2980, 2853, 1601, 1468, 1345, 1284, 1210, 1077, 982, 921, 778 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 154.84, 129.87, 126.96, 118.99, 116.95, 113.21, 43.49, 40.43, 19.59; EIMS, *m/z* (relative intensity) 149 (75.8, M^+), 148 (84.7), 132 (25.8), 120 (100), 91 (40.0). Anal. ($C_9H_{11}NO$·HCl) C, H, N.

Radiochemical Assay for PNMT Activity. The assay employed in this investigation has been described elsewhere.[32,48] Briefly, a typical assay mixture consisted of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of a 10 μM solution of unlabeled AdoMet, 5 μL of [*methyl*-^{3}H]AdoMet, containing approximately 2×10^6 dpm (specific activity approximately 15 mCi/mmol), 25 μL of substrate solution, 25 μL of inhibitor solution (if added), 25 μL of the enzyme preparation, and sufficient water to achieve a final volume of 250 μL. After incubation for 30 min at 37 °C, the reaction was terminated by the addition of 250 μL of 0.5 M borate buffer (pH 10) and extracted with 2 mL of toluene/isoamyl alcohol (7:3). The organic layer was removed and transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition was ascertained by inspection of the $1/V$ vs $1/S$ plot of the data.

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Registry No. **12**, 31404-61-2; **13**, 91-21-4; **14**, 102877-50-9; **14**·HCl, 102879-34-5; **15**, 14446-24-3; **15**·HBr, 59839-23-5; **16**, 30798-64-2; **16**·HBr, 110192-19-3; **17**, 32999-37-4; **17**·HBr, 110192-20-6; **18**, 103030-70-2; **18**·HCl, 103030-69-9; **19**, 42923-77-3; **19**·HCl, 57196-62-0; **20**, 43207-78-9; **21**, 34146-68-4; **22**, 103904-80-9; **23**, 88081-58-7; **24**, 103904-88-7; **25**, 103904-81-0; **26**, 103904-85-4; **27**, 103904-89-8; **28**, 2039-67-0; **29**, 110192-21-7; **30**, 22246-62-4; **31**, 74904-29-3; **32**, 55-81-2; **33**, 91247-71-1; **34**, 22246-04-4; **35**, 2439-04-5; **36**, 110192-22-8; **37**, 110192-23-9; **38**, 110192-24-0; PNMT, 9037-68-7.

Novel 1*H*-Benzimidazol-4-ols with Potent 5-Lipoxygenase Inhibitory Activity

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The synthesis and structure–activity profile of 2-substituted benzimidazol-4-ols as inhibitors of cell-free RBL-1 5-lipoxygenase are discussed, and their potency is compared with that of the standard inhibitors phenidone, AA 861, BW 755C, and nordihydroguaiaretic acid. In contrast to the standard compounds, most did not inhibit the release of slow-reacting substance of anaphylaxis (SRS-A) in vivo when administered at 200 μM ip to rats subjected to peritoneal anaphylaxis, although five compounds containing a methoxylated benzyl group (compounds **36**, **39**, **42**, and **43**) or hydroxylated benzyl group (**41**) showed similar activity to that of phenidone, nordihydroguaiaretic acid, and AA 861. Of the many compounds tested, two, 5-*tert*-butyl-7-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-ol (**57**) and 2-(4-methoxybenzyl)-7-methyl-1*H*-benzimidazol-4-ol (**36**), like dexamethasone, inhibited monocyte accumulation in a pleural exudate model of inflammation. Standard lipoxygenase inhibitors such as phenidone, BW 755C, and AA 861 were inactive in this system.

