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Communications to the Editor

Tetrahydrobenzindoles: Selective Antagonists of the 5-HT$_7$ Receptor

Chika Kikuchi,* Hiroshi Nagaso, Toyokazu Hiranuma, and Masao Koyama

Pharmaceutical Research Center, Meiji Seika Kaisha Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan

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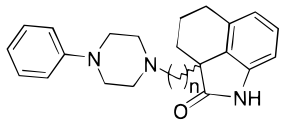
The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) plays important roles in a variety of physiological and pathophysiological processes through the activation of seven types of 5-HT receptors, 5-HT$_1$−5-HT$_7$. The 5-HT$_7$ receptor was found by the application of molecular cloning and has been identified in the rat,[1−3] mouse,[4] human,[5] and guinea pig.[6] The deduced amino acid sequences of 5-HT$_7$ receptors show a high degree of interspecies homology, but only a limited homology with those of other types of 5-HT receptors. All four species homologues of the 5-HT$_7$ receptor have high affinity for 5-HT, 5-carboxyamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT), and methiothepin and moderate affinity for 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), clozapine, and a number of other psychoactive drugs.[7] Four 5-HT$_7$ splice variants exist in human and rat. Both the long (5-HT$_{7a}$) and short (5-HT$_{7b}$) forms of the human receptor exhibit similar distribution patterns and pharmacology.[8−10] 5-HT$_7$ receptors are coupled to stimulation of adenylyl cyclase, and 5-HT, 5-CT, and 5-MeOT display agonist activity. 8-OH-DPAT is a partial agonist, and methiothepin and clozapine are antagonists. Recently, the first selective 5-HT$_7$ receptor antagonist, SB-258719, was reported,[11] but no selective agonist for the 5-HT$_7$ receptor is yet available.

The biological functions of the 5-HT$_7$ receptor have not been fully clarified. Early pharmacological data suggested that the 5-HT$_7$ receptor may be involved in the vasodilation of blood vessels.[12−15] Indeed, the highest levels of 5-HT$_7$ receptor mRNA have been found in human coronary arteries.[5] High levels of 5-HT$_7$ receptor mRNA have also been observed in the brain (hypothalamus, thalamus, brain stem, and hippocampus),[1,3,5,16] and the distribution of 5-HT$_7$ receptor binding sites in rat and guinea pig brain was essentially the same as the mRNA distribution.[16−18] The 5-HT$_7$ receptor is involved in the control of circadian rhythms of spontaneous electrical activity in the suprachiasmatic nucleus (SCN) of the hypothalamus.[3,19−21] The 5-HT$_7$ receptor may be involved in disturbance of circadian rhythms, such as jet lag, delayed sleep-phase syndrome (DSPS), and non-24-h sleep−wake disorder (non-24).[22] The affinity of a number of antipsychotic agents for the 5-HT$_7$ receptor also suggests that this receptor may mediate the therapeutic actions of these compounds.[7] Clearly, the 5-HT$_7$ receptor may be a valuable novel drug target. To examine this possibility, the development of potent and selective ligands for the 5-HT$_7$ receptor is highly desirable.

In the present study, we have synthesized highly potent and selective 5-HT$_7$ receptor ligands. Screening of our compound library against the cloned human 5-HT$_7$ receptor resulted in the identification of tetrahydrobenzindole derivatives as ligands.[23] Although these compounds have high affinity for the 5-HT$_7$ receptor, they also show high affinity for the 5-HT$_2$ receptor. To find derivatives with lower affinity for the 5-HT$_2$ recep-

Table 1. 5-HT$_7$ and 5-HT$_2$ Receptor Affinities of Compounds **1**−**3**



		pK_i ± SE[a]	
compd	*n*	5-HT$_7$[b]	5-HT$_2$[c]
1	2	6.99 ± 0.14	8.27 ± 0.19
2	3	8.28 ± 0.15	7.79 ± 0.08
3	4	8.48 ± 0.02	7.37 ± 0.05

[a] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean. [b] Binding affinity (human recombinant receptors in mammalian cells; [^{3}H]5-CT). [c] Binding affinity (rat cerebral cortex membranes; [^{3}H]ketanserin).

