Novel 5-Hydroxytryptamine (5-HT$_3$) Receptor Antagonists. IV.[1] Synthesis and Pharmacological Evaluation of the Oxidation Products of (−)-(*R*)-5-[(1-Methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole Hydrochloride (YM060: Ramosetron)

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In physicochemical and pharmacokinetic evaluations of (−)-(*R*)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole hydrochloride 1 (YM060: ramosetron), which is a highly potent 5-hydroxytryptamine (5-HT$_3$) receptor antagonist, 4-hydroxy-6-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole 2 was identified as a degradation product and metabolite of 1. The (−)-(4*R*,6*S*)-isomer 2 was synthesized from the diketone derivative 3, *via* the stereoselective reduction of 3 followed by the stereocontrolled epimerization of the (−)-(4*S*,6*S*)-isomer 10, the epimer of 2. The stereochemistry of 2 and 10 was determined by NMR and HPLC studies. Compounds 2 and 10 were found to be potent 5-HT$_3$ receptor antagonists, like 1. Among the other oxidation products, the diketone derivatives 3 and 7 and the dihydroxylated derivative 4 retained antagonistic activity similar to that of ondansetron. This is of interest, because they do not possess the amine group which is known to be necessary for high affinity to the 5-HT$_3$ receptor.

Key words 4,5,6,7-tetrahydro-1*H*-benzimidazole; YM060 (ramosetron); 5-hydroxytryptamine receptor antagonist; degradation product; metabolite

In the preceding papers,[1] we reported a novel series of the 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives to be 5-hydroxytryptamine (5-HT$_3$) receptor antagonists. Among them, (−)-(*R*)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole hydrochloride **1** (YM060: ramosetron, Chart 1) was found to be a highly potent inhibitor of cisplatin-induced emesis in ferrets and restraint stress-induced increase in fecal pellet output in rats, thought to be mediated by endogenous 5-HT through the 5-HT$_3$ receptor.[1c] The compound **1** is under clinical trial.

In the physicochemical and pharmacokinetic evaluations of **1**, 4-hydroxy-6-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole **2** was found as one of the major degradation products under a variety of conditions, as well as one of the major metabolites in animals (Chart 1). For example, 0.08 or 0.3% (based on peak area in HPLC) of **2** was observed after irradiation of **1** (0.2 mg of **1** in 1 ml of 0.1 N NaOH) with a high-pressure mercury lamp for 1 h at 15 °C or the exposure of **1** (0.15 mg of **1** in 1 ml of H_2O, with NaCl (9.0 mg) and lactic acid (1.13 mg) as the other ingredients) to a near-ultraviolet fluorescent lamp for 6 d at room temperature, respectively. However, the stereochemistry of **2** was not determined completely.

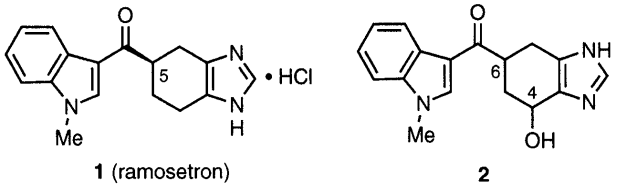

Chart 1

In this paper, we report the synthesis of the optically pure enantiomer **2** and related oxidized compounds, and the evaluation of their 5-HT$_3$ receptor-antagonistic activities.

Chemistry

Synthesis Oxidation at the benzylic position of the imidazole ring in **1** has been widely investigated for the production of a more oxidized diketone derivative **3** as well as the target compound **2** (Chart 2). Compound **3** was also found as a thermal or photolytic degradation product of **1**, and was expected to provide **2** upon stereoselective reduction. The oxidation was examined under several conditions: (1) with chemical oxidants [a) ceric ammonium nitrate (CAN), b) *N*-methylmorpholine *N*-oxide (NMO) or c) 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)], (2) by photolytic oxidation [d) without or e) with sensitizer], (3) by pyrolysis with O_2 and (4) by autoxidation with 2,2′-azobisisobutyronitrile (AIBN).

