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Imidazole derivatives as new potent and selective 20-HETE synthase inhibitors

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Abstract—In a previous paper, we reported that an imidazole derivative **1** exhibited a potent inhibitory activity of 20-HETE synthase (**1**; IC_{50} value of 5.7 nM), but this compound also exhibited little selectivity for cytochrome P450s (CYPs). We examined some derivatives of imidazole **1** which had an amino group on the side chain, and found that a dimethylaminohexyloxy derivative (**3g**; IC_{50} value of 8.8 nM) showed potent and selective inhibitory activity.

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 catalyzed by cytochrome P450 (CYP) 4A isozymes (CYP4A1, 4A2, 4A3 and 4A8) in rat kidney[2] and cerebral artery,[3] and by CYP4A11 and 4F2 in human liver and kidney.[4] 20-HETE plays an important role in the regulation of renal vascular and tubular functions,[5–7] and it also regulates vascular tone in the cerebral circulation. Therefore, the inhibition of 20-HETE is now considered a promising new target for the treatment of renal and cerebrovascular diseases. 17-Octadecynoic acid (17-ODYA),[8] 1-aminobenzotriazole (1-ABT),[9] *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), 12,12-dibromododec-11-enoic acid (DBDD)[10] and sodium 10-undecynyl sulfate (10-SUYS)[11] are known to inhibit the production of 20-HETE (Fig. 1). However, these compounds are not potent and specific inhibitors of 20-HETE formation. 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.[12] The IC_{50} value of HET0016 for the production of 20-HETE by human renal microsomes was 8.9 ± 2.7 nM, while its IC_{50} value for inhibiting the formation of EETs was 2800 nM.[13] HET0016 had very little inhibitory activity for CYP2C9, CYP2D6, CYP3A4 or cyclooxygenase (COX), even at a higher concentration (100 μM).[13] HET0016 prevented the acute fall in cerebral blood flow following subarachnoid hemorrhage (SAH) in the rat.[14] Despite its promising pharmacological properties, HET0016 is not soluble enough for injectable formulations under neutral conditions. While the solubility of HET0016 is increased in acidic conditions due to the basicity of the *N*-hydroxyformamidine moiety, this is accompanied by rapid decomposition of the *N*-hydroxyformamidine moiety. Recently, we found that replacement of the *N*-hydroxyformamidine moiety of HET0016 by an imidazole ring could retain the potent inhibitory activity of 20-HETE synthase and improve its acid stability (compound **1**; IC_{50} value of 5.7 nM, Fig. 1).[15] However, some imidazole derivatives are known to show high affinity for the heme iron of major drug-metabolizing CYP enzymes,[16] and **1** also strongly inhibited the activities of drug-metabolizing CYP enzymes (CYP 1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4; IC_{50} values of 7, 96, 7, 464 and 348 nM, respectively).[15] Compounds like **1**, which strongly inhibit the activities of drug-metabolizing CYP enzymes may cause unfavorable drug–drug metabolizing interactions. We sought to improve the selectivity of **1** toward drug-metabolizing CYP enzymes by evaluating the residues on the 1-imidazolylphenyl ring.

Compounds **1** and **3a**–**g** were prepared from 1-(4-hydroxyphenyl)imidazole **2** by the method shown in Scheme 1. Reaction of **2** with 1-butanol under Mitsu-

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nobu conditions afforded the imidazole **3a**. Imidazole derivatives **3b–e** were prepared from the corresponding alcohols under the same conditions as for **3a**. Compounds **6a** and **6b** were prepared according to the method shown in Scheme 2. Alkylation of 4-fluoronitrobenzene **4** with 3-(2-pyridyl)propanol and sodium hydride gave 4-[3-(2-pyridyl)propyloxy]nitrobenzene **5a**, and the use of 2-(dimethylamino)ethanol instead of 3-(2-pyridyl)propanol in the same reaction gave 4-[2-(dimethylamino)ethoxy]nitrobenzene **5b**. The nitrobenzene derivatives **5a** and **5b** were treated by the method reported by Gall et al.[17] to give the imidazole derivatives **6a** and **6b**. Compound **9** was prepared according to the method shown in Scheme 3. Treatment of 2,5-dibromopyridine **7** with *n*-heptylalcohol and sodium hydride in *N,N*-dimethylformamide afforded 5-bromo-2-heptyloxypyridine **8**, successive treatment of which with imidazole by the method reported by Buchwald et al.[18] gave the imidazole derivative **9**.

