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Discovery of a *N*′-Hydroxyphenylformamidine Derivative HET0016† as a Potent and Selective 20-HETE Synthase Inhibitor

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Abstract—*N*-(4-Butyl-2-methylphenyl)-*N*′-hydroxyformamidine (HET0016) was evaluated as the first potent and selective inhibitor of 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) synthase. The IC_{50} value of HET0016 for the production of 20-HETE from arachidonic acid (AA) by human renal microsomes was 8.9 ± 2.7 nM, with over 200 times the selectivity of xenobiotic-metabolizing cytochrome P450 enzymes. An examination of the structure–activity relationship revealed that the unsubstituted hydroxyformamidine moiety and the substituent at the *para*-position of the *N*-hydroxyformamidine moiety are necessary for the potent activity of HET0016.

Introduction

Recently, several studies have been conducted on the biological properties of 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE), which is a major metabolite of arachidonic acid (AA) produced in the kidney.[2,3] 20-HETE plays an important role in the regulation of renal vascular and tubular functions,[4–6] and contributes to the control of arterial 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 autoregulation of cerebral blood flow.[8] Therefore, the regulation of 20-HETE is now considered a promising new therapeutic target for renal and cerebral diseases. The formation of 20-HETE from AA is catalyzed by cytochrome P450 (CYP) 4A isozymes (CYP4A1, 4A2, 4A3 and 4A8) in rat kidney,[3] and CYP4F2 and 4F11 in human liver.[9] Some AA analogues [i.e., 17-octadecynoic acid (17-ODYA)[10] and *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS)[11]] and 1-aminobenzotriazole (1-ABT)[12] have been reported to inhibit 20-HETE synthase. However, their IC_{50} values for 20-HETE formation are on the order of μM orders, and some lack selectivity for xenobiotic-metabolizing CYPs. HET0016 is the first reported potent and selective 20-HETE synthase inhibitor. Its IC_{50} value was 8.9 ± 2.7 nM for the formation of 20-HETE by human renal microsomes, while its IC_{50} values for human recombinant CYP2C9-, 2D6- and 3A4-catalyzed oxidative metabolism of substrate were 3.3 ± 0.2, 83.9 ± 7.0 and 71.0 ± 21 μM, respectively.[1] In comparison, 1-ABT inhibited CYP2C9-, 2D6- and 3A4 with IC_{50} values of 42.9 ± 1.6, 10.5 ± 0.1 and 0.45 ± 0.01 μM, these were similar concentrations to those needed to inhibit the formation of 20-HETE.[1] Epoxyeicosatrienoic acids (EETs) are produced by epoxyganases (CYP1A, 2B, 2C and 2J families) from AA. IC_{50} value for HET0016 for inhibition of the formation of EETs was 2.8 μM,[1] in contrast, it is known that 17-ODYA is a non-selective inhibitor of the formation of 20-HETE and EETs.[1,11] These results suggest that HET0016 is not a non-selective inhibitor of CYPs like 1-ABT and 17-ODYA. HET0016 had a selective inhibitory effect on CYP that produces 20-HETE from AA without affecting other CYP isoforms. AA is also metabolized by cyclo-oxyganase (COX), but HET0016 had very little effect on the activity of COX (IC_{50} 2.3 μM).[1] Therefore, we synthesized a series of HET0016 derivatives and elucidated their ability to inhibit 20-HETE formation. In this paper, we discuss the structure–activity relationships of these new potent inhibitors (Fig. 1).

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†See ref 1.

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Chemistry

HET0016 (**3a**) was synthesized from commercially available 4-butyl-2-methylaniline (**1a**) by successive treatment with a slight excess of dimethylformamide dimethyl-acetal in refluxing toluene and hydroxylamine hydrochloride in MeOH at room temperature.[13] Compounds **3b**–**3m** were synthesized from the corresponding anilines in the same way. The introduction of a methyl group to each atom of the hydroxyformamidine moiety of **3a** was accomplished by treating the intermediate **2a** with *O*-methyl and *N*-methylhydroxylamine for **4** and **5**, respectively, in refluxing MeOH, by treating **1a** with triethylorthoacetate (neat at 60 °C) for **6**, and by the *N*-methylation of **1a** via trifluoroacetamide for **7** (Scheme 1). Introduction of a hydroxyformamidine group for **3a**–**3m** via an iminoether intermediate like **2b** gave lower yields due to the formation of *N*,*N*′-diarylamidine by-products.

