selective, and orally active 5-HT$_{1F}$ receptor agonist with a long duration of action.

Chemistry. Compound **4** was prepared in a four-step sequence shown in Scheme 1 starting from 4-nitroaniline **5**. Coupling reaction of **5** with 4-fluorobenzoyl chloride **6** gave an arylamide intermediate whose nitro group was then reduced by hydrogenation to afford compound **7**. Diazotization of aniline **7** with sodium nitrite in HCl solution followed by an in situ reduction using $SnCl_2$ yielded arylhydrazine **8** in good yield.[9] When the Fischer indole synthesis is applied,[10] the reaction of hydrazine **8** with 1-*N*,*N*-dimethylaminopentan-4-one **9** gave rise to 2,3,5-trisubstituted indole **4** in good overall yield (44% from **5**). It should be noted that the Fischer indolization with an asymmetrically substituted ketone usually gives a mixture of two possible indole products.[11] The reaction of **8** with asymmetrically substituted ketone **9**, however, is chemoselective to give only **4**. No other possible indole product was isolated.

In Vitro and in Vivo Pharmacological Results. The affinity of **4** for cloned human serotonin receptors in vitro (Tables 2 and 3) was determined by radioligand binding studies using membranes from transfected cells.[12−14] Studies examining compound **4** binding to the

* To whom correspondence should be addressed. Phone: (317) 277-2284. Fax: (317) 277-1125. E-mail: Xu_Yao-Chang@Lilly.com.
† Eli Lilly and Company.
‡ Synaptic Pharmaceutical Corporation.

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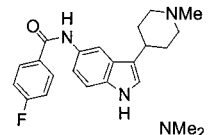
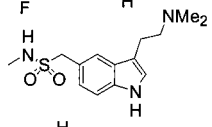
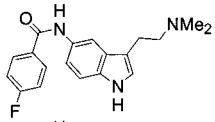
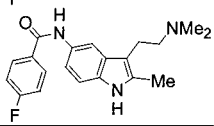
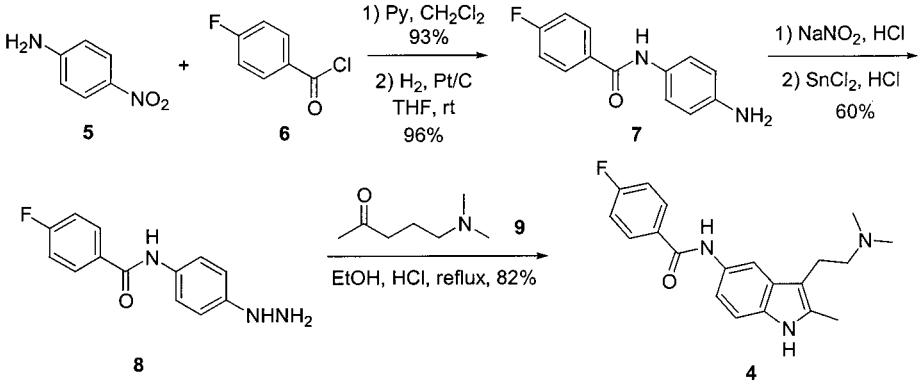
Table 1. Binding Affinity and Selectivity of 5-HT_{1F} Receptor Agonists at Human 5-HT_1 Receptors

Compound	K_i[a] (nM) 5-HT_{1F}	Selectivity[b] (5-HT_{1A}/5-HT_{1F})	(5-HT_{1B}/5-HT_{1F})	(5-HT_{1D}/5-HT_{1F})
 **1**	1.6 ± 0.4	7	85	86
 **2** (sumatriptan)	25.7[5]	9	0.4	0.2
 **3**	3.8 ± 1.1	3	9	3
 **4**	8.2 ± 1.2	32	130	200

[a] The K_i values are expressed as the mean values ± SEM of two to eight experiments. [b] The K_i valus for 5-HT1A, 5-HT1B, and 5-HT1D used for calculation of the selectivity are derived from at least two separate experiments.

Scheme 1. Synthesis of *N*-[3-(2-Dimethylaminoethyl)-2-methyl-1*H*-indol-5-yl]-4-fluorobenzamide **4**



native rat adrenergic, dopaminergic, benzodiazepine, GABA, histamine H_1, and muscarinic receptors were conducted using methods referenced in Table 2. All other binding assays (Table 3) were conducted by Novascreen (Hanover, MD) using their protocols.

