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New Tetrahydrobenzindoles as Potent and Selective 5-HT$_7$ Antagonists with Increased In Vitro Metabolic Stability

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Abstract—Chemical modifications of compound **1** (DR4004), a potent, selective antagonist of the 5-HT$_7$ receptor, were conducted with the aim of improving its metabolic stability. Halogenation of putative sites of oxidative metabolism afforded compounds **7**–**10**, which retained high affinity and selectivity for the 5-HT$_7$ receptor, and showed increased in vitro metabolic stability. Compound **10** (DR4485) showed oral bioavailability, and should be a useful tool for evaluating the therapeutic potential of 5-HT$_7$ antagonists.
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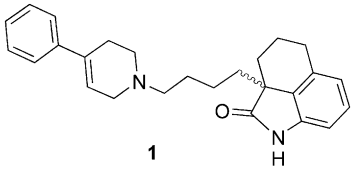
The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) plays important roles in a variety of physiological and pathophysiological processes through the activation of seven types of 5-HT receptors, 5-HT$_1$–5-HT$_7$. The 5-HT$_7$ receptor is the most recent addition to the family of 5-HT receptors.[1–6] Its biological functions are still poorly understood. Early pharmacological data suggested that the 5-HT$_7$ receptor may be involved in the vasodilation of blood vessels.[7–10] This receptor is involved in the control of circadian rhythms of spontaneous electrical activity in the suprachiasmatic nucleus (SCN) of the hypothalamus,[3,11–13] and may be involved in disturbance of circadian rhythms.[14,15] A recent study provided preliminary evidence that 5-HT$_7$ receptor antagonists show an antidepressant-like effect on sleep parameters in animals and it was suggested that they might have therapeutic utility in sleep disorders and depression.[16] The affinity of a number of antipsychotic agents for the 5-HT$_7$ receptor also led to the speculation that this receptor may mediate the therapeutic actions of these compounds.[17] Thus, the 5-HT$_7$ receptor may be of value as a novel therapeutic target.

Only a few selective antagonists for the 5-HT$_7$ receptor have been reported to date,[18–23] and no selective agonist is yet available (Chart 1). In previous papers, we reported the synthesis and affinities for the 5-HT$_7$ receptor and other receptors of a series of tetrahydrobenzindoles.[18-20] Among them, compound **1** is highly potent antagonist for the 5-HT$_7$ receptor, with selectivity over the 5-HT$_2$ receptor and other related receptors.[18]

The purpose of this study is to improve the oral bioavailability of compound **1**.[24] As shown in Chart 1, most 5-HT$_7$ receptor antagonists contain a phenyl ring, and might be expected to be rapidly metabolized in vivo. Therefore, we modified compound **1** by protection of putative metabolic sites. On the basis of superior in vitro metabolic stability and relative affinity for the 5-HT$_7$ and 5-HT$_2$ receptors, compound **10** was selected for further evaluation of binding property for other related receptors. We also evaluated its in vitro agonist or antagonist activity.



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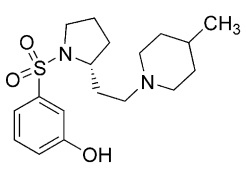

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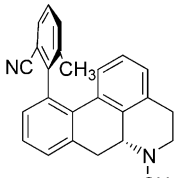

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Chart 1. 5-HT$_7$ antagonists **1**–**3**.

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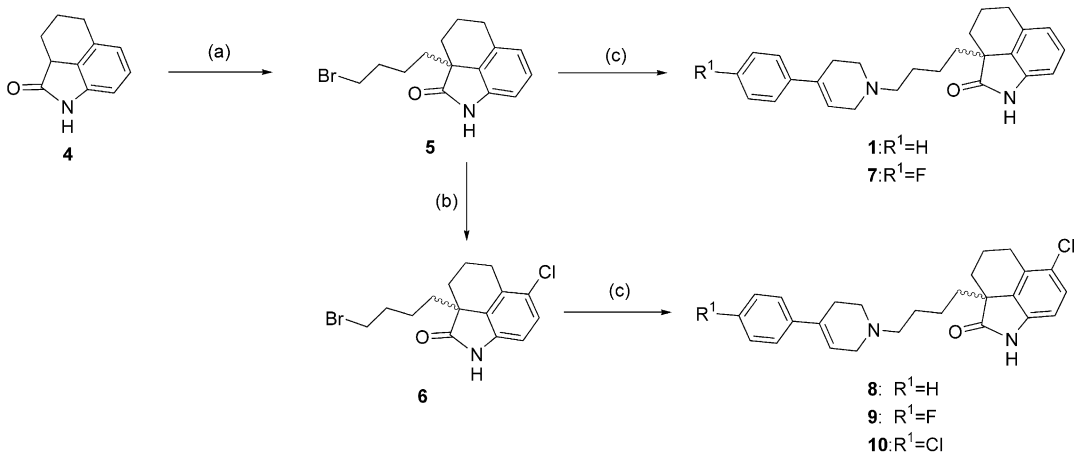
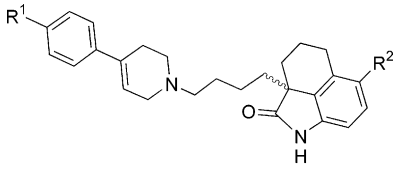


Scheme 1. (a) 1,4-Dibromobutane, 55% NaH, DMF, −40 to 0 °C (54%); (b) SO_2Cl_2, CH_2Cl_2, 0 °C (87%); (c) corresponding amine, K_2CO_3, DMF, 60 °C (**7** = 32%, **8** = 64%, **9** = 60%, **10** = 64%).