The chemical oxidants and the photolysis did not afforded **2** or **3** in sufficient yield. In the photolytic oxidation

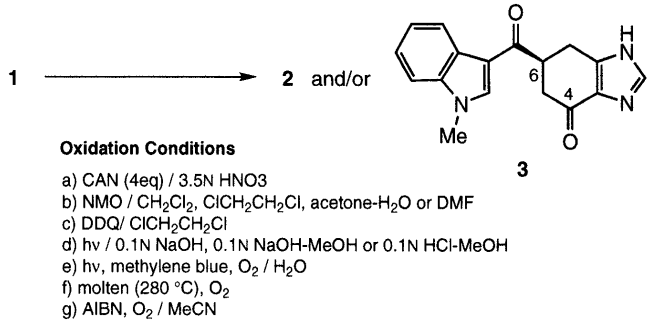

Chart 2

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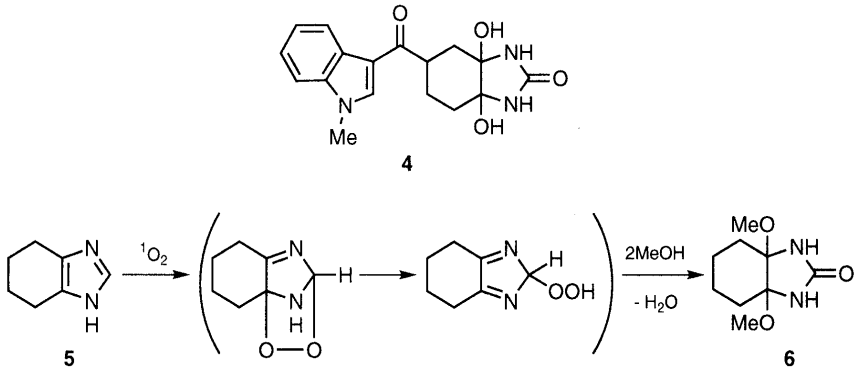


Chart 3

Table 1. Analytical Data

Compd. No.	HPLC analysis[a] Retention time (min)	HPLC analysis[a] Purity (area %)	mp (°C) (Recryst. solv.)	$[\alpha]_D^{20}$ (°) (Solv., *c*)	Formula	Elemental analysis Calcd (Found) C	H	N	Cl
4[b]	10.9	95.0	195—201 (H_2O)	—	$C_{17}H_{19}N_3O_4 \cdot 0.4H_2O$	60.67 (60.66)	5.93 5.81	12.49 12.30)	
3	13.0	96.4	237—239 (MeOH–MeCN)	+17.8 (DMF, 1.0)	$C_{17}H_{15}N_3O_2 \cdot 0.15H_2O$	68.98 (69.01)	5.21 5.13	14.19 14.31)	
7	10.8	99.7	242—244 (EtOH–AcOEt)	—	$C_{17}H_{15}N_3O_2 \cdot 0.4H_2O$	67.94 (67.99)	5.30 5.10	13.98 13.98)	
8	26.4	99.5	206—209 (MeOH–MeCN)	—	$C_{17}H_{13}N_3O \cdot HCl \cdot 0.8H_2O$	62.60 (62.55)	4.82 4.50	12.88 12.92	10.87 11.17)
9	30.6	99.4	>300 (dec.) (EtOH–AcOEt)	—	$C_{17}H_{15}N_3O_2 \cdot 0.4H_2O$	68.40 (68.41)	4.66 4.78	14.08 13.79)	
10	7.4 (5.6)[c]	99.2 (99.3)[c]	188—192 (EtOH–MeCN)	−28.2 (MeOH, 0.73)	$C_{17}H_{17}N_3O_4 \cdot HCl \cdot H_2O$	58.37 (58.29)	5.76 5.66	12.01 12.21	10.13 9.76)
2	7.2 (7.3)[c]	99.4 (99.5)[c]	209—211 (aq. HCl)	−41.5 (MeOH, 1.0)	$C_{17}H_{17}N_3O_2 \cdot HCl \cdot 0.75H_2O$	59.13 (59.08)	5.69 5.35	12.17 12.17	10.27 10.39)

a) Column, Nomura Chemical Develosil C8-5 (4.0 mm i.d. × 150 mm); temperature, 40 °C; detection, UV 254 nm; flow rate, 0.9 ml/min; eluent, 0.05 M KH_2PO_4 (pH 4.0)–MeOH–THF (8 : 3 : 1.5). *b*) Racemate. *c*) For separation of the epimers: Column, YMC A-K03 (4.6 mm i.d. × 250 mm); temperature, 34 °C; detection, UV 311 nm; flow rate, 1 ml/min; eluent, 0.05 M Na_2HPO_4–H_3PO_4 (pH 5.2)–MeCN (6 : 5).

of the racemate of **1** in H_2O with methylene blue as a sensitizer (condition e), 1,6-dihydroxy-3-[(1-methyl-1*H*-indol-3-yl)carbonyl]-8-oxo-7,9-diazabicyclo[4.3.0]nonane **4** was obtained as a major product (Chart 3). The mechanism of this reaction may be similar to that of formation of the dimethoxy derivative **6** from 4,5,6,7-tetrahydrobenzimidazole **5** in MeOH.[2] It was postulated that **5** is oxidized to endoperoxide by singlet oxygen, followed by ring-opening to form the hydroperoxide, then methanolysis to produce **6**.

