Table II. Physical Data of New Synthesized Compounds

no.	formula	R	mp, °C	crystn solv	yield, %
1b	$C_{18}H_{14}N_2O_2$	4-Me	191–192	EtOH	78
1c	$C_{18}H_{14}N_2O_2$	3-Me	202–203	EtOH	60
1d	$C_{18}H_{14}N_2O_2$	2-Me	186–187	EtOH	58
1e	$C_{17}H_{11}ClN_2O_2$	4-Cl	208–209	AcOEt	60
1f	$C_{17}H_{11}ClN_2O_2$	3-Cl	213–215	AcOEt	60
1g	$C_{17}H_{11}ClN_2O_2$	2-Cl	209–210	EtOH	55
1h	$C_{17}H_{11}BrN_2O_2$	3-Br	220–221	EtOH	75
1i	$C_{17}H_{11}N_3O_4$	4-NO_2	255–256	DMF	63
1j	$C_{17}H_{11}N_3O_4$	3-NO_2	277–278	DMF	30
1k	$C_{17}H_{11}N_3O_4$	2-NO_2	211–213	EtOH	65
1l	$C_{18}H_{14}N_2O_3$	4-OMe	199–200	AcOEt	52
1m	$C_{18}H_{14}N_2O_3$	3-OMe	202–203	EtOH	95
1n	$C_{18}H_{14}N_2O_3$	2-OMe	202–204	AcOEt	56
1o	$C_{19}H_{16}N_2O_2$	3,5-Me_2	238–240	MeOH	28
1p	$C_{17}H_{13}N_3O_2$	4-NH_2	258–259	DMSO	30
1q	$C_{17}H_{13}N_3O_2$	3-NH_2	228–230	acetone	30
1r	$C_{17}H_{13}N_3O_2$	2-NH_2	232–233	EtOH	83
2c	$C_{18}H_{14}N_2O_2$	3-Me	158–159	EtOH	75
2h	$C_{17}H_{11}BrN_2O_2$	3-Br	208–210	EtOH	35
2m	$C_{18}H_{14}N_2O_3$	3-OMe	184–185	EtOH	30
3b	$C_{18}H_{16}N_2O_3$	4-Me	207–208 dec	EtOH	85
3c	$C_{18}H_{16}N_2O_3$	3-Me	175–176 dec	EtOH	61
3d	$C_{18}H_{16}N_2O_3$	2-Me	163–164 dec	EtOH	82
3e	$C_{17}H_{13}ClN_2O_3$	4-Cl	200–202 dec	AcOEt	35
3f	$C_{17}H_{13}ClN_2O_3$	3-Cl	236–237 dec	EtOH	75
3g	$C_{17}H_{13}ClN_2O_3$	2-Cl	150–151 dec	EtOH	75
3h	$C_{17}H_{13}BrN_2O_3$	3-Br	233–234 dec	EtOH	61
3i	$C_{17}H_{13}N_3O_5$	4-NO_2	263–264 dec	DMF	75
3j	$C_{17}H_{13}N_3O_5$	3-NO_2	248–249 dec	EtOH	70
3k	$C_{17}H_{13}N_3O_5$	2-NO_2	208–210 dec	AcOEt	96
3l	$C_{18}H_{16}N_2O_4$	4-OMe	211–212 dec	AcOEt	57
3m	$C_{18}H_{16}N_2O_4$	3-OMe	187–189 dec	EtOH	55
3n	$C_{18}H_{16}N_2O_4$	2-OMe	144–146 dec	EtOH	75
3o	$C_{19}H_{18}N_2O_3$	3,5-Me_2	216–217 dec	EtOH	40

of 1i–k (0.500 g) in AcOEt (250 mL) was added 10% Pd/C (0.250 g). The mixture was hydrogenated in a Parr apparatus at 50 psi at room temperature for 24 h. The catalyst was filtered off, and the solution was evaporated to give a residue.

Binding Studies. Tritiated flunitrazepam was obtained from New England Nuclear (Dreieichenhain, West Germany) and had a specific activity of 78 Ci/mmol and a radiochemical purity >99%. All the other chemicals were reagent grade and obtained from commercial suppliers.

Membranes from bovine brains were prepared as described in ref 3.

Benzodiazepine receptor binding activity was determined as follows: 100 μL of diluted membranes (0.4–0.5 mg of proteins) was incubated in triplicate with 0.6 nM [^{3}H]flunitrazepam at 0 °C (90 min) in 50 mM Tris·HCl buffer in a final volume of 500 μL. After incubation, the samples were diluted at 0 °C with 5 mL of assay buffer and were immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B). Afterwards the samples were washed with 5 mL of the same buffer, dried, and added to 8 mL of HP Beckman scintillation liquid containing 0.4 mL of a solution of 0.01 M KOH in plastic vials.

