Communications to the Editor

5-(Nonyloxy)tryptamine: A Novel High-Affinity 5-HTIDβ Serotonin Receptor Agonist

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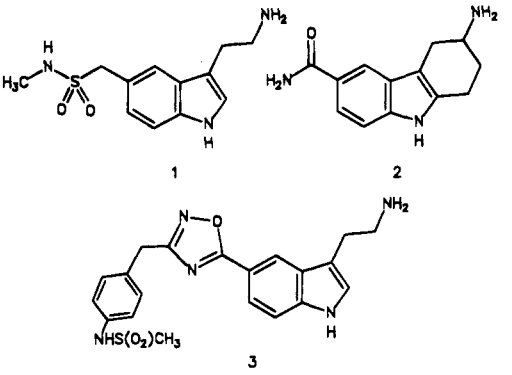
The cloning of more than a dozen serotonin (5-HT) receptors has led to a greater understanding of their structural similarities and differences.[1] For example, rat 5-HT1B receptors and human 5-HT1Dβ receptors represent species homologs that display 95% sequence homology in their transmembrane domains.[2,3] 5-HT1B receptors correspond to rodent 5-HT autoreceptors, whereas 5-HT1D receptors play a parallel role in humans. Specifically, 5-HT1Dβ receptors appear to constitute the human counterpart of rodent 5-HT1B receptors.[2,3] To date, no 5-HT1D-selective agents have been identified (reviewed in ref 4). Sumatriptan (**1**), the first agent demonstrated to bind with high affinity and some selectivity for 5-HT1D receptors, was recently introduced for the treatment of migraine headaches. Although there is some controversy regarding its exact mechanism of action,[5] 5-HT1D receptors are thought to be involved. Sumatriptan, though fairly selective for 5-HT1D versus most other populations of 5-HT receptors, reportedly displays only about 10–50-fold selectivity for 5-HT1D versus 5-HT1A receptors.[4] Its affinity for 5-HT1A receptors detracts somewhat from its use as a pharmacological tool, and certain of sumatriptan's side effects, as noted in clinical studies, may be mediated via activation of central 5-HT1A receptors.[6]

The proven efficacy of sumatriptan in the management of migraine,[7] regardless of its specific mechanism of action, has spurred the development of newer 5-HT1D agents. Compound **2**, for example, binds at 5-HT1D receptors with twice the affinity of sumatriptan,[8] and the conformationally-restricted analog **3** (IC_{50} = 0.3 nM) represents one of the highest affinity 5-HT1D ligands reported to date.[9] However, neither agent displays >50-fold selectivity for 5-HT1D versus 5-HT1A receptors.[8,9]

In the course of our work with 5-HT1D receptors, we noted the possible existence of a hydrophobic binding region in the proximity of the 5-position of serotonin.[10] We had earlier identified a corresponding hydrophobic region on 5-HT1A receptors.[11] However, because there is relatively little (ca. 50%) sequence homology between 5-HT1D and 5-HT1A receptors,[2] we felt that it might be possible to take advantage of this structural difference and develop an agent that would bind at 5-HT1D receptors with greater selectivity than sumatriptan, that



is, incorporation of an appropriate hydrophobic substituent might span the transmembrane helices in such a manner so as to take advantage of different distant amino acid environments. We report here the synthesis, radioligand binding, and functional data for such a compound: 5-(nonyloxy)tryptamine (NOT; **4**).

The synthesis of **4** is shown in Scheme 1. 5-(Benzyloxy)tryptamine (**5**) was N-protected as its *N*-acetyl derivative **6** (mp 133–134 °C), and the benzyl group was removed by hydrogenolysis to afford *N*-acetyl-5-HT (**7**) as an oil. O-Alkylation of **7** with *n*-nonyl bromide followed by hydrolysis of the amide under acidic conditions provided an overall 20% yield of **4** (from **5**) as its free base, which was converted to its hydrogen oxalate salt (mp 148–150 °C).[12]