Subsequently, the pyrolytic oxidation of **1** was examined in the molten state at 280 °C under O_2, which afforded *ca.* 1% of **3** in a small-scale experiment (see footnote in Table 1 for HPLC conditions). As a result of further investigation of the autoxidation of **1**, **3** was obtained in 20% (by HPLC) yield by the reaction of a suspension of **1** in MeCN with bubbling O_2 in the presence of a catalytic amount of AIBN, though *ca.* 60% of **1** remained and other oxidized derivatives **7**, **8** and **9** were also observed as major side reaction products (Chart 4). Pure **3** and **8** was obtained by column chromatography and crystallization. However, compounds **7** and **9** were not separated from **3**. Under this condition, **1** was recovered without racemization. Thus, compound **3** was speculated to be the *S*-isomer, showing a specific rotation of +17.8° in dimethylformamide (DMF).

Treatment of a mixture of **3**, **7** and **9** with $NaBH_4$ in EtOH gave a reduced compound **10** together with unreacted **7** and **9**, which were separable as pure compounds (Chart 4). However, the HPLC and NMR studies suggested **10** to be different from the target compound **2**; thus, **10** and **2** were concluded to be *syn*- and *anti*-isomer, respectively (see NMR studies and HPLC data in Tables 1 and 3). Although further approaches to the stereoselective reduction of the diketone derivative **3** were investigated, the *syn*-isomer **10** was exclusively produced [reagents: a) $NaBH_4$, b) Et_2BOMe–$NaBH_4$ or c) $LiEt_3BH$], or over-reduction was observed [reagent: d) $CeCl_3$–$NaBH_4$] (Chart 5).

The *syn*-isomer **10** was not detectable among metabolites or degradation products by HPLC analysis. Considering the mechanism of the stereoselective production of the *anti*-isomer **2** under metabolic or degradative conditions, active oxygen atom appears to react from the α-face of an intermediate such as A (Chart 6). This selectivity may be induced by the steric and/or electronic influence of the (1-methyl-3-indolyl)carbonyl group at the 5β-position of the sterically restricted 4,5,6,7-tetrahydro-1*H*-benzimidazole ring. Similarly, the exclusive α-attack of a hydride on the diketone derivative **3** might give the *syn*-isomer **10**, as