The synthetic procedures are shown in Scheme 1. Compound **5** was prepared by treating tetrahydrobenzindole **4** with sodium hydride and 1,4-dibromobutane.[19] Compound **6** was obtained from compound **5** by reaction with SO_2Cl_2 at 0 °C. The reaction furnished the 6-chlorobenzindole **6** in a high yield, with no detectable formation of the 8-chlorobenzindole. Chlorination of compound **5** at room temperature mainly provided the 6,8-dichlorinated compound. Compounds **1** and **7–10**[25] were obtained from compounds **5** and **6** by the reaction with the corresponding amines in the presence of K_2CO_3.

Compounds **1** and **7–10** were evaluated for metabolic stability in the presence of rat liver microsomes.[26] These results are summarized in Table 1. Compound **1** was almost completely lost after 30-min incubation with rat microsomes, and its several metabolites were detected. Some metabolites were hydroxylated derivatives (M + 16), and one of the major metabolites was identified as the 6-hydroxy-tetrahydrobenzindole derivative.[27] These results suggested the need of blocking the metabolic oxidation, and we modified compound **1** by hologenation of putative metabolic sites. The monohalogenated compounds **7** and **8** were slightly more stable than compound **1**, and the dihalogenated compounds **9** and **10** were more stable than compounds **7** and **8**. Compounds **1** and **7–10** were further evaluated for metabolic stability in the presence of human liver microsomes.[26] As can be seen Table 1, compounds **9** and **10** were more stable than compound **1** for both rat and human liver microsomes. Increased stability of compounds **9** and **10** could be explained by the protection of metabolic hydroxylation. As we had expected, the in vitro metabolic stability was improved by the protection of putative sites of oxidative metabolism.

Table 1. Metabolic stability and 5-HT_7 and 5-HT_2 receptor affinities of compounds **1**, **7–10**



Compd	R^1	R^2	Metabolic rate (pmol/min/mg protein)		pK_i[d]	
			Rat	Human	$5HT_7$[e]	$5HT_2$[f]
1	H	H	635–885[a]	141–213[a]	8.67 ± 0.07	7.01 ± 0.05
7	F	H	493[b]	131[b]	8.45 ± 0.05	7.18 ± 0.04
8	H	Cl	418, 423[c]	103, 150[c]	8.30 ± 0.05	6.50 ± 0.02
9	F	Cl	323[b]	93[b]	8.69 ± 0.06	6.64 ± 0.13
10	Cl	Cl	228[b]	51[b]	8.14 ± 0.06	< 6

[a] $n = 4$.
[b] $n = 1$.
[c] $n = 2$.
[d] The pK_i values are means ± SE of 8–12 values.
[e] Binding affinity (human recombinant receptors in mammalian cells; [^{3}H]5-CT).
[f] Binding affinity (rat cerebral cortex membranes; [^{3}H]ketanserin).[28]

Next, compounds **7–10** 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-carboxyamidotryptamine ([^{3}H]5-CT) from cloned human 5-HT_7 receptor expressed in COS-7 cells. The results, expressed as pK_i, are also summarized in Table 1.

As we have reported in the previous paper,[19] the 4-fluorophenyl derivative **7** is as potent as compound **1**. The 6-chloro-tetrahydrobenzindole **8** was as potent and selective as compound **1**, and had slightly higher affinity for the 5-HT_7 receptor than the 6-bromo-tetrahydrobenzindole analogue[19] (pK_i = 7.91 for the 5-HT_7 receptor). This result is consistent with our previous speculation that a large substituent at the 6-position of tetrahydrobenzindole reduces the affinity for the 5-HT_7 receptor. The dihalogenated compounds **9** and **10** also showed high affinity for the 5-HT_7 receptor. Moreover, compounds **9** and **10** were at least 2-fold more selective for the 5-HT_7 receptor than compound **1**.

