Articles

Bicyclic N-Hydroxyurea Inhibitors of 5-Lipoxygenase: Pharmacodynamic, Pharmacokinetic, and *in Vitro* Metabolic Studies Characterizing *N*-Hydroxy-*N*-(2,3-dihydro-6-(phenylmethoxy)-3-benzofuranyl)urea†

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A series of *N*-hydroxyurea derivatives have been prepared and examined as inhibitors of 5-lipoxygenase. Oral activity was established by examining the inhibition of LTB_4 biosynthesis in an *ex vivo* assay in the mouse. The pharmacodynamic performance in the mouse of selected compounds was assessed using an *ex vivo* LTB_4 assay and an adoptive peritoneal anaphylaxis assay at extended pretreat times. Compounds with an extended duration of action were re-examined as the individual enantiomers in the *ex vivo* assay, and the (*S*) enantiomer of *N*-hydroxy-*N*-[2,3-dihydro-6-(phenylmethoxy)-3-benzofuranyl]urea, (+)-**1a** (SB 202235), was selected as the compound with the best overall profile. Higher plasma concentrations and longer plasma half-lives were found for (+)-**1a** relative to its enantiomer in the mouse, monkey, and dog. *In vitro* metabolic studies in mouse liver microsomes established enantiospecific glucuronidation as a likely mechanism for the observed differences between the enantiomers of **1a**. Enantioselective glucuronidation favoring (−)-**1a** was also found in human liver microsomes.

Introduction

The 5-lipoxygenase enzyme (5-LO) transforms arachidonic acid into leukotriene A_4 (LTA_4) which can be further metabolized to the peptidoleukotrienes (LTC_4 and LTD_4), potent vasoactive and spasmogenic mediators capable of inducing increased vascular permeability and bronchoconstriction, as well as leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (HETE), which function as chemotactic and chemokinetic mediators playing a central role in inflammatory cell infiltration. Current evidence suggests that several inflammatory diseases are associated with the presence and actions of leukotrienes. First, bronchoalveolar lavages and sputum from patients with asthma are replete with lipid-derived mediators including peptidoleukotrienes and LTB_4.[1] Elevated levels of these mediators have been associated with the key features of the disease including, increased vascular permeability, airway obstruction, mucus hypersecretion, and leukocytic infiltration of pulmonary tissue.[2] The improvement of acute airway function in asthmatic patients upon administration of either a peptidoleukotriene antagonist or a 5-LO inhibitor is strong evidence supporting the importance of peptidoleukotrienes in this disease.[3] Likewise, the biochemical pharmacology of inflammatory bowel disease (IBD) suggests an important role for 5-LO products in the pathophysiology of this group of diseases. As is the case with asthma, supporting evidence for the role of leukotrienes has been obtained in the clinic. The efficacy of sulphasalazine and the active principle, 5-aminosalicylic acid, has been suggested to be due to inhibition of leukotriene biosynthesis.[4] Perhaps most cogent are the recent findings that the 5-LO inhibitor, zileuton, reduced colonic levels of LTB_4 in ulcerative colitis patients. Since these results were encouraging, a preliminary clinical trial was conducted with zileuton in which significant symptomatic improvement was noted.[5] Lastly, leukotrienes are believed to be contributing mediators to the pathophysiology of rheumatoid arthritis, and clinical results with zileuton indicate significant improvement in some disease parameters.[6] These results suggest that blockade of the leukotriene cascade will be of therapeutic benefit in the treatment of a number of inflammatory diseases for which the current therapeutic modalities have proven inadequate. Recognition that the reduction of multiple lipid mediators via the inhibition of 5-LO theoretically offers a greater therapeutic benefit than a selective leukotriene antagonist has made the goal of developing effective 5-LO inhibitors an important therapeutic target. Within the field of 5-lipoxygenase inhibition, multiple strategies have been developed, and compounds derived from these efforts are currently being tested in the clinic.[7]

We were particularly intrigued by the observation that lipophilic *N*-hydroxyureas which are orally active inhibitors of leukotriene biosynthesis can be prepared.[8] The mechanistic rationale for these compounds first

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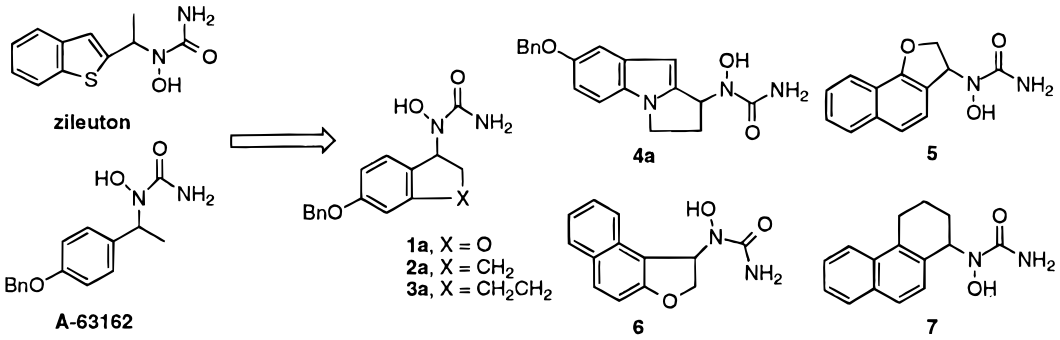


Figure 1. Bicyclic and tricyclic templates.

Table 1. Yield and Analytical Data

no.	R_1	R_2	formula	mp (°C)	yield (%)[a,b]
1a	O	β-OCH_2Ph	$C_{16}H_{16}N_2O_4$	175−6	45
1b	O	β-OCH_2(4-MeOPh)	$C_{17}H_{18}N_2O_5$	181−2	13
1c	O	β-OPh	$C_{15}H_{14}N_2O_4$	168−9	12
1d	O	γ-OPh	$C_{15}H_{14}N_2O_4$	170−1	16
2a	CH_2	β-OCH_2Ph	$C_{17}H_{18}N_2O_3$	167	48
2b	CH_2	β-OCH_2(4-MePh)	$C_{18}H_{20}N_2O_4$	166−7	10
2c	CH_2	β-OPh	$C_{16}N_{16}N_2O_3$	164−5	26
2d	CH_2	δ-OPh	$C_{16}H_{16}N_2O_3$	161−2	27
3a	$(CH_2)_2$	β-OCH_2Ph	$C_{18}H_{20}N_2O_3$	160−2	26
3b	$(CH_2)_2$	β-OMe	$C_{12}H_{16}N_2O_3$	163−4	27
3c	$(CH_2)_2$	H	$C_{11}H_{14}N_2O_2$	168−9	33
3d	$(CH_2)_2$	α-OCH_2Ph	$C_{18}H_{20}N_2O_3$	187−8	35
3e	$(CH_2)_2$	β-OCH_2(4-MePh)	$C_{19}H_{22}N_2O_4$	165−6	22
3f	$(CH_2)_2$	γ-OCH_2Ph	$C_{18}H_{20}N_2O_3$	160−1	24
3g	$(CH_2)_2$	β-Ph	$C_{17}H_{18}N_2O_2$	175−6	44
3h	$(CH_2)_2$	α-OPh	$C_{17}H_{18}N_2O_3$	168−9	46
3i	$(CH_2)_2$	β-OCH_2(4-ClPh)	$C_{18}H_{19}N_2O_3Cl$	166−7	21
3j	$(CH_2)_2$	β-$(CH_2)_2Ph$	$C_{19}H_{22}N_2O_2$	162−3	30
3k	$(CH_2)_2$	β-OCH_2-(2-naphthyl)	$C_{22}H_{22}N_2O_3$	169	28
4a	H	OCH_2Ph	$C_{18}H_{19}N_3O_3$	199−200	40
4b	H	H	$C_{12}H_{13}N_3O_3$	190−2	30
4c	H	Cl	$C_{12}H_{12}N_3O_2Cl$	186−9	45
4d	H	Et	$C_{14}H_{17}N_3O_2$	209−10	7
4e	Me	*i*-Pr	$C_{16}H_{21}N_3O_2$	175−7	15
4f	H	OPh	$C_{18}H_{17}N_3O_3$	186−8	35
4g	Me	OPh	$C_{19}H_{19}N_3O_3$	181−2	22
5			$C_{13}H_{12}N_2O_3$	195−6	18
6			$C_{13}H_{12}N_2O_3$	191−2	12
7			$C_{15}H_{16}N_2O_2$	192−3	60

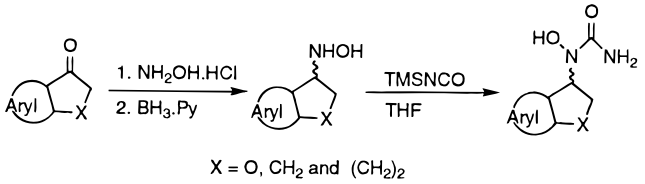
[a] Elemental analyses were within ±0.4% of the calculated value except where stated otherwise. [b] Conversion of the ketone to the hydroxyurea (three steps, Scheme 1).

proposed by Corey was bidentate binding of the hydroxamate to the ferric ion of 5-LO.[9] Recent studies on the mechanism of the hydroxamic acid/hydroxyurea class suggest that reduction of ferric to ferrous enzyme, and not the formation of a stable enzyme inhibitor complex, may be the actual mechanism of inhibition.[10] As mentioned above, initial clinical studies with the *N*-alkyl-*N*-hydroxyurea, zileuton, suggest that it will have therapeutic utility in the treatment of asthma and perhaps IBD and rheumatoid arthritis. However, the pharmacodynamics of zileuton in humans may require dosing four times daily for maximum efficacy.[7a] Hence, there exists a need to develop 5-lipoxygenase inhibitors which will have a more extended duration of action in humans. This paper outlines our efforts to identify an *N*-hydroxyurea with the potential for an extended duration of action in humans.