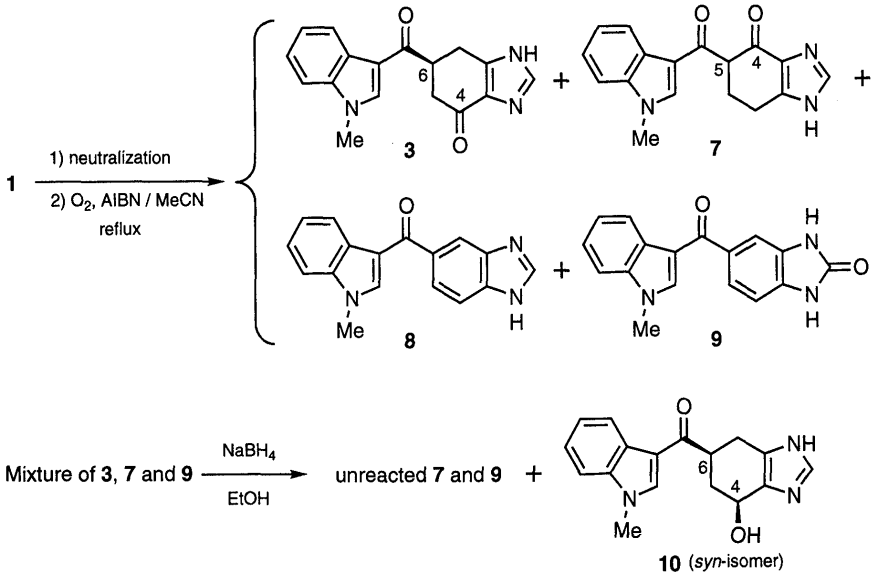


Chart 4

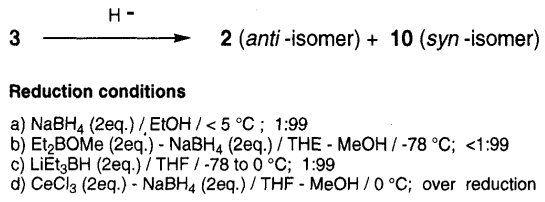


Chart 5

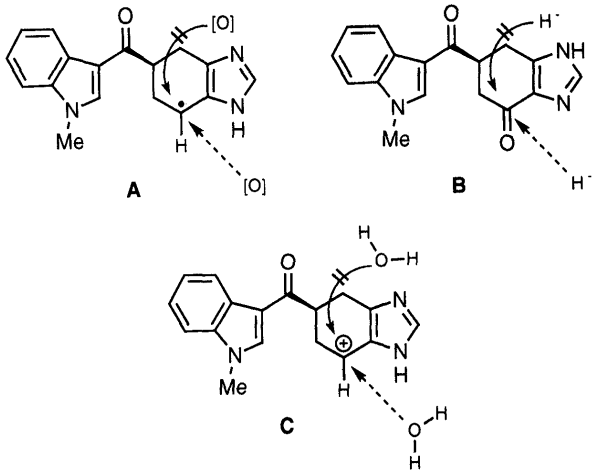


Chart 6

depicted by B in Chart 6.

These led us to attempt stereoselective epimerization of **10** to the *anti*-isomer **2** by the hydroxylation of a benzylic cation *via* an *S*N1-like transition state such as C (Chart 6). The reaction of *ca.* 0.15 M **10** in 1 N HCl at 70 °C in a sealed tube yielded a precipitate of the HCl salt of the *anti*-isomer **2** as a single isomer in 56% yield (Chart 7). Even under this condition, no racemization of **1** was observed. Consequently, the absolute configuration of **2** was supposed to be 4*R* (at the α-position of the hydroxy group) and 6*S* (at the α-position of the carbonyl group). The absolute configuration of **10** was also supposed to be 4*R* and 6*R*. Furthermore, the isomer **2** was shown to be identical with metabolite and degradation product by HPLC and NMR studies.

NMR Studies Detailed NMR studies (in DMSO-d_6) were performed to determine the conformation of **10** and **2**. Examination of the 1-D (^{1}H- and ^{13}C-NMR) and 2-D (correlation spectroscopies (COSY), heteronuclear multiple-bond correlation (HMBC) and heteronuclear single-quantum coherence (HSQC)) spectral data for **10** and **2** allowed a complete and unambiguous assignment of all protons and carbons. Observed coupling constants and nuclear Overhauser effect (NOE) data obtained by nuclear Overhauser enhancement spectroscopy (NOESY) for the protons at the cyclohexene ring of the 4,5,6,7-tetrahydro-1*H*-benzimidazole moiety in **10** and **2** are summarized in Chart 8.

In both **10** and **2**, coupling constants between $H^{6\alpha}$ and each of $H^{7\alpha}$, $H^{7\beta}$, $H^{5\alpha}$ or $H^{5\beta}$ suggest that $H^{7\beta}$ and $H^{5\beta}$ exist *anti*-periplanar to $H^{6\alpha}$, and that $H^{7\beta}$, $H^{6\alpha}$ and $H^{5\beta}$ have axial or *pseudo*-axial orientation. In addition, NOE was observed between $H^{6\alpha}$ and each of $H^{2'}$, $H^{7\alpha}$ or $H^{5\alpha}$. These observations indicate that the cyclohexene ring in the 4,5,6,7-tetrahydro-1*H*-benzimidazole moiety of **10** and **2** takes the twist conformation, and that the indole-carbonyl moiety is in the 6β (equatorial) position (Chart 8). Similar results were observed for **1** in the NMR study, and twist conformation for **1** was confirmed by X-ray analysis.[1c]

It is noteworthy that NOE between $H^{4\alpha}$ and each of $H^{6\alpha}$ and $H^{5\alpha}$ was observed for **10**. In contrast, for **2**, NOE between $H^{4\beta}$ and $H^{6\beta}$ was observed, but not between $H^{4\beta}$ and either of $H^{6\alpha}$ or $H^{5\alpha}$. In addition, the coupling constant between $H^{4\alpha}$ and $H^{5\alpha}$ or $H^{5\beta}$ for **10** was larger than that between $H^{4\beta}$ and $H^{5\alpha}$ or $H^{5\beta}$ for **2**. Downfield shifts of $H^{5\alpha}$ for **10** relative to **2** and of $H^{6\alpha}$ for **2** relative to **10** were found, due to the influence of the oxygen atom. These results identify **10** as the *syn*-isomer and **2** as the *anti*-isomer.