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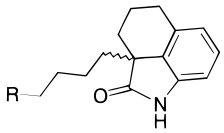
Table 2. 5-HT_7 and 5-HT_2 Receptor Affinities of Compounds **4**−**8**



compd	R	pK_i ± SE[a] 5-HT_7[b]	pK_i ± SE[a] 5-HT_2[c]
4	4-(2-methoxphenyl)piperazinyl	8.29 ± 0.08	6.95 ± 0.10
5	4-(2-cyanophenyl)piperazinyl	8.42 ± 0.07	6.98 ± 0.07
6	4-(2-pyridyl)piperazinyl	8.73 ± 0.09	7.27 ± 0.06
7	4-phenyl-1,2,3,6-tetrahydropyridyl	8.67 ± 0.07	7.01 ± 0.05
8	4-cyclohexylpiperazinyl	<6	<6

[a] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean. [b] Binding affinity (human recombinant receptors in mammalian cells; [^{3}H]5-CT). [c] Binding affinity (rat cerebral cortex membranes; [^{3}H]ketanserin).

Scheme 1

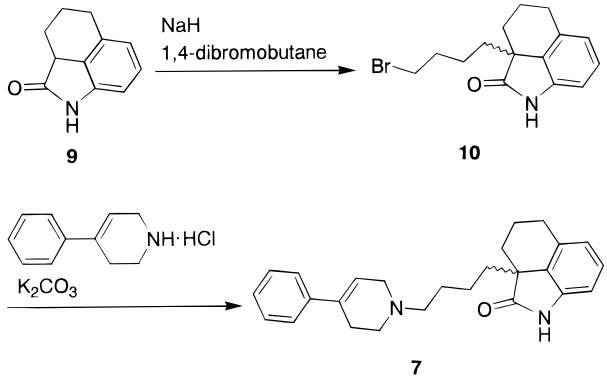


Table 3. Receptor Binding Profile of **7**[a]

receptor	affinity (pK_i ± SE)[b]
5-HT_{1A}	6.77 ± 0.05
5-HT_2	7.01 ± 0.05
5-HT_4	<6
5-HT_6	6.28 ± 0.07
5-HT_7	8.67 ± 0.07
dopamine D_2	6.98 ± 0.05

[a] Binding experiments were conducted as follows. Receptors and radioligands used in the binding assay: 5-HT_{1A} (human recombinant (mammalian); [^{3}H]8-OH-DPAT); 5-HT_2 (rat cerebral cortex; [^{3}H]ketanserin); 5-HT_4 (guinea-pig striatum; [^{3}H]GR-113808); 5-HT_6 (human recombinant (mammalian); [^{3}H]LSD); 5-HT_7 (human recombinant (mammalian); [^{3}H]5-CT); dopamine D_2 (rat striatum; [^{3}H]spiperone). [b] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean.

tor, we synthesized and tested a range of modified compounds, including the phenylpiperazine derivatives **1**−**3**. The substituted phenylpiperazine derivatives **4** and **5** and the pyridylpiperazine derivative **6** were also investigated. Preparation of the tetrahydropyridine analogue, corresponding to **3**, led to the identification of **7** (DR4004) as a highly potent and selective 5-HT_7 receptor ligand. A cyclohexylpiperazine derivative **8** was also synthesized.

Compounds **1**−**8** were evaluated for affinity for the 5-HT_7 and 5-HT_2 receptors. The affinity for the 5-HT_7 receptor was assayed in terms of the ability to displace the radioligand [^{3}H]5-CT from cloned human 5-HT_7 receptor expressed in COS-7 cells. The results, expressed as pK_i, are summarized in Tables 1 and 2. Compound **3** was a potent ligand for the *5*-HT_7 receptor, being at least 4-fold more selective than compounds **1**

