Pyrazole and Isoxazole Derivatives as New, Potent, and Selective 20-Hydroxy-5,8,11,14-eicosatetraenoic Acid Synthase Inhibitors

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In a previous paper, we reported the *N*-hydroxyformamidine derivative HET0016 as a potent and selective 20-HETE synthase inhibitor. Despite its attraction as a potential therapeutic agent for cerebral diseases, the preparation of an injectable formulation of HET0016 was limited by its poor solubility under neutral conditions and instability under acidic conditions. The instability of HET0016 in acidic conditions is due to the *N*-hydroxyformamidine moiety, which is considered to be essential for the potent and selective activity seen in our previous study. The activity was maintained when the *N*-hydroxyformamidine moiety was replaced by an imidazole ring (**3a**; $IC_{50} = 5.7 \pm 1.0$ nM), but this was associated with a loss of selectivity for cytochrome P450s (CYPs). However, other azole derivatives such as isoxazole derivative **23** (IC_{50} value 38 ± 10 nM) and pyrazole derivative **24** (IC_{50} value 23 ± 12 nM) showed potent and selective activities with improved stability.

Introduction

20-Hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) is a major metabolite of arachidonic acid (AA) produced in the kidney,[1] and its biological properties have recently been extensively studied. The formation of 20-HETE from AA is reportedly catalyzed by cytochrome P450 (CYP) 4A isozymes (CYP4A1, CYP4A2, CYP4A3, and CYP4A8) in rat kidney,[2] and by CYP4A11 and CYP4F2 in human liver and kidney.[3] 20-HETE plays an important role in the regulation of renal vascular and tubular functions[4−6] and contributes to the control of arterial blood pressure.[7] More recent studies have indicated that 20-HETE is also produced in the brain, where it regulates vascular tone and contributes to the regulation of cerebral blood flow.[8] Therefore, the inhibition of 20-HETE is now considered a promising new target for the treatment of renal and cerebral diseases. Some compounds are known to inhibit the production of 20-HETE (Figure 1). These compounds are not potent or specific inhibitors for 20-HETE formation. 17-Octadecynoic acid (17-ODYA)[9] inhibits the ω-hydroxylation of AA; however, its inhibitory effect is not specific for 20-HETE formation, since it also inhibits the formation of epoxyeicosatrienoic acids (EETs), which are produced by epoxyganases (CYP1A, CYP2B, CYP2C, and CYP2J families) from AA.[10,11] 1-Aminobenzotriazole (1-ABT)[12] inhibits the catalytic activity of CYPs and also inhibits the formation of 20-HETE. *N*-Methylsulfonyl-12,12-dibromododec-11-enamide (DDMS)[13] and 12,12-dibromododec-11-enoic acid (DBDD)[13] inhibit the formation of 20-HETE with an IC_{50} value of 2 μM, whereas the IC_{50} values for epoxidation of AA were 60 and 51 μM. Sodium 10-undecynyl sulfate (10-SUYS)[14] inhibits the formation of 20-HETE with an IC_{50} value of 10.1 ± 2.6 μM, whereas it does not affect epoxigenase activity at concentrations up to 50 μM.

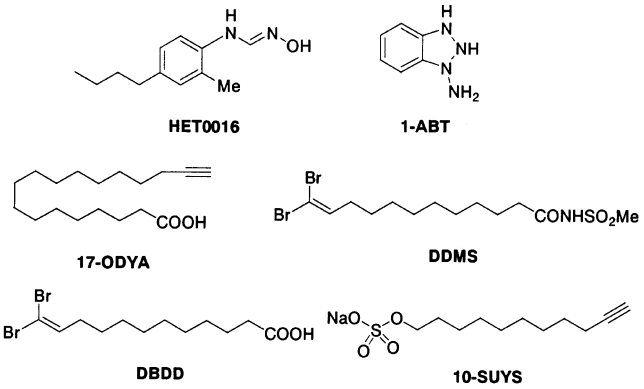

Figure 1. Structures of HET0016, 1-ABT, 17-ODYA, DDMS, DBDD, and 10-SUYS.

In a previous paper, we reported HET0016 (*N*-hydroxy-*N*′-(4-*n*-butyl-2-methylphenyl)formamidine) as the first potent and selective 20-HETE synthase inhibitor.[15] The IC_{50} value of HET0016 for the formation of 20-HETE by rat renal microsomes was 35.2 ± 4.4 nM, while its IC_{50} value for inhibition of the formation of EETs was 2800 nM.[11] HET0016 had very little effect on the activities of CYP2C9, CYP2D6, CYP3A4, or cyclooxygenase (COX), even at higher concentrations (100 μM).[11] HET0016 prevented the acute fall in cerebral blood flow following subarachnoid hemorrhage (SAH) in the rat.[16] These results indicate that HET0016 is a potent and selective inhibitor of the CYP enzymes that catalyze the formation of 20-HETE from AA and possesses attractive properties for the treatment of cerebrovascular diseases.

Despite its promising pharmacological properties, HET0016 is not soluble enough for injectable formulations under neutral conditions. The solubility of HET0016 is increased under acidic conditions due to the basicity

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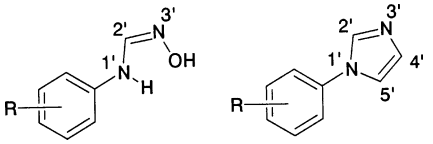


Figure 2. Comparison of the *N*-hydroxyformamidine derivative and the imidazole derivative.

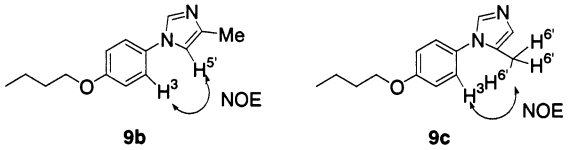


Figure 3. Observed NOE enhancements between C$^{5'}$H and C^{3}H in **9b**, and observed NOE enhancements between C$^{6'}$H and C^{3}H in **9c**.

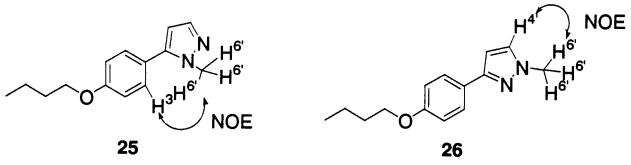


Figure 4. Observed NOE enhancements between C$^{6'}$H and C^{3}H in **25**, and observed NOE enhancements between C$^{6'}$H and C$^{4'}$H in **26**.

of the *N*-hydroxyformamidine moiety; however, this is accompanied by rapid decomposition of the *N*-hydroxyformamidine moiety. Therefore, we have attempted to replace the *N*-hydroxyformamidine moiety with some other acid-stable pharmacologically isostatic moiety. According to previous X-ray analytic reports on some *N*-hydroxyformamidine derivatives,[17] the amidine moiety of HET0016 may exist in a cis configuration, which is structurally similar to a 1,3-azole ring (Figure 2). Among various 1,3-azoles, imidazole derivatives are known to be potent CYP inhibitors, and an imidazole ring may be more stable than an *N*-hydroxyformamidine moiety under acidic conditions. Therefore, we examined replacement of the *N*-hydroxyformamidine moiety of HET0016 by an imidazole ring and evaluated its biological properties in vitro.

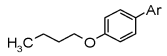
Chemistry

To begin the synthesis of azole derivatives, the substituent on the phenyl ring of HET0016 was changed to a 4-*n*-butoxy moiety for ease of synthesis. *N*-(4-*n*-Butoxyphenyl)-*N*′-hydroxyformamidine **1** shows potent and selective 20-HETE synthase inhibitory activity, like HET0016 (Table 1), and is suitable as a template compound instead of HET0016.

Compounds **3a**−**e** were prepared according to the method shown in Scheme 1. Reaction of 1-(4-hydroxyphenyl)imidazole **2a** with 1-iodobutane and potassium carbonate in *N*,*N*-dimethylformamide afforded the imidazole **3a**, and phenol derivatives **2b**, **2c**,[18] **2d**,[19] and **2e**[20] were treated under the same conditions to give the corresponding 4-*n*-butoxyphenyl derivatives **3b**−**e**.

Compounds **9a**−**d** were prepared according to the method shown in Scheme 2. 1-(4-Methoxyphenyl)-2-methylimidazole **7a** was obtained from 2-methylimidazole **4** by a diamine−copper complex-catalyzed coupling reaction with 4-methoxyboronic acid.[21] 1-(4-Methoxyphenyl)-4-methylimidazole **7b** and 1-(4-methoxyphenyl)-5-methylimidazole **7c** were obtained as a mixture by a reaction similar to that for **7a** from 4-methylimidazole **5**. 2-(4-Methoxyphenyl)pyrazine **7d** was obtained from 2-chloropyrazine **6** by Suzuki coupling.[22] 4-Methoxyphenyl derivatives **7a**−**d** were demethylated by heating with HBr, and subsequent alkylation with 1-iodobutane gave the 4-*n*-butoxyphenyl derivatives **9a**−**d**. A mixture of **7b** and **7c** was converted to a mixture of **8b** and **8c**, and **8c** was isolated by recrystallization from methanol. Alkylation of a mixture of **8b** and **8c** gave a mixture of **9b** and **9c**, and **9b** was isolated

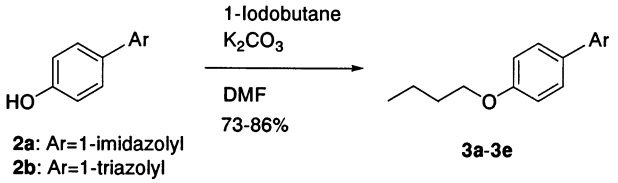
Table 1. Inhibition of AA Metabolism Involving Human 20-HETE Synthesizing Enzyme and Inhibitory Activities against Drug-Metabolizing CYPs by New Heterocyclic Compounds (1)



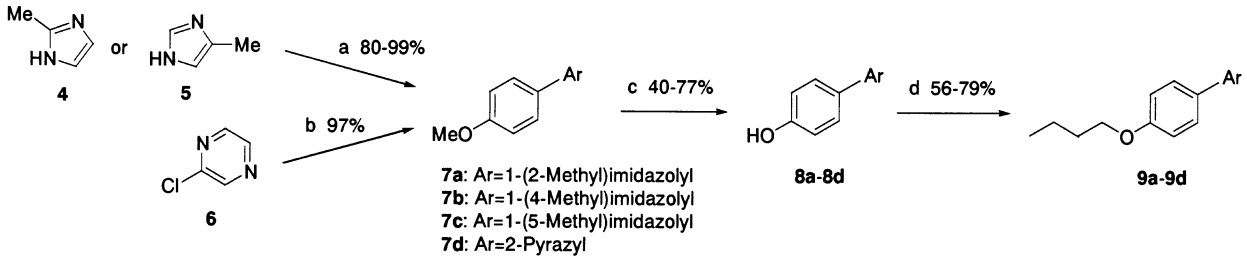
compd	Ar	IC_{50} (nM)[a]	P450 inhibition IC_{50} (nM)[b]				
			CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
HET0016		3.5 ± 0.7	461	4 170	272	84 500	65 400
1		2.3 ± 0.7	9 980	19 900	270	>100 000	>100 000
3a		5.7 ± 1.0	7.0	96	7.0	460	348
9a[c]		>300	8 120	10 200	770	39 800	50 900
9b[c]		143 ± 17	386	6 880	360	7 810	9 170
9c[c]		5.9 ± 2.6	<46	88	<46	120	116
3b		177 ± 60	283	2 800	68	9 540	>100 000
3c		>1 000	487	26 400	3 570	88 700	>100 000
3d[c]		27 ± 9.6	<46	7 520	8 440	12 550	29 300
3e		803 ± 100	<46	<46	<46	398	51 100
11		23 ± 14	1 100	>100 000	<46	6 000	883
12		>1 000	25 500	27 200	18 700	940	44 300
13		32 ± 25	<46	<46	<46	1 010	58 100
21		>1 000	NT	NT	NT	NT	NT
23		38 ± 10	3 230	8 000	5 510	86 350	>100 000
16		>1 000	2 550	43 300	3 680	85 500	>100 000
29		98 ± 24	1 260	<46	<46	10 800	22 900
24		23 ± 12	5 650	19 600	3 160	93 500	70 600
9d		115 ± 35	<46	36 800	7 860	6 590	>100 000
30		>1 000	<46	<46	<46	500	>100 000
18		>300	5 850	25 000	6 080	53 300	7 520
35		>1 000	>100 000	10 200	316	>100 000	>100 000

[a] IC_{50} value for 20-HETE production from AA by human renal microsome. Data are calculated from at least triplicate observations and are presented as a mean ± SE. [b] IC_{50} was estimated for each test substance and each enzyme according to the method of Crespi et al.[36] Data are calculated from triplicate observations. [c] These compounds were evaluated as a *p*-toluenesulfonic acid salt.