Pharmacological Results

We evaluated the 5-HT_3 receptor-antagonistic activities of **1**—**4** and **7**—**10** by making use of the von Bezold-Jarisch

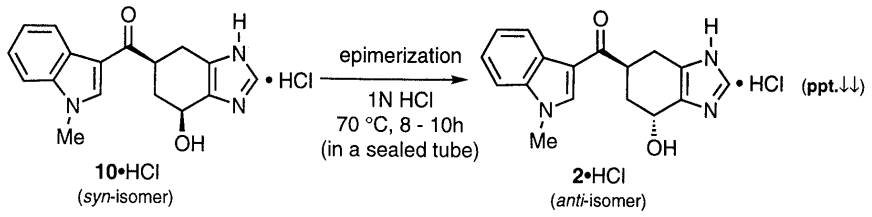
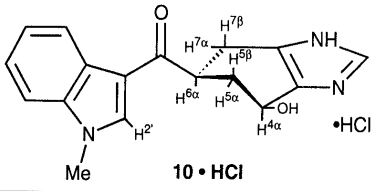
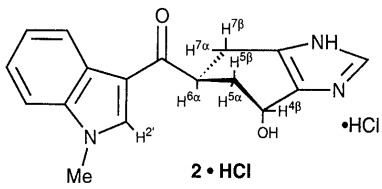


Chart 7



δ (ppm)	J (Hz)	NOE
1.78 ($H^{5\beta}$, ddd)	$H^{7\beta}$ - $H^{7\alpha}$: 16.8	$H^{7\beta} \leftrightarrow H^{7\alpha}$
2.34 ($H^{5\alpha}$, ddd)	$H^{7\beta}$ - $H^{6\alpha}$: 10.9	$H^{7\beta} \leftrightarrow H^{5\beta}$
2.81 ($H^{7\alpha}$, dd)	$H^{7\alpha}$ - $H^{6\alpha}$: 5.5	$H^{7\alpha} \leftrightarrow H^{6\alpha}$
2.91 ($H^{7\beta}$, dd)	$H^{6\alpha}$ - $H^{5\beta}$: 12.8	$H^{6\alpha} \leftrightarrow H^{5\alpha}$
3.82 ($H^{6\alpha}$, m)	$H^{6\alpha}$ - $H^{5\alpha}$: 1.5	$H^{6\alpha} \leftrightarrow H^{4\alpha}$
5.00 ($H^{4\alpha}$, dd)	$H^{5\beta}$ - $H^{5\alpha}$: 12.8	$H^{5\beta} \leftrightarrow H^{5\alpha}$
	$H^{5\beta}$ - $H^{4\alpha}$: 9.7	$H^{5\alpha} \leftrightarrow H^{4\alpha}$
	$H^{5\alpha}$ - $H^{4\alpha}$: 6.7	$H^{6\alpha} \leftrightarrow H^{2'}$



δ (ppm)	J (Hz)	NOE
2.04 ($H^{5\beta}$, ddd)	$H^{7\beta}$ - $H^{7\alpha}$: 16.4	$H^{7\beta} \leftrightarrow H^{7\alpha}$
2.17 ($H^{5\alpha}$, ddd)	$H^{7\beta}$ - $H^{6\alpha}$: 10.0	$H^{7\beta} \leftrightarrow H^{5\beta}$
2.86 ($H^{7\alpha}$, dd)	$H^{7\alpha}$ - $H^{6\alpha}$: 5.0	$H^{7\alpha} \leftrightarrow H^{6\alpha}$
2.89 ($H^{7\beta}$, dd)	$H^{6\alpha}$ - $H^{5\beta}$: 12.0	$H^{6\alpha} \leftrightarrow H^{5\alpha}$
3.98 ($H^{6\alpha}$, m)	$H^{6\alpha}$ - $H^{5\alpha}$: 2.7	$H^{5\beta} \leftrightarrow H^{5\alpha}$
4.81 ($H^{4\beta}$, dd)	$H^{5\beta}$ - $H^{5\alpha}$: 14.0	$H^{4\beta} \leftrightarrow H^{5\beta}$
	$H^{5\beta}$ - $H^{4\beta}$: 3.7	$H^{6\alpha} \leftrightarrow H^{2'}$
	$H^{5\alpha}$ - $H^{4\beta}$: 3.0	

Chart 8. Chemical Shift, Coupling Constant and NOE Data by NOESY for **10** and **2** in DMSO-d_6