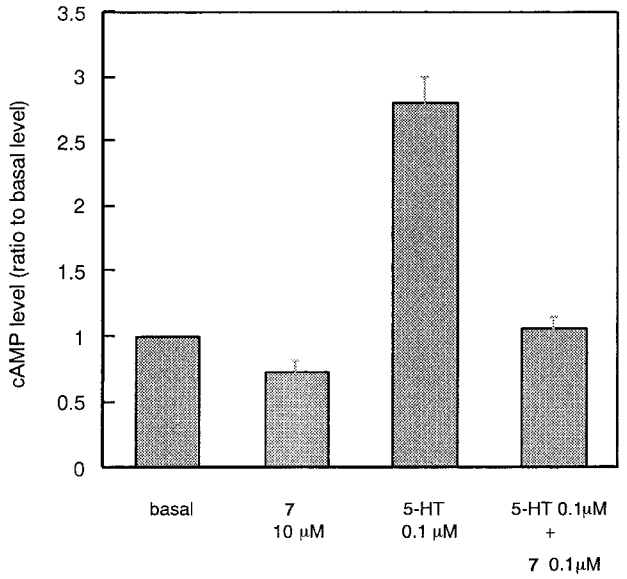


Figure 1. 5-HT-induced stimulation of cAMP accumulation in COS-7 cells expressing the 5-HT_7 receptor and its inhibition by compound **7**. Data represent the mean ± SE of at least three determinations.

and **2**. This result suggested that the carbon chain length of these compounds is very important in determining the affinity for the 5-HT_7 receptor. As the next step, we fixed the carbon chain length at the optimum value (n = 4) and modified the cyclic amine moiety. The *o*-substituted phenylpiperazines **4** and **5** showed high affinity for the 5-HT_7 receptor, with about 20-fold selectivity over the 5-HT_2 receptor. The pyridylpiperazine **6** also had high affinity for the 5-HT_7 receptor, and the 4-phenyltetrahydropyridine derivative **7** showed both high affinity and high selectivity. Compound **7** had a pK_i of 8.67 for the 5-HT_7 receptor, with 47-fold selectivity over the 5-HT_2 receptor. Thus, high affinity for the 5-HT_7 receptor did not require both nitrogen atoms. The cyclohexylpiperazine derivative **8** did not show affinity for either the 5-HT_7 or the 5-HT_2 receptor. An aromatic moiety would thus appear to be required for affinity for the 5-HT_7 and 5-HT_2 receptors.

The method of synthesis of **7** is shown in Scheme 1. Compound **10** was prepared by reacting tetrahydrobenzindole **9** with sodium hydride and 1,4-dibromobutane. Structure of compound **10** was confirmed by ^{1}H and ^{13}C NMR. Compound **7** was obtained by allowing compound

10 to react with the corresponding amine in the presence of K_2CO_3. Compounds **1**−**6** and **8** were synthesized in a similar manner to that described above. The synthesis of compound **7** was accomplished in 30% overall yield by a two-step procedure.

On the basis of its relative affinity for the 5-HT_7 and 5-HT_2 receptors, the 4-phenyltetrahydropyridine derivative **7** was selected for further evaluation. As can be seen from Table 3, compound **7** was found to be highly selective for the 5-HT_7 receptor compared with the 5-HT_{1A}, 5-HT_2, 5-HT_4, 5-HT_6, and dopamine D_2 receptors. Thus, compound **7** was confirmed to be a high-affinity ligand for the 5-HT_7 receptor with high selectivity.

Compound **7** was evaluated for influence on 5-HT-induced stimulation of cAMP accumulation in COS-7 cells transfected with an expression vector containing human 5-HT_7 receptor cDNA. Intracellular cAMP formation was measured by enzyme-immunoassay (Amersham cAMP EIA kit). Compound **7** did not stimulate basal activity on its own; i.e., it lacked agonist activity, but it inhibited 5-HT-induced stimulation of cAMP accumulation (Figure 1). Compound **7** is thus a 5-HT_7 receptor antagonist.

In summary, we have described the synthesis and the affinity for the 5-HT_7 receptor and other receptors of a novel series of tetrahydrobenzindoles. Some of the compounds showed high affinity and high selectivity for the 5-HT_7 receptor. Compound **7** was a highly potent ligand for the 5-HT_7 receptor, with at least 47-fold selectivity over the 5-HT_2 receptor and other receptors. A limited structure−activity relationship study for these derivatives indicated that an aromatic ring is required for affinity for the 5-HT_7 and 5-HT_2 receptors. Compound **7** was evaluated in a functional model of the 5-HT_7 receptor activation and confirmed to be a 5-HT_7 receptor antagonist. This compound should be a useful tool for examining the feasibility of targeting the 5-HT_7 receptor with new drugs to obtain novel pharmacological effects.

Supporting Information Available: Experimental procedure, including analytical and spectral data, for the preparation of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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