Generally, CYP oxidizes lipophilic xenobiotics to introduce a polar substituent so that they can be excreted, and it has been shown that the increased lipophilicity of such compounds is associated with an increased catalytic efficiency for CYP.[19] Therefore, we examined the introduction of some polar functional groups to the alkoxy side chain of **1**, which may decrease the affinity toward drug-metabolizing CYPs. For example, we introduced some polar groups, like an amino group, to the side chain of **1**. All of the synthesized compounds were evaluated with regard to their inhibitory activity toward the human 20-HETE synthesizing enzyme[15] and the major drug-metabolizing CYP enzymes, CYP 1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.[20] The observed IC_{50} values are shown in Table 1 along with the calculated ACD-logD values. Replacement of the butoxy group of **1** with a pyridin-2-ylmethoxy group did not affect the inhibitory activity toward 20-HETE synthase (compound **3a**; IC_{50} value of 4.5 nM). While compound **3a** showed better selectivity than **1** toward CYP1A2 (compounds **1** and **3a**; IC_{50} values of 7.0 and 120 nM, respectively), a similar advantage was not seen for CYP2C19. While extending the methylene chain of **3a** to a propylene group (compounds **6a**, **3b** and **3c**) did not affect the inhibitory activity toward 20-HETE synthase, it tended to increase the inhibitory activity toward CYP1A2, probably due to an increase in lipophilicity (compounds **3a**, **6a**, **3b** and **3c** had ACD-logD values of 1.99, 2.84, 2.85 and 2.81, respectively). Replacement of the benzene ring of **1** with a pyridine ring maintained inhibitory activity toward 20-HETE synthase (compound **9**; IC_{50} value of 7.7 nM). While compound **9** showed lower inhibitory activity than **1** toward CYP2C9 (IC_{50} value of $>100{,}000$ nM), it showed strong inhibitory activities toward CYP1A2 and 2C19. The introduction of a cyclic amino substituent to the butoxy side chain of **1** maintained the inhibitory activity toward 20-HETE synthase (compounds **3d**, **3e**; IC_{50} values of 8.6 and 16.2 nM, respectively), and gave lower inhibitory activities toward drug-metabolizing CYP enzymes. While replacement of the butoxy group of **1** with a 2-dimethylaminoethoxy group greatly decreased the inhibitory activities toward the drug-metabolizing CYP enzymes (compound **6b**), and improved the selectivity toward 20-HETE synthase, the inhibitory activity toward 20-HETE synthase itself was decreased to less than 1/60 that of **1** (compound **6b**; IC_{50} value of 387 nM). Changing the dimethylaminoethoxy side chain of **6b** to a dimethylaminopropoxy substituent increased the inhibitory activity toward 20-HETE synthase (compound **3f**; IC_{50} value of 51 nM). This result suggests that a longer side chain may be preferable for potent 20-HETE synthase inhibitory activity. Therefore, we examined dimethylaminohexyloxy derivative **3g**, which has a longer alkoxy side chain than **3f**, and found that it showed potent inhibitory activity toward 20-HETE synthase (IC_{50} value of 8.8 nM). It showed very little inhibitory activity toward the drug-metabolizing CYP enzymes (CYP 1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4; IC_{50} values of 410, 4460,

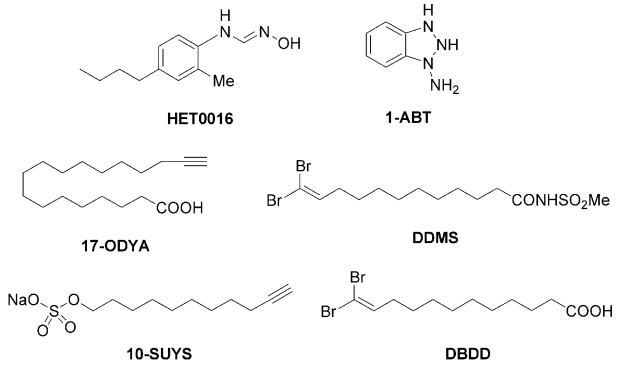
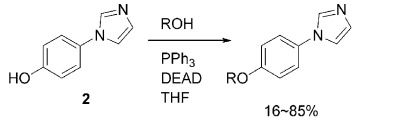
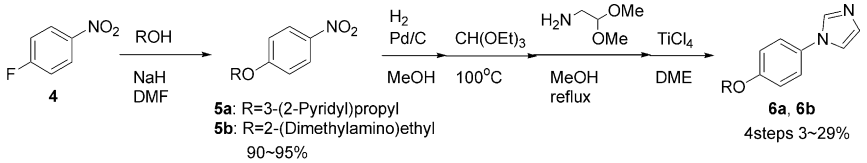


Figure 1. Structures of HET0016, 1-ABT, 17-ODYA, DDMS, DBDD and 10-SUYS.