Chemistry

Hydroxyureas (Figure 1, **1**−**7**, and Table 1) were prepared from the corresponding ketones in three steps,

Scheme 1



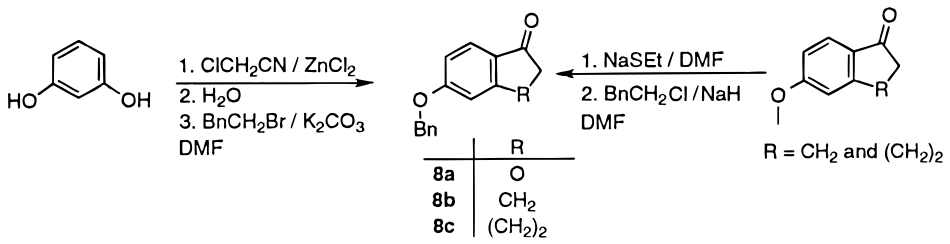
viz. oximation, reduction, and hydroxyurea formation (Scheme 1).[11] The 6-alkoxybenzofuranones **8a** (X = O) were efficiently synthesized from resorcinol in three steps[12] (Scheme 2). The 5-alkoxyindanones (**8b**, X = CH_2) and 6-alkoxytetralones (**8c**, X = $(CH_2)_2$) were prepared from the commercially available 5-methoxyindanone and 6-methoxytetralone, respectively, by demethylation and realkylation with the appropriate alkyl halide. 6-Phenoxybenzofuranone (**10**) was prepared by an intramolecular Friedel−Crafts reaction of (3-phenoxyphenoxy)acetyl chloride **9** (Scheme 3). 5-Phenoxybenzofuranone (**12**) was prepared by an extension of a procedure published by Jung et al.[13] starting from the methoxymethyl ether of 4-phenoxyphenol which was converted to the benzoyl chloride **11** by the directed metalation strategy.[14] The benzoyl chloride **11** was transformed to the corresponding diazo ketone, which was treated with glacial acetic acid to afford **12**. The hydroxyureas **1a**, **2a**, and **3a** were resolved by the previously published procedure from our laboratories.[15] The preparation of 5- and 7-phenoxyindanones (**14** and **15**) was accomplished by an intramolecular Friedel−Crafts cyclization of 3-(3-phenoxyphenyl)propionic acid (**13**).

Ullman coupling was used to synthesize 5-phenoxytetralone (**17**) from the corresponding phenol (Scheme 4). 6-Phenyltetralone (**16**) was prepared by a Pd-catalyzed coupling of 6-hydroxytetralone triflate and phenylzinc chloride. Pyrrolo[1,2-*a*]indole skeleton **19** was prepared from the readily available ethyl indole-2-carboxylates **18** by standard procedures[16] involving condensation with ethyl acrylate and decarbethoxylation in refluxing acetic acid. The furanones required for the synthesis of hydroxyureas **5** and **6** were prepared from α- and β-naphthol, respectively, e.g., the naphthols were reacted with chloroacetic acid to afford the naphthoxyacetic acids **20** and **22** which underwent a clean intramolecular Friedel−Crafts reaction to afford **21** and **23**. The hydroxyurea **7** was prepared from the commercially available 1,2,3,4-tetrahydrophenanthren-1-one.

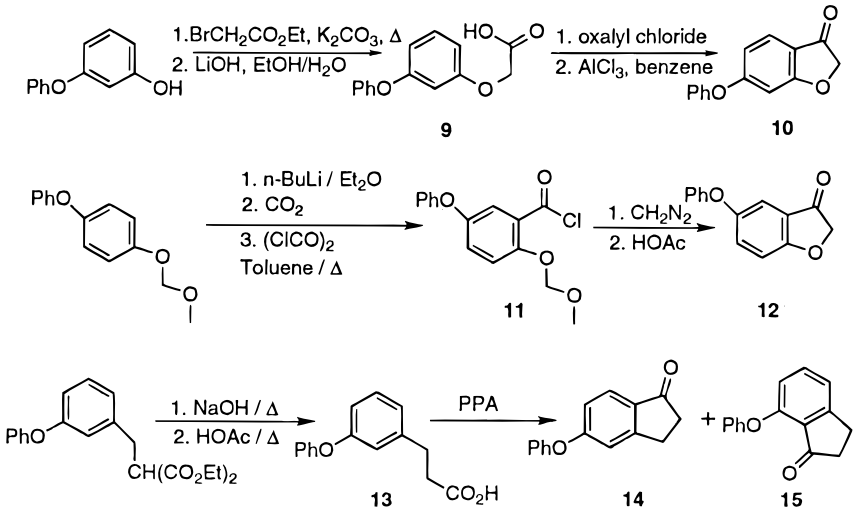
Biological Results and Discussion

In order to probe the structural requirements for binding, the templates chosen for investigation were

Scheme 2



Scheme 3



conformationally restricted analogs of the benzylic hydroxamic acids and hydroxyureas that had been previously identified by the work of Summers and co-workers at Abbott as orally active 5-LO inhibitors.[11,17] In addition to providing some knowledge of the binding, we thought that conformational constriction might afford enhanced potency relative to the known compounds in which the *N*-hydroxyurea was not constrained in a ring. The investigation was further restricted to the synthesis of *N*-hydroxyureas as workers at both Abbott and Wellcome had demonstrated that the *N*-hydroxyurea group afforded superior pharmacokinetic and hence pharmacological behavior relative to the acetohydroxaminc acids.[8] Because lipophilicity was known to be very important for good 5-LO inhibition,[18] the *N*-hydroxyureas chosen for the initial study were prepared with substituents which would afford compounds of similar lipophilicity. This was achieved by preparing the benzyloxy analogs **1a**−**4a** and the parent heterocycles for the tricyclic analogs **5**−**7** (Figure 1, Table 1).

The IC_{50} for the inhibition of 5-lipoxygenase was determined for the semipurified enzyme from a rat basophilic leukemia cell line (RBL-1) using a continuous assay based upon oxygen consumption. Previous studies in our labs employing the oxygen electrode assay had demonstrated that the rat and recombinant human enzyme afford similar IC_{50} values for a number of structurally diverse 5-LO inhibitors (ref 19 and unpublished observations). Moreover, we have shown that the oxygen consumption assay affords results comparable to those obtained by monitoring 5-HPETE production in a 5-LO-containing supernatant prepared from human monocytes.[19] The benzyloxy analogs **1a**−**4a**, as expected on the basis of the findings of Summers[18] and their similarity to Abbott-63162 and zileuton (Scheme 1), demonstrated *in vitro* IC_{50} values of ~1−2 μM. The unsubstituted tricyclic compounds **5**−**7** were slightly less active, a result that for compounds **6** and **7** could be related to reduced lipophilicity (Clog*P*, Table 2). An additional assay which was examined early on as an indicator of the potential efficacy of these compounds in humans was inhibition of leukotriene biosynthesis in calcium ionophore-stimulated human whole blood. This assay has the additional complexities of protein binding and partitioning that are encountered in the *in vivo* setting and has been used as a criteria to predict the potential utility in humans.[20] When examined in human whole blood, compounds **1a**−**3a** performed well, displaying IC_{50} values similar to those obtained on the isolated enzyme, whereas the activity of **4a** was notably attenuated. The remaining tricyclic compounds, **5**−**7**, which were weaker inhibitors of the isolated enzyme, had IC_{50} values in excess of 10 μM in human whole blood. These studies established the 5-LO inhibitory activity of all seven cyclic templates to which the hydroxyurea moiety was attached. Subsequent studies focused on the templates represented by compounds **1a**−**4a**.