Table 2. 5-HT_3 Receptor-Antagonistic Activities

Compd. No.	ID_{50} (μg/kg i.v.) of B. J. reflex[a] (95% confidence limit)	IC_{50} (μM) of colon contraction[b] (95% confidence limit)
4[c]	4.2 (3.5—5.2)	3.7 (2.8—4.9)
3	1.9 (1.2—3.1)	2.1 (1.5—3.0)
7	2.8 (1.6—5.1)	8.8 (7.7—10.0)
8	>30	8.1 (6.8—9.6)
9	>30	>10
10	0.063 (0.055—0.072)	0.098 (0.081—0.11)
2	0.040 (0.029—0.053)	0.18 (0.13—0.24)
1	0.036 (0.031—0.041)	0.012 (0.005—0.028)
Ondansatron	1.9 (1.5—2.4)	0.78 (0.43—1.4)

a) Vagally-mediated bradycadia induced by 5-HT (30 μg/kg, i.v.) in rats. *b*) Contraction of isolated guinea-pig colon induced by 5-HT (5×10^5 M). *c*) Racemate.

reflex (B.J. reflex) induced by 5-HT[3] and the contractile responses to 5-HT in the isolated distal colon[4] (Table 2). These effects are mediated by activation of the neuronal 5-HT_3 receptor. Data are presented as the ID_{50} values (μg/kg) on the B.J. reflex induced by 5-HT (30 μg/kg, iv) in anesthetized rats and as the IC_{50} values (μM) on the contraction of the isolated guinea-pig colon by 5-HT (50 μM).

The *anti*-isomer **2** and its epimer **10** were found to be highly potent antagonists, like **1**, of the B.J. reflex, whereas they were 15 and 8 times less potent than **1** in terms of effect on colon contraction, respectively. The diketone derivatives **3** and **7** showed weaker antagonistic activity than that of **1**, but similar activity on the B.J. reflex to that of ondansetron,[5] which is in clinical use to inhibit nausea and vomiting associated with cancer chemotherapy. In general, 5-HT_3 receptor antagonists seem to require a strongly basic amine (or quarternary salt) to exhibit the activity.[6] It is of interest that the active compounds **3** and **7** do not possess strong basicity in the imidazole ketone part (*e.g.*, pK_a values of 2.90 and 7.56 for 4-formylimidazole and 4-methylimidazole, respectively).[7] More interestingly, the dihydroxylated compound **4**, which has no basic amine, retained antagonistic activity on the B.J. reflex with an ID_{50} value of 4.2 μg/kg.

Conclusion

We prepared the *anti*-(4*R*,6*S*)-isomer **2** from the diketone derivative **3**, *via* the stereoselective reduction of **3** followed by the stereocontrolled epimerization of the *syn*-(4*S*,6*S*)-isomer **10**. Compound **2** was shown to be identical with the degradation product as well as the metabolite of optically active **1** (YM060: ramosetron). Compounds **2** and **10** were found to be highly potent 5-HT_3 receptor antagonists, like **1**. Among the other oxidized compounds, **3**, **7** and **4** interestingly retained antagonistic activity similar to that of ondansetron, even though they do not possess the amine group as a strong base believed to be necessary for high affinity to the 5-HT_3 receptor.

Experimental

All melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. NMR spectra were measured with a JEOL JNM-A500 FT NMR spectrometer. Chemical shifts are recorded in δ units from tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, d = doublet, m = multiplet, dd = double