The benzopyranopyrazole derivatives, unless otherwise stated, were dissolved in EtOH and added to the assay mixture to a final volume of 500 μL. Blank experiments were carried out to determine the effect of the solvent (2%) on the binding.

Specific binding was obtained by subtracting nonspecific binding from total binding and approximated to 85–90% of the total binding. The amount of nonspecific binding was determined by incubating membranes and [^{3}H]flunitrazepam in the presence of 10 μM diazepam.

Protein estimation was based on the method of Lowry et al.[11] after membrane solubilization with 0.75 N NaOH. Bovine serum albumin was utilized as the standard.

The concentrations of the test compounds that inhibited specific [^{3}H]flunitrazepam binding by 50% (IC_{50}) were determined by log–probit analysis with seven concentrations of the displacers, each performed in triplicate.

Registry No. 1a, 2764-19-4; 1b, 110570-18-8; 1c, 110570-19-9; 1d, 110570-20-2; 1e, 110570-21-3; 1f, 110613-13-3; 1g, 110570-22-4; 1h, 110570-23-5; 1i, 110570-24-6; 1j, 110570-25-7; 1k, 110570-26-8; 1l, 110570-27-9; 1m, 110570-28-0; 1n, 110570-29-1; 1o, 110570-30-4; 1p, 110570-31-5; 1q, 110570-32-6; 1r, 110570-33-7; 2a, 86100-07-4; 2c, 110570-34-8; 2h, 110613-14-4; 2m, 110570-35-9; 3a, 2587-10-2; 3b, 110570-04-2; 3c, 110570-05-3; 3d, 110570-06-4; 3e, 110570-07-5; 3f, 110570-08-6; 3g, 110570-09-7; 3h, 110570-10-0; 3i, 110570-11-1; 3j, 110570-12-2; 3k, 110570-13-3; 3l, 110570-14-4; 3m, 110570-15-5; 3n, 110570-16-6; 3o, 110570-17-7; 4, 100008-84-2; 4-$MeC_6H_4NHNH_2$·HCl, 637-60-5; 3-$MeC_6H_4NHNH_2$·HCl, 637-04-7; 2-$MeC_6H_4NHNH_2$·HCl, 635-26-7; 4-$ClC_6H_4NHNH_2$·HCl, 1073-70-7; 3-$ClC_6H_4NHNH_2$·HCl, 2312-23-4; 2-$ClC_6H_4NHNH_2$·HCl, 41052-75-9; 3-$BrC_6H_4NHNH_2$·HCl, 27246-81-7; 4-$O_2NC_6H_4NHNH_2$·HCl, 636-99-7; 3-$O_2NC_6H_4NHNH_2$·HCl, 636-95-3; 2-$O_2NC_6H_4NHNH_2$·HCl, 6293-87-4; 4-$MeOC_6H_4NHNH_2$·HCl, 19501-58-7; 3-$MeOC_6H_4NHNH_2$·HCl, 39232-91-2; 2-$MeOC_6H_4NHNH_2$·HCl, 57396-67-5; 3,5-$Me_2C_6H_3NHNH_2$·HCl, 60481-36-9; 3-acetyl-4-hydroxycoumarin, 2555-37-5.

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Orally Active Hydroxamic Acid Inhibitors of Leukotriene Biosynthesis

Sir:

5-Lipoxygenase has been the subject of intense study since its identification as the first enzyme involved in the biosynthesis of the leukotrienes. Because the leukotrienes have been suggested to be important mediators in a variety of diseases including asthma, arthritis, and psoriasis, inhibition of 5-lipoxygenase is a promising therapeutic target for the development of new, potentially more effective treatments for these conditions.