The importance of optimizing lipophilicity (log *P*) for *in vitro* 5-LO inhibitor activity is illustrated in Table 3 for a series of tetraline derivatives. The less lipophilic methoxy and unsubstituted compounds (**3b** and **3c**) were weak inhibitors. For the compounds having the more lipophilic substituents (**3a** and **3d**−**k**), similar IC_{50} values were obtained regardless of the nature of this substituent, suggesting that lipophilicity was the main factor governing affinity at this site remote from the hydroyurea. The inhibition of LTB_4 production in an *ex vivo* assay in the mouse was used to examine the potential of these compounds to inhibit leukotriene biosynthesis *in vivo* following oral administration. The

Scheme 4

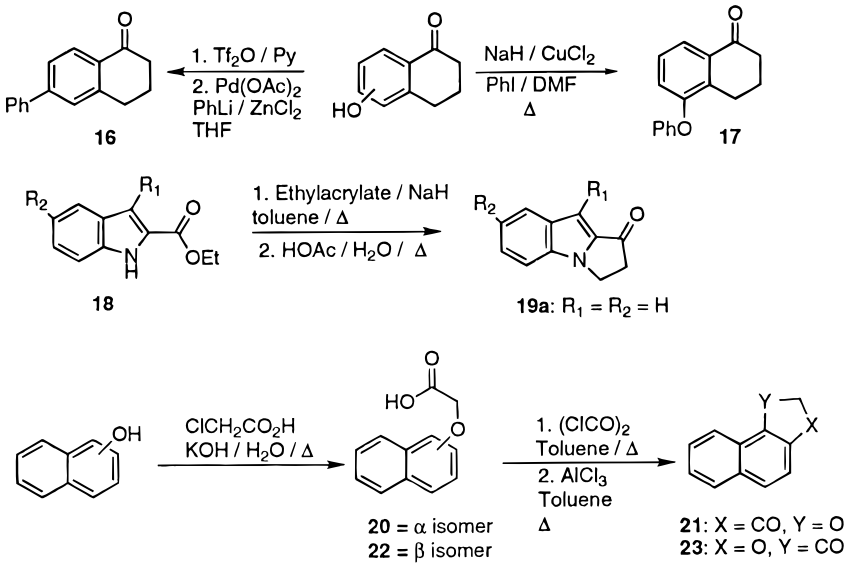


Table 2. *In Vitro* Evaluation of Hydroxyurea Templates

	IC$_{50}$ (μM)		
compound	5-LO	human whole blood[a]	calcd log P[b]
1a	2.3	4.3 (3.2−6.8)	3.6
2a	0.78	0.64 (0.41−0.80)	4.7
3a	1.8	1.2 (0.76−1.8)	5.3
4a	0.65	4.5 (4.0−4.9)	4.6
5	4.2	29% at 10 μM	4.7
6	8.0	27% at 10 μM	2.9
7	5.6	31% at 10 μM	2.9
zileuton	3.2	2.4 (1.2−5.5)	3.6

[a] Numbers in parentheses are 95% confidence limits calculated by linear regression analysis of percent inhibition at the highest concentration tested. [b] Numbers calculated using the Pomona Clog*P* program and do not include hydroxyurea fragment.

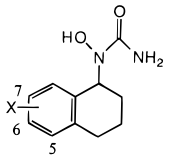
optimum oral activity was achieved with benzyloxy and phenoxy substituents (**3a**, **3e**, **3h**, and **3i**). A further increase in lipophilicity (**3j** and **3k**) slightly enhanced *in vitro* activity, but was deleterious to *ex vivo* activity. Our findings on the optimization of aryl substituent group closely parallel the observations made by Summers in the corresponding A-63162 series.[17] These hydroxyureas are poorly water soluble (the measured aqueous solubility for **3a** was 0.67 μg/mL). One possible explanation for the reduced *in vivo* activity of **3j** and **3k** may be that the increased lipophilicity further decreases aqueous solubility, resulting in solubility-limited absorption. In order to minimize any variation due to solubility-limited absorption, all compounds were dosed in solution (DMA/sesame oil) and not as suspensions. Oral dosing with solutions does not ensure that the compounds will remain in solution, and it is possible that **3j** and **3k** precipitated from solution in the stomach. Interestingly, the 5- and 6-benzyloxy-substituted tetralins, but not the 7-benzyloxy-substituted tetralin (**3d** and **3a**, but not **3f**) were orally active. Because all three compounds were equally effective as 5-LO inhibitors, this result also suggests differences in absorption and metabolism.

The relationship between lipophilicity and *in vitro*/*ex vivo* activity seen in the pyrroloindole series is similar to that seen for the tetralins (Table 4). As was found for the tetralins, compounds with benzyloxy (**4a**) and phenoxy (**4f**) substituents demonstrated good *ex vivo* activity. In addition the unsubstituted compound (**4b**) which had modest activity in the 5-LO assay was active in the *ex vivo* assay. Alkyl substitution of the pyrroloindole decreased oral activity for both the 9-unsubstituted and 7-phenoxy compounds (compare **4b** to **4d** and **4f** to **4g**). Finally, analogues of the indan (**2**) and dihydrobenzofuran (**1**) possessing the preferred benzyloxy and phenoxy substituents performed well in the mouse *ex vivo* assay for oral activity (Table 5).

Having established oral activity for a number of compounds, we next examined the pharmacological duration of action. Summers[17] had reported data in rats that demonstrates that a hydroxamic acid inhibitor of 5-LO containing a benzyl ether linkage (Abbott-63162) was metabolically inactivated by oxidative cleavage of this linkage. A similar experience was also reported in the rat for BW-A137C.[21] In this latter example, substitution with an aryl ether improved duration of action.[22] Because these examples suggested that oxidative metabolism would limit the duration of action of compounds containing the benzyl ether linkage, representative examples of benzyl and phenyl ethers were chosen for pharmacodynamic comparison.

Duration of action was examined using the mouse *ex vivo* assay, but now extending pretreatment time from 0.5 to 6.0 h. To make this comparison more representative of the individual pharmacodynamic performance of each compound, animals were dosed at 1.5 times the ED$_{50}$ determined with the initial protocol (0.5 h pretreatment). Time courses were performed at these doses to determine the maximal inhibition achievable with the compound. Test compounds were then examined to determine those which best retained their maximal potency in the *ex vivo* assay at the 6 h time point. To our surprise, the duration of pharmacological action in mice for the benzyl esters, **1a** and **3a**, was superior to that of the phenyl ethers, **1d** and **2c** (Table 6). Additional compounds of interest having the benzyl ether linkage were evaluated in the adoptive peritoneal anaphylaxis (APA) reaction in the mouse using a pretreatment time of 7 h. The APA model, which is complementary to the *ex vivo* assay, utilizes antigen-specific IgE to stimulate leukotriene biosynthesis *in vivo*.[23] While not statistically significant at a $p < 0.05$ due to abnormally high variability in the vehicle control group,

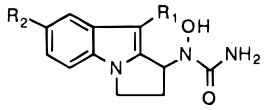
Table 3. Tetralins: Correlation of Lipophilicity with *in Vitro* and *ex Vivo* 5-LO Inhibition



compound	position of X	X	calcd log P^a	5-LO IC_{50} (μM)	LTB_4 mouse *ex vivo* % of control [ED_{50}]^b
3a	6	OCH_2Ph	5.3	1.8	−67***[9.9 (7.9−14.4)]
3b	6	OMe	3.6	48	NS
3c		H	3.6	29	−30*
3d	5	OCH_2Ph	5.3	0.91	−44***
3e	6	OCH_2(4-OMePh)	5.3	0.5	−81***[4.8 (4.0−5.8)]
3f	7	OCH_2Ph	5.3	1.0	NS
3g	6	Ph	5.5	3.0	−52***
3h	5	OPh	5.7	0.84	−57***
3i	6	OCH_2(4-ClPh)	6.0	2.2	−70*** [4.5 (1.8−8.6)]
3j	6	$(CH_2)_2Ph$	6.2	0.6	NS
3k	6	OCH_2(2-naphthyl)	6.4	0.5	NS
zileuton			3.6	3.2	−78***[1.0 (0.05−1.8]

^{a} Numbers calculated using the Pomona Clog*P* version 3.4 program and do not include hydroxyurea fragment. ^{b} Compounds screened at a dose of 10 mg/kg, po; ED_{50} values (mg/kg) along with 95% confidence limits (shown in parentheses) were determined for compounds demonstrating 60% or greater inhibition at the screen dose and were calculated by linear regression analysis; statistical significance, which was judged by Student's *t* test versus vehicle control, is given as *$p < 0.05$; **$p > 0.01$; ***$p < 0.001$ or NS for no stastistically significant inhibition at the screen dose.

Table 4. Pyrrolo[1,2-*a*]indole Inhibitors of 5-LO



compound	R_1	R_2	calcd log P^a	5-LO IC_{50} (μM)	LTB_4 mouse *ex vivo* % of control [ED_{50}]^b
4a	H	OCH_2Ph	4.6	0.65	−68***[1.8 (0.9−55)]
4b	H	H	2.8	10	−74***^{c}
4c	H	C;	3.6	1.3	not tested
4d	H	Et	4.0	1.7	NS
4e	Me	i-pr	5.0	1.7	NS
4f	H	OPh	5.0	0.52	−58***
4g	Me	OPh	5.7	0.45	−26***

^{a,b} Same as Table 3. ^{c} ED_{50} not determined because of poor IC_{50} for 5-LO inhibition.

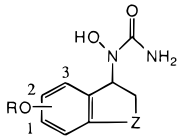
2a showed a clear inhibitory trend and approached statistical significant inhibition at $p < 0.10$. This trend was not seen with any of the compounds tested in the APA model, other than **1a**, which strongly inhibited the response (55%, $p < 0.01$). In addition to confirming the duration of action demonstrated for **1a** in the *ex vivo* LTB_4 assay, the APA model identified the (benzyloxy)-dihydroindan (**2a**) as a compound with an extended duration of action (Table 7). In summary these two *in vivo* models identified three compounds (**1a**, **2a**, and **3a**) with an improved pharmacodynamic profile relative to the standard, zileuton.