On the basis of its metabolic stability and relative affinity for the 5-HT_7 and 5-HT_2 receptors, the dihalogenated compound **10** was selected for evaluation of binding ability to other 5-HT receptor subtypes. As can

Table 2. Receptor binding profile of **10**[a]

Receptor	Affinity (pK_i)[b]
5-HT_{1A}	6.50±0.14
5-HT_{1B}	<6
5-HT_{2C}	<6
5-HT_2	<6
5-HT_3	<6
5-HT_4	<6
5-HT_6	<6
5-HT_7	8.14±0.06

[a]Receptors and radioligands used in binding assay were as follows: 5-HT_{1A} [human recombinant (mammalian); [^{3}H]8-OH-DPAT];[29] 5-HT_{1B} [human recombinant (mammalian); [^{3}H]5-CT];[30] 5-HT_{2C} [human recombinant (mammalian); [^{3}H]mesulergine];[31] 5-HT_2 [rat cerebral cortex; [^{3}H]ketanserin];[28] 5-HT_3 [human recombinant (mammalian); [^{3}H]GR65630);[32,33] 5-HT_4 (guinea-pig striatum; [^{3}H]GR-113808);[34] 5-HT_6 [human recombinant (mammalian); [^{3}H]LSD);[35] 5-HT_7 [human recombinant (mammalian); [^{3}H]5-CT].
[b]The pK_i values are means±SE of 8–12 values.

be seen in Table 2, compound **10** showed low affinity for the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, 5-HT_4 and 5-HT_6 receptors. Thus, compound **10** was confirmed to be a high-affinity ligand for the 5-HT_7 receptor with high selectivity.

We next examined the effect of compound **10** on 5-HT-induced stimulation of cAMP accumulation in HEK293 cells expressing the human 5-HT_7 receptor. Intracellular cAMP formation was measured by enzyme-immunoassay. Compound **10** on its own did not stimulate basal activity, that is it lacked agonist activity, but it inhibited 5-HT-induced stimulation of cAMP accumulation (Fig. 1). Compound **10** is thus a 5-HT_7 receptor antagonist.

In summary, we have improved the in vitro metabolic stability of compound **1** by the chemical modification of sites expected to be susceptible to oxidative metabolism, with retention of high affinity and selectivity for the 5-HT_7 receptor. Among the compounds synthesized, compound **10**, an orally available 5-HT_7 antagonist (bioavailability = 18% in rats), with high selectivity over the 5-HT_2 receptor and other receptors, should be a useful tool for evaluating the therapeutic potential of 5-HT_7 antagonists.

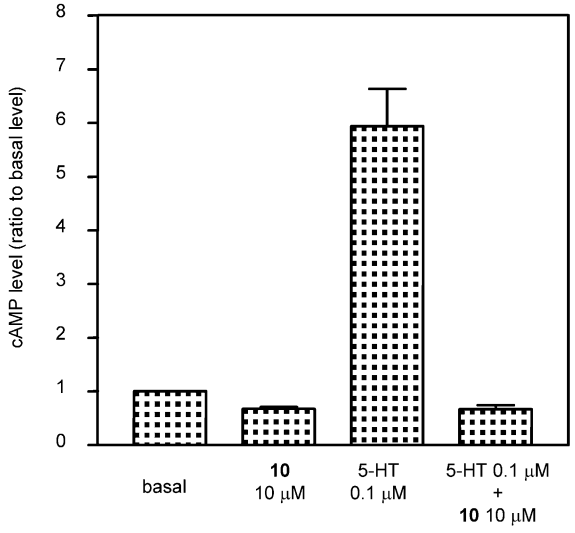


Figure 1. 5-HT-induced stimulation of cAMP accumulation in HEK273 cells expressing the 5-HT_7 receptor and its inhibition by compound **10**. Data represent the mean±SE of at least three determinations.

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24. The oral bioavailability of compound **1** was $<10\%$ in rats.
25. New compounds were characterized by ^1H NMR and MS. 2a-[4-[4-(4-Chlorophenyl)-1,2,3,6-tetrahydropyridyl]butyl]-6-chloro-2a,3,4,5-tetrahydrobenzo[*cd*]indol-2(1*H*)-one (**10**): ^1H NMR ($CDCl_3$) δ 1.06–1.18 (1H, m), 1.30–1.53 (4H, m), 1.71–2.00 (3H, m), 2.08–2.20 (2H, m), 2.30–2.42 (2H, m), 2.47–2.53 (2H, m), 2.59–2.66 (2H, m), 2.73–2.85 (2H, m), 3.04–3.11 (2H, m), 6.02 (1H, br s), 6.62 (1H, d, $J=8.0$ Hz), 7.15 (1H, d), 7.26 (1H, br s), 7.26–7.28 (4H, m); EIMS m/z 454, 456, 458 (M^+).
26. All incubations [rat liver or human liver microsomes, substrate, glucose-6-phosphate, β-$NADP^+$, G-6-P dehydrogenase, $MgCl_2$ $6H_2O$, phosphate buffer (pH 7.4) and EDTA Na_2] were performed on a gently shaking platform maintained at 37 °C. Incubations were started by the addition of substrate and were stopped after 0, 10, 30 and 60 min by addition of DMF. Precipitated proteins were removed by centrifugation and supernatants were injected into the HPLC system to determine the remaining amount of each compound.
27. This product was confirmed by LC–MS/MS. The 6-hydroxy-tetrahydrobenzindole derivative showed high affinity for the 5-HT_7 receptor, but this derivative had lower selectivity than compound **1**. See ref 19.
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