Oxidative metabolites of arachidonic acid and their subsequent derivatives have been implicated in the pathology of a variety of inflammatory and allergic diseases,[1] and modulation of their formation has attracted considerable attention.[2] While the early work of Vane and others[3] showed that the nonsteroidal antiinflammatory drugs owed their activity to the inhibition of cyclooxygenase and the consequent reduction in the formation of thromboxanes and prostaglandins, little interest was shown in other oxidative pathways until the late 1970s. Indeed, it was the characterization of the slow-reacting substance of anaphylaxis (SRS-A), a potent bronchoconstrictor, as a mixture of the leukotrienes LTC_4, LTD_4, and LTE_4[1] and the identification of LTB_4, a potent chemotaxin for mononuclear cells[4] and polymorphonuclear cells,[5] that focused attention on the 5-lipoxygenase pathway of arachidonic acid metabolism and the range of products available through it. It was this increased awareness of the breadth of the arachidonic acid cascade and the enzymes involved that led to the development of inhibitors of leukotriene synthesis. Early inhibitors such as phenidone[6] and BW 755C[7] were shown to exhibit activity on both the major oxidative pathways, although subsequent work has identified an ever-increasing number of more selective inhibitors of 5-lipoxygenase.[8] Few of these, however, have shown useful in vivo activity in potentially relevant animal models of allergic or inflammatory disease.[9]

† Biosciences Research Centre.
‡ Medicinal Research Centre.

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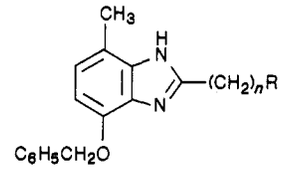
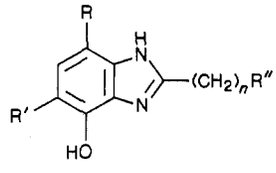
Table I. 4-(Benzyloxy)-7-methyl-1*H*-benzimidazoles



no.	R	*n*	method[a]	yield, %	crystn solvent	mp, °C	formula	anal.
10	C_6H_5	0	B	50	xylene	221–223	$C_{21}H_{18}N_2O$	C, H, N
11	C_6H_5	1	A	55	EtOAc–hexane	198–200	$C_{22}H_{20}N_2O$	C, H, N
12	C_6H_5	2	A	65	EtOH	203–204	$C_{23}H_{22}N_2O$	C, H, N
13	4-$CH_3C_6H_4$	1	A	67	EtOAc	184–186	$C_{23}H_{22}N_2O$	C, H, N
14	4-$CH_3OC_6H_4$	0	A	64	EtOAc	223–225	$C_{22}H_{22}N_2O_2$	C, H, N
15	4-$CH_3OC_6H_4$	1	A	56	EtOAc	181–183	$C_{23}H_{22}N_2O_2$	C, H, N
16	3-$CH_3OC_6H_4$	1	A	80	EtOAc	144–145	$C_{23}H_{22}N_2O_2$	C, H, N
17	2-$CH_3OC_6H_4$	1	A	73	EtOAc	174–176	$C_{23}H_{22}N_2O_2$	C, H, N
18	4-$C_2H_5OC_6H_4$	1	A	72	EtOAc–Et_2O	161–162	$C_{24}H_{24}N_2O_2$	C, H, N
19	4-HOC_6H_4	1	A	22	Me_2CO	121–123	$C_{22}H_{20}N_2O_2{\cdot}0{\cdot}5C_2H_5OH$	C, H, N
20	3,4-$(CH_3O)_2C_6H_3$	1	A	71	EtOAc	140–143	$C_{24}H_{24}N_2O_3$	C, H, N
21	3,4,5-$(CH_3O)_3C_6H_2$	1	A	86	EtOAc	165–167	$C_{25}H_{26}N_2O_4$	C, H, N
22	3-$CF_3C_6H_4$	0	B	31[b]	–	166–167	$C_{22}H_{17}F_3N_2O$	C, H, N
23	4-$N(CH_3)_2C_6H_4$	0	B	52	–	260–262	$C_{23}H_{23}N_3O$	
24	1-$C_{10}H_7$	1	A	71	EtOAc	171–172	$C_{26}H_{22}N_2O$	C, H, N
25	2-$C_{10}H_7$	1	A	62	EtOAc	172–174	$C_{26}H_{22}N_2O$	C, H, N
26	2-furyl	0	B	91[c]	–		$C_{19}H_{16}N_2O_2$	
27	2-thienyl	1	A	52	EtOAc	194–196	$C_{20}H_{18}N_2OS$	H, N, C[d]
28	3-pyridyl	1	A	*e*			$C_{21}H_{19}N_3O$	

[a] See text and Experimental Section. [b] Purified by column chromatography; CH_2Cl_2 on SiO_2. [c] Crude yield after partial chromatographic purification; CH_2Cl_2 on SiO_2. [d] C: calcd, 71.82; found, 71.37. [e] Not isolated.