Simple, stable molecules containing the hydroxamic acid moiety that are potent in vitro inhibitors of 5-lipoxygenase have been identified.[1-5] The hydroxamate unit appears to be required for the inhibition observed with these molecules since replacement by closely related functional groups leads to inactive compounds.[1]

All of the inhibitors that we previously described[1,6] have

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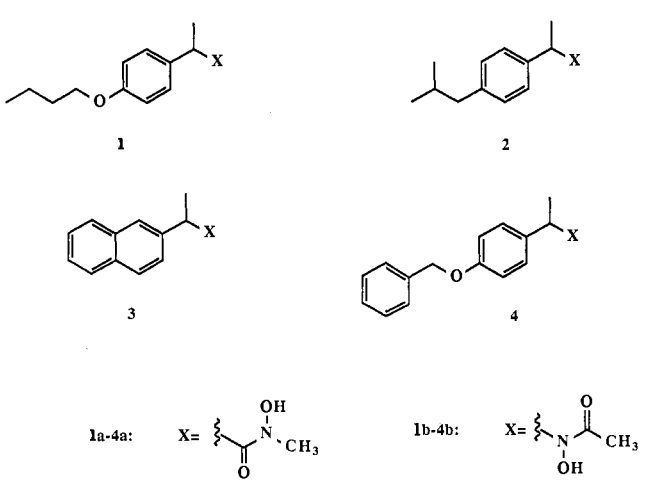
Table I. Inhibitory Activities and Plasma Concentrations for Pairs of Type A and Type B Hydroxamic Acids

no.	X	analysis[a]	mp, °C	in vitro 5-LO inhibn: IC_{50},[b] μM	plasma concn,[c] μM following 100 mg/kg, po: 1 h	3 h	6 h	in vivo LT inhibn: ED_{50},[d] mg/kg
1a	$CON(OH)CH_3$	$C_{14}H_{21}NO_3$	oil	0.78 (0.70–0.88)	58 ± 11	7.3 ± 0.3	3.2 ± 1.2	62 ± 5% at 100 mg/kg[e,f]
1b	$N(OH)COCH_3$	$C_{14}H_{21}NO_3$	68–70	0.79 (0.55–1.1)	92 ± 11	71 ± 4	13 ± 1.0	17 (11–31)
2a	$CON(OH)CH_3$	$C_{14}H_{21}NO_2$	oil	0.29 (0.23–0.35)	<9[g]	<9[g]	<9[g]	65 ± 6% at 100 mg/kg[e,f]
2b	$N(OH)COCH_3$	$C_{14}H_{21}NO_2$	101–103	0.42 (0.34–0.51)	21 ± 3	10 ± 2	5.0 ± 3.0	24 (1–135)
3a	$CON(OH)CH_3$	$C_{14}H_{15}NO_2$	113–115	0.59 (0.52–0.67)	37 ± 3	13 ± 12	1.4 ± 0.7[h]	54 ± 6% at 30 mg/kg[f]
3b	$N(OH)COCH_3$	$C_{14}H_{15}NO_2$	115–116	0.54 (0.46–0.60)	91 ± 37	26 ± 6	33 ± 1[h]	19 (10–34)
4a	$CON(OH)CH_3$	$C_{17}H_{19}NO_3$	121–125	0.28 (0.27–0.28)	8.0 ± 0.6	5.8 ± 1.7	3.8 ± 0.9[h]	40 (18–300)
4b	$N(OH)COCH_3$	$C_{17}H_{19}NO_3$	115–117	0.37 (0.24–0.51)	110 ± 15	140 ± 21	95 ± 23[h]	8 (5–13)

[a] Elemental analysis (C, H, N) within ±0.4% of theoretical value. [b] IC_{50} with 95% confidence limits in parentheses for the in vitro inhibition of 5-lipoxygenase from the 20000*g* supernatant of RBL-1 cells.[1] [c] Average plasma concentration ± the range of values from two determinations. [d] ED_{50} with 95% confidence limits in parentheses for inhibition in the rat peritoneal anaphylaxis model. [e] Nonsignificant ($p > 0.05$) inhibition (<40%) at 30 mg/kg. [f] Average percent inhibition ± SEM ($n = 8$). [g] Plasma concentrations for all determinations were less than the indicated level of detection. [h] Plasma concentration at 5 h after dosing.

small substituents (hydrogen or methyl) attached to the hydroxamic acid nitrogen and relatively large groups (aryl ring systems) appended to the carbonyl group. We have arbitrarily designated this structural arrangement as "type A hydroxamic acids". We now wish to describe compounds in which the substitution pattern is reversed. In these so-called "type B hydroxamic acids", a small group like methyl is attached to the carbonyl of the hydroxamate and a relatively large substituent is appended to the nitrogen. Although this reversal produces nearly isosteric compounds, it results in profoundly different properties in vivo. Type B hydroxamic acids show high bioavailablity and long plasma duration, and many are potent orally active inhibitors of leukotriene biosynthesis.