As shown in Tables 2 and 3, **4** possessed high affinity and selectivity for the 5-HT_{1F} receptor. With K_i being 8.2 nM at the 5-HT_{1F} receptor, **4** displayed more than 32-fold selectivity versus the 44 binding sites examined. Minimizing the affinity at the 5-HT_{1B} and 5-HT_{1D} receptors was most important to potentially avoid the cardiovascular side effects associated with current triptan agents. **4** showed greater than 100-fold selectivity for the 5-HT_{1F} receptor relative to both 5-HT_{1B} and 5-HT_{1D} receptors.

To determine the functional properties of **4**, the compound was evaluated for its ability to inhibit forskolin-stimulated adenylate cyclase in cell lines expressing the human 5-HT_{1F} receptor. Responses were compared to those elicited by 5-HT and sumatriptan.[12] As shown in Table 4, compound **4** was a potent (EC_{50} = 6.0 nM) 5-HT_{1F} receptor agonist (E_{max} = 98%). The rank order of agonist potency in this functional assay was **4** ≈ 5-HT > sumatriptan.

Neurogenic dural extravasation and subsequent inflammation have been used as an animal model of migraine. Inhibition of the inflammation by compounds has been suggested to predict their clinical efficacy in

Table 2. Binding Profile of **4** to Neurotransmitter Receptor Sites

binding site	K_i[a] (nM)	species	radioligand
5-HT_{1F}	8.2 ± 1.2	human	[^{3}H] serotonin[12]
5-HT_{1A}	265 ± 99	human	[^{3}H] serotonin[13]
5-HT_{1B}	1060 ± 204	human	[^{3}H] serotonin[14]
5-HT_{1D}	1620 ± 100	human	[^{3}H] serotonin[14]
5-HT_{1E}	>4830	human	[^{3}H] serotonin[13]
5-HT_{2A}	1000 ± 85	human	[^{125}I] DOI[15]
5-HT_{2B}	676 ± 118	human	[^{3}H] serotonin[15]
5-HT_{2C}	2200 ± 390	human	[^{125}I] DOI[15]
5-HT_4	380 ± 40	human	[^{3}H] serotonin
5-HT_6	1770 ± 130	human	[^{3}H] LSD
5-HT_7	1460 ± 102	human	[^{3}H] serotonin[16]
α_1-adrenergic	15600	rat	[^{3}H] prazosin[17]
α_2-adrenergic	6000	rat	[^{3}H] rauwolscine[18]
β-adrenergic	38000	rat	[^{3}H] DHA[19]
dopamine D_1	>100000	rat	[^{3}H] SCH23390[20]
dopamine D_2	38000	rat	[^{3}H] raclopride[21]
benzodiazepine	>100000	rat	[^{3}H] flunitrazepam[22]
GABA	>10000	rat	[^{3}H] muscimol[23]
histamine H_1	12000	rat	[^{3}H] pyrilamine[24]
muscarinic	>100000	rat	[^{3}H] QNB[25]

[a] The K_i values are expressed as the mean ± SEM of two to eight experiments. Single values indicate the average results of two separate binding experiments.

Table 3. Low Binding Activity (K_i > 1000 nM) of **4** at Additional Sites

5-HT_4	melatonin	substance P
5-HT uptake	kainate	NK-3
NE uptake	opoid	somatostatin
DA uptake	angiotensin i	neurotensin
adenosine	angiotensin ii	VIP
GABAA	arg-vasopressin 1	galanin
GABAB	arg-vasopressin 2	calcium channels (T and L)
histamine (H2 and H3)	bradykinin	chloride channels
nicotinic	CCK central	potassium channels

Table 4. Functional Properties of **4**, Serotonin, and Sumatriptan at the Cloned Human 5-HT_{1F} Receptor in Vitro

compound	EC_{50} [a] (nM)	E_{max} [a] (% of 5-HT)
4	6.0 ± 0.7	98 ± 1.4
serotonin[12b]	7.9 ± 0.8	100
sumatriptan[12b]	35 ± 5.0	98 ± 2.0

[a] EC_{50} and E_{max} values are expressed as mean values ± SEM from at least two separate experiments performed in triplicate.