In this paper we present data relating to a series of 1*H*-benzimidazol-4-ols I with potent 5-lipoxygenase activity in vitro. Several compounds have been shown to inhibit

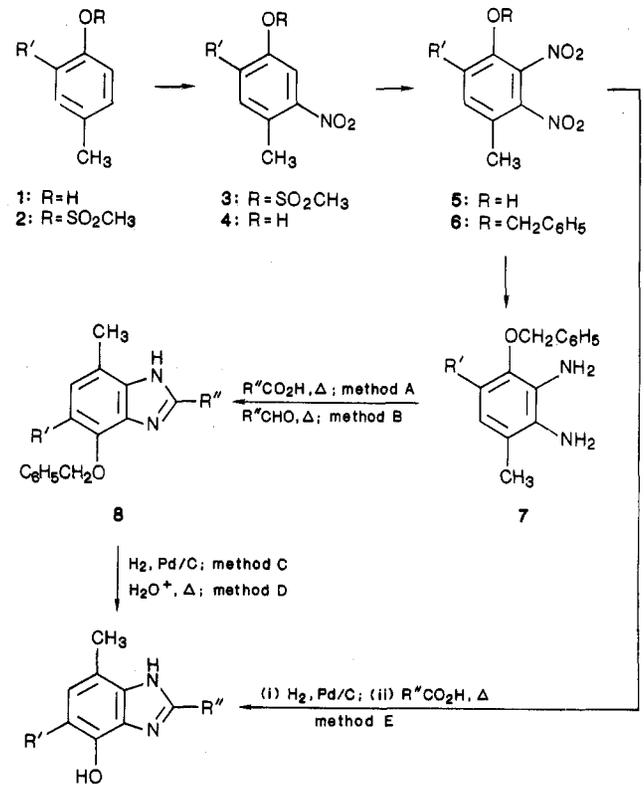


I: R, R′=H, alkyl; R″=H, CF_3, aryl, heteroaryl; *n* = 0–3

the release of SRS-A in vivo in a rat model of immediate hypersensitivity. Furthermore, representatives of the series have shown the ability to inhibit the accumulation of monocytes in a rat pleural exudate model of inflammation. The monocyte is thought to play a key role in chronic inflammation through its interactions with other cells and the release of soluble mediators such as interleukin 1.[10] Compounds such as the 1*H*-benzimidazol-4-ols that inhibit SRS-A release and monocyte accumulation could prove useful therapy in allergic and inflammatory conditions in humans.

Chemistry. Benzimidazoles were prepared by the classic route[11] from the appropriate *o*-phenylenediamines as shown in Scheme I. Thus, prior mesylation of the readily available phenols **1** to give **2** allows nitration to proceed at the meta position to give good yields of the mononitro derivatives **3**. Alkaline hydrolysis of the mesyl protecting group and further nitration result in the predominant formation of the ortho dinitro compounds **5**. Further protection of the phenolic moiety as its benzyl ether **6** is generally essential at this stage in order to

Scheme I[a]



[a] R′ = H, *t*-Bu.

prevent competitive cyclization to benzoxazoles in subsequent steps.[12] This is not a requirement, however, when the C-6 substituent (of **6**) is a sterically demanding group such as *tert*-butyl, as shown by the synthesis of compounds