Type A hydroxamic acids are potent in vitro inhibitors of 5-lipoxygenase (Table I). However, they generally possess little or no activity in vivo.[6] This discrepancy between in vitro and in vivo activity can be attributed, at least in part, to the rapid metabolism of type A hydroxamic acids to the corresponding carboxylic acids, which are inactive. This metabolism is slower in some type A hydroxamic acids. For instance, several 2-arylpropanohydroxamic acids have been shown to have moderate plasma levels and durations and in turn exhibit modest in vivo 5-lipoxygenase inhibitory activity.[4] Among the type A hydroxamic acids studied, compound **4a** was one of the most potent. When administered orally, **4a** inhibited



leukotriene biosynthesis in vivo as measured by the rat peritoneal anaphylaxis model[7-10] and had an ED_{50} of 40 mg/kg. Nonetheless, hydroxamate **4a** is extensively metabolized to the corresponding carboxylic acid. In the rat, 15 min following an oral dose of 100 mg/kg, twice as much carboxylate as hydroxamate was found in the plasma[11] (16 μM vs 8.4 μM, respectively). At 1 h the level of metabolite was nearly sevenfold higher than that of the hydroxamic acid (57 μM vs 7.9 μM, respectively).

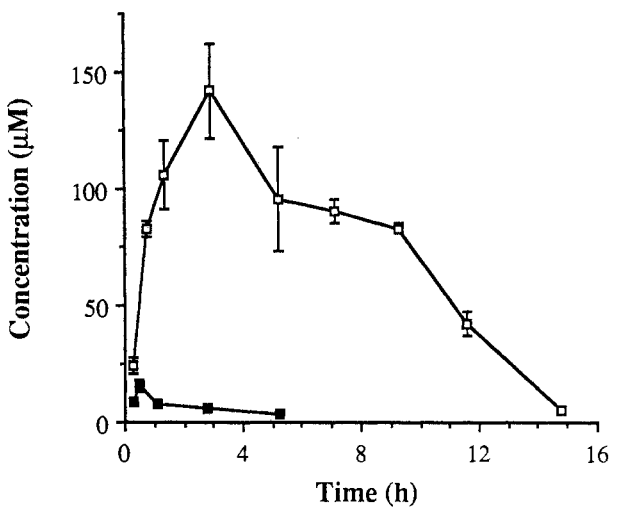


Figure 1. Rat plasma concentration of type A hydroxamic acid **4a** (■) and its type B analogue **4b** (□) following an oral dose of 100 mg/kg. Error bars show the range of two determinations.

When the carbonyl and nitrogen substituents of type A hydroxamic acids are transposed, the resulting type B hydroxamates are also potent inhibitors of RBL-1 5-lipoxygenase in vitro. Their activities are often quite similar

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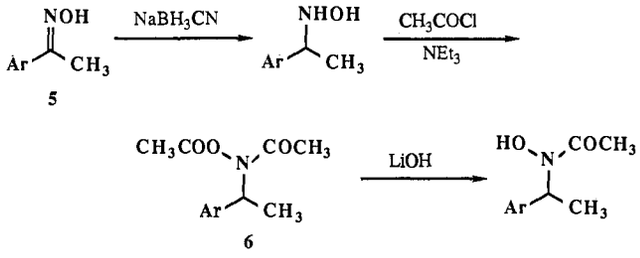
(10) In the rat peritoneal anaphylaxis model, rats were injected intraperitoneally (ip) with rabbit antibody to bovine serum albumin (BSA) followed 3 h later by an ip injection of BSA. Rats were sacrificed 15 min after this challenge and the peritoneal fluids collected and processed. Leukotriene levels were determined by radioimmunoassay. To evaluate oral effectiveness, we administered inhibitors by gavage 1 h prior to antigen challenge.

(11) Plasma concentrations were determined as follows: Rats were dosed orally by gavage or intravenously via a jugular cannula, and blood samples were removed at various times from a lateral tail vein. The plasma was separated from whole blood by centrifugation, and proteins were precipitated by addition of methanol. Plasma samples were analyzed by HPLC, and compounds were detected by UV spectroscopy at their absorbance maxima.