For these initial studies all of the compounds had been examined as their racemates. As there are many potential advantages to developing homochiral therapeutic agents, we chose to examine the importance of chirality on both the *in vitro* and *in vivo* potency of the three lead compounds before proceeding further. No greater than a 2-fold difference was observed in the oxygen electrode assay IC_{50} values of the individual enantiomers of the three enantiomeric pairs (Table 8). These findings are in accordance with previous observations demonstrating that the chirality of the asymmetric center adjacent to the hydroxyurea does not substantially influence the 5-LO inhibitory activity of these compounds.[21] However, in the *ex vivo* LTB_4 assay of the individual enantiomers, notable differences were seen. The separation of activity was most clearly seen for the (+) and (−) enantiomers of **1a** which produced ED_{50} values of 0.9 and 22 mg/kg, respectively. On the basis of these findings for the individual enantiomers and the duration of action studies, (+)-**1a** (SB 202235) was chosen as the best overall compound. In order to determine if the profile of (+)-**1a** met our criteria of the potential for improved pharmacodynamic performance in humans, more detailed studies focusing on the pharmacokinetics and metabolism of (+)-**1a** were initiated.

Pharmacokinetic Characterization of the Enantiomers of 1a. Assuming that **1a** has no pharmacologically active metabolites, the inhibition results from the *ex vivo* LTB_4 assay should correlate with the pharmacokinetics of the compound. To test this the oral pharmacokinetics of (+)-**1a** and (−)-**1a** (20 mg/kg) were characterized following oral administration of a suspension in 0.5% tragacanth to mice. The maximal plasma concentrations and AUCs achieved for (+)-**1a** were 2.6 μg/mL and 21 μg·h/mL, and for (−)-**1a** these values were 1.0 μg/mL and 7.5 μg·h/mL (Figure 2). The approximately 60% lower C_{max} and AUC for (−)-**1a** relative to (+)-**1a** following oral administration explains, at least in part, the differences in the oral pharmacology (*ex vivo* LTB_4) of the two compounds in the mouse. Furthermore, the difference in oral pharmacokinetics appears to be due to enantioselective differences in clearance as intravenous studies indicated that (+)-**1a** was cleared from plasma much slower than (−)-**1a** (data not shown). The oral pharmacokinetics of (+)-**1a** compares favorably with published data on zileuton in the mouse.[8a] Similar AUCs and half-lives were observed for both compounds.

Differences favoring (+)-**1a** were also seen in the dog following iv administration of the enantiomers (Table 9). As the clinical data for zileuton[24] suggested that the pharmacokinetics in the dog was the least predictive of the four species examined preclinically,[8a] further phar-

Table 5. Benzyl and Phenyl Ethers of Indanyl and Dihydrobenzofuranyl 5-LO inhibitors



compound	position of subst	R	Z	5-LO IC_{50} (μM)	LTB_4 mouse *ex vivo* % of control[ED_{50}][a]
1a	1	CH_2Ph	O	2.3	−100***[3.9 (3.1−4.9)]
1b	1	CH_2(4-MeOPh)	O	2.7	−73***[9.8 (5.6−90)]
1c	1	Ph	O	3.3	−56***
1d	2	Ph	O	3.5	−64***[1.9 (1.0−3.8)]
2a	1	CH_2Ph	CH_2	0.78	−85***[2.6 (1.803.4)]
2b	1	CH_2(4-MeOPh)	CH_2	1.4	NS
2c	1	Ph	CH_2	0.85	−88***[6.4 (4.7−9.0)]
2d	3	Ph	CH_2	5.6	NT

[a] Same as Table 3.

Table 6. Duration of Action Comparison in the Mouse Using the *ex Vivo* LTB_4 Assay

compound	dose (mg/kg)	maximum inhibition of LTB_4[a]	time of maximum LTB_4 inhibition	% inhibition of LTB_4 of 6 h[a]
1a	6	91***	1	87***
3a	15	61***	2	67***
1d	3	67***	0.5	0 NS
2c	10	88***	1	57***
zileuton	2	70***	1	40***

[a] ***$p = <0.001$.

Table 7. Duration of Action Comparison in the Mouse Adoptive Peritoneal Anaphylaxis Assay

compound	5-LO IC_{50} (μM)	*ex vivo* screen ED_{50}[a]	APA % change from controls[b]
1a	2.3	3.9	−55**
2a	0.78	4.0	−31[c]
4a	0.65	1.8	−8 NS
3e	0.5	4.8	−13 NS
3i	2.2	4.5	0 NS
zileuton	3.2	1.0	−10 NS

[a] Data from Tables 3−5. [b] Following a 7 h treatment with the compounds dosed at 1.5 times the *ex vivo* ED_{50} or vehicle (DMA/oil), sensitized 1BD8 cells were injected ip. Five minutes later ARS-BSA was injected, and following an additional 5 min the animals were sacrificed and the LTB_4 levels of the peritoneal determined according to published procedures.[22] [c] Approached statistical significance at a $p < 0.10$ ($t = 1.3353$, df = 15).

Table 8. Selection of Preferred Enantiomer

compound	5-LO IC_{50} (μM)	LTB_4 mouse *ex vivo*, ED_{50} (mg/kg)[a]
(+)-**1a**	1.9	0.9 (0.5−1.3)
(−)-**1a**	2.0	22 (10.3−170)
(+)-**2a**	0.88	1.9 (1.6−2.1)
(−)-**2a**	0.62	0.9 (0.57−1.2)
(+)-**3a**	0.76	28 % inhibition at 10 mg/kg
(−)-**3a**	1.3	6.6 (4.7−9.7)

[a] 95% confidence limits (shown in parentheses) were calculated by linear regression analysis.

macokinetic characterization of (+)- and (−)-**1a** in the dog was not pursued.

Unlike mouse and dog, the differences in plasma clearance between (+)-**1a** and (−)-**1a** in the monkey were small (6.1 and 10.3 (mL/min)/kg, respectively). However, a longer half-life was observed for (+)-**1a** (6 h) when compared with that of (−)-**1a** (1 h) following iv administration presumably due to a larger volume of distribution for (+)-**1a**. The oral pharmacokinetics of (+)-**1a**, the more active enantiomer, were characterized

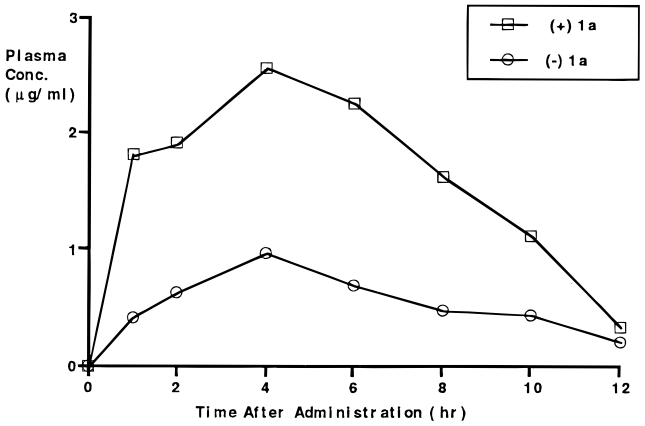


Figure 2. Mean plasma concentrations of (+)- and (−)-**1a** in male CD-1 mice following oral administration (20 mg/kg).

Table 9. Plasma Clearance Values and Half-Lives following Intravenous Administration[a]

	(+)-**1a**		(−)-**1a**		zileuton[b]
	CL_p	$t_{1/2}$	CL_p	$t_{1/2}$	$t_{1/2}$
mouse	8.6	1.0	ID[c]	ID	0.9
dog	3.5	2.9	10.9	1.1	7.5
monkey	6.1	6.0	10.3	1.1	0.3

[a] Abbreviations and units of pharmacokinetic parameters are as follows: CL_p ((mL/min)/kg), apparent plasma clearance; $t_{1/2}$ (h), apparent terminal half-life. Compounds were administered intravenously as a solution. [a] Obtained from ref 8a. [b] ID, insufficient data to calculate parameter.

following administration of an aqueous solution in 30% (w/v) (2-hydroxypropyl)-β-cyclodextrin. Maximal plasma concentrations (1.3 μg/mL) were reached approximately 2 h after drug administration, and (+)-**1a** disappeared from plasma with an apparent terminal half-life of approximately 10 h. The longer half-life following oral administration for (+)-**1a** when compared to the half-life measured after iv administration suggested that the apparent terminal half-life is a reflection of prolonged absorption rather than elimination. The mean AUC_{0-t} for (+)-**1a** was approximately 13 μg·h/mL. Comparison of the oral pharmacokinetic data of (+)-**1a** (10 mg/kg) with published data on zileuton (20 mg/kg) indicates that systemic exposure of (+)-**1a**, based upon AUCs of parent drug, was almost 3-fold greater than that of zileuton even though a higher dose of zileuton was administered.[8a] The mean bioavailability was good, but somewhat variable (>50% in two monkeys and approximately 20% in a third).

Table 10. Glucuronidation Rates for (+)- and (−)-**1** and Zileuton in Mouse and Human Liver Microsomes

test system	compound	rate of formation ((nmol/mg of protein)/h)[a]
mouse	(+)-**1a**	none detected
	(−)-**1a**	5.9
	zileuton	0.7
human	(+)-**1a**	2.5
	(−)-**1a**	60.3
	zileuton	6.8 (0.7)[b]

[a] Values are the mean of duplicate determinations. [b] Along with the major glucuronide evidence for a formation of a second glucuronide at a reduced rate was seen.