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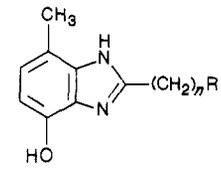
Table II. 7-Methyl-1*H*-benzimidazol-4-ols



no.	R	*n*	method[a]	yield, %	crystn solvent	mp, °C	formula	anal.	*inhibn of* RBL-1 5-lipoxygenase: IC_{50},[b] μM
29	C_6H_5	0	C	73	EtOH–Et_2O	252–256	$C_{14}H_{12}N_2O$·HCl	C, H, N, Cl	1.2 (1.0–1.5)
30	C_6H_5	1	C	85	EtOH–Et_2O	204–205	$C_{15}H_{14}N_2O$	C, H, N	1.2 (0.4–2.2)
31[c]	C_6H_5	1	E	67	$CHCl_3$–EtOAc–pentane	226–228	$C_{19}H_{22}N_2O$	C, H, N	1.3 (1.1–1.6)
32	C_6H_5	2	C	86	EtOH–Et_2O	238–240	$C_{16}H_{16}N_2O$·HCl	C, H, N	2.1 (1.3–2.8)
33	4-$CH_3C_6H_4$	1	C	19	EtOH	217–218	$C_{16}H_{16}N_2O$	C, H, N	2.54 (2.46–2.63)
34	4-ClC_6H_4	1	–	67	EtOH–Et_2O	275–277	$C_{15}H_{13}ClN_2O$·HBr	C, H, N	1.0 (0.3–1.6)
35	4-$CH_3OC_6H_4$	0	C	51	EtOH	283–285	$C_{15}H_{14}N_2O_2$·HCl·C_2H_5OH	C, H, N, Cl	2.2 (1.4–3.2)
36	4-$CH_3OC_6H_4$	1	C	87	EtOH	232–235	$C_{16}H_{16}N_2O_2$·HCl	C, H, N, Cl	2.1 (1.7–2.6)
37[c]	4-$CH_3OC_6H_4$	1	E	38	EtOH–Et_2O	232–235	$C_{20}H_{24}N_2O_2$·HCl	C, H, N, Cl	2.2 (0.9–4.0)
38	3-$CH_3OC_6H_4$	1	C	48	EtOH	239–241	$C_{16}H_{16}N_2O_2$·HCl·C_2H_5OH	C, H, N, Cl	1.7 (1.0–2.4)
39	2-$CH_3OC_6H_4$	1	C	54	EtOH	244–245	$C_{16}H_{16}N_2O_2$·HCl	C, H, N	3.4 (3.3–3.5)
40	4-$C_2H_5OC_6H_4$	1	C	88	EtOH	227–229	$C_{17}H_{18}N_2O_2$·HCl	C, H, N, Cl	3.0 (2.3–3.8)
41	4-HOC_6H_4	1	C	32	EtOH	273–275	$C_{15}H_{14}N_2O_2$·HCl	C, H, N	6.9 (4.9–11.6)
42	3,4-$(CH_3O)_2C_6H_3$	1	C	52	EtOH	264–265	$C_{17}H_{18}N_2O_3$·HCl	C, H, N, Cl	5.2 (3.8–7.1)
43	3,4,5-$(CH_3O)_3C_6H_2$	1	C	87	EtOH	250–251	$C_{18}H_{20}N_2O_4$·HCl	H, N, Cl, C[d]	9.7 (7.5–11.8)
44	3-$CF_3C_6H_4$	0	C	59	EtOH–H_2O	>280	$C_{15}H_{11}F_3N_2O$·HCl	C, H, N, Cl	*e*
45	4-$N(CH_3)_2C_6H_4$	0	C	82	EtOH	194	$C_{16}H_{17}N_3O$·HCl	C, H, Cl, N[f]	1.3 (1.0–1.7)
46	1-$C_{10}H_7$	1	C	69	EtOH	262–263	$C_{19}H_{16}N_2O$·HCl	C, H, N, Cl[g]	2.3 (1.8–2.8)
47	2-$C_{10}H_7$	1	C	92	EtOH	160–165	$C_{19}H_{16}N_2O$·HCl	C, H, N, Cl	*e*
48	2-furyl	0	C	30	EtOH	256–258	$C_{12}H_{10}N_2O_2$·1.1 HCl·0.5C_2H_5OH	C, H, N, Cl	3.3 (1.8–5.7)
49	2-thienyl	1	D	98	–	206–208	$C_{13}H_{12}N_2OS$·HCl	C, H, N, Cl, S	2.1 (1.9–2.4)
50	3-pyridyl	1	D	4	MeOH–EtOAc–Et_2O	264–268	$C_{14}H_{13}N_3O$	C, H, N	5.0 (4.0–6.4)