Scheme I



to those of their type A congeners (Table I). The greatest distinction between the two classes of compounds is observed in vivo. Whereas the type A hydroxamates are rapidly converted to inactive carboxylic acids, type B compounds are generally resistant to this route of metabolism. As a result, the type B hydroxamates circulate in the plasma longer than their type A congeners. For example, **4b** (Abbott-63162) exhibited a plasma half-life of about 2.5 times that of its type A analogue **4a** (1.1 and 0.4 h, respectively, following a 20 mg/kg dose, iv). Even more impressive is the difference between **4a** and **4b** in plasma concentrations when orally dosed at 100 mg/kg. Type A analogue **4a** reached peak plasma levels of approximately 15 μM at about 30 min, while **4b** reached its maximum concentration of 140 μM between 2 and 3 h (Figure 1). The low levels of **4a** could not be detected in the plasma more than 5 h after dosing, while **4b** was still circulating after 15 h. While **4a** is rapidly converted to the corresponding carboxylic acid, the major route of metabolism for **4b** is glucuronide conjugation followed by biliary excretion.

The superior pharmacokinetic properties of type B hydroxamates relative to their type A counterparts can result in greater in vivo potency. For example, in the rat peritoneal anaphylaxis model, **4b** was fivefold more potent in inhibiting leukotriene biosynthesis than **4a**. Type B hydroxamic acid **4b** had an ED_{50} in this model of 8 mg/kg when administered orally. The duration of this inhibition was consistent with the duration of **4b** in the plasma. A 30 mg/kg oral dose of the compound produced 67% and 57% inhibition of leukotriene biosynthesis 2 and 4 h after administration, respectively. At 8 h, this dose no longer produced significant inhibition (36%).

This difference between the in vivo properties of type A and type B hydroxamic acids has been a consistent observation. In each of the examples listed in Table I, the type B hydroxamate exhibited higher plasma concentrations and longer duration than its type A counterpart. The type B configuration consistently produced orally active inhibitors of leukotriene biosynthesis. Although the difference in the two series can be attributed to different rates of metabolism, the underlying molecular basis for this remains to be determined.

The type B hydroxamic acids described here can be prepared as illustrated in Scheme I. Oxime **5** is reduced with sodium cyanoborohydride (methanol, pH 3, 3 h) and converted to the diacetate **6** (2.2 equiv of acetyl chloride and 3 equiv of Et_3N in CH_2Cl_2, 30 min). The *O*-acetate is then removed with lithium hydroxide (5 equiv in 2:1 2-propanol–water, 30 min).

On the basis of these results, hydroxamic acids of structural type B appear to be valuable tools for the evaluation of the role of leukotrienes in animal models and human disease.

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[^{125}I]-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane: An Iodinated Radioligand That Specifically Labels the Agonist High-Affinity State of 5-HT_2 Serotonin Receptors

Sir:

The neurotransmitter serotonin (5-HT) is currently receiving renewed interest and widespread attention due to the recent identification of multiple populations of 5-HT binding/receptor sites (see ref 1 for a review). In order to further characterize these sites and to determine their physiological/pharmacological significance, it is necessary to develop site-selective agonists and antagonists. We have demonstrated that certain phenalkylamine derivatives possess a high affinity and selectivity for a particular population of 5-HT sites (i.e., 5-HT_2 sites); such agents include 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropane where X = Br (DOB; 1, R = Br) and X = iodo (DOI; 1, R = I).[2] Recently, we reported that [^{3}H]DOB specifically labels a guanine nucleotide-sensitive state of the 5-HT_2 receptor in rat brain homogenates.[3] However, because the agonist high-affinity state of the 5-HT_2 receptors labeled by [^{3}H]DOB represents only about 5% of the total 5-HT_2 receptor population in rat frontal cortical homogenates,[4] it is necessary to use a relatively large amount of tissue (20 mg wet weight) to produce a reliable signal. Furthermore, no specific signal was detectable when [^{3}H]DOB was incubated with 10-μm slices of rat frontal cortex (ca. 1 mg wet weight of tissue); this precludes the use of [^{3}H]DOB as a useful tool for autoradiographic studies. In order to use less tissue in the binding studies, and because one of the ultimate goals of this work is to perform autoradiographic studies on 5-HT_2 receptors, it became necessary to prepare a radioligand that would overcome these problems. Radioiodinated ligands are generally far superior to tritiated ligands because of their extremely high specific activities; such ligands allow for the use of small amounts of tissue and far shorter time periods to produce significant grain densities in autoradiographic studies.[5] We report here the synthesis and preliminary evaluation of a radioiodinated ligand, [^{125}I]DOI (1, R = ^{125}I), that should prove useful for future 5-HT_2 studies.

Chemistry.[6] Synthesis of [^{125}I]DOI was achieved by using the triazene method[7] (Scheme I). Direct nitration

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(6) Proton NMR and infrared spectra are consistent with assigned structures. Satisfactory (±0.4%) elemental analyses were obtained for compounds **4–7** (Atlantic Microlab; Atlanta, GA).

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