Table 3. Mass and ^{1}H-NMR Spectral Data

Compd. No.	FAB-MS m/z ($M^+ + 1$)	^{1}H-NMR (DMSO-d_6) δ (ppm) and J (Hz)
4	330	1.52 (1H, m), 1.65 (1H, t, J=9 Hz), 1.74 (2H, m), 1.93 (1H, m), 2.13 (1H, d, J=9 Hz), 3.20 (1H, m), 3.88 (3H, s), 5.04 (1H, s), 5.61 (1H, s), 6.84 (1H, s), 6.86 (1H, s), 7.23 (1H, t, J=6 Hz), 7.28 (1H, t, J=6 Hz), 7.53 (1H, d, J=6 Hz), 8.20 (1H, d, J=6 Hz), 8.33 (1H, s)
3	294	2.56 (1H, dd, J=4, 16 Hz), 2.77 (1H, m), 3.08 (2H, br d), 3.87 (3H, s), 4.17 (1H, m), 7.23—7.31 (2H, m), 7.55 (1H, d, J=8 Hz), 7.78, 7.92 (1H, br), 8.20 (1H, d, J=8 Hz), 8.55 (1H, s), 12.55, 13.10 (1H, br)
7	294	2.31 (1H, m), 2.45 (1H, m), 2.87 (1H, m), 2.96 (1H, m), 3.88 (3H, s), 4.43 (1H, m), 7.24 (1H, t, J=8 Hz), 7.30 (1H, t, J=8 Hz), 7.56 (1H, d, J=8 Hz), 7.87 (1H, s), 8.20 (1H, d, J=8 Hz), 8.44 (1H, s), 13.00 (1H, br)
8	276	3.90 (3H, s), 7.32 (1H, t, J=8 Hz), 7.37 (1H, t, J=8 Hz), 7.61 (1H, d, J=8 Hz), 7.97 (1H, d, J=8 Hz), 7.99 (1H, d, J=8 Hz), 8.09 (1H, s), 8.21 (1H, s), 8.27 (1H, d, J=8 Hz), 9.66 (1H, s)
9	292	3.86 (3H, s), 7.06 (1H, d, J=8 Hz), 7.26 (1H, t, J=8 Hz), 7.32 (1H, t, J=8 Hz), 7.36 (1H, s), 7.50 (1H, d, J=8 Hz), 7.57 (1H, d, J=8 Hz), 8.03 (1H, s), 8.23 (1H, d, J=8 Hz), 10.82 (1H, s), 0.94 (1H, s)
10	296	1.78 (1H, ddd, J=10, 13, 13 Hz), 2.34 (1H, ddd, J=2, 7, 13 Hz), 2.81 (1H, dd, J=6, 17 Hz), 2.91 (1H, dd, J=11, 17 Hz), 3.82 (1H, m), 3.90 (3H, s), 5.00 (1H, dd, J=7, 9 Hz), 7.26 (1H, t, J=8 Hz), 7.31 (1H, t, J=8 Hz), 7.57 (1H, d, J=8 Hz), 8.23 (1H, d, J=8 Hz), 8.58 (1H, s), 9.01 (1H, s)
2	296	2.04 (1H, ddd, J=4, 12, 14 Hz), 2.17 (1H, ddd, J=3, 3, 14 Hz), 2.86 (1H, dd, J=5, 16 Hz), 2.89 (1H, dd, J=10, 16 Hz), 3.90 (3H, s), 3.98 (1H, m), 4.81 (1H, dd, J=3, 4 Hz), 7.26 (1H, t, J=8 Hz), 7.31 (1H, t, J=8 Hz), 7.57 (1H, d, J=8 Hz), 8.24 (1H, d, J=8 Hz), 8.47 (1H, s), 9.01 (1H, s)

doublet and ddd=double double doublet. Coupling constants were evaluated by first-order rules with an estimated accuracy of 0.5 Hz. Mass spectra were recorded with a JEOL JMS-DX300 (FAB) spectrometer. Elemental analyses were performed with a Yanaco MT-5. Specific rotations were measured with a Horiba SEPA-200 polarimeter. HPLC analysis was performed using a Nomura Chemical Co., Ltd. Develosil C8-5 (4.0 mm i.d. × 15 cm) column with 0.05 M KH_2PO_4 (pH 4.0)–MeOH–THF (8:3:1.5) as the eluent and, for the separation of the epimers, an optically active YMC A-K03 (4.6 mm i.d. × 25 cm) column with 0.05 M Na_2HPO_4–H_3PO_4 (pH 5.2)/CH_3CN as the eluent. All organic solvent extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator under reduced pressure. Analytical data for **2**, **3**, **4** and **7**—**10** are summarized in Tables 1 and 3.

1,6-Dihydroxy-3-[(1-methyl-1*H*-indol-3-yl)carbonyl]-8-oxo-7,9-diazabicyclo[4.3.0]nonane (4) The photooxidation reaction of (±)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole hydrochloride (racemate of **1**)[1b] (10.0 g, 31.7 mmol) in H_2O (1150 ml) was carried out at 0—5 °C with methylene blue (30 mg) as sensitizer for 23 h. Oxygen gas was passed through the solution during irradiation with a high-pressure mercury lamp. The resulting solid mass, sticking to the walls of the lamp, was collected and washed with $CHCl_3$ to give 0.50 g (4.9% yield) of **4** as a single isomer. HPLC analysis: 95.0 area %.