Pharmacokinetic studies in the rat following the oral administration of (+)- and (−)-**1a** (20 mg/kg as a suspension in 0.5% tragacanth) were also performed. Low (generally <200 ng/mL) and erratic plasma concentrations were noted up to 24 h after dosing, which made it impossible to calculate any relevant pharmacokinetic parameters. Similarly, both (+)- and (−)-**1a** rapidly disappeared from plasma following iv administration, and again no relevant pharmacokinetics could be calculated. The lack of significant plasma concentrations for the enantiomers of **1a** is consistent with the performance in the rat *ex vivo* LTB_4 assay in which poor and erratic inhibition was observed. Of the four species in which pharmacokinetics were examined, the rapid clearance of **1a** proved unique to the rat.

***In Vitro* Metabolism.** The selection of (+)-**1a** as the preferred enantiomer was initially based upon the results of the *ex vivo* LTB_4 assay in the mouse. The subsequent pharmacokinetic data from three species provided further support for this choice (Table 9). Nevertheless, without any understanding of the origin of this difference, one could not predict with confidence which would be the preferred enantiomer in humans. Experiments in mouse hepatic microsomes were conducted to investigate if the difference in clearances of (+)- and (−)-**1a** in the mouse were the result of enantioselective metabolism. Originally, cytochrome P-450-catalyzed oxidation of (+)- and (−)-**1a** was investigated; however, no evidence was found for the involvement of P-450 enzymes in the metabolism of these enantiomers. Glucuronidation was examined next as this mechanism had been reported as an important pathway of metabolism for zileuton.[25] Incubating mouse microsomes with (−)-**1a** and [^{14}C]UDP-glucuronic acid (UDPGA) resulted in the formation of a labeled metabolite that was identified as a glucuronide of (−)-**1a** using quadrupole MS−MS. This metabolite was produced at an apparent rate of 5.9 (nmol/mg of protein)/h (Table 10). In contrast, there was very little, if any, ^{14}C-labeled glucuronide of (+)-**1a** formed under identical incubation conditions. Appropriate controls were run to verify that the formation of the glucuronide metabolite was not an artifact of the *in vitro* incubations, i.e., the metabolite was not produced in the absence of (−)-**1a** or microsomes. These studies suggest that the difference in clearances of (+)- and (−)-**1a** in the mouse could be at least partially due to metabolism via hepatic stereoselective glucuronidation.

Since the ultimate goal of this work was to select the best compound for use in humans, the glucuronidation of (+)- and (−)-**1a** was also examined in human liver microsomes. As was found with the mouse microsomes, the (+)-enantiomer of **1a** was a poor substrate for glucuronidation forming a small amount of the ^{14}C-labeled (+)-**1a** glucuronide at a rate of 2.5 (nmol/mg of protein)/h. However, the (−) enantiomer of **1a** formed the glucuronide at a much higher rate of 60.3 (nmol/mg of protein)/h. The rate with (−)-**1a** was approximately 10-fold higher than was observed in mouse microsomes. On the basis of the *in vitro* data, enantioselective differences in metabolic clearances of (+)- and (−)-**1a** in humans may be similar to or even larger than in the mouse. The metabolism of zileuton was also examined in mouse and human liver microsomes. An apparent glucuronide of zileuton was formed in mouse microsomes, at a rate of 0.7 (nmol/mg of protein)/h. This glucuronide was also formed in human liver microsomes, at a rate of 6.8 (nmol/mg of protein)/h.

Conclusions

The 5-LO inhibitory properties of seven cyclic templates possessing the hydroxyurea moiety have been examined. Although not rigorously evaluated, it appears that attaching the hydroxyurea moiety to a ring did not enhance 5-LO inhibition relative to less conformationally restricted compounds, such as zileuton and A-63162. Moreover, the enzyme showed very little discrimination between the individual enantiomers in the three cases examined (**1a**−**3a**). These results are consistent with a redox mechanism of inhibition, as this would require only transient association of the inhibitor with the enzyme.[10] A nonredox mechanism involving formation of a stable complex, presumably with the active-site iron, would be expected to be more sensitive to the immediate environment of the hydroxyurea. The lag phase seen in the kinetics of inhibition using the oxygen electrode assay is further support for the involvement a redox active species in the mechanism of inhibition.[19]

The protocol for selection of a compound of potential therapeutic interest began with the determination of IC_{50} for inhibition of the semipurified 5-LO enzyme from RBL-1 cells in combination with the inhibition of ionophore-induced LTB_4 generation in whole human blood. Analogues of those bicyclic systems (**1a**−**4a**) having good activity in both assays were examined for their ability to block LTB_4 production in a murine *ex vivo* assay, and the best compounds from this assay were then chosen based upon duration of action studies. From an evaluation of individual enantiomers of the most promising racemic compounds, (+)-**1a** (SB 202235) was selected, and further characterization was undertaken to evaluate the compound's suitability as a potential clinical development candidate. Pharmacokinetic studies in three species demonstrated enantiospecific differences between (+)-**1a** and (−)-**1a**. In all three species, (+)-**1a** was eliminated more slowly and was clearly superior to (−)-**1a**. In the mouse a good correspondence between and pharmacokinetics and pharmacodynamics (*ex vivo* assay) was established. The demonstration of a much higher rate of glucuronidation for (−)-**1a** when compared to that for (+)-**1a** in mouse hepatic microsomes supports enantiospecific glucuronidation as the probable mechanism to explain the enantioselective clearance. Enantioselective glucuronidation favoring (−)-**1a** was also found in human liver microsomes. These data clearly pointed to the selection of (+)-**1a** over (−)-**1a** as the preferred enantiomer for clinical development.[26]

Pharmacodynamic studies in the mouse demonstrated that compounds containing a benzyl ether linkage were equivalent, if not superior, to compounds possessing the phenyl ether linkage. The superior *in vivo* activity of the benzyl ethers in the mouse was unexpected in light of the reported metabolic lability of related benzyl ether hydroxyureas in the rat. In agreement with these reports, the benzyl ether **1a** was rapidly cleared following iv administration in the rat. However, **1a** was not rapidly inactivated in the two additional species which were examined (dog and monkey). These data suggest that oxidative cleavage of the benzylic ether is the rate-limiting metabolic pathway in the rat for clearance of **1a**, but that oxidation of the benzylic linkage may be less important in other species. In support of this proposal, glucuronidation and not cytochrome P-450 oxidation was the predominant metabolic pathway demonstrated in both mouse and human liver microsomes.

Glucuronidation is the major clearance pathway of zileuton in humans, accounting for as much as 70% of the administered dose. If the relative rates of glucuronidation demonstrated for (+)-**1a** and zileuton *in vitro* are reflective of the relative ease of glucuronidation in humans, then (+)-**1a** (SB 202235) should demonstrate an improved duration of action relative to that of zileuton.[27,28]

Experimental Section

Chemistry: General Procedures. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 783 or 683 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Model AM 250. Chemical shifts are reported as parts per million on the δ scale relative to tetramethylsilane as internal standard. Data are presented as follows: chemical shift (multiplicity, integrated intensity, coupling constants, assignment). Unless otherwise noted, the NMR solvent was $CDCl_3$. Chromatography refers to purification by flash chromatography on E. Merck silica gel 60 (230−400 mesh). When air or moisture sensitive reagents were used, reactions were run under argon, and all aqueous workups culminated in washing the organic layer with water and brine, drying over magnesium sulfate, filtering, and concentrating at reduced pressure.

General Procedure for Conversion of Cycloalkanones to Hydroxyureas (Scheme 1). To a solution of the cycloalkanone in anhydrous pyridine (0.5−1.0 M solution) was added hydroxylamine hydrochloride (2.0 equiv), and the mixture was stirred for at 50 °C for 1−2 h. The solution was cooled to room temperature and poured into water. The thick solid, which forms slowly, is filtered, washed with water, and dried to afford the required oxime usually as a mixture of *E* and *Z* isomers. The oximino compound obtained above was dissolved in a suitable solvent (such as EtOH, CH_2Cl_2/EtOH or CH_2Cl_2/MeOH, 0.1−0.5 M solution), and BH_3−pyridine (3−7 equiv) was added. Hydrogen chloride was added as an aqueous or ethanolic solution (6 N), and the resulting solution was stirred at room temperature until the starting oxime was completely consumed. The product was isolated either by extraction or as the hydrochloride salt. The hydroxylamine was dissolved in THF (0.1−0.4 M solution), and trimethylsilyl isocyanate (2 equiv) was added. The reaction was stirred at 55 °C until the starting hydroxylamine was consumed. The reaction mixture was cooled, and the product was isolated by crystallization or extraction. A final recrystallization from a suitable solvent mixture afforded pure hydroxyurea.