[a] See text and Experimental Section. [b] IC_{50} values for standard compounds: NDGA, 0.14 (0.03–0.29) μM; BW 755C, 0.89 (0.72–1.07) μM; AA 861, 2.0 (1.9–2.1) μM; phenidone, 2.1 (1.07–5.54) μM; 95% confidence limits in brackets. [c] 5-$C(CH_3)_3$. [d] C: calcd, 57.14; found, 57.65. [e] Inhibition >90% at 20 μM; IC_{50} not determined. [f] N: calcd, 13.83; found, 13.40. [g] Cl: calcd, 10.92; found, 11.49.

Table III. Rat Passive Peritoneal Anaphylaxis Data

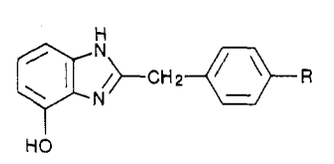
compound	% inhibn ± SEM of SRS-A release at compd concn of 2×10^{-4} M ip (n)[a]
36	58 ± 8** (6)
39	64 ± 12** (7)
41	58 ± 6*** (6)
42	55 ± 12*** (7)
43	59 ± 7*** (6)
phenidone	70 ± 8*** (7)
AA 861	77 ± 5*** (7)
BW 755C	25 ± 15* (14)
NDGA	64 ± 9*** (21)

[a] Asterisks denote differences from controls as assessed by Student's *t* test; (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$; n = number of animals used. Concentrations are those in the 6 mL injected. Compounds **29**, **30**, **32–35**, **38**, **40**, **45**, **46**, **48**, **49**, **51**, **52**, **56**, and **57** were tested and shown to have no effect on the release of SRS-A at this dose.

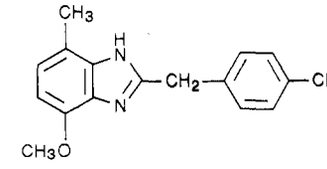
31 and **37** (method E). Raney nickel reduction of the protected dinitro derivatives **6** then proceeds well to give the requisite diamines **7**, which were generally used without further purification. The transformation of **7** to the required benzimidazol-4-ols may be effected by either of two standard routes: by the reaction with a suitable carboxylic acid in the absence of solvent at elevated temperatures (method A) or, alternatively, with an aryl carboxaldehyde in xylene at reflux (method B). Moderate to good yields of the protected benzimidazol-4-ols **8** (Table I) are obtained via either route. Reductive or hydrolytic cleavage of the *O*-benzyl groups from **8** (methods C and D respectively) gives the unprotected benzimidazol-4-ols **9** (Table II).

Results and Discussion

Early studies with the first member of the series, **30**, showed it to be a potent inhibitor of 5-lipoxygenase in vitro (IC_{50} = 1.2 μM). In contrast it was a poor inhibitor of bovine seminal vesicle cyclooxygenase, having little activity at 20 μM.[13] The interesting activity found with **30** prompted a program in which compounds bearing a variety of substituents in the benzimidazole ring were prepared (Table II). A free hydroxyl group appeared essential for good activity (cf. **30** with **15**, **25**, and **53**, which had IC_{50} values in excess of 20 μM), as did the presence of an alkyl group at C-7 (cf. **30** with **51** and **52**, which have IC_{50} values of ca. 20 μM).



51: R = H
52: R = OCH_3



53

Similar in vitro potency was observed when the C-2 substituent contained an aromatic ring either directly bonded to the imidazole nucleus or one or two carbon atoms removed from it (cf. **29**, **30**, and **32**). In vivo results suggested that the C-2 benzyl moieties were preferred. With the possible exception of the 4-hydroxy and trimethoxy compounds, **41** and **43** respectively, there was a

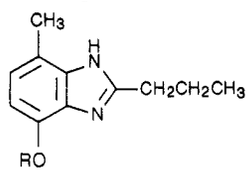
(13) Thody, V. E., unpublished results.