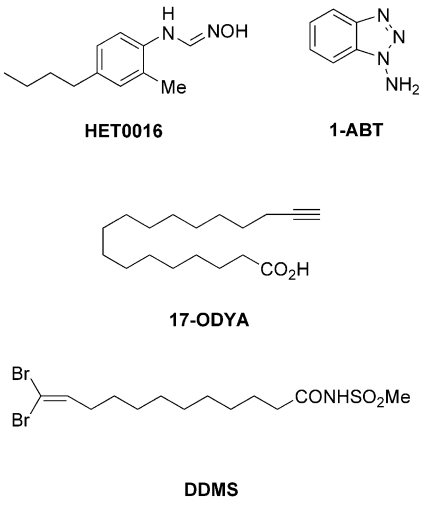
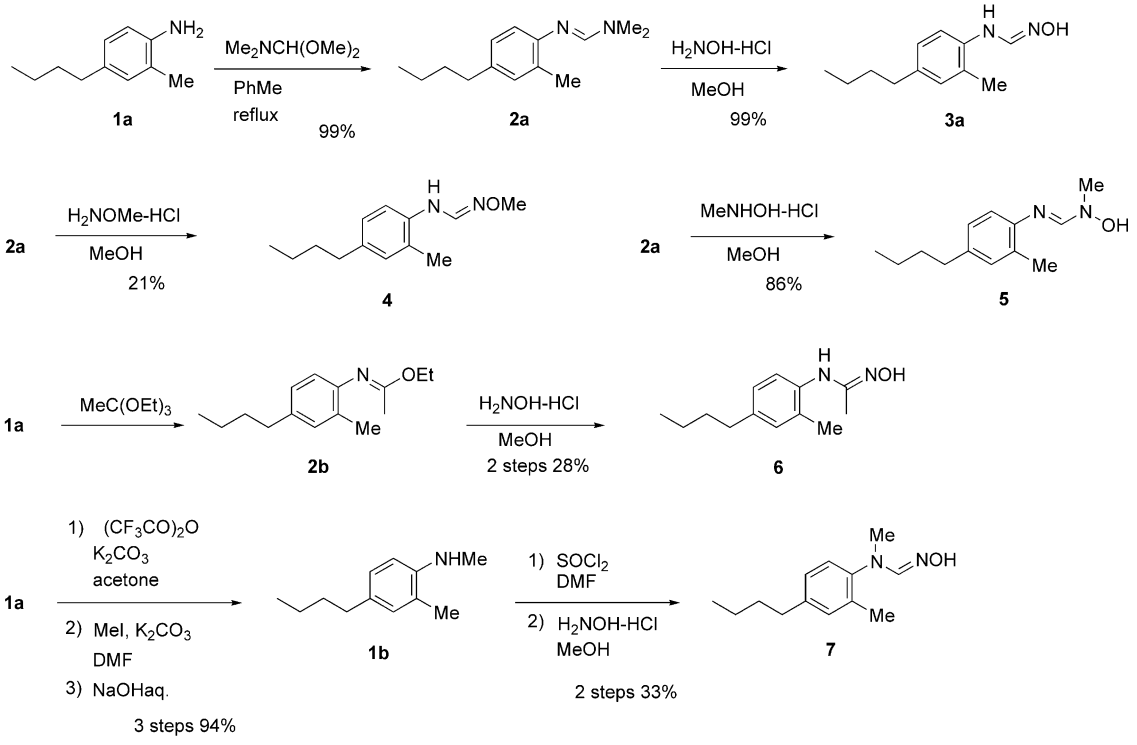


Figure 1.