Scheme 1

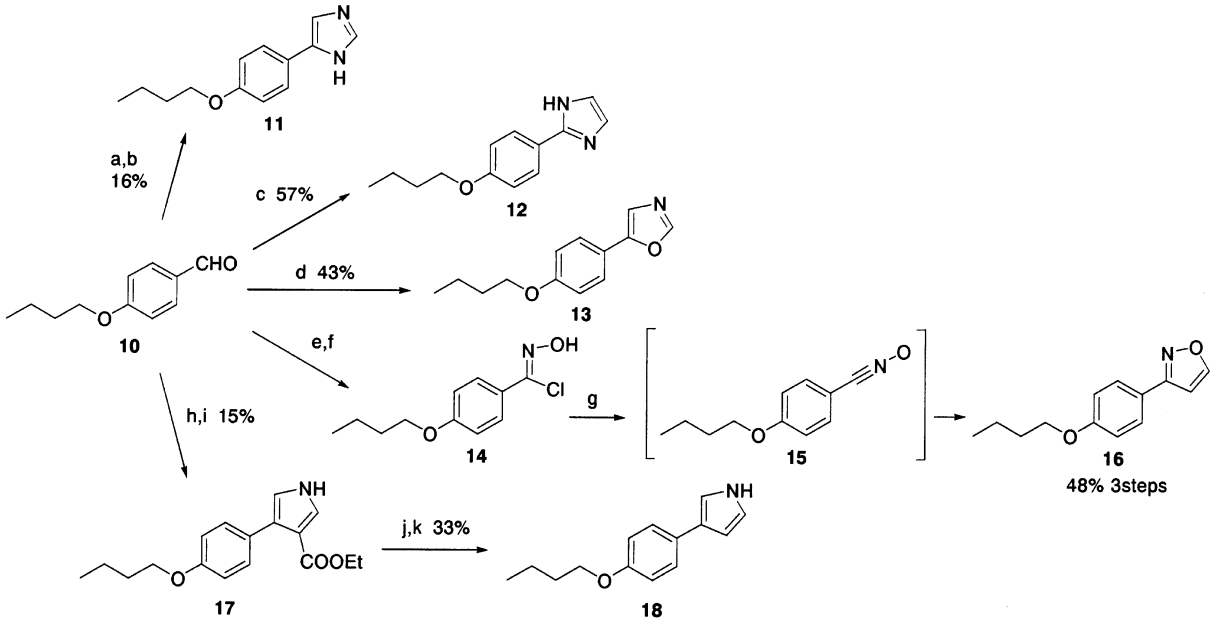


Scheme 2



[a] Reagents: (a) 4-methoxyphenylboronic acid, O_2, $(Cu(OH)\text{-}TMEDA)_2Cl_2$, CH_2Cl_2; (b) 4-methoxyphenylboronic acid, $Pd(OAc)_2$, PPh_3, K_2CO_3, DME; (c) HBr, reflux; (d) 1-iodobutane, K_2CO_3, DMF.

Scheme 3



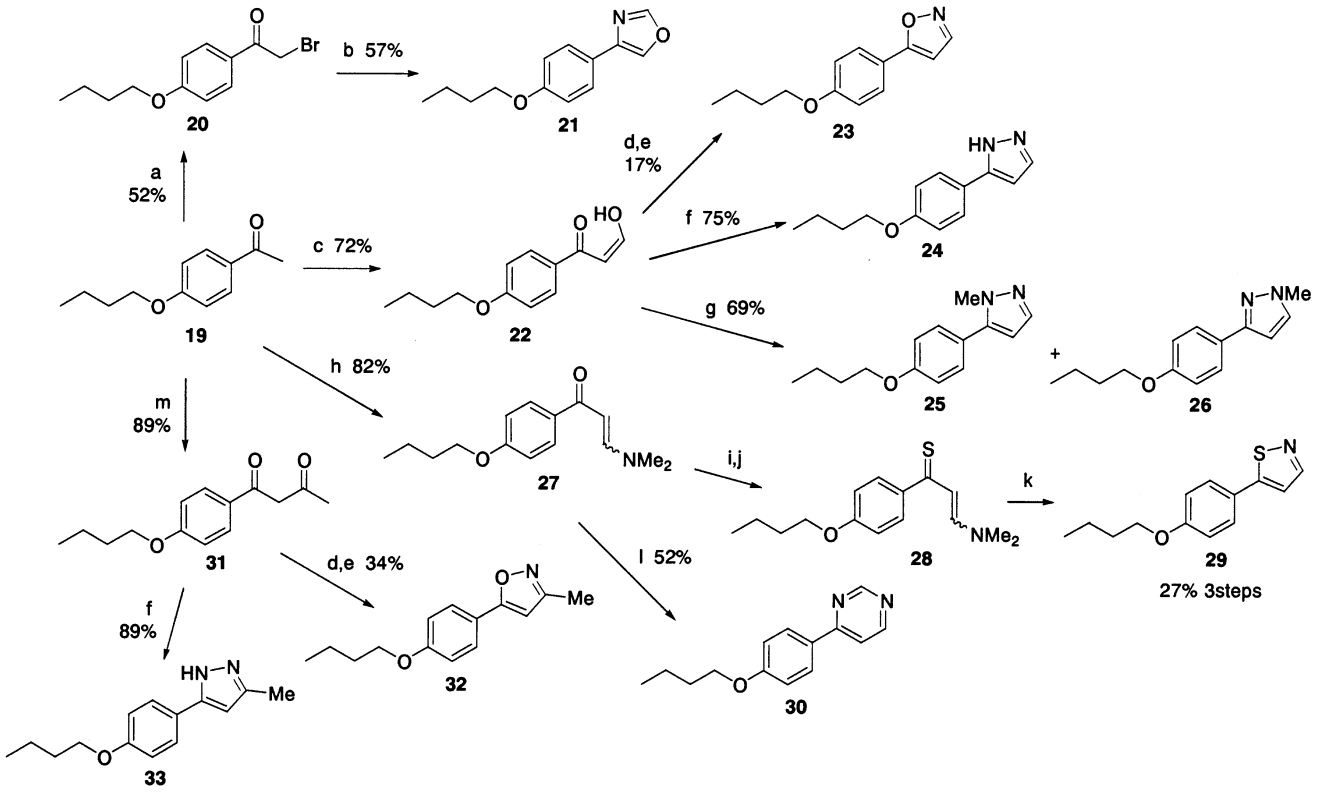
[a] Reagents: (a) p-tosylmethyl isocyanide, NaCN, EtOH; (b) NH_3, MeOH, 110 °C; (c) glyoxal, NH_4OH, $EtOH/H_2O$; (d) *p*-tosylmethyl isocyanide, NaOMe, MeOH, reflux; (e) NH_2OH, $MeOH/H_2O$; (f) tBuOCl, CCl_4; (g) acetylene, Et_3N, benzene; (h) diethyl cyanomethylphosphonate, NaH, toluene; (i) *p*-tosylmethyl isocyanide, NaH, Et_2O/DMSO, reflux; (j) KOH, $EtOH/H_2O$; (k) ethanolamine, 180 °C.

as a *p*-toluenesulfonic acid salt by recrystallization from ethyl acetate. The regiochemistries of **9b** and **9c** were confirmed by an NOE study (Figure 3).[23]

Compounds **11**−**13**, **16**, and **18** were prepared according to the method shown in Scheme 3. Treatment of 4-*n*-butoxybenzaldehyde **10** with tosylmethylisocyanide (TosMIC) and a catalytic amount of sodium cyanide in ethanol afforded 5-(4-*n*-butoxyphenyl)-4-tosyloxazoline,[24] successive treatment of which with NH_3 in methanol at 110 °C in a sealed tube without purification gave 4-(4-*n*-butoxyphenyl)imidazole **11**. Reaction of **10** with glyoxal and ammonium hydroxide solution gave 2-(4-*n*-butoxyphenyl)imidazole **12**.[25] Treatment of **10** with TosMIC and sodium methoxide in methanol under reflux afforded 5-(4-*n*-butoxyphenyl)oxazole **13**.[26] 3-(4-*n*-Butoxyphenyl)isoxazole **16** was synthesized by [3 + 2] addition of nitrile oxide **15** with acetylene.[27] The aldehyde **10** was converted into the corresponding 4-(4-*n*-butoxyphenyl)benzoxime, and a subsequent reaction with *tert*-butyl hypochlorite in carbon tetrachloride gave the corresponding hydroxamyl chloride **14**.[28] The hydroxamyl chloride **14** was treated with triethylamine to generate the corresponding nitrile oxide **15** in situ, and subsequent cycloaddition with acetylene gas afforded the isoxazole derivative **16**. 3-(4-*n*-Butoxyphenyl)-pyrrole **18** was synthesized by the method reported by Trudell et al.[29] The aldehyde **10** was converted into ethyl 3-(4-*n*-butoxyphenyl)acrylate using the Horner−Emmons olefination procedure, and subsequent treatment with TosMIC afforded 3-ethoxycarbonyl-4-(4-*n*-butoxyphenyl)pyrrole **17**. The ester moiety of **17** was hydrolyzed with excess potassium hydroxide in 50% methanol and then decarboxylated by heating in 2-ethanolamine to give the pyrrole derivative **18**.

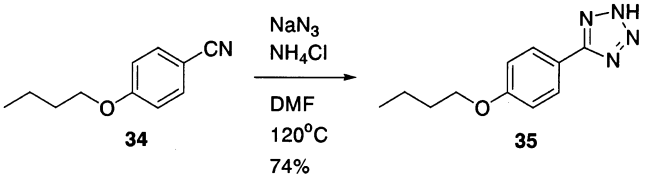
Compounds **21**, **23**−**26**, **29**−**30**, and **32**−**33** were prepared according to the method shown in Scheme 4. 4-(4-*n*-Butoxyphenyl)oxazole **21** was obtained by heating 2-bromo-4′-*n*-butoxyacetophenone **20**, which was prepared by 2-bromination of commercially available 4′-*n*-butoxyacetophenone **19**, in formamide.[30] 2-Formylation of **19** with ethyl formate and sodium hydride gave 4′-*n*-butoxy-2-formylacetophenone **22**, and subsequent treatment of **22** with hydroxylamine followed by concentrated HCl afforded the isoxazole derivative **23**. Treatment of **22** with hydrazine afforded 3-(4-*n*-butoxyphenyl)pyrazole **24**. Treatment of **22** with *N*-methylhydrazine gave 5-(4-*n*-butoxyphenyl)-1-methylpyrazole **25** and 5-(4-*n*-butoxyphenyl)-2-methylpyrazole **26**, which were easily separated by silica gel column chromatography. The regiochemistries of **25** and **26** were determined by an NOE study (Figure 4).[31] 5-(4-*n*-Butoxyphenyl)thiazole **29** was obtained from 1-(4-*n*-butoxyphenyl)-3-(dimethy-

Scheme 4

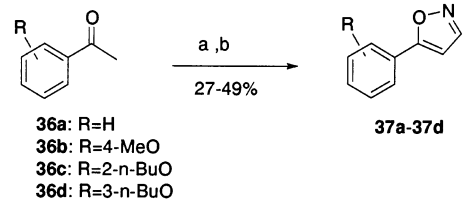


[a] Reagents: (a) Br_2, $CHCl_3$; (b) H_2NCHO; 180 °C; (c) HCOOMe, NaH, THF; (d) H_2NOH, pyridine; (e) concd HCl, THF; (f) H_2NNH_2, MeOH; (g) H_2NNHMe, MeOH; (h) $tBuOCH(NMe_2)_2$, 90 °C; (i) $POCl_3$, $NaClO_4$, CH_2Cl_2; (j) $Na_2S{\cdot}9H_2O$, DMF; (k) NH_2OSO_3H, pyridine, EtOH; (l) formamidine hydrochloride, tBuOK, tBuOH; (m) AcOEt, NaH, 18-crown-6-ether, THF/EtOH.