Table IV. Rat Pleural Exudate Model

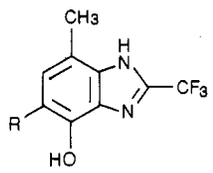
compound[a]	dose, mg/kg po	n[b]	% inhibition[c] DNA content	% inhibition[c] exudate volume
57	24	10	38*	48*
	12	10	57***	23
	6	10	57***	49*
	3	10	54**	52**
dexamethasone	45 μg	10	71***	80***
36	12	9	33*	35*
dexamethasone	45 μg	10	59***	56***

[a] Compounds **29–32**, **37**, **42–45**, and **47** were tested and shown to be inactive. [b] Number of animals used. [c] Asterisks denote differences from controls as assessed by Student's t test; (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

tendency for similar potency to be shown in vitro regardless of the nature of the aromatic substitution at C-2 (cf. **30**, **33**, **34**, **36**, and **46**). Potency was also retained by replacement of the benzenoid ring at C-2 by both thiophene, as in **49**, and pyridine, as in **50**. However, complete removal of the aromatic appendage and substitution by *n*-propyl or trifluoromethyl (compounds **54** and **56** respectively) resulted in a reduction in potency ($IC_{50} \sim 10$ μM). Interestingly, while introduction of a *tert*-butyl group at C-5 had little effect on potency in compounds **31** and **37**, the 5-*tert*-butyl homologue **57** of the trifluoromethyl compound **56** was the most potent inhibitor of this series, with $IC_{50} = 0.25$ (0.18–0.31) μM, comparable to that of nordihydroguaiaretic acid (NDGA), the most potent of the standard compounds in this screen (Table II).



54: R = H
55: R = $CH_2C_6H_5$



56: R = H
57: R = *t*-Bu

Significant differences between the more potent members of the series were seen in vivo, with only a narrow band of compounds (Table III) showing activity against the release of SRS-A in a rat passive peritoneal model of anaphylaxis using homocytotropic antibody.[14] Thus, the 2- and 4-methoxybenzyl derivatives, **39** and **36** respectively, significantly inhibited the release of SRS-A when administered at 200 μM ip while two close analogues, the 3-methoxy (**38**) and 4-ethoxy (**40**) compounds, did not show in vivo activity. It is of some interest that the 4-hydroxy compound **41** and the two multiply methoxylated analogues **42** and **43** were also active whereas the (methoxyphenyl)benzimidazol-4-ol **35** was inactive at the doses tested. Phenidone, NDGA and AA 861 show similar potency when administered ip in this model, but BW 755C was markedly less potent (Table III).

Compound **36** and the trifluoromethyl analogue **57** were also active in the 72-h pleural exudate model given orally, showing the same profile as dexamethasone (Table IV), although all other compounds tested were inactive. At this time point the monocyte is the predominant cell type.[15,16] Cyclooxygenase inhibitors such as indomethacin and the mixed cyclooxygenase/lipoxygenase inhibitors such as phenidone[17] and BW 755C[18] have been shown to exacerbate the cellular reaction measured at 72 h post carrageenan injection.[19] The selective 5-lipoxygenase inhibitor AA 861[8f] has been shown to be inactive in this model.[19] The low number of compounds active in the passive peritoneal anaphylaxis model that are also active in the pleural model may, of course, simply reflect the absence of oral absorption or different metabolism or pharmacokinetics, following oral administration in the latter system. (The compounds are given intraperitoneally in the anaphylaxis model and are thus immediately at the site of the inflammatory response.) Compound **57**, however, proved active in the pleural model in spite of being inactive in the anaphylaxis system. This may suggest that activities of some of these compounds other than inhibition of 5-lipoxygenase may play a role in reducing monocyte accumulation.

While the importance of 5-lipoxygenase products in allergic disease and chronic inflammation must await the successful progression and evaluation of a selective inhibitor in humans, the design of relevant animal models remains a major priority. The compounds described herein might be of value as pharmacological tools in the development of such animal models.