***N*-Hydroxy-*N*-[2,3-dihydro-6-(benzyloxy)benzofuran-3-yl]urea (1a).** Using the above general procedure, **8a** (116 g, 0.5 mol) was converted to the oxime as a yellow solid (118 g, 92%). ^{1}H NMR ($CDCl_3$): δ 7.45 (d, 1H), 7.40 (m, 5H), 6.64 (dd, 1H), 6.55 (d, 1H), 5.17 (s, 2H), 5.07 (s, 2H), 4.07 (br s, 1H). The oxime (118 g, 0.45 mol) in 1:1 MeOH/CH_2Cl_2 (2.3 L) was reduced to the hydroxylamine (100 g, 86%), which was reacted with trimethylsilyl isocyanate (77 mL, 0.57 mol) to afford **1a** as a white powder (62 g, 53%). Mp: 174.5−175.5 °C; ^{1}H NMR ($CDCl_3$, MeOH-d_4): δ 7.45−7.25 (m, 5H), 7.14 (d, 1H), 6.51 (dd, 1H), 6.41 (d, 1H), 5.87 (t, 1H), 5.03 (s, 2H), 4.53 (d, 1H). IR (cm^{-1}): 3460, 3320, 3180, 2880, 1650−1620.

(*S*)-(+)- and (*R*)-(−)-*N*-Hydroxy-*N*-[2,3-dihydro-6-(benzyloxy)benzofuran-3-yl]urea [(+)-1a and (−)-1a]. To a solution of (±)-*N*-hydroxy-*N*-[2,3-dihydro-6-(benzyloxy)benzofuran-3-yl]urea (37.87 g, 0.12 mol) in CH_2Cl_2 (750 mL) at 0 °C was added triethylamine (32 mL, 0.23 mol). After 10 min of stirring, (*R*)-4-benzyl-2-oxazolidinone 3-carbamoyl chloride (43.80 g, 0.18 mol) was added, and the mixture was stirred at 0 °C for 1 h. Water was added, and the organic phase was washed with brine. The solvent was evaporated, and the residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to give the desired diasteriomers (47.04 g, 76% yield) that were separated by preparative HPLC (Chiralcel OJ, 4.6 mm × 250 mm, 1:1 EtOH/hexane, 1.0 mL/min, UV 210 nm), with diasteriomer I having a retention time of 9.72 min and diasteriomer II having a retention time of 13.68 min.

To a solution of diasteriomer I from the above procedure (15.3 g, 29.6 mmol) in THF/H_2O (4:1, 300 mL) at 0 °C was added dropwise hydrogen peroxide (12.5 mL, 116.1 mmol, 30%). Lithium hydroxide (0.716 g, 45.5 mmol) dissolved in H_2O (15 mL) was added, and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous $NaHSO_3$, and the mixture was diluted with EtOAc. The organic phase was washed with brine, and the solvent was evaporated. The residue was triturated with Et_2O and then recrystallized from EtOH to give **(+)-1a** (3.27 g, 35% yield). Mp: 189−190 °C. $[\alpha]_D$: +10.1° in methanol. Following the same procedure the diastereomer II yielded **(−)-1a**. Mp: 188−189 °C. $[\alpha]_D$: −10.7° in methanol.

***N*-Hydroxy-*N*-[2,3-dihydro-5-(benzyloxy)-1*H*-inden-1-yl]urea (2a).** Using the above general procedure **8b** was converted to hydroxyurea **2a**. Mp: 167 °C. ^{1}H NMR (250 MHz, DMSO): δ 8.89 (s, 1H), 7.40 (m, 4H), 7.31 (m, 1H), 7.05 (d, 1H), 6.84 (s, 1H), 6.78 (dd, 1H), 6.36 (s, 2H), 5.58 (m, 1H), 5.07 (s, 1H), 2.88 (m, 1H), 2.71 (m, 1H), 2.17 (m, 1H), 2.09 (m, 1H).

(+)- and (−)-*N*-Hydroxy-*N*-[2,3-dihydro-5-(benzyloxy)-1*H*-inden-1-yl]urea [(+)-2a and (−)-2a]. The resolution was performed using the above general procedure described above for **1a**. Data for **(+)-2a**. Mp: 167−168 °C. $[\alpha]_D$: +36.02° in methanol. Data for **(−)-2a**. Mp: 165.6−166.5 °C. $[\alpha]_D$: −39.2° in methanol.

***N*-Hydroxy-*N*-[1,2,3,4-tetrahydro-6-(benzyloxy)-1-naphthalenyl]urea (3a).** Using the above general procedure, **8c** was converted to hydroxyurea **3a**. Mp: 160−162 °C. ^{1}H NMR (250 MHz, MeOD): δ 7.37 (m, 5H), 7.20 (d, 1H), 6.80 (dd, 1H), 6.71 (d, 1H), 5.43 (t, 1H), 5.05 (s, 2H), 2.90−2.63 (m, 2H), 2.00 (m, 3H), 1.78 (m, 1H).

(+)- and (−)-*N*-Hydroxy-*N*-[1,2,3,4-tetrahydro-6-(benzyloxy)-1-naphthalenyl]urea [(+)-3a and (−)-3a]. The resolution was performed using the above general procedure described above for **1a**. Data for **(+)-3a**. Mp: 154.5−155.5 °C. $[\alpha]_D$: +29.8° in methanol. Data for **(−)-3a**. Mp: 146−147 °C. $[\alpha]_D = -15.3°$ in methanol.

6-(Benzyloxy)-3-oxo-2,3-dihydrobenzofuran (8a). The requisite 2,3-dihydro-6-hydroxy-3-oxobenzofuran was prepared using a modification of the procedure of Shriner.[12] To a solution of 2,3-dihydro-6-hydroxy-3-oxobenzofuran (363 g, 2.42 mol) in DMF (4 L) was added anhydrous potassium carbonate (668 g, 4.84 mol). After 5 min of stirring at room temperature, benzyl bromide (582 g, 3.40 mol) was added to the mixture dropwise over 15 min. The resulting mixture was stirred at room temperature for 18 h, at which time the potassium carbonate was removed by filtration and washed with DMF. The combined organic material was poured into cold H_2O (12 L) and stirred. The solid was collected by filtration, washed with H_2O (4 L), and dried to provide the benzyl ether (554 g, 100%).

5-(Benzyloxy)-1-indanone (8b). To a solution of 5-hydroxy-1-indanone[29] (8.02 g, 54.2 mmol) in dry DMF (120 mL) under an argon atmosphere was added slowly sodium hydride (1.64 g of 80% suspension in mineral oil, 59.6 mmol). After the evolution of hydrogen ceased, benzyl chloride (7.12 mL, 60.0 mmol) was added, and the resulting mixture was stirred for 15 min. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between EtOAc and 1:1 saturated aqueous NaCl/3 N HCl. The organic extract was wsahed with saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was used without further purification.

6-(Benzyloxy)-1-tetralone (8c). To a solution of 6-hydroxy-1-tetralone[29] (9.2 g, 56.8 mmol) in DMF (150 mL) was added potassium hydride (2.49 g, 62 mmol). When the evolution of hydrogen subsided, benzyl bromide (10.6 g, 62 mmol) was added. After 2 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc and washed successively with 3 N HCl, H_2O, and saturated aqueous NaCl. Removal of the solvent *in vacuo* and purification by flash chromatography eluting with a gradient of 0−100% CH_2Cl_2/hexanes yielded the desired product (9.7 g, 73%). The infrared spectrum of the product indicated a conjugated ketone at 1665−1685 cm^{-1}. The NMR spectrum indicated the presence of the benzyl methylene at δ 5.0 and aromatic benzyl protons at δ 7.4.

(3-Phenoxyphenoxy)acetic acid (9). To a solution of 3-phenoxyphenol (4.48 g, 24.1 mmol) in acetone (20 mL) was added potassium carbonate (4.00 g, 29.0 mmol) and ethyl bromoacetate (4.84 g, 29.0 mmol). After overnight reflux, the mixture was cooled and then filtered. The solvent was evaporated, and the residue was dissolved in EtOAc and washed with H_2O and brine. The solvent was evaporated, yielding a yellow oil (5.14 g) that was used without further purification. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.31 (m, 2H), 7.20 (t, 1H), 7.10 (m, 1H), 7.00 (m, 2H), 6.60 (m, 3H), 4.54 (s, 2H), 4.19 (q, 2H), 1.22 (t, 3H). To a solution of this compound in 1:4 H_2O/THF (10 mL) was added lithium hydroxide (0.188 g, 7.83 mmol). After 1 h of stirring the mixture was neutralized with 3 N HCl and diluted with H_2O. The product was extracted from the aqueous phase with EtOAc. The organic phase was washed with brine and evaporated, yielding a red oil (0.739) that was used without further purification. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.30 (m, 2H), 7.21 (t, 1H), 7.10 (m, 1H), 7.00 (m, 2H), 6.60 (m, 3H), 4.61 (s, 2H).

(6-Phenoxy)benzofuran-3-one (10). To a solution of (3-phenoxyphenoxy)acetic acid (0.739 g, 2.61 mmol) in benzene (3 mL) was added oxalyl chloride (3 mL, 45.8 mmol). The mixture was refluxed 0.5 h. The solution was concentrated, and the residue was dissolved in benzene (10 mL). Aluminum chloride was added (0.46 g, 3.48 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was poured over ice, and the organic layer was separated and washed with brine. The solvent was evaporated, and the residue was purified with flash chromatography (1:1 hexane/CH_2Cl_2) to give the desired product (0.12 g, 20% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.60 (d, 1H), 7.42 (m, 2H), 7.24 (m, 2H), 7.09 (m, 1H), 6.70 (dd, 1H), 6.50 (d, 1H), 4.60 (s, 2H).