(+)-(*S*)-6-[(1-Methyl-1*H*-indol-3-yl)carbonyl]-6,7-dihydro-1*H*-benzimidazol-4(5*H*)-one (3), 5-[(1-Methyl-1*H*-indol-3-yl)carbonyl]-6,7-dihydro-1*H*-benzimidazol-4(5*H*)-one (7), 5-[(1-Methyl-1*H*-indol-3-yl)-carbonyl]-1*H*-benzimidazole (8), 5-[(1-Methyl-1*H*-indol-3-yl)carbonyl]-2,3-dihydro-1*H*-benzimidazol-2-one (9), and (−)-(4*S*,6*S*)-4-Hydroxy-6-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole Hydrochloride (10) (i) Oxygen gas was bubbled through a mixture of (−)-(*R*)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole (free base of **1**)[1c] (27.9 g, 100 mmol) and AIBN (0.5 g, 3.0 mmol) in MeCN (500 ml) for 10 h under reflux, and the mixture was left overnight at room temperature without bubbling of O_2. After addition of AIBN (1.5 g, 9.0 mmol), the mixture was heated again under reflux for 12 h with bubbling of O_2 and concentrated. The residue was subjected to silica gel column chromatography ($CHCl_3$–MeOH). First, each fraction that contained >75% (as peak area in HPLC) of the free base of **8** was collected, concentrated and treated with 4 N HCl–MeOH in MeCN to give 1.42 g (4.6% yield) of **8** as a solid. HPLC analysis: 99.5%. Second, each fraction that contained >70% (HPLC) of **3** was collected, concentrated and crystallized with MeCN to give 1.35 g (4.6% yield) of **3** as a solid. HPLC analysis: 96.4%. Third, the fractions that contained **3**, **7** and **9** were collected and concentrated to give 3.9 g of a mixture of **3**, **7** and **9** (53, 32 and 7% based on peak area in HPLC, respectively).

(ii) The mixture of **3**, **7** and **9** (2.0 g) obtained above was suspended in EtOH (40 ml), and $NaBH_4$ (0.16 g, 4.2 mmol) was added portionwise to the suspension at below 5 °C. The mixture was stirred for 30 min, then 1 N HCl (20 ml), 1 N NaOH (20 ml) and $CHCl_3$ (100 ml) were added. The organic layer was dried and concentrated, and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH). The product was treated with 8.5 N HCl–EtOH in MeCN to give 407 mg of **10** as a solid. HPLC analysis: 99.2%.

Unreacted **7** and **9** (0.70 g) were also recovered as a mixture from the above column chromatography treatment, and were separated by another silica gel column chromatography (AcOEt–MeOH–conc. NH_4OH), then crystallized from EtOH–AcOEt to give 155 mg of **7** as a solid (HPLC analysis: 99.7%) and 70 mg of **9** as a solid (HPLC analysis: 99.4%).

(−)-(4*R*,6*S*)-4-Hydroxy-6-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole Hydrochloride (2) A solution of **10** (108 mg, 0.309 mmol) in 1 N HCl (2 ml) was heated for 8 h at 70 °C in a sealed tube, then cooled to below 5 °C. The precipitates were collected by filtration and washed with MeCN, EtOH–MeCN and EtOH to give 60 mg (56% yield) of **2** as a solid. HPLC analysis: 99.4%.

Biological Methods Doses are expressed in terms of free base. 5-HT was purchased from E. Merck (Darmstadt, FRG) as creatinine sulfate.

B. J. Reflex Test[3] Male Wistar rats weighing 200 to 250 g were anesthetized with urethane (1.25 g/kg i.p.), and the trachea was cannulated. Arterial blood pressure and heart rate were recorded on a polygraph through a pressure transducer and a cardiotachometer, respectively, connected to a catheter placed in the carotid artery. The femoral vein was also cannulated for drug injection. 5-HT at a dose of 30 μg/kg was intravenously administered to rats at intervals of 15 min. After a stable response to 5-HT had been obtained, drugs were intravenously administered to rats 10 min before 5-HT injection.

Contraction of Isolated Guinea Pig Colon[4] The distal portion of the colon was removed from a Hartley guinea pig (300 to 500 g), cleaned in fresh Krebs–bicarbonate buffer at room temperature and then divided into segments of approximately 20 mm. Isomeric contraction under a loading tension of 1 g was recorded. Submaximal contraction was first elicited by repeated application of 10^{-6} M 5-HT until a constant response was obtained. A test compound was added to the bath after the concentration-response curve for 5-HT had been obtained. The tissue was exposed to the test compound for 30 min before rechallenge with 5-HT (control). Each test compound was examined at one or two concentrations in the same preparation.

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