Scheme 5



Scheme 6



[a] Reagents: (a) HCOOEt, NaH, THF; (b) H_2NOH, H_2O.

lamino)-2-propen-1-thione **28** by the procedure of Lin et al.[32] Treatment of 4′-*n*-butoxyacetophenone **19** with *tert*-butoxybis(dimethylamino)methane afforded 1-(4-*n*-butoxyphenyl)-3-(dimethylamino)-2-propen-1-one **27**. Thioenaminone **28** was prepared by the reaction of the enaminone **27** with phosphorus oxychloride in dichloromethane followed by treatment with sodium perchlorate in water and reaction with sodium sulfide in aqueous *N*,*N*-dimethylformamide. The thioenaminone **28** was converted into the isothiazole derivative **29** by treatment with hydroxylamine-*O*-sulfonic acid (HSA). Reaction of the enaminone **27** with formamidine and potassium *tert*-butoxide in *tert*-butyl alcohol at 50 °C gave 4-(4-*n*-butoxyphenyl)pyrimidine **30**.[33] 5-(4-*n*-Butoxyphenyl)-3-methylisoxazole **32** and 3-(4-*n*-butoxyphenyl)-5-methylisoxazole **33** were obtained from 2-acetyl-4′-*n*-butoxyacetophenone **31** by treatment with hydroxylamine hydrochloride and hydrazine. The 2-acetylacetophenone derivative **31** was prepared by acetylation of the acetophenone derivative **19** with ethyl acetate and sodium hydride in tetrahydrofuran.[34]

5-(4-*n*-Butoxyphenyl)tetrazole **35** was prepared according to the method shown in Scheme 5. Reaction of nitrile **34** with sodium azide and ammonium hydrochloride in *N*,*N*-dimethylformamide at 120 °C afforded the tetrazole derivative **35**.[35]

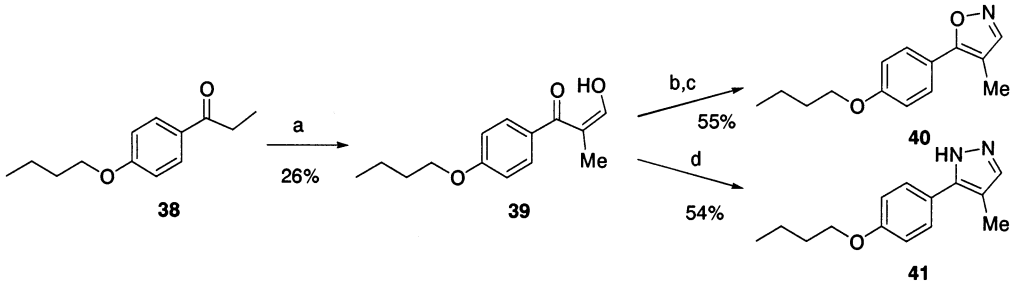
Compounds **37a**−**d** were prepared according to the method shown in Scheme 6. Acetophenone derivatives **36a**−**d** were formylated with ethyl formate and sodium hydride to give the corresponding 2-formyl ketone derivatives, and subsequent treatment with hydroxylamine followed by concentrated HCl afforded the isoxazole derivatives **37a**−**d**.

4-Methyl-5-(4-*n*-butoxyphenyl)isoxazole **40** and 4-methyl-3-(4-*n*-butoxyphenyl)pyrazole **41** were prepared according to the method shown in Scheme 7. Formylation of **38** with ethyl formate and sodium hydride gave 4′-*n*-butoxy-2-formylpropiophenone **39**, and subsequent treatment of **39** with hydroxylamine followed by concentrated HCl afforded the isoxazole derivative **40**. Reaction of 2-formyl-4′-*n*-butoxypropiophenone **39** with hydrazine in methanol afforded the pyrazole derivative **41**.

Results and Discussion

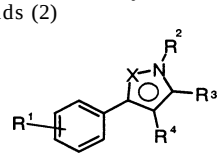
All of the compounds synthesized were evaluated with regard to their ability to inhibit microsomal synthesis

Scheme 7[a]



[a] Reagents: (a) HCOOEt, NaH, THF; (b) H_2NOH, pyridine; (c) concd HCl, THF; (d) H_2NNH_2, MeOH.

Table 2. Inhibition of AA Metabolism Involving Human 20-HETE Synthesizing Enzyme and Inhibitory Activities against Drug-Metabolizing CYPs by New Heterocyclic Compounds (2)



							P450 inhibition IC_{50} (nM)[b]				
compd	R^1	X	R^2	R^3	R^4	IC_{50} (nM)[a]	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
37a	H	O	H	H	H	>1 000	NT	NT	NT	NT	NT
37b	4-MeO	O	H	H	H	>1 000	NT	NT	NT	NT	NT
37c	2-BuO	O	H	H	H	>300	120	6 090	14 200	>100 000	>100 000
37d	3-BuO	O	H	H	H	>300	570	7 740	1 630	>100 000	>100 000
32	4-BuO	O	H	Me	H	>1 000	14 200	5 220	6 070	>100 000	>100 000
40	4-BuO	O	H	H	Me	>1 000	<46	8 180	480	>100 000	>100 000
23	4-BuO	O	H	H	H	38 ± 10	3 230	8 000	5 500	86 400	>100 000
25[c]	4-BuO	NMe	H	H	H	>1 000	69 900	6 480	1 970	96 500	93 800
26	4-BuO	NH	Me	H	H	>1 000	8 650	19 200	23 500	>100 000	>100 000
33	4-BuO	NH	H	Me	H	>300	21 100	17 800	16 000	81 600	>100 000
41	4-BuO	NH	H	H	Me	44 ± 2.9	<46	8 700	3 100	21 400	26 800
24	4-BuO	NH	H	H	H	23 ± 12	560	19 600	3 160	93 500	70 600

[a] IC_{50} value for 20-HETE production from AA by human renal microsome. Data are calculated from at least triplicate observations, and are presented as a mean ± SE. [b] IC_{50} was estimated for each test substance and each enzyme, according to the method of Crespi et al.[36] Data are calculated from triplicate observations. [c] This compound was evaluated as a HCl salt.

of 20-HETE, and those compounds were also evaluated for their ability to inhibit the major drug-metabolizing P450 (CYP) enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.[36] The methods of these assays are described in the Experimental Section. The obtained IC_{50} values are shown in Tables 1 and 2.

Imidazole derivative **3a** showed potent inhibitory activity toward 20-HETE synthase (IC_{50} value for **3a** was 5.7 ± 1.0nM); however, like other imidazole analogues, **3a** also strongly inhibited the drug-metabolizing CYP enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4; IC_{50} values of 7, 96, 7, 460, and 348 nM, respectively). Since some imidazole derivatives show high affinity to the heme iron atom of CYPs, the loss of the selective inhibition of CYPs upon replacement of the *N*-hydroxyformamidine moiety of **1** with an imidazole ring may be due to the character of imidazole. To improve the selectivity, a methyl group was introduced to the imidazole ring of **3a**, based on an observation in an evaluation of antifungal agents.[37] The introduction of a methyl group at the 2′- or 4′-position of the imidazole ring of **3a** (**9a**, **9b**) decreased the CYP inhibitory activity as expected; however, this was accompanied by a loss of 20-HETE synthase inhibitory activity [IC_{50} values for **9a** and **9b** were >300 nM ($n = 3$) and 143 ± 17 nM ($n = 3$), respectively, and there was a significant difference in IC_{50} values between **3a** ($n = 4$) and **9b** ($p < 0.05$)]. Meanwhile, the introduction of a methyl group at the 5′-position (**9c**) did not affect the inhibitory activity toward either 20-HETE synthase or CYPs [IC_{50} value for **9c** was 5.9 ± 2.6 nM ($n = 3$) and there was no significant difference in IC_{50} values between **3a** and **9c** ($p > 0.05$)]. These results suggest that the range of spatial tolerance around the nitrogen atom at the 3′-position of imidazole derivatives was narrow in coordination to heme iron.

Next, replacement of the hydroxyformamidine moiety of **1** with other heteroaromatic rings was examined. Triazole derivative **3b** showed a lower inhibitory activity toward drug-metabolizing CYPs, but its inhibitory activity toward 20-HETE synthase was also decreased. 3-Pyridyl derivative **3d** showed as potent an inhibitory activity as **3a** to 20-HETE synthase [IC_{50} value for **3d** was 27 ± 9.6 nM ($n = 3$) and there was no significant difference in IC_{50} values between **3a** and **3d** ($p > 0.05$)], with elevated selectivity to CYP2C9, CYP2C19, CYP2D6, and CYP3A4. On the other hand, 4-pyridyl derivative **3e** was less active than 3-pyridyl derivative **3d** [IC_{50} value for **3e** was 803 ± 100 nM ($n = 5$) and there was a significant difference in IC_{50} values between **3d** and **3e** ($p < 0.05$)], and 2-pyridyl derivative **3c** showed almost no activity (IC_{50} value for **3c** was >1000 nM). These results suggest that the presence of a nitrogen atom at the 3′-position of pyridine derivatives is essential for potent inhibitory activity toward 20-HETE synthase.

The position of the nitrogen atom on the imidazole ring also affected the inhibitory activity toward 20-

Table 3. Stability and Water Solubility of HET0016 and Compounds **23** and **24**

compd	solubility in water (μg/mL)	stability (% remaining) pH 4.0, 50 °C, 1 day
HET0016	3.7	43.2
23	5.6	100
24[a]	37.2	100

[a] $MeSO_3H$ salt.

HETE synthase. 4-Imidazolyl derivative **11**, which has a nitrogen atom at the 3′-position, showed as potent an inhibitory activity toward 20-HETE synthase as **3a** (IC_{50} value for **11** was 23 ± 14 nM), while 2-imidazolyl derivative **12** showed almost no inhibitory activity (IC_{50} value for **12** was >1000 nM). These results show that the nitrogen atom at the 3′-position of imidazole derivatives is also essential for potent 20-HETE synthase inhibitory activity, as seen with pyridine derivatives.