5-Phenoxybenzofuranone (12). To a solution of methoxymethyl 4-phenoxyphenyl ether (2.9 g, 12.6 mmol) in anhydrous THF (20 mL) was added *n*-BuLi (6.0 mL, 2.5 M in hexane) at −78 °C over 10 min. The mixture was stirred at −78 °C for 2 h, and dry CO_2 was bubbled through the reaction mixture for 10 min. The reaction mixture was allowed to warm to room temperature over 2 h and was partitioned between saturated NH_4Cl and EtOAc. The organic extract was dried and concentrated to afford the product (2.4 g, 70%), which was used without any purification.

A solution of the benzoic acid (2.0 g, 7.3 mmol) in oxalyl chloride (15 mL) was refluxed for 40 min. The excess oxalyl chloride was removed *in vacuo* to afford the required benzoyl chloride which was used in the next step without any further purification. IR (neat): 3010, 2970, 1775, 1470, 1310. Freshly prepared ethereal diazomethane solution was added to a solution of the benzoyl chloride in CH_2Cl_2, and the mixture was stirred until the gas evolution ceased. After evaporation of the solvent, the residue was dissolved in glacial acetic acid (20 mL) and stirred for 30 min at room temperature. The solvent was removed, and the crude product was purified by flash chromatography on silica gel (1:10 hexane/EtOAc) to afford the benzofuranone (0.83 g, 50%). ^{1}H NMR ($CDCl_3$): δ 7.40−6.96 (m, 8H), 4.65 (s, 2H).

3-(3-Phenoxyphenyl)propanoic Acid (13). To a suspension of sodium hydride (1.36 g of 60% dispersion in mineral oil, 33.9 mmol) in DMF (30 mL) under an argon atmosphere at 0 °C was added, over 10 min, a solution of diethyl malonate (5.42 mL, 35.7 mmol) in DMF (20 mL). The reaction mixture was stirred at 0 °C for 30 min, at which time a solution of 3-phenoxybenzyl bromide[30] (4.7 g, 17.9 mmol) in DMF (25 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was partitioned between Et_2O and aqueous NH_4Cl, and the organic extract was washed successively with H_2O and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was purified by flash chromatography, eluting with 10% EtOAc/hexanes to afford diethyl (3-phenoxybenzyl)-malonate as a colorless oil (4.17 g, 68%).

To a solution of diethyl (3-phenoxybenzyl)malonate (4.17 g, 12.2 mmol) in EtOH (100 mL) was added 1 M NaOH (61 mL, 60.9 mmol). The mixture was heated at reflux overnight and then allowed to cool, and the pH was adjusted to pH 4 by the addition of 1 M HCl. The mixture was diluted with CH_2Cl_2, and the organic layer was washed successively with H_2O and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was dissolved in acetic acid and heated at reflux overnight. The reaction mixture was allowed to cool and was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic extracts were washed successively with H_2O and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo* to afford **13** as a pale yellow oil (2.67 g, 90%).

5-Phenoxy-1-indanone and 7-Phenoxy-1-indanone (14 and 15). To a round-bottom flask fitted with a mechanical stirrer was added polyphosphoric acid (600 g). To this was added, over 1 h, 3-(3-phenoxyphenyl)propanoic acid (49 g, 0.202 mol), maintaining the temperature at 75 °C. Upon completion of the addition, the mixture was stirred for 1 h at 80 °C, then cooled to 0 °C, and diluted with ice cold H_2O. Ether was added, and the mixture was stirred for 30 min. The layers were separated, and the aqueous phase was extracted with Et_2O (2 × 200 mL). The combined organic extracts were washed successively with H_2O, saturated K_2CO_3, H_2O, and saturated aqueous NaCl and dried (K_2CO_3). The solvent was removed *in vacuo*, and the residue was purified in portions by flash chromatography, eluting with 10% EtOAc/hexanes. The major component was recrystallized from cyclohexane to afford 5-phenoxy-1-indanone **14** (4.63 g, 10%). Mp: 67−69 °C. ^{1}H NMR ($CDCl_3$): δ 7.72 (d, 1H), 7.42 (t, 2H), 7.22 (m, 1H), 7.10 (d, 1H), 6.98 (d, 1H), 6.93 (s, 1H), 3.06 (dd, 2H), 2.71 (dd, 2H). A minor component was also isolated and recrystallized from cyclohexane to afford 7-phenoxy-1-indanone (**15**) (548 mg, 1%). Mp: 102−103 °C.

6-Phenyl-1-tetralone (16). To a solution of zinc chloride (5.1 mL of 1.0 M solution, 5.1 mmol) in dry THF (20 mL) was added phenyllithium (2.6 mL of 2.0 M solution, 5.1 mmol). The mixture was stirred at room temperature for 30 min and added to a solution containing 6-(1-tetralonyl (trifluoromethyl)-sulfonate (1.09 g, 3.7 mmol), palladium acetate (7.6 mg, 0.03 mmol), and 1,3-bis(diphenylphosphino)propane (14 mg, 0.03 mmol) in dry THF (50 mL). The mixture was stirred at room temperature for 1 h and then partitioned between EtOAc and 3 N HCl. The organic extract was washed with saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was purified by flash chromatography, eluting with 10% EtOAc/hexanes to provide the desired product which was recrystallized from hexanes (0.42 g, 51%). ^{1}H NMR ($CDCl_3$): δ 8.10 (d, 1H), 7.66−7.35 (m, 7H), 3.03 (t, 2H), 2.70 (t, 2H), 2.20 (m, 2H).

5-Phenoxy-1-tetralone (17). To a solution containing 5-hydroxy-1-tetralone (970 mg, 6.0 mmol) and iodobenzene (4.0

mL, 35.7 mmol) in DMF (12 mL) was added slowly with cooling sodium hydride (150 mg, 6.25 mmol). The mixture was heated until dissolution occurred and then allowed to cool. To the mixture was added slowly with cooling cuprous chloride (600 mg, 6.1 mmol), followed by tris[2-(2-methoxyethoxy)ethyl]-amine (0.68 mL, 2.1 mmol). The mixture was heated at 145−150 °C overnight and then allowed to cool. The reaction mixture was partitioned between 3 N HCl and EtOAc was filtered. The organic extract was washed successively with H_2O and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was purified by flash chromatography, eluting with a solvent gradient of 0−5% EtOAc/hexanes to afford the title compound (300 mg, 21%). ^{1}H NMR ($CDCl_3$): δ 7.89 (dd, 1H), 7.40−7.25 (m, 3H), 7.10 (m, 2H), 6.94 (m, 2H), 2.93 (apparent t, 2H), 2.67 (dd, 2H), 2.14 (apparent quintet, 2H).

General Procedure for the Conversion of Indoles to 19, Illustrated for 2,3-Dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-one (19a). To a suspension of sodium hydride (2.65 g of 60% dispersion in mineral oil, 66 mmol) was added ethyl 2-indolecarboxylate (10.0 g, 53 mmol). The mixture was stirred for 15 min, at which time ethyl acrylate (6.3mL, 58 mmol) was added. Heating was continued for 1 h, at which time additional sodium hydride (1.5g of 60% dispersion in mineral oil, 37.5 mmol) and ethyl acrylate (1.0 mL, 9.2 mmol) were added. The reaction mixture was heated at reflux for an additional 4 h and allowed to cool to room temperature. Ethanol was added to the reaction mixture, followed by saturated aqueous NH_4Cl. The pH was adjusted to 4 with 5% HCl, and the mixture was extracted with CH_2Cl_2. The organic extract was washed with saturated aqueous NaCl. The solvent was removed *in vacuo*, and the residue was dissolved in acetic acid (750 mL) containing H_2O (40 mL). The reaction was heated at reflux for 14 h and then allowed to cool to room temperature. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with aqueous $NaHCO_3$ and brine. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography, eluting with a solvent gradient of 10-100% EtOAc/hexane to provide 2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-one (8.00 g, 89%). ^{1}H NMR ($CDCl_3$): δ 7.74 (apparent dd, 1H), 7.41−7.30 (m, 2H), 7.19 (apparent dt, 1H), 6.97 (s, 1H), 4.34 (t, J = 8 Hz, 2H), 3.14 (t, J = 8 Hz, 2H).

1-Naphthoxyethanoic Acid (20). Chloroacetic acid (2.3 g, 24.7 mmol) and 1-naphthol (3.1g, 21.4 mmol) were added to a solution of potassium hydroxide (3.0 g, 52.1 mmol) in H_2O (35 mL). After 3 h of refluxing and then cooling, the solution was extracted with EtOAc to remove the remaining 1-naphthol. The remaining aqueous phase was made acidic with 3 N HCl and was extracted with EtOAc. The organic phase was washed with brine, and the solvent was removed *in vacuo*, affording the title compound (2.8 g, 64%). ^{1}H NMR ($CDCl_3$/MeOD): δ 8.35 (m, 1H), 7.77 (m, 1H), 7.47 (m, 3H), 7.33 (t, 1H), 6.77 (d, 1H), 4.71 (s, 2H), 4.62 (s, 1H).