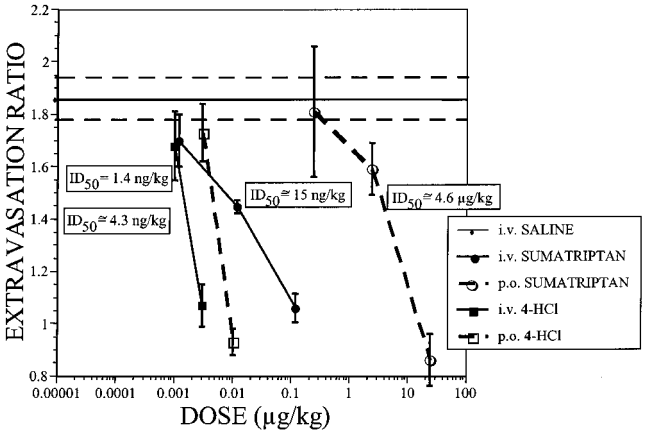


Figure 1. Dose response of **4**·HCl and sumatriptan to inhibit trigeminal stimulation induced dural extravasation in guinea pig.

treating acute migraine.[26] Several compounds such as sumatriptan, naratriptan, and rizatriptan, being active in the dural extravasation model,[5] were eventually found to be effective drugs for migraine with the exception of CP-122288.[27] **4** was examined for its ability to inhibit neurogenic dural inflammation following either oral or intravenous administration in guinea pigs.[28] As shown in Figure 1, **4** potently inhibited neurogenic dural inflammation by both routes with an intravenous ID_{50} value of 1.4 ng/kg and an oral ID_{50} value of 4.3 ng/kg. Sumatriptan was less potent than **4** with ID_{50} values of 15 and 4600 ng/kg under the same conditions following intravenous and oral administration, respectively.

Sumatriptan displayed a high oral-to-intravenous ratio of ID_{50} values ($^{4600}/_{15}$) in the dural inflammation model in guinea pig. In contrast **4** displayed a much lower ratio ($^{4.3}/_{1.4}$), indicative of the high oral bioavailability of this compound. Clinically, two doses of sumatriptan are often required to treat a migraine attack. Figure 2 compares the duration of action for both sumatriptan and **4** using the ID_{100} value following oral administration from the dural inflammation model in the guinea pig. Sumatriptan was fully effective at 1 h but no longer active at 4 h after administration. In

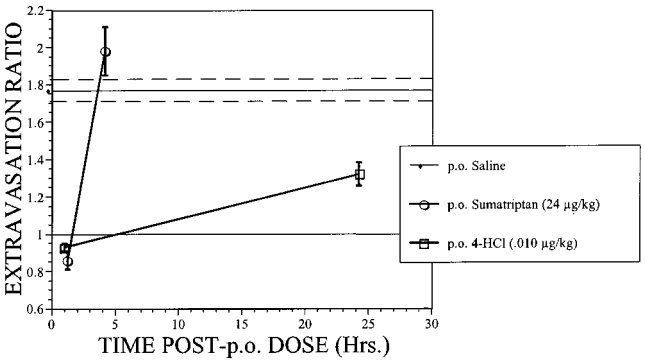


Figure 2. Duration of action of **4**·HCl and sumatriptan to inhibit trigeminal stimulation induced dural extravasation in the guinea pig.

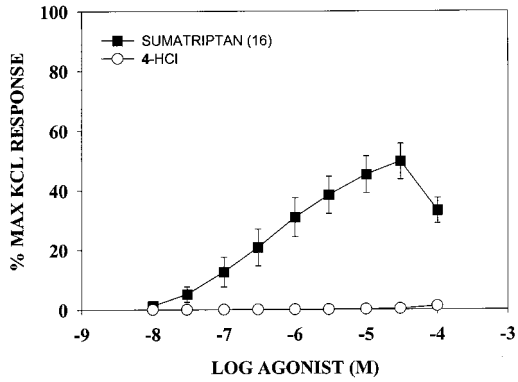


Figure 3. Effects of **4**·HCl and sumatriptan to contract the rabbit saphenous vein.

contrast, **4** was fully effective at 1 h and remained partially effective at 24 h after oral administration.

Sumatriptan is a known vasoconstrictor and was developed on the basis of the vascular theory of migraine.[29] It is not known which serotonin receptor (5-HT_{1B}, 5-HT_{1D}, or 5-$HT_{1D-like}$) mediates the vasoconstriction,[4b] which might be related to the cardiovascular side effects of the drug. **4** was examined in vitro and compared to sumatriptan using the isolated rabbit saphenous vein to evaluate contractile activity (Figure 3). While sumatriptan produced concentration-dependent contractile activity in this tissue, **4** did not constrict this tissue (up to 10^{-4} M). These in vitro results suggest that **4** does not possess the vasoconstrictive properties in rabbit saphenous vein that might be potentially linked to the cardiovascular side effects associated with sumatriptan.[6]

In conclusion, **4** represents the discovery of a novel, potent, and selective 5-HT_{1F} receptor agonist. The compound lacks the vasoconstriction properties in the isolated rabbit saphenous vein and has good oral activity and a long duration of action in the guinea pig neurogenic dural inflammation model of migraine.

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Supporting Information Available: NMR and MS data for compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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