According to these indications, various heterocyclic derivatives including a nitrogen atom, namely oxazole, isoxazole, isothiazole, pyrazole, pyrazine, pyrimidine, pyrrole, and tetrazole derivatives, were synthesized. Among these derivatives, compounds with a nitrogen atom at the 3′-position (pyrazine derivative **9d**, oxazole derivative **13**, isoxazole derivative **23**, pyrazole derivative **24**, and isothiazole derivative **29**) showed inhibitory activity toward 20-HETE synthase, as expected (IC_{50} values were 115 ± 35, 32 ± 25, 38 ± 10, 23 ± 12, and 98 ± 24 nM, respectively). In contrast, compounds that lacked a nitrogen atom at the 3′-position (isoxazole derivative **16**, oxazole derivative **21**, and pyrimidine derivative **30**) did not show inhibitory activity toward 20-HETE synthase, even at 1000 nM. These results strongly support the notion that the nitrogen atom at the 3′-position of various heterocyclic rings is essential for the potent inhibitory activity of these derivatives toward 20-HETE synthase. In contrast to these heteroaryl derivatives, pyrrole derivative **18** and tetrazole derivative **35** showed less inhibitory activity toward 20-HETE synthase. This difference may be due to the difference in the coordination ability of the nitrogen atoms of **18** and **35** to the heme iron.

Isoxazole derivative **23** and pyrazole derivative **24** showed potent 20-HETE synthase inhibitory activity with much less inhibitory activity toward the CYP enzymes (more than 80-fold to over 10 000-fold selectivity), and the selectivities of **23** and **24** to CYP 1A2 and 2C19 were $^1/_7$ to $^1/_{30}$ the magnitude of that of HET0016. As expected, **23** and **24** were very stable under acidic conditions (in both cases 100% remained under pH 4.0, 50 °C, 1 day, Table 3), while HET0016 was unstable under the same conditions (only 43% remained). Thus, replacement of the *N*-hydroxyformamidine moiety of HET0016 by an isoxazole or pyrazole ring improved the stability of the compound in acid, and the methanesulfonic acid salt of **24** was more soluble than HET0016 in water. The stability and solubility of HET0016 and compounds **23** and **24** are shown in Table 3.

The SAR of the substituents on the phenyl ring in **23** and **24** is shown in Table 2. Replacement of the 4-*n*-butoxy group of **23** by a hydrogen atom (**37a**), 4-methoxy group (**37b**), or 2- and 3-(4-*n*-butoxy) group (**37c**, **37d**) resulted in a loss of activity, as seen in the case of *N*-hydroxyformamidine derivatives.[14] The similarity of the SAR of the substituent on the phenyl ring of heteroaryl derivatives in corresponding formamidine derivatives strongly suggests that the isoxazole ring of **23** and the pyrazole ring of **24** mimic the *N*-hydroxyformamidine moiety. In addition, the introduction of a methyl group at the 4′- or 5′-position of the isoxazole ring of **23** (**32**, **40**) and the 2′-, 3′-, or 4′-position of the pyrazole ring of **24** (**25**−**26**, **33**) resulted in a loss of activity, while introduction of a methyl group at the 5′-position of a pyrazole ring (**41**) did not drastically affect the activity (IC_{50} value for **41** was 44 ± 2.9 nM). These results suggest that the spatial tolerance around the nitrogen atom at the 3′-position of **23** and **24** is narrow in coordination to the heme iron, as seen in the case of the imidazole ring.

Conclusion

Replacement of the *N*-hydroxyformamidine moiety of compound **1** by some heterocyclic rings gave the isoxazole derivative **23** and the pyrazole derivative **24** as potent 20-HETE synthase inhibitors (IC_{50} values of 38 ± 10 and 23 ± 12 nM, respectively). These compounds showed improved stability under acidic conditions and exhibited much less inhibitory activity toward drug-metabolizing CYPs CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. The SAR of these newly synthesized heterocyclic compounds is quite similar to that of *N*-hydroxyformamidine derivatives. This result suggests that the structural and electrical properties of azole rings in heterocyclic compounds such as **23** and **24** are equivalent to those of the *N*-hydroxyformamidine moiety of HET0016.

Experimental Section

Chemistry. Melting points were determined on a Yanaco MP-500D melting point apparatus. NMR spectra were recorded at 200 or 300 MHz using a Varian Instruments Gemini 2000 or a Varian Instruments INOVA 300 with tetramethylsilane as an internal standard. Electron impact (EI) mass spectra were taken on a Perkin-Elmer Sciex API-300 mass spectrometer. Electrospray ionization (ESI) mass spectra were taken on a Micromass Platform LC mass spectrometer. Elemental analyses were performed on EA2400 elemental analyzers, and the results were within 0.4% of calculated values. Reactions were monitored by TLC analysis using Merck silica gel 60F-254 thin-layer plates. Column chromatography was carried out on silica gel Wako Pure Chemical C-200 and NH silica gel Fuji Silicia chromatorex DM1020.

***N*-(4-*n*-Butoxyphenyl)-*N*′-hydroxyformamidine (1).** This compound was synthesized as described in the previous paper:[15] mp 131.5−133.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.92 (t, J = 7.3 Hz, 3H), 1.35−1.48 (m, 2H), 1.59−1.73 (m, 2H), 3.88 (t, J = 6.4 Hz, 2H), 6.79 (m, J_{AB} = 8.8 Hz, 2H), 7.06 (m, J_{AB} = 8.8 Hz, 2H), 7.32 (m, J_{AB} = 10.8 Hz, 1H), 8.33 (m, J_{AB} = 11.0 Hz, 1H), 9.68 (s, 1H); MS (ESI) m/z 207 (M − H, 70%). Anal. ($C_{11}H_{16}N_2O\cdot^2/_3H_2O$) C, H, N.

1-(4-*n*-Butoxyphenyl)imidazole (3a). To a mixture of 1-(4-hydroxyphenyl)imidazole **2a** (0.5 g, 3.12 mmol) and potassium carbonate (0.52 g, 3.12 mmol) in *N*,*N*-dimethylformamide (6 mL) was added 1-iodobutane (0.57 g, 3.12 mmol), and the reaction mixture was stirred for 16 h at room temperature. After stirring, the mixture was diluted with water, and a colorless precipitate was filtered and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 4/1) to give a colorless solid. Recrystallization of the solid from hexane gave 0.57 g (84%) of **3a** as a colorless powder: mp 47−50 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 3H), 1.41−1.60 (m, 2H), 1.73−1.87 (m, 2H), 4.00 (t, J = 6.4 Hz, 2H), 6.97 (m, J_{AB} = 8.8 Hz, 2H), 7.18 (s, 1H), 7.20 (s, 1H), 7.29 (m,

J_{AB} = 8.8 Hz, 2H), 7.76 (s, 1H); MS (ESI) *m/z* 217 (M + H, 62%). Anal. ($C_{13}H_{16}N_2O$) C, H, N.

1-(4-*n*-Butoxyphenyl)triazole (3b). This compound was prepared from 1-(4-hydroxyphenyl)tridazole **2b** (0.52 g, 3.12 mmol) as described in the procedure for synthesizing **3a** to yield 0.49 g (73%) of colorless crystals: mp 59−60.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.4 Hz, 3H), 1.43−1.61 (m, 2H), 1.74−1.88 (m, 2H), 4.02 (t, J = 6.5 Hz, 2H), 7.01 (m, J_{AB} = 9.0 Hz, 2H), 7.56 (m, J_{AB} = 9.0 Hz, 2H), 8.09 (s, 1H), 8.45 (s,1H); MS (ESI) *m/z* 218 (M + H, 100%). Anal. ($C_{12}H_{15}N_3O$) C, H, N.

2-(4-*n*-Butoxyphenyl)pyridine (3c). This compound was prepared from 2-(4-hydroxyphenyl)pyridine **2c** (0.46 g, 2.69 mmol) as described in the procedure for synthesizing **3a** to yield 0.449 g (73%) of colorless crystals: mp 47−48.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.42−1.61 (m, 2H), 1.73−1.88 (m, 2H), 4.03 (t, J = 6.5 Hz, 2H), 7.00 (m, J_{AB} = 8.8 Hz, 2H), 7.13−7.21 (m, 1H), 7.64−7.78 (m, 2H), 7.95 (m, J_{AB} = 8.8 Hz, 2H), 8.62−8.68 (m, 1H); MS (ESI) *m/z* 228 (M + H, 53%). Anal. ($C_{15}H_{17}NO$) C, H, N.

3-(4-*n*-Butoxyphenyl)pyridine (3d). This compound was prepared from 3-(4-hydroxyphenyl)pyridine **2d** (0.2 g, 1.17 mmol) as described in the procedure for synthesizing **3a** to yield 0.21 g (79%) of a colorless oil: (*p*-toluenesulfonic acid salt) mp 132−133.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.95 (t, J = 7.3 Hz, 3H), 1.38−1.56 (m, 2H), 1.64−1.80 (m, 2H), 2.29 (s, 3H), 4.06 (t, J = 6.5 Hz, 2H), 7.08−7.18 (m, 4H), 7.48 (m, J_{AB} = 8.0 Hz, 2H), 7.88 (m, J_{AB} = 9.0 Hz, 2H), 7.93−8.02 (m, 1H), 8.67−8.80 (m, 2H), 9.15 (d, J = 2.2 Hz, 1H); MS (ESI) *m/z* 228 (M + H, 100%). Anal. ($C_{22}H_{25}NO_4S{\cdot}C_7H_8O_3S$) C, H, N.

4-(4-*n*-Butoxyphenyl)pyridine (3e). This compound was prepared from 4-(4-hydroxyphenyl)pyridine **2e** (0.2 g, 1.17 mmol) as described in the procedure for synthesizing **3a** to yield 0.230 g (86%) of a light brown powder: mp 58.5−59.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.3 Hz, 3H), 1.42−1.61 (m, 2H), 1.73−1.88 (m, 2H), 4.03 (t, J = 6.5 Hz, 2H), 7.01 (m, J_{AB} = 8.8 Hz, 2H), 7.48 (m, J_{AB} = 6.4 Hz, 2H), 7.60 (m, J_{AB} = 8.8 Hz, 2H), 8.62 (m, J_{AB} = 6.4 Hz, 2H); MS (ESI) *m/z* 228 (M + H, 100%). Anal. ($C_{15}H_{17}NO$) C, H, N.

1-(4-Methoxyphenyl)-2-methylimidazole (7a). A mixture of 2-methylimidazole (1 g, 12.2 mmol), 4-methoxyphenylboronic acid (3.7 g, 24.4 mmol), and $[Cu(OH)\text{-}TMEDA]_2Cl_2$ (0.57 g, 1.22 mmol) in dichloromethane (48 mL) was stirred for 18 h at room temperature under an atmosphere of O_2. The mixture was then filtered through Celite and the filtrate was concentrated to give a crude oil, which was purified by NH silica gel column chromatography (eluent: hexane/ethyl acetate = 4/1 to ethyl acetate) to give 2.15 g (94%) of **7a** as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.45 (s, 3H), 3.88 (s, 3H), 6.93−7.05 (m, 4H), 7.20 (m, J_{AB} = 9.9 Hz, 2H).

1-(4-Hydroxyphenyl)-2-methylimidazole (8a). 1-(4-Methoxyphenyl)-2-methylimidazole **7a** (2 g, 10.6 mmol) was added to 48% hydrobromic acid (20 mL) and the mixture was stirred for 16 h at 100 °C. After heating, the reaction mixture was cooled to room temperature. To the reaction mixture was added 6 M sodium hydroxide (10 mL) and an aqueous solution of sodium bicarbonate, and a precipitated colorless solid was filtered and dried to give 0.745 g (40%) of **8a** as a colorless powder: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.21 (s, 3H), 6.83−6.93 (m, 3H), 7.13−7.26 (m, 3H), 9.80 (s, 1H).