As shown in Table 1, compound **4** binds at human 5-HT1Dβ receptors with about 5 times higher affinity than sumatriptan (1) (K_i = 1.2 and 5.5 nM, respectively). The 5-HT1Dβ affinity of sumatriptan is consistent with what has been previously reported (K_i = 7.7 nM).[3] Furthermore, unlike serotonin which displays no selectivity for 5-HT1Dβ versus 5-HT1A receptors and sumatriptan which displays only 60-fold selectivity (Table 1), compound **4** binds with 260-fold selectivity. As such, **4** is the most 5-HT1Dβ versus 5-HT1A-selective agent reported to date. Although **4** binds with higher affinity than sumatriptan at 5-HT2A and 5-HT2C receptors, it still retains >200-fold selectivity. Agents that bind at 5-HT1Dβ sites typically possess little selectivity relative to 5-HT1Dα sites. Sumatriptan, for example, reportedly binds with about a 2-fold selectivity at 5-HT1Dα versus 5-HT1Dβ receptors.[3] Compound 4 binds with about 10-fold selectivity for 5-HT1Dβ versus 5-HT1Dα (K_i = 16 ± 1 nM) receptors. The significance of these differences will need to be further explored. It has been speculated that these two types of 5-HT1D receptors may be differentially expressed in different brain regions or in different cells within the same region.[3]

It has been suggested that an indole 5-position substituent capable of participating in at least one, and perhaps two, hydrogen-bond interactions is important for binding at 5-HT1D receptors; the second hydrogen-bonding site supposedly enhances 5-HT1D agonist potency. In order to determine if **4** is a 5-HT1D agonist or antagonist, its effects on forskolin-stimulated cAMP production were examined. Compound **4** did not display

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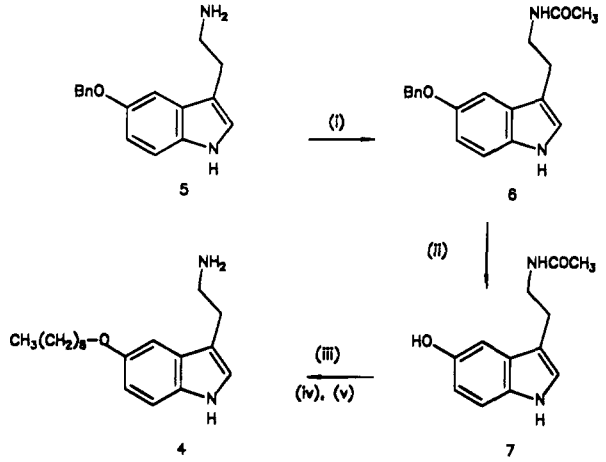
Scheme 1. Synthesis of 5-(Nonyloxy)tryptamine 4[a]



[a] (i) Ac_2O, NaOAc; (ii) H_2/Ra Ni; (iii) $CH_3(CH_2)_8Br$, K_2CO_3; (iv) 2 N HCl; (v) $(COOH)_2/Et_2O$.

Table 1. Binding of 5-HT, Sumatriptan (1), and NOT (4) at 5-HT1A, 5-HT1B, 5-HT1Dβ, 5-HT1Dα, 5-HT2A, 5-HT2C, and 5-HT3 Receptors[a]

receptor population	K_i, nM (±SEM)		
	5-HT	sumatriptan (1)	NOT (4)
5-HT1A	1.7 (±0.4)	330 (±5)	315 (±50)
5-HT1B	5.4 (±0.5)	23 (±3)	13 (±3)
5-HT1Dβ	4.0 (±0.2)	5.5 (±0.1)	1.2 (±0.1)
5-HT1Dα	5.5 (±0.8)		16 (±1)
5-HT2A	510 (±30)	>1000	260 (±50)
5-HT2C	23 (±2)	>1000	270 (±45)
5-HT3	320 (±35)	>1000	>1000

[a] Binding assays were performed as cited below. Data are expressed as K_i values and represent the mean and SEM of at least three experiments each performed on two separate occasions. 5-HT1A receptors in AK cells transfected with the human 5-HT1A gene[13] were labeled with 0.4 nM [^{3}H]-8-OH-DPAT; 5-HT1B receptors in rat striatum,[14] 5-HT1Dβ receptors in CHO cells transfected with the human 5-HT1Dβ gene,[15] and 5-HT1Dβ receptors in COS cells transfected with the human 5-HT1Dα gene[13] were labeled with 2 nM [^{3}H]-5-HT. 5-HT2A receptors in GF6 cells transfected with the human 5-HT2A gene[16] were labeled with 0.4 nM [^{3}H]ketanserin, and 5-HT2C receptors in J1 cells transfected with the rat 5-HT1C gene[16] were labeled with 1 nM [^{3}H]mesulergine. 5-HT3 receptors in NG-108 cells[17] were labeled with 0.6 nM [^{3}H]GR65630. Cell membranes for binding assays were prepared as previously described.[16] Radioligands and competing drugs were incubated with homogenates at 37 °C for 30 min, filtered through Schleicher & Schuell glass fiber filters, and counted in ecoscint (National Diagnostics) in a Beckman 3801 liquid scintillation counter.