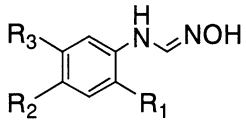
Results and Discussion

Compounds **3a**–**3m** and **4**–**7** were evaluated for their ability to inhibit the catalytic activity of human cytochrome enzymes; their IC_{50} values are shown in Table 1. Compounds **4**–**7**, which have a methyl group on the *N*-hydroxyformamidine moiety, showed lower inhibitory activity than **3a**. This suggests that the *N*-hydroxyformamidine moiety might be essential for the potent inhibitory activity of **3a**. Next, modification of the substituents on the phenyl ring of **3a** was examined. Displacement of the *para*-Bu group (**3b**) resulted in dramatic decrease in activity (about 1/400), whereas displacement of the *ortho*-Me group (**3g**) did not affect the activity. These observations suggest that the butyl group at the *para*-position of the *N*-hydroxyformamidine moiety is also essential for the potent activity of **3a**. Indeed, moving the *para*-Bu group to the *meta*-position (**3c**) also reduced the activity (about 1/80). Introduction of various alkyl groups (**3e**–**3k**) at the *para*-position of *N*-hydroxyformamidine indicated that an alkyl group larger than a methyl group is sufficient for potent activity (**3f**–**3k**), and branched alkyl groups such as isopropyl (**3i**), *sec*-butyl (**3j**) and *tert*-butyl (**3k**) also give tolerable results. Introduction of a benzyl group (**3l**) somewhat decreased the activity (about 1/6), but replacing the Bu group of **3g** with a propoxy group (**3m**) did not affect the activity.



Scheme 1.

Table 1.



Compd	R_1	R_2	R_3	IC_{50} (nM)[a]	IC_{50} for CYPs (μM)[1] 2C9	2D6	3A4
3a	Me	Bu	H	8.9±2.7	3.3	83.9	71
3b	Me	H	H	3625	77	>100	>100
3c	Me	H	Bu	720	24	98	94
3d	Bu	H	H	20% at 1 μM	42	32	51
3e	H	Me	H	669	79	52	>100
3f	H	Et	H	6.6	17	>100	>100
3g	H	Bu	H	3.9	8.8	83	65
3h	H	hexyl	H	4.9	0.3	32	50
3i	H	*i*-Pr	H	2.4	30	>100	>100
3j	H	*s*-Bu	H	3	11	>100	>100
3k	H	*t*-Bu	H	7.8	>100	>100	>100
3l	H	$PhCH_2$	H	52	17	>100	>100
3m	H	PrO	H	3.5	35	>100	>100
4				6812			
5				1845			
6				3286			
7				759			

[a]IC_{50} value for 20-HETE production from AA by human renal microsome.[1]

We also examined the effects of compounds **3a**–**3m** on CYP isoforms (CYP 2C9, CYP 2D6 and CYP 3A4) that could be important enzymes for drug metabolism. The results are shown in Table 1. Compound **3a** and its derivatives inhibited CYP 2C9-, CYP 2D6- and CYP 3A4-catalyzed oxidative metabolism of substrate with IC_{50} values on the order of μM. The inhibitory effects on CYP 2C9, CYP 2D6 and CYP 3A4 were 1000 times weaker than those on CYPs that catalyzed ω-hydroxylation of AA. Compounds **3a**–**3m** were not non-selective inhibitors of CYPs like 1-ABT. This means that *N*-hydroxyformamidine derivatives can be the selective inhibitors of CYPs that produce 20-hydroxy-5,8,11,14-eicosatetraenoic acid from AA.

Conclusions

N-Phenyl-*N*′-hydroxyformamidine derivatives exhibited potent inhibitory activity against 20-HETE synthase. Unsubstituted *N*-hydroxyformamidine was essential for the potent activity, and the substituent at the *para*-position of *N*-hydroxyformamidine had large effect on the potent inhibitory activity, while those at the *ortho*- and *meta*-position did not.

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