1-(4-*n*-Butoxyphenyl)-2-methylimidazole (9a). This compound was prepared from 1-(4-hydroxyphenyl)-2-methylimidazole **8a** (0.2 g, 1.15 mmol) as described in the procedure for synthesizing **3a** to yield 0.17 g (64%) of a light brown oil: (*p*-toluenesulfonic acid salt) mp 148−149 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.00 (t, J = 7.3 Hz, 3H), 1.41−1.60 (m, 2H), 1.65−1.84 (m, 2H), 2.37 (s, 3H), 2.68 (s, 3H), 4.04 (t, J = 6.4 Hz, 2H), 7.18−7.27 (m, 4H), 7.42 (s, 1H), 7.88 (m, J_{AB} = 8.1 Hz, 2H); MS (ESI) *m/z* 231 (M + H, 100%). Anal. ($C_{21}H_{26}N_2O_4S{\cdot}C_7H_8O_3S$) C, H, N.

4-Methyl-1-(4-methoxyphenyl)imidazole (7b) and 5-Methyl-1-(4-methoxyphenyl)imidazole (7c). These compounds were prepared from 4-methylimidazole (5 g, 60.9 mmol) as described in the procedure for synthesizing **7a** to yield 9.23 g (81%) of a mixture of **7b** and **7c** as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.14 and 2.30 (2s, 3H), 3.85 and 3.88 (2s, 3H), 6.80−7.05 (m, 3H), 7.15−7.34 (m, 2H), 7.53 and 7.66 (2s, 1H).

1-(4-Hydroxyphenyl)-4-methylimidazole (8b) and 1-(4-Hydroxyphenyl)-5-methylimidazole (8c). A mixture of 1-(4-methoxyphenyl)-4-methylimidazole **7b** and 1-(4-methoxyphenyl)-5-methylimidazole **7c** (4 g, 21.2 mmol) was added to 48% hydrobromic acid (40 mL), and the mixture was stirred for 54 h at 100 °C. After heating, the reaction mixture was cooled to room temperature, and 6 M sodium hydroxide (20 mL) and saturated sodium bicarbonate were added. A colorless solid precipitate was filtered and recrystallized two times from ethyl acetate/methanol to give 0.26 g (7%) of **8c** as light brown crystals: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.08 (s, 3H), 6.77 (s, 1H), 6.88 (m, J_{AB} = 9.7 Hz, 2H), 7.20 (m, J_{AB} = 9.7 Hz, 2H), 7.62 (s, 1H).

The mother liquor was concentrated and recrystallized from methanol to give 1.97 g (53%) of a 3:2 mixture of **8b** and **8c** as a colorless amorphism: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.08 (s, 1.2H), 2.14 (s, 1.8H), 6.77 (s, 0.4H), 6.79−6.95 (m, 2H), 7.13−7.41 (m, 1H), 7.25 (s, 0.6H), 7.62 (s, 0.4H), 7.91 (s, 0.6H), 9.73 (br s, 1H).

1-(4-*n*-Butoxyphenyl)-5-methylimidazole (9b). To a 3:2 mixture of 1-(4-hydroxyphenyl)-4-methylimidazole **8b** and 1-(4-hydroxyphenyl)-5-methylimidazole **8c** (0.2 g, 1.15 mmol) in *N*,*N*-dimethylformamide (2 mL) was added potassium carbonate (0.19 g, 1.38 mmol) and 1-iodobutane (0.21 g, 1.38 mmol). The reaction mixture was stirred for 18 h at room temperature. After stirring, the mixture was diluted with water and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated, and the residue was purified by NH silica gel column chromatography (eluent: hexane/ethyl acetate = 1/1) to give a mixture of 0.15 g (57%) of **9b** and **9c** as a colorless oil. Compound **9b** was isolated as a salt with *p*-toluenesulfonic acid by recrystallization from ethyl acetate: (*p*-toluenesulfonic acid salt) mp 163−164 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.95 (t, J = 7.3 Hz, 3H), 1.38−1.56 (m, 2H), 1.67−1.80 (m, 2H), 2.17 (d, J = 0.9 Hz, 3H), 2.29 (s, 3H), 4.07 (t, J = 6.5 Hz, 2H), 7.09−7.21 (m, 4H), 7.48 (m, J_{AB} = 8.1 Hz, 2H), 7.55 (m, J_{AB} = 8.8 Hz, 2H), 9.25 (d, J = 1.5 Hz, 1H); MS (ESI) *m/z* 231 (M + H, 90%). Anal. ($C_{21}H_{26}N_2O_4S{-}C_7H_8O_3S$) C, H, N.

1-(4-*n*-Butoxyphenyl)-4-methylimidazole (9c). This compound was prepared from 1-(4-hydroxyphenyl)-4-methylimidazole **8c** (0.15 g, 0.86 mmol) as described in the procedure for synthesizing **3a** to yield 0.11 g (56%) of **8c** as a brown oil: (*p*-toluenesulfonic acid salt) mp 130−130.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.95 (t, J = 7.2 Hz, 3H), 1.35−1.56 (m, 2H), 1.64−1.80 (m, 2H), 2.30 (s, 1H), 2.35 (d, J = 0.9 Hz, 3H), 4.05 (t, J = 5.5 Hz, 2H), 7.07−7.22 (m, 4H), 7.48 (m, J_{AB} = 8.5 Hz, 2H), 7.67 (m, J_{AB} = 8.5 Hz, 2H), 7.93 (t, J = 1.5 Hz, 1H), 9.46 (d, J = 1.5 Hz, 2H); MS (ESI) *m/z* 231 (M + H, 100%). Anal. ($C_{21}H_{26}N_2O_4S{\cdot}C_7H_8O_3S$) C, H, N.

2-(4-Methoxyphenyl)pyrazine (7d). To a mixture of 2-chloropyrazine (1 g, 8.7 mmol), 4-methoxyphenylboronic acid (1.6 g, 10.56 mmol), triphenylphosphine (0.23 g, 0.88 mmol), and palladium(II) acetate (0.05 g, 0.22 mmol) in 1,2-dimethoxyethane (10 mL) was added a 2 M aqueous solution of potassium carbonate (12 mL, 24 mmol), and the mixture was stirred for 5 h at 100 °C under an atmosphere of N_2. The mixture was then diluted with water and extracted with ethyl acetate, and the extract was dried over magnesium sulfate and concentrated to give a crude oil, which was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 3/1 to 1/1) to give 1.57 g (97%) of **7d** as a colorless solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.89 (s, 3H), 7.05 (m, J_{AB} = 9.0 Hz, 2H), 8.00 (m, J_{AB} = 9.0 Hz, 2H), 8.45 (d, J = 2.5 Hz, 1H), 8.60 (t, J = 1.5 Hz, 1H), 9.00 (d, J = 1.5 Hz, 1H).

2-(4-Hydroxyphenyl)pyrazine (8d). This compound was prepared from 1-(4-hydroxyphenyl)-4-methylimidazole **7b** (1.5 g, 8.06 mmol) as described in the procedure for synthesizing **8a** to yield 1.06 g (77%) of an orange powder: mp 130−130.5

°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (m, J_{AB} = 9.7 Hz, 2H), 7.95 (m, J_{AB} = 9.7 Hz, 2H), 8.45 (d, J = 2.5 Hz, 1H), 8.59 (t, J = 1.4 Hz, 1H), 8.98 (d, J = 1.4 Hz, 1H).

2-(4-*n*-Butoxyphenyl)pyrazine (9d). This compound was prepared from 1-(4-hydroxyphenyl)-4-methylimidazole **8b** (0.2 g, 1.16 mmol) as described in the procedure for synthesizing **3a** to yield 0.21 g (79%) of a colorless powder: mp 72−74.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.3 Hz, 3H), 1.43−1.61 (m, 2H), 1.72−1.88 (m, 2H), 4.05 (t, J = 6.5 Hz, 2H), 7.03 (m, J_{AB} = 8.6 Hz, 2H), 7.98 (m, J_{AB} = 8.6 Hz, 2H), 8.45 (d, J = 2.4 Hz, 1H), 8.57−8.61 (m, 1H), 8.99 (d, J = 1.4 Hz, 1H); MS (ESI) *m*/*z* 229 (M + H, 65%). Anal. ($C_{14}H_{16}N_2O$) C, H, N.

4-(4-*n*-Butoxyphenyl)imidazole (11). To a stirred suspension of tosylmethylisocyanide (TosMIC) (1.07 g, 5.5 mmol) and 4-*n*-butoxybenzaldehyde **10** (1 g, 5.6 mmol) in dry ethanol (16.5 mL) was added finely powdered sodium cyanide (0.027 g, 0.55 mmol). The reaction mixture was stirred for 20 min and the resulting clear solution was concentrated in vacuo. The residue was dissolved in a saturated solution of ammonia in dry methanol (44 mL) and heated at 110 °C for 18 h in a sealed tube. After heating, the reaction mixture was cooled to room temperature and concentrated to give a crude oil, which was purified by silica gel column chromatography (eluent: ethyl acetate) to give a colorless solid. Recrystallization of the solid from hexane/ethyl acetate gave 0.188 g (16%) of **11** as a colorless powder: mp 120−122 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (t, J = 7.3 Hz, 3H), 1.41−1.60 (m, 2H), 1.71−1.86 (m, 2H), 3.99 (t, J = 6.5 Hz, 2H), 6.94 (m, J_{AB} = 8.6 Hz, 2H), 7.25 (s, 1H), 7.64 (m, J_{AB} = 8.6 Hz, 2H), 7.69 (s, 1H); MS (ESI) *m*/*z* 217 (M + H, 100%). Anal. ($C_{13}H_{16}N_2O$) C, H, N.

2-(4-*n*-Butoxyphenyl)imidazole (12). To a solution of 4-*n*-butoxybenzaldehyde **10** (1 g, 5.61 mmol) in ethanol (7.5 mL) at 0 °C was added a solution of 40% glyoxal in water (1.3 mL) and 20 M ammonium hydroxide (1.9 mL). The reaction mixture was stirred for 30 min at 0 °C and then at room temperature overnight. The reaction mixture was concentrated and the residue was purified by NH silica gel column chromatography (eluent: ethyl acetate) to give a colorless solid. Recrystallization of the solid from hexane/ethyl acetate gave 0.69 g (57%) of **12** as colorless crystals: mp 172−173 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.41−1.60 (m, 2H), 1.72−1.86 (m, 2H), 4.01 (t, J = 6.6 Hz, 2H), 6.96 (m, J_{AB} = 8.7 Hz, 2H), 7.13 (s, 2H), 7.75 (m, J_{AB} = 8.7 Hz, 2H); MS (ESI) *m*/*z* 217 (M + H, 86%). Anal. ($C_{13}H_{16}N_2O$) C, H, N.

5-(4-*n*-Butoxyphenyl)oxazole (13). To a mixture of 4-*n*-butoxybenzaldehyde **10** (1 g, 5.61 mmol) and sodium methoxide (0.91 g, 16.8 mmol) in methanol (5 mL) was added TosMIC (1.30 g, 6.73 mmol). The reaction mixture was refluxed for 16 h with stirring. The mixture was diluted with water and extracted with chloroform, and the extract was dried over magnesium sulfate and concentrated to give a crude oil, which was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 7/3) to give a colorless solid. Recrystallization of the solid from hexane/ethyl acetate gave 0.63 g (43%) of **13** as colorless crystals: mp 57.5−59 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (t, J = 7.2 Hz, 3H), 1.40−1.60 (m, 2H), 1.71−1.86 (m, 2H), 4.00 (t, J = 6.4 Hz, 2H), 6.95 (m, J_{AB} = 8.6 Hz, 2H), 7.67 (m, J_{AB} = 8.6 Hz, 2H), 7.86 (s, 1H), 7.92 (s, 1H); MS (EI) *m*/*z* 217 (M^+, 27%). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

4-*n*-Butoxybenzohydroxamyl Chloride (14). To a solution of 4-*n*-butoxybenzaldehyde **10** (2 g, 11.2 mmol) in methanol (33 mL) was added a mixture of hydroxylamine hydrochloride (0.93 g, 13.5 mmol) and sodium acetate trihydrate (1.83 g, 13.5 mmol) in water (17 mL). The reaction mixture was stirred for 16 h at room temperature. The mixture was then diluted with water and extracted with ethyl acetate, and the extract was dried over magnesium sulfate and concentrated to give a colorless oil. Without further purification, the oil was dissolved in carbon tetrachloride (30 mL) and added to a solution of *tert*-butyl hypochlorite (1.37 g, 12.6 mmol) in carbon tetrachloride (10 mL). The reaction mixture was stirred for 1 h at room temperature and concentrated, and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 9/ 1) to give 2.48 g (98%) of **14** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, J = 7.5 Hz, 3H), 1.43−1.58 (m, 2H), 1.73−1.88 (m, 2H), 4.00 (t, J = 6.2 Hz, 2H), 6.99 (m, J_{AB} = 9.0 Hz, 2H), 7.76 (m, J_{AB} = 9.0 Hz, 2H).