Naphtho[1,2-*a*]-1*H*-furan-3-one (21). To a solution of 1-naphthoxyethanoic acid (2.26 g, 11.2 mmol) in benzene (20 mL) was added oxalyl chloride (5 mL, 57.3 mmol), and the solution was refluxed 30 min. The mixture was cooled and concentrated. Additional benzene (20 mL) was added followed by aluminum chloride (1.8 g, 13.9 mmol). The mixture was stirred at room temperature 1 h and then poured over ice. The organic phase was separated and washed with brine, and the solvent was removed *in vacuo* to afford the title compound (1.74 g, 84%). ^{1}H NMR ($CDCl_3$): δ 8.23 (d, 1H), 7.89 (d, 1H), 7.72 (t, 1H), 7.60 (m, 2H), 7.46 (d, 1H), 4.83 (s, 2H).

2-Naphthoxyethanoic Acid (22). This compound was prepared using the procedure described above for 1-naphthoxyethanoic acid (**20**). ^{1}H NMR (CD_3OD): δ 8.4 (m, 1H), 7.7 (m, 1H), 7.41 (m, 2H), 7.34 (m, 2H), 6.70 (d, 1H), 4.65 (s, 2H).

Naphtho[2,1-*b*]fur-1-one (23). Oxalyl chloride (5 mL, 56 mmol) was added to a solution of 2-naphthoxyacetic acid (0.29 g, 1.4 mmol) in toluene (5 mL). The solution was refluxed for 1 h, cooled, and concentrated. Aluminum chloride (0.37 g, 2.8 mmol) in toluene (5 mL) was added, and the reaction was stirred for 2 h before being poured over ice. The organic phase was separated, washed with brine, and concentrated, yielding the desired product (0.24 g, 93% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.74 (d, 1H), 8.06 (d, 1H), 7.82 (d, 1H), 7.66 (ddd, 1H), 7.50 (ddd, 1H), 7.25 (d, 1H), 4.54 (s, 2H).

Biology. The IC_{50} values for inhibition of 5-lipoxygenase by test compounds were determined using a continuous oxygen consumption assay of Breton et al. using a semipurified cytosolic preparation of 5-LO dervied for RBL-1 cells.[19] The IC_{50}s for inhibition of leukotriene formation in A23187-stimulated human whole blood were determined using a LTB_4 specific radioimmunoassay as previously reported by Chabot-Fletcher.[27]

(A) Animals. Male CD-1 mice were obtained from Charles River Breeding Laboratories (Kingston, NY). Within a single experiment, mice were age-matched. The *ex vivo* experiments involved the use of five animals per treatment and control groups.

(B) *Ex Vivo* Mouse Whole Blood LTB_4 Assay. The 5-lipoxygenase product LTB_4 was extracted from mouse whole blood following A23187 stimulation. Aliquots of pooled heparinized mouse blood (1 mL each aliquot) from male CD1 mice were placed into 4 mL polypropylene tubes. The samples were preincubated for 5 min at 37 °C. A23187 (60 μM) was added to stimulate eicosanoid production. All samples were incubated for 30 min at 37 °C. The blood samples were centrifuged at 2500*g* for 15 min, and the plasma was recovered for extraction. One volume of chilled acetonitrile was added to all at 4 °C. The supernatants were recovered after centrifugation at 2500*g* for 15 min and diluted with 1% formic acid to achieve a final concentration of 20% acetonitrile. These supernatants were then loaded onto the extraction cartridges that have been conditioned according to the manufacturer's instructions (solid phase extraction columns, J. T. Baker, C18 3 mL size). The samples were washed with 3 mL of 1% formic acid, air-dried, and then washed with 3 mL of petroleum ether. After air-drying, the samples were eluted with methyl formate. The eluents were concentrated under vacuum. The concentrates were resuspended in 1 mL of enzyme immunoassay (EIA) buffer. Leukotriene B_4 was quantitated by EIA (Cayman Chemical Co., Ann Harbor, MI).

Pharmacokinetics. In mice (N = 3/time point), (+)- and (−)-**1a** (in ethanol/propylene glycol, 30:70, v/v) were administered intravenously (0.5 mg/kg) by lateral tail vein injection over a 5−10 s period or orally (20 mg/kg) by gavage (as a suspension in 0.5% tragacanth). At various times after drug administration, mice were anesthetized with ether and terminal blood samples were taken via the vena cava or by cardiac puncture and immediately chilled.

Pharmacokinetic experiments with (+)- and (−)-**1a** (in 7.5% (w/v) aqueous (2-hydroxypropyl)-β-cyclodextrin, Molecusol, Pharmtec, Inc., Alachua, FL) in male cynomolgus monkeys and beagle dogs (N = 3) were performed using intravenous (1−1.4 mg/kg) infusion (15 min) via a catheter placed in the saphenous (monkeys) or cephalic (dogs) vein. In monkeys, (+)-**1a** was also administered orally (approximately 10 mg/kg) by nasogastric intubation (in 30% (w/v) aqueous Molecusol). At various times after drug administration, blood samples were taken from the opposite cepahilic vein (dogs) or from a vascular access port with the catheter tip in the vena cava (monkeys) and immediately chilled. In all cases, plasma was separated by centrifugation at 4 °C and stored at −20 °C until HPLC analysis.

For dogs and monkeys, noncompartmental pharmacokinetic parameters for (+)- and (−)-**1a** were first estimated for individual animals and then averaged to obtain mean estimates. Because destructive sampling was used for mice, a mean plasma concentration was calculated for each time point prior to pharmacokinetic analysis. The area under the plasma concentration time curve was determined using linear/log-linear trapezoidal rule. The half-life of (+)- and (−)-**1a** was obtained from the slope of the log transformed plasma concentration time profile; the appropriate number of points used to calculate the half-life in an individual animal was determined by visual inspection of the data. Maximal (C_{max}) plasma concentrations and the time C_{max} was achieved (T_{max}) were determined from actual data. The plasma clearance (CL_p) was

obtained by dividing the dose administered by the AUC. The bioavailability (F) of (+)-**1a** was calculated by dividing the dose-normalized AUC obtained after iv administration by the AUC obtained following oral administration.

HPLC Assay. Plasma concentrations of (+)- and (−)-**1a** were quantified by HPLC from standard curves dissolved in plasma. The assay had a limit of quantitation of 0.05 μg/mL and was linear up to 15 μg/mL for each enantiomer. Samples and standards (50 μL) were analyzed after addition of the internal standard (**3a**, 10 μL of a 50 μg/mL solution in acetonitrile/water, 1:1) and 2.5 mL of 30% methanol in 1% aqueous acetic acid using the following procedure. Following mixing, samples were passed through conditioned (methanol followed by 1% aqueous acetic acid) Baker 3 mL LD C_{18}-bonded phase extraction columns, washed with 2 mL of 1% aqueous acetic acid, and eluted with methanol (2 × 0.5 mL). The extracts were evaporated under nitrogen, redissolved in 100 μL of mobile phase, and analyzed by HPLC at 215 nm using a Waters NOVAPAK C_{18} column (5 × 100 mm). Chromatography was carried out isocratically at a flow rate of 2 mL/min with a mobile phase of 34% acetonitrile in 10 mM aqueous ammonium formate.

***In Vitro* Metabolism. Sources of Materials.** [^{14}C]UDP-glucuronic acid was purchased from DuPont NEN (Boston, MA). All other materials were of the highest quality from commercial suppliers.

Tissue Source and Preparation. Livers were obtained from male CD-1 mice [(ICR)Br OUTBRED] greater than 10 weeks of age. Human livers were obtained from organ donors through the Association of Human Tissue Users (Tucson, AZ). Microsomes were prepared from fresh mouse liver and frozen human liver using a standard method [Guengerich, In *Principles and Methods of Toxicology*; Hayes, A. W., Ed.; Raven Press, New York, 1989; pp 777−814.

Enzyme Assay Conditions. Glucuronidation reaction mixtures contained 2.5 mM substrate [(+)- and (−)-**1a**, zileuton], 15 mM [^{14}C]UDP-glucuronic acid (0.3 mCi/mmol), 0.25−1.0 mg/mL microsomal protein, 10 mM $MgCl_2$, 2% bovine serum albumin, and 100 mM sodium phosphate buffer (pH 7.4) in a final volume of 250 mL. Reaction mixtures were incubated at 37 °C for 1−4 h, and reactions were terminated by adding acetonitrile to a final concentration of 20% (v/v). Samples were centrifuged at 14 000 rpm for 10 min, and supernatant was analyzed by HPLC.

HPLC Conditions. Samples were analyzed using a NOVA-PAK C18 column (4 m, 5 × 100 mm) in a radial compression module (Millipore, Milford, MA). The column was equilibrated with water/acetonitrile/trifluoroacetic acid (84.9:15.0:0.1, v/v) and was eluted by linearly increasing the concentration of acetonitrile to 50% over 15 min, with a flow rate of 2 mL/min. Eluent from the HPLC was monitored at 215 nm and with a Radiomatic Flo-One\Beta radioflow detector (Packard, Meriden, CT).

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