any antagonist properties when 5-HT was used as agonist (data not shown), and like serotonin (ED_{50} = 1.2 nM), compound 4 (ED_{50} = 68 ± 19 nM) was found to be a 5-HT1D agonist (Figure 1). For purpose of comparison, the ED_{50} value for sumatriptan under similar conditions is 317 nM. Thus, although a second hydrogen-bonding site may result in enhanced affinity and/or potency, it would not seem to be an absolute requirement. Although compound 4 binds at 5-HT1A receptors only with modest affinity, its effects on 5-HT1A cyclase were also examined. Compound 4 did not act as a 5-HT antagonist (up to 1 μM) and, as an agonist, has an EC_{50} > 1 μM (data not shown); solubility problems precluded an examination of higher concentrations.

In summary, we have found that 4 is a novel example of a high-affinity 5-HT1Dβ-selective agonist that possesses only a single hydrogen-bonding substituent at the indole 5-position. It is unique from other 5-HT1D ligands in that it possesses a hydrophobic substituent at the 5-position; it also binds with higher affinty at human 5-HT1Dβ receptors and displays greater 5-HT1Dβ versus 5-HT1A selectivity than sumatriptan. It behaves as a 5-HT1D full agonist and, at concentrations of up to 1 μM, lacks 5-HT1A agonist and antagonist activity.

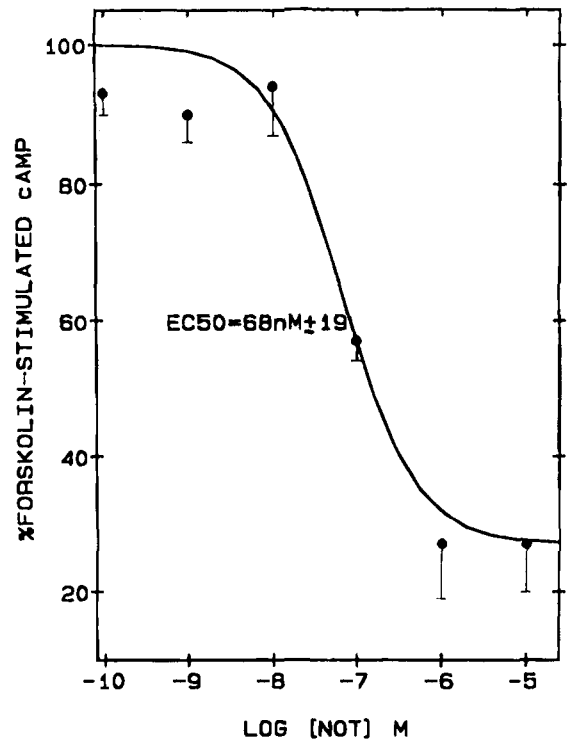


Figure 1. Inhibition of forskolin-stimulated cAMP production by NOT (4) in CHOKM 6 cells transfected with the human 5-HT1Dβ receptor gene. Percent maximal response was normalized to the degree of inhibition produced by 10^{-5} M serotonin (approximately 30% of the forskolin stimulation). Results are the mean and SEM of a typical experiment performed three times in duplicate.[18]

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(12) Anal. ($C_{21}H_{32}N_2O_5$). Found (calcd): C, 64.36 (64.25); H, 8.25 (8.22); N, 7.15 (7.14).

(13) AK cells transfected with 5-HT1A receptors and COS cells with 5-HT1Dα receptors were generously provided by Allelix Biopharmaceuticals.

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(17) NG-108 cells were generously provided by Dr. Marshall Nirenberg.

(18) 5-HT1Dβ cyclase assay: CHOMK 6 cells transfected with the human 5-HT1Dβ gene[15] were grown in MEM-α media (GIBCO) with 10% FBS and geneticin. For the cAMP RIA, 10^5 cells/well (24-well plates) were incubated in serum-free media for 18 h, washed twice, and incubated at 37 °C for 5 min in 0.25 mL of MEM-α with the phosphodiesterase inhibitor RO 20-1724 (0.25 mM). After 5 min, the media was replaced with MEM-α containing forskolin (25 μM) or drugs to be tested and cells were incubated at 37 °C for 10 min. Following this 10-min incubation, 0.25 mL of ice-cold stop solution (0.1 N HCl/0.1 mM $CaCl_2$) was added to each well to lyse the cells. cAMP was measured in the cell lysate according to the manufacturers (DuPont-NEN) protocol. The 5-HT1A cyclase assay was conducted in a similar manner using AK cells transfected with the human 5-HT1A gene.