3-(4-*n*-Butoxyphenyl)isoxazole (16). A solution of 4-*n*-butoxybenzohydroxamyl chloride **14** (2.4 g, 10.5 mmol) in benzene (105 mL) was bubbled with acetylene gas for 10 min. After bubbling, triethylamine (2.12 g, 21.0 mmol) was added dropwise at 0 °C, and the mixture was stirred for 1 h at 60 °C. The reaction mixture was then cooled, diluted with brine, and extracted with ethyl acetate, and the extract was dried over magnesium sulfate and concentrated to give a light yellow residue. The crude oil was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 13/1) to give 1.29 g (57%) of **16** as a colorless solid: mp 34.5−35.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.44−1.60 (m, 2H), 1.74−1.85 (m, 2H), 4.02 (t, J = 6.6 Hz, 2H), 6.60 (d, J = 1.5 Hz, 1H), 6.97 (m, J_{AB} = 9.0 Hz, 2H), 7.75 (m, J_{AB} = 9.0 Hz, 2H), 8.41 (d, J = 1.5 Hz, 1H); MS (ESI) *m*/*z* 240 (M + Na, 73%). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

4-(4-*n*-Butoxyphenyl)-3-ethoxycarbonylpyrrole (17). To a stirred suspension of sodium hydride (60% in oil, 0.90 g, 22.4 mmol) in toluene (23 mL) was added diethylphosphonoacetic acid ethyl ester (5.02 g 22.4 mmol) at 0 °C, and the mixture was stirred for 1 h at room temperature. 4-*n*-Butoxybenzaldehyde **10** (4 g, 22.4 mmol) was then added and the reaction mixture was stirred for 30 min at room temperature. After stirring, the reaction mixture was diluted with water and extracted with ethyl acetate, and the extract was dried over magnesium sulfate and concentrated to give a colorless oil which was used without further purification. A mixture of the oil, TosMIC (4.44 g, 22.8 mmol), diethyl ether (16 mL), and dimethyl sulfoxide (8 mL) was added dropwise to a stirred suspension of sodium hydride (60% in oil, 1.15 g, 28.7 mmol) in diethyl ether (29 mL). The mixture was stirred for 16 h at 60 °C and then cooled to room temperature. The reaction mixture was diluted with water and extracted with ethyl acetate, and the extract was dried over magnesium sulfate and concentrated to give a brown oil. The crude oil was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 4/1 to 1/1) to give 0.96 g (15%) of **14** as a colorless powder:^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 6.5 Hz, 3H), 1.26 (t, J = 6.5 Hz, 3H), 1.38−1.61 (m, 2H), 1.58−1.86 (m, 2H), 3.99 (t, J = 6.5 Hz, 2H), 4.22 (q, J = 6.5 Hz, 2H), 6.76 (t, J = 1.5 Hz, 1H), 6.90 (m, J_{AB} = 8.2 Hz, 2H), 7.41 (m, J_{AB} = 8.2 Hz, 2H), 7.49 (t, J = 1.5 Hz, 1H); MS (ESI) *m*/*z* 310 (M + Na, 100%). Anal. ($C_{17}H_{21}NO_3$) C, H, N.

3-(4-*n*-Butoxyphenyl)pyrrole (18). To a mixture of 4-(4-*n*-butoxyphenyl)-3-ethoxycarbonylpyrrole **17** (0.2 g, 0.694 mmol) dissolved in ethanol (1 mL) was added potassium hydroxide (0.39 g, 6.94 mmol), and the mixture was stirred for 4 h at 80 °C. After stirring, the reaction mixture was cooled to room temperature, diluted with water, acidified with 12 M HCl, and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to give a brown oil, which was dissolved in 2-ethanolamine (3 mL) and refluxed for 3 h with stirring. After heating, the reaction mixture was cooled to room temperature and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 1/1) to give 0.05 g (33%) of **18** as a brown amorphism: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 3H), 1.43−1.57 (m, 2H), 1.72−1.82 (m, 2H), 3.97 (t, J = 6.6 Hz, 2H), 6.48 (dd, J = 2.7 Hz, 4.2 Hz, 1H), 6.82 (dd, J = 2.7 Hz, 4.8 Hz, 1H), 6.88 (m, J_{AB} = 8.9 Hz, 2H), 7.01 (dd, J = 2.0 Hz, 4.2 Hz, 1H), 7.44 (m, J_{AB} = 8.9 Hz, 2H); MS (EI) *m*/*z* 217 (M^+, 48%). Anal. ($C_{14}H_{17}NO\cdot{}^1/_4H_2O$) C, H, N.

2-Bromo-4′-*n*-butoxyacetophenone (20). To a solution of 4′-*n*-butoxyacetophenone **19** (2.5 g, 13.0 mmol) in chloroform (75 mL) was added bromine (2.75 g, 14.3 mmol) dropwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature and then diluted with an aqueous solution of sodium thiosulfate and extracted with chloroform. The organic layer was dried over magnesium sulfate and concentrated to give a

yellow oil, which was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 19/1) to give 1.85 g (52%) of **20** as a light yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.41−1.60 (m, 2H), 1.72−1.87 (m, 2H), 4.05 (t, J = 6.5 Hz, 2H), 4.40 (s, 2H), 6.95 (m, J_{AB} = 8.8 Hz, 2H), 7.97 (m, J_{AB} = 8.8 Hz, 2H).

4-(4-*n*-Butoxyphenyl)oxazole (21). 2-Bromo-4′-*n*-butoxyacetophenone **20** (0.2 g, 0.74 mmol) was dissolved in formamide (2.5 mL) and stirred for 1 h at 180 °C. After stirring, the reaction mixture was cooled to room temperature, diluted with water, and extracted with ethyl acetate. The extract was dried over magnesium sulfate and concentrated to give a brown oil. The crude oil was purified by silica gel column chromatography (eluent: hexane) to give 0.091 g (57%) of **21** as a colorless powder: mp 52−52.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 3H), 1.41−1.62 (m, 2H), 1.72−1.87 (m, 2H), 4.01 (t, J = 6.5 Hz, 2H), 6.96 (m, J_{AB} = 8.8 Hz, 2H), 7.23 (s, 1H), 7.58 (m, J_{AB} = 8.8 Hz, 2H), 7.88 (s, 1H); MS (EI) *m*/*z* 217 (M^+, 51%). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

4′-*n*-Butoxy-2-hydroxyacrylophenone (22). To a stirred suspension of sodium hydride (60% in oil, 0.51 g, 14.3 mmol) in tetrahydrofuran (13 mL) and methyl formate (0.859 g, 14.3 mmol) was added 4′-*n*-butoxyacetophenone **19** (2.5 g, 13.0 mmol) in tetrahydrofuran (2.5 mL). The reaction mixture was stirred for 2 h at room temperature and then diluted with 0.5 M HCl and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated, and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 8/ 1) to give 2.05 g (72%) of **22** as a yellow oil:^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.40−1.61 (m, 2H), 1.72−1.87 (m, 2H), 4.04 (t, J = 6.5 Hz, 2H), 6.16 (d, J = 4.8 Hz, 1H), 6.94 (m, J_{AB} = 8.8 Hz, 2H), 7.89 (m, J_{AB} = 9.0 Hz, 2H), 8.14 (d, J = 4.4 Hz, 1H).

5-(4-*n*-Butoxyphenyl)isoxazole (23). To a mixture of 4′-*n*-butoxy-2-hydroxyacrylophenone **22** (1 g, 4.54 mmol) dissolved in pyridine (4.5 mL) was added hydroxylamine hydrochloride (0.379 g, 5.45 mmol). The reaction mixture was stirred for 1 h at 80 °C and then poured into water, acidified with 6 M HCl, and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to give a colorless solid. Without further purification, the solid was dissolved in tetrahydrofuran (1.6 mL) and 12 M HCl (0.8 mL) was added. The reaction mixture was stirred for 1 h at room temperature, and then diluted with water and extracted with ethyl acetate. The extract was dried over magnesium sulfate and concentrated to give a light yellow oil. The crude oil was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 19/1) to give 0.168 g (17%) of **23** as a colorless powder: mp 48−49.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 3H), 1.46−1.56 (m, 2H), 1.73−1.87 (m, 2H), 4.02 (t, J = 6.6 Hz, 2H), 6.39 (d, J = 2.0 Hz, 1H), 6.97 (m, J_{AB} = 8.6 Hz, 2H), 7.72 (m, J_{AB} = 8.6 Hz, 2H), 8.26 (d, J = 2.0 Hz, 1H); MS (ESI) *m*/*z* 240 (M + Na, 39%). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

3-(4-*n*-Butoxyphenyl)pyrazole (24). To a solution of 4′-*n*-butoxy-2-hydroxyacrylophenone **22** (1 g, 4.54 mmol) in methanol (27 mL) was added hydrazine monohydrate (0.273 g, 5.45 mmol). The reaction mixture was stirred for 1 h at room temperature and then diluted with water. The solid yellow precipitate was filtered and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 2/1) to give 0.740 g (75%) of **24** as a light yellow powder: mp 72−76 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.41−1.61 (m, 2H), 1.72−1.87 (m, 2H), 4.00 (t, J = 6.2 Hz, 2H), 6.54 (d, J = 2.2 Hz, 1H), 6.95 (m, J_{AB} = 9.0 Hz, 2H), 7.60 (d, J = 2.2 Hz, 1H), 7.65 (m, J_{AB} = 9.0 Hz, 2H); MS (ESI) *m*/*z* 240 (M + Na, 38%). Anal. ($C_{13}H_{16}N_2O\cdot{}^1/_2H_2O$) C, H, N. Methanesulfonic acid salt: mp 130.5−131.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.94 (t, J = 11.0 Hz, 3H), 1.38−1.52 (m, 2H), 1.63−1.80 (m, 2H), 2.38 (s, 3H), 4.00 (t, J = 9.8 Hz, 2H), 6.69 (d, J = 3.3 Hz, 1H), 6.98 (m, J_{AB} = 12.9 Hz, 2H), 7.71 (m, J_{AB} = 12.9 Hz, 2H), 7.77 (d, J = 3.3 Hz, 1H). Anal. ($C_{13}H_{16}N_2O\cdot CH_4O_3S$) C, H, N.