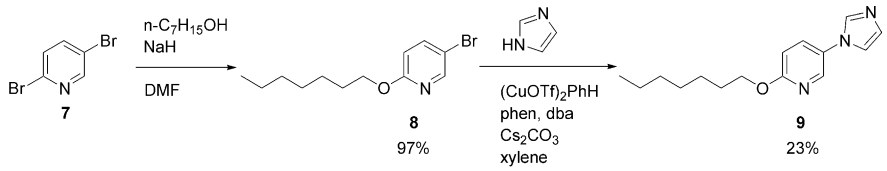


1: R=1-*n*-Butyl
3a: R=2-Pyridylmethyl
3b: R=3-(3-Pyridyl)propyl
3c: R=3-(4-Pyridyl)propyl
3d: R=2-(4-Morfolyno)ethyl
3e: R=2-{1-(4-Ethoxycarbonyl)piperazino}ethyl
3f: R=3-(Dimethylamino)propyl
3g: R=6-(Dimethylamino)hexyl

Scheme 1.

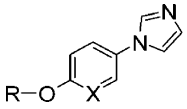


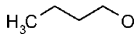
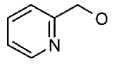
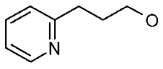
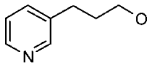
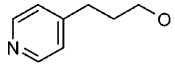
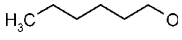
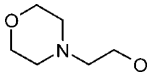
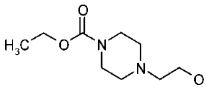
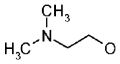
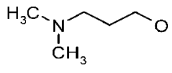
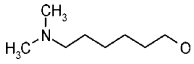
Scheme 2. Reagents: (a) ROH, NaH, DMF; (b) H_2, Pd/C, MeOH; (c) $CH(OEt)_3$, 100 °C; (d) 1-(2,2-dimethoxy)ethylamine, MeOH, reflux; (d) $TiCl_4$, DME.



Scheme 3.

Table 1. Inhibition of AA metabolism involving human 20-HETE synthesizing enzyme and inhibitory activities against drug-metabolizing CYPs by new heterocyclic compounds



Compd	RO	X	IC_{50} (nM)[a]	P450 inhibition IC_{50} (nM)[b]					ACD-LogD
				1A2	2C9	2C19	2D6	3A4	pH 6.8
1		CH	5.7	7.0	96	7.0	460	348	3.38
3a		CH	4.5	120	130	<46	1040	630	1.99
6a		CH	2.1	<46	129	<46	620	252	2.84
3b[c]		CH	1.1	<46	185	<46	1300	417	2.85
3c		CH	1.1	<46	101	<46	1040	344	2.81
9[c]		N	7.7	<46	>100,000	<46	1000	76,900	3.74
3d		CH	8.6	1480	1810	91	399	5670	0.91
3e[c]		CH	16	2240	1440	469	3200	>100,000	2.27
6b[d]		CH	387	15,000	40,000	2960	1050	>100,000	−0.08
3f[d]		CH	51	3400	32,800	9700	400	39,000	−0.41
3g[c]		CH	2.2	800	33,200	6220	410	4460	0.51

[a] IC_{50} value for 20-HETE production from AA by human renal microsome.
[b] IC_{50} was estimated for each test substance and each enzyme, according to the method of Crespi et al.[20]
[c] These compounds were evaluated as the 2HCl salt.
[d] These compounds were evaluated as the 2 *p*-toluenesulfonic acid salt.

33,200, 6220 and 800 nM, respectively). It is quite unique for an imidazole analogue like **3g** to have such selective inhibitory activity toward CYP enzymes.

In conclusion, the introduction of a dialkylamino group to the butoxy side chain of **1** greatly improved its CYP selectivity without effecting its inhibitory activity toward 20-HETE synthase. 2-Dimethylaminohexyloxy derivative **3g** exhibited strong inhibitory activity against 20-HETE synthase with an IC_{50} value of 8.8 nM, and more than 180-fold to over 15,000-fold selectivity toward CYP1A2, 2C9, 2C19, 2D6 and 3A4.

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