3-(4-*n*-Butoxyphenyl)-2-methylpyrazole (25) and 3-(4-*n*-Butoxyphenyl)-1-methylpyrazole (26). To a solution of 4′-*n*-butoxy-2-hydroxyacrylophenone **22** (0.5 g, 2.27 mmol) in tetrahydrofuran (5 mL) was added a solution of *N*-methylhydrazine (0.115 g, 2.50 mmol) in tetrahydrofuran (5 mL). The reaction mixture was stirred for 2 h at room temperature and then diluted with brine and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated, and the residue was purified by NH silica gel column chromatography (eluent: hexane/ethyl acetate = 8/1) to give 0.100 g (19%) of **25** as a colorless oil and 0.170 g (33%) of **26** as a colorless powder. **25** as the hydrochloride salt: mp 151.5−154 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.95 (t, J = 7.2 Hz, 3H), 1.37−1.55 (m, 2H), 1.66−1.80 (m, 2H), 3.82 (s, 3H), 4.03 (t, J = 6.4 Hz, 2H), 6.32 (d, J = 2.0 Hz, 1H), 7.04 (m, J_{AB} = 8.8 Hz, 2H), 7.43 (m, J_{AB} = 8.8 Hz, 2H), 7.44 (d, J = 2.0 Hz, 1H); MS (ESI) *m*/*z* 231 (M + H, 100%). Anal. ($C_{14}H_{18}ClN_2O$) C, H, N. **26:** mp 57−59.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (t, J = 7.3 Hz, 3H), 1.41−1.60 (m, 2H), 1.70−1.84 (m, 2H), 3.94 (s, 3H), 3.99 (t, J = 6.5 Hz, 2H), 6.46 (d, J = 2.2 Hz, 1H), 6.92 (m, J_{AB} = 8.7 Hz, 2H), 7.35 (d, J = 2.2 Hz, 1H), 7.71 (m, J_{AB} = 8.7 Hz, 2H); MS (ESI) *m*/*z* 253 (M + Na, 100%). Anal. ($C_{13}H_{16}N_2O$−HCl) C, H, N.

4′-*n*-Butoxy-3-(dimethylamino)acrylophenone (27). 4′-*n*-Butoxyacetophenone **19** (0.5 g, 2.6 mmol) was dissolved in *tert*-butoxybis(dimethylamino)methane (1.36 g, 7.8 mmol) and stirred for 1 h at 90 °C. After stirring, the reaction mixture was diluted with a mixture of ethyl acetate/hexane = 2/1, and a solid yellow precipitate was collected by filtration and dried to give 0.52 g (81%) of **27** as a light yellow powder: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (t, J = 7.2 Hz, 3H), 1.40−1.61 (m, 2H), 1.67−1.88 (m, 2H), 3.02 (br s, 6H), 4.01 (t, J = 6.5 Hz, 2H), 5.72 (d, J = 12 Hz, 1H), 6.91 (m, J_{AB} = 8.8 Hz, 2H), 7.80 (d, J = 12 Hz, 1H), 7.90 (m, J_{AB} = 8.8 Hz, 2H).

5-(4-*n*-Butoxyphenyl)isothiazole (29). To a mixture of 4′-*n*-butoxy-3-(dimethylamino)acrylophenone **27** (1 g, 4.04 mmol) dissolved in anhydrous dichloromethane (4 mL) was added a solution of phosphorus oxychloride (0.62 g, 4.04 mmol) in anhydrous dichloromethane (1 mL) at 0 °C. After the reaction mixture was stirred at room temperature for 30 min, the reaction mixture was concentrated to give a yellow solid. The solid was added to a stirred solution of sodium perchlorate monohydrate (1.7 g, 12.1 mmol) in water (3 mL). The reaction mixture was vigorously stirred at 0 °C for 30 min, and a solid yellow precipitate was collected by filtration and added to a stirred ice-cold mixture of *N*,*N*-dimethylformamide (8.3 mL), sodium sulfide nonahydrate (1.02 g, 4.24 mmol), and water (1 mL). The reaction mixture was stirred at room temperature for 2 h, diluted with water and extracted with chloroform, and the extract was washed with brine, dried over magnesium sulfate, and concentrated to give 4′-*n*-butoxy-3-(dimethylamino)thioacrylophenone **28** as a red-brown oil.

Without further purification, the oil was dissolved in ethanol (30 mL), and pyridine (0.626 g, 8.08 mmol) and a solution of hydroxylamine-*O*-sulfonic acid (0.53 g, 4.65 mmol) in ethanol (5 mL) were added at 0 °C. The reaction mixture was stirred at room temperature for 30 min, and the solvents were then removed under reduced pressure to give a residue. Water was added to the residue, the mixture was extracted with ethyl acetate, and the extract was washed with saturated aqueous solution of sodium bicarbonate, dried over magnesium sulfate, and concentrated to give a brown oil. The crude oil was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 9/1) to give a yellow solid. Recrystallization of the solid from hexane gave 0.255 g (27%) of **29** as a yellow powder: mp 75−76.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.40−1.61 (m, 2H), 1.72−1.87 (m, 2H), 4.01 (t, J = 6.3 Hz, 2H), 6.95 (m, J_{AB} = 8.8 Hz, 2H), 7.31 (d, J = 1.6 Hz, 1H), 7.54 (m, J_{AB} = 8.8 Hz, 2H), 8.43 (d, J = 1.6 Hz, 1H); MS (ESI) *m*/*z* 234 (M + H, 100%). Anal. ($C_{13}H_{15}NOS$) C, H, N.

4-(4-*n*-Butoxyphenyl)pyrimidine (30). To a mixture of formamidine hydrochloride (029 g, 3.60 mmol) suspended in *tert*-butyl alcohol (11 mL) was added a warm solution of

potassium *tert*-butoxide (0.63 g, 7.20 mmol) in *tert*-butyl alcohol (11 mL). The mixture was stirred vigorously for 45 min at room temperature. To this mixture was then added 4′-*n*-butoxy-3-(dimethylamino)acrylophenone **27** (0.5 g, 2.02 mmol) in *tert*-butyl alcohol (11 mL). The reaction mixture was stirred for 16 h at 50 °C. The mixture was cooled, poured into ice water, and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated, and the residue was purified by NH silica gel column chromatography (eluent: hexane/ethyl acetate = 8/1 to 1/1) to give a colorless solid. Recrystallization of the solid from hexane gave 0.24 g (52%) of **30** as a colorless powder: mp 81.5−82.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.4 Hz, 3H), 1.42−1.62 (m, 2H), 1.74−1.89 (m, 2H), 4.05 (t, J = 6.5 Hz, 2H), 7.02 (m, J_{AB} = 8.8 Hz, 2H), 7.65 (m, J_{AB} = 5.4 Hz, 1H), 8.07 (m, J_{AB} = 8.8 Hz, 2H), 8.70 (m, J_{AB} = 5.4 Hz, 1H), 9.21 (d, J = 1.0 Hz, 3H); MS (ESI) m/z 251 (M + Na, 100%). Anal. ($C_{14}H_{16}N_2O$) C, H, N.

2-Acetyl-4′-*n*-butoxyacetophenone (31). A mixture of two drops of dry ethanol, 4′-*n*-butoxyacetophenone **19** (2 g, 10.4 mmol), and dibenzo-18-crown-6 (0.062 g, 0.166 mmol) in tetrahydrofuran (13 mL) was added to a stirred mixture of sodium hydride (60% in oil, 0.853 g, 21.8 mmol) and ethyl acetate (1.83 g, 20.8 mmol) in tetrahydrofuran (13 mL) at room temperature. The mixture was stirred for 3 h at 80 °C and then cooled to room temperature and diluted with 1 M HCl. The mixture was extracted with ethyl acetate and the extract was dried over magnesium sulfate and concentrated, and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 8/1) to give 2.17 g (89%) of **31** as yellow crystals:^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.40−1.60 (m, 2H), 1.72−1.84 (m, 2H), 2.17 (s, 3H), 4.03 (t, J = 6.5 Hz, 2H), 6.11 (s, 1H), 6.93 (m, J_{AB} = 9.0 Hz, 2H), 7.86 (m, J_{AB} = 9.0 Hz, 2H).

5-(4-*n*-Butoxyphenyl)-3-methylisoxazole (32). To a solution of 2-acetyl-4′-*n*-butoxyacetophenone **31** (0.3 g, 1.28 mmol) in pyridine (3 mL) was added hydroxylamine hydrochloride (0.098 g, 1.41 mmol). The mixture was stirred for 30 min at room temperature. To this mixture was then added 6 M HCl (7 mL), and the result was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated to give a light yellow solid. The crude solid was recrystallized from hexane/ethyl acetate to give 0.100 g (34%) of **32** as a colorless powder: mp 77.5−79 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 3H), 1.40−1.61 (m, 2H), 1.72−1.84 (m, 2H), 2.33 (s, 3H), 4.01 (t, J = 6.4 Hz, 2H), 6.23 (s, 1H), 6.95 (m, J_{AB} = 8.8 Hz, 2H), 7.68 (m, J_{AB} = 8.8 Hz, 2H); MS (ESI) m/z 254 (M + Na, 73%). Anal. ($C_{14}H_{17}NO_2$) C, H, N.

3-(4-*n*-Butoxyphenyl)-5-methylpyrazole (33). To a solution of 2-acetyl-4′-*n*-butoxyacetophenone **31** (0.2 g, 0.85 mmol) in methanol (5 mL) was added hydrazine monohydrate (0.047 g, 0.94 mmol). The mixture was stirred for 1 h at room temperature. This mixture was then concentrated and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 3/1) to give 0.175 g (89%) of **33** as a colorless powder: mp 100−101 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 3H), 1.41−1.61 (m, 2H), 1.70−1.83 (m, 2H), 2.35 (s, 3H), 4.00 (t, J = 6.5 Hz, 2H), 6.28 (s, 1H), 6.93 (m, J_{AB} = 8.7 Hz, 2H), 7.61 (m, J_{AB} = 8.7 Hz, 2H); MS (ESI) m/z 231 (M + H, 100%). Anal. ($C_{14}H_{18}N_2O$) C, H, N.

5-(4-*n*-Butoxyphenyl)tetrazole (35). To a mixture of 4-*n*-butoxybenzonitrile **34** (5 g, 28.5 mmol) and ammonium chloride (1.68 g, 31.4 mmol) in *N*,*N*-dimethylformamide (57 mL) was added sodium azide (2.04 g, 31.4 mmol). The reaction mixture was stirred for 30 h at 120 °C. After stirring, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The mixture was diluted with 1 M HCl and a colorless solid precipitate was filtered and recrystallized from water/ethanol to give 4.60 g (74%) of **35** as colorless crystals: mp 198.5−200 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.95 (t, J = 7.3 Hz, 3H), 1.36−1.57 (m, 2H), 1.66−1.81 (m, 2H), 4.07 (t, J = 6.3 Hz, 2H), 7.15 (m, J_{AB} = 8.9 Hz, 2H), 7.97 (m, J_{AB} = 8.9 Hz, 2H); MS (ESI) m/z 217 (M − H, 100%). Anal. ($C_{11}H_{14}N_4O$) C, H, N.

2′-*n*-Butoxyacetophenone (36c). This compound was prepared from 2′-hydroxyacetophenone (3 g, 22.0 mmol) as described in the procedure for synthesizing **3a** to yield 3.07 g (73%) of a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.0 Hz, 3H), 1.42−1.63 (m, 2H), 1.76−1.94 (m, 2H), 2.64 (s, 3H), 4.08 (t, J = 6.0 Hz, 2H), 6.90−7.04 (m, 2H), 7.38−7.51 (m, 1H), 7.70−7.79 (m, 1H).

3′-*n*-Butoxyacetophenone (36d). This compound was prepared from 3′-hydroxyacetophenone (3 g, 22.0 mmol) as described in the procedure for synthesizing **3a** to yield 3.99 g (94%) of a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.2 Hz, 3H), 1.40−1.63 (m, 2H), 1.70−1.89 (m, 2H), 2.60 (s, 3H), 4.03 (t, J = 6.2 Hz, 2H), 7.06−7.16 (m, 1H), 7.37 (t, J = 8.5 Hz, 1H), 7.46−7.59 (m, 2H).

5-(4-Methoxyphenyl)isoxazole (37b). To a stirred mixture of sodium hydride (60% in oil, 14.6 g, 385.4 mmol), ethyl formate (43.8 g, 730.8 mmol), and tetrahydrofuran (300 mL) was added 4′-methoxyacetophenone **36b** (30 g, 182.7 mmol) in tetrahydrofuran (100 mL) at 0 °C. The reaction mixture was stirred for 2.5 h at room temperature, and then diluted with water (200 mL) and washed with ethyl acetate. The aqueous layer was separated and hydroxylamine hydrochloride (12.7 g, 182.7 mmol) was added. The mixture was stirred for 17 h at room temperature, and then diluted with 1 M HCl and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to give a yellow solid. The crude solid was recrystallized from diethyl ether to give 15.8 g (49%) of **37b** as a light yellow powder: mp 62−64 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.40 (d, J = 2.0 Hz, 1H), 6.99 (m, J_{AB} = 9.0 Hz, 2H), 7.74 (m, J_{AB} = 8.8 Hz, 2H), 8.25 (d, J = 1.6 Hz, 1H); MS (ESI) m/z 198 (M + Na, 70%). Anal. ($C_{10}H_9NO_2$) C, H, N.

5-Phenylisoxazole (37a). This compound was prepared from acetophenone (2.47 g, 20.5 mmol) as described in the procedure for synthesizing **37b** to yield 1.70 g (57%) of a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (d, J = 1.2 Hz, 1H), 7.43−7.52 (m, 3H), 7.76−7.83 (m, 2H), 8.29 (d, J = 1.2 Hz, 1H); MS (EI) m/z 145 (M^+, 100%). Anal. (C_9H_7NO) C, H, N.

5-(2-*n*-Butoxyphenyl)isoxazole (37c). This compound was prepared from 2′-*n*-butoxyacetophenone **36c** (1.75 g, 9.10 mmol) as described in the procedure for synthesizing **37b** to yield 0.930 g (35%) of a colorless powder: mp 33−34.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (t, J = 7.2 Hz, 3H), 1.43−1.63 (m, 2H), 1.82−1.94 (m, 2H), 4.12 (t, J = 6.3 Hz, 2H), 6.82 (d, J = 1.8 Hz, 1H), 6.97−7.12 (m, 2H), 7.34−7.44 (m, 1H), 8.01 (dd, J = 1.8 Hz, 7.8 Hz, 1H), 8.31 (d, J = 1.8 Hz, 1H); MS (ESI) m/z 240 (M + Na, 100%). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

5-(3-*n*-Butoxyphenyl)isoxazole (37d). This compound was prepared from 3′-*n*-butoxyacetophenone **36d** (3 g, 15.6 mmol) as described in the procedure for synthesizing **37b** to yield 0.910 g (27%) of a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.4 Hz, 3H), 1.45−1.62 (m, 2H), 1.73−1.85 (m, 2H), 4.03 (t, J = 6.3 Hz, 2H), 6.51 (d, J = 2.0 Hz, 1H), 6.94−7.02 (m, 1H), 7.30−7.42 (m, 3H), 8.29 (d, J = 2.0 Hz, 1H); MS (EI) m/z 217 (M^+, 36%). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

4′-*n*-Butoxypropiophenone (38). This compound was prepared from 4′-hydroxypropiophenone (3.3 g, 22.0 mmol) as described in the procedure for synthesizing **3a** to yield 4.20 g (93%) of a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.21 (t, J = 7.2 Hz, 3H), 1.40−1.62 (m, 2H), 1.70−1.90 (m, 2H), 2.96 (q, J = 7.2 Hz, 2H), 4.03 (t, J = 5.6 Hz, 2H), 6.91 (m, J_{AB} = 8.8 Hz, 2H), 7.95 (m, J_{AB} = 8.8 Hz, 2H).

4′-*n*-Butoxy-2-hydroxy-1-methylacrylophenone (39). To a stirred suspension of sodium hydride (60% in oil, 1.94 g, 48.4 mmol) in ethyl formate (19.26 g, 260 mmol) was added 4′-*n*-butoxypropiophenone **38** (3.8 g, 18.4 mmol) in tetrahydrofuran (24 mL). The reaction mixture was stirred for 20 min at room temperature, and then diluted with 0.5 M HCl and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated, and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 9/ 1) to give 1.12 g (26%) of **39** as a colorless

powder: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, J = 7.2 Hz, 3H), 1.40−1.62 (m, 2H), 1.70−1.89 (m, 2H), 2.02 (s, 3H), 4.03 (t, J = 6.5 Hz, 2H), 6.95 (m, J_{AB} = 9.2 Hz, 2H), 7.67 (m, J_{AB} = 9.2 Hz, 2H), 8.53 (d, J = 4.8 Hz, 1H).

5-(4-*n*-Butoxyphenyl)-4-methylisoxazole (40). This compound was prepared from 4′-*n*-butoxy-2-hydroxy-1-methylacrylophenone **39** (0.7 g, 3.0 mmol) as described in the procedure for synthesizing **23** to yield 0.38 g (55%) of a light yellow powder: mp 71−72 °C; ^{1}H NMR (200 MHz, $CDCl_3$) ? 0.99 (t, J = 7.2 Hz, 3H), 1.45−1.61 (m, 2H), 1.73−1.84 (m, 2H), 2.24 (s, 3H), 4.02 (t, J = 6.5 Hz, 2H), 7.00 (m, J_{AB} = 8.8 Hz, 2H), 7.66 (m, J_{AB} = 8.8 Hz, 2H), 8.12 (s, 1H); MS (ESI) *m*/*z* 254 (M + Na, 100%). Anal. ($C_{14}H_{17}NO_2$) C, H, N.

3-(4-*n*-Butoxyphenyl)-4-methylisoxazole (41). This compound was prepared from 4′-*n*-butoxy-2-hydroxy-1-methylacrylophenone **39** (0.2 g, 0.85 mmol) as described in the procedure for synthesizing **24** to yield 0.105 g (54%) of a light yellow powder: mp 71.5−72.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 3H), 1.42−1.60 (m, 2H), 1.72−1.85 (m, 2H), 2.21 (s, 3H), 4.01 (t, J = 6.5 Hz, 2H), 6.97 (m, J_{AB} = 8.8 Hz, 2H), 7.43 (s, 1H), 7.48 (m, J_{AB} = 8.8 Hz, 2H); MS (ESI) *m*/*z* 253 (M + Na, 100%). Anal. ($C_{14}H_{18}N_2O$) C, H, N.

Biological Evaluation: Arachidonic Acid (AA) Metabolism. The compounds were dissolved in 100% DMSO and diluted with reaction buffer. The final concentration of DMSO was 1% and did not affect 20-HETE synthesis enzyme activity. To examine the effect of the compounds on the activity of 20-HETE synthesizing enzyme, the compounds (10^{-10}−10^{-6} mol/L, five concentrations) were incubated for 6 h at 37 °C with human renal microsome (HCCC, Laurel, MD) (100 μg/mL), tritiated arachidonic acid (2 μCi/mL), and NADPH (1 mM) in reaction buffer (50 mM MOPS/5 mM $MgCl_2$/1 mM EDTA, pH 7.4). Reaction was terminated by addition of formic acid (pH 3.5). Reaction mixtures were separated on Unifilter2000 (Whatman Japan KK, Tokyo, Japan) filled with ODS−SS (Sensyu Scientific Co., Ltd., Tokyo, Japan). Tritiated 20-HETE was eluted with 70% acetonitrile and the radioactivity of the eluate was measured by liquid scintillation counting. Before starting the above-mentioned experiments, we have completed the validation of this assay system by using the radio HPLC and have confirmed that we could exactly measure the production of 20-HETE. In the present study, we found that human renal microsomes produced only tritiated 20-HETE peak from tritiated arachidonic acid under our present experimental conditions by the use of HPLC according to the procedure of Miyata et al.[11] The retention time of this peak was corresponded to that of the cold 20-HETE (Sigma Chemical Co., St. Louis, MO), and any peaks of other metabolites including EETs and DHETs were not detected. Curve-fitting and parameter estimation were carried out by using Origin 6.0J (OriginLab Corp., MA).

Biological Evaluation: Drug-Metabolizing Cytochrome P450 (CYP) Inhibition. Baculovirus/insect cell-expressed human CYP enzymes were obtained from Gentest Corp. (Wirburn, MA). The CYP-specific substrates used were as follows: CYP1A2, 3-cyano-7-ethoxycoumarin; CYP2C9, 7-methoxy-4-trifluoromethylcoumarin; CYP2C19, 3-cyano-7-ethoxycoumarin; CYP2D6; 3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7methoxy-4-methylcoumarin; CYP3A4, 7-benzyloxyquinoline. The enzyme/substrate contained buffer, cDNA-expressed P450, the CYP-specific substrate, and the amount was adjusted to give the final concentration [CYP1A2, 0.5 pmol(enzyme) and 5 μM (substrate); CYP2C9, 1.0 pmol (enzyme) and 75 μM (substrate); CYP2D6, 1.5 pmol (enzyme) and 1.5 μM (substrate); CYP2C19, 0.5 pmol (enzyme) and 25 μM (substrate); CYP3A4: 3.0 pmol (enzyme) and 40 μM (substrate)] in a reaction volume of 200 μL.

The compounds were dissolved in 100% acetonitrile and diluted with reaction buffer. The final concentration of acetonitrile was 2% and did not affect CYP enzyme activity. Each enzyme and its substrate were incubated in the presence or absence of test compounds. The reactions were terminated at 15 min for CYP1A2, 30 min for CYP2C19 and CYP2D6, and 45 min for CYP2C9 and CYP3A4, and metabolite concentrations were measured.

Fluorescence per well was measured using a fluorescent plate scanner (ARVO 1420 multilabel counter, Wallac, Turku, Finland). Metabolite concentrations were measured using the excitation and emission wavelength (CYP1A2, 405 and 460 nm; CYP2C9, 405 and 535 nm; CYP2C19, 405 and 460 nm; CYP2D6, 390 and 460 nm; CYP3A4, 405 and 535 nm, respectively). Detection of the products of either assay was linear over the range used for these assays.

IC_{50} values were estimated for each test compounds and each enzyme, according to the method of Crespi et al. (1997).[36]

Statistical Analysis. Data are reported as the mean ± SE. Significance was evaluated by the unpaired *t*-test. The level of significance was $p<0.05$.

Measurement of Solubility. About 2 mg of each compound was added to 2 mL of water, and the mixture was shaken by using a shaker (model SA31, Yamato Kagaku) at room temperature. Then the suspension was centrifuged for 10 min at 25 °C, 11 000 rpm by using a centrifugal separator (model CF 15R, Hitachi). The supernatant was diluted with a mixed solvent of water/acetonitrile = 1/1. The concentration was measured by HPLC.

The HPLC analysis was performed with a Shimadzu HPLC system composed of a LC-10AD, SPD-10AV, and SIL-10A. The condition for HPLC was as follows: mobile phase, 10 mmol/L aqueous ammonium acetate solution/acetonitrile = 45/55; flow rate, 1.0 mL/min; column, reverse-phase (Capcell Pak UG120, 4.6 mm i.d. + 150 mm; Shiseido) at 40 °C; and detection wavelength, 255 nm.

Measurement of Stability. About 0.5 mg of each compound was added to 5 mL of pH 4.0 Briton−Robinson buffer, and the mixture was shaken by using a shaker (model SA31, Yamato Kagaku) at room temperature for 2 h. Then the suspension was filtrated with membrane filter (0.45 μm, PALL). The filtrate was stored at 50 °C in an oven (model DS-44, Yamato Kagaku). After 24 h the sample solution was diluted with a mixed solvent of water/acetonitrile = 1/1 and analyzed by HPLC. The HPLC method was same as that for solubility measurement.

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