Experimental Section

Melting points were taken on a Büchi melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL JMN-GX270 270-MHz spectrometer using Me_4Si as internal standard. All compounds had spectra consistent with their assigned structures and had elemental analyses within ±0.4% of the calculated values except where noted.

2-*tert*-Butyl-4-methyl-5-nitrophenyl Methanesulfonate (3) [R′ = $C(CH_3)_3$]. Mesyl chloride (147 g, 1.28 mol) was added dropwise over 30 min to a solution of 2-*tert*-butyl-4-methylphenol (1) [R′ = $C(CH_3)_3$] (200 g, 1.22 mol) in pyridine (500 mL) at 0 °C. The solution was stirred at 10 °C for 1 h and room temperature for 3 h. It was poured into excess 5 M HCl and extracted with CH_2Cl_2. The organic layer was washed with 5 M HCl and H_2O and dried (Na_2SO_4). Evaporation to dryness in vacuo gave crude 2-*tert*-butyl-4-methylphenyl methanesulfonate (2) [R′ = $C(CH_3)_3$] (250 g, 1.03 mol), which was dissolved in concentrated H_2SO_4 (750 mL) and cooled to 0 °C. A mixture of concentrated HNO_3 (93 g, 1.03 mol) and concentrated H_2SO_4 (175 mL) was added dropwise over 1 h at 0 °C, and the solution was poured onto ice. The resulting solid was collected, washed with H_2O, and dried to afford 260 g (74%) of **3** [R′ = $C(CH_3)_3$]: mp 91–92 °C ($CHCl_3$–pentane); ^1H NMR ($CDCl_3$) δ 1.4 (9 H, s, CH_3), 2.55 (3 H, s, Ar CH_3), 3.30 (3 H, s, SO_2CH_3), 7.40 (1 H, s), 8.15 (1 H, s). Anal. ($C_{12}H_{17}NO_5S$) C, H, N.

2-*tert*-Butyl-4-methyl-5-nitrophenol (4) [R′ = $C(CH_3)_3$]. A suspension of **3** [R′ = $C(CH_3)_3$] (260 g, 0.906 mol) in 4.06 M aqueous NaOH (800 mL) was stirred at 60–70 °C for 1.5 h. The solution was filtered and poured onto excess iced 5 M HCl, and the mixture was extracted with CH_2Cl_2. The organic layer was washed (H_2O), dried (Na_2SO_4), and evaporated to dryness to give 140 g (74%) of **4** [R′ = $C(CH_3)_3$]: mp 96–98 °C (CH_2Cl_2–pentane); ^1H NMR ($CDCl_3$) δ 1.40 (9 H, s, CH_3), 2.50 (3 H, s, Ar CH_3), 5.20 (1 H, br, exchangeable OH), 7.20 (1 H, s), 7.43 (1 H, s). Anal. ($C_{11}H_{15}NO_3$) C, H, N.

6-*tert*-Butyl-2,3-dinitro-4-methylphenol (5) [R′ = $C(CH_3)_3$]. A solution of fuming HNO_3 (10 mL, 0.22 mol) in glacial AcOH (15 mL) was added dropwise over 15 min to a solution of **4** [R′ = $C(CH_3)_3$] (35 g, 0.17 mol) in AcOH (60 mL), with a temperature of 18–20 °C maintained. After 10 min, the solution was poured onto ice and the solid was collected, washed with H_2O, dried (Na_2SO_4), and evaporated in vacuo. The residue was separated

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by column chromatography on silica eluting with CH_2Cl_2 to afford 25 g (59%) of the title compound after recrystallization from CH_2Cl_2–pentane: mp 104–105 °C; ^1H NMR ($CDCl_3$) δ 1.45 (9 H, s, CH_3), 2.30 (3 H, s, Ar CH_3), 7.50 (1 H, s, H-6), 11.20 (1 H, s, exchangeable OH). Anal. ($C_{11}H_{14}N_2O_5$) C, H, N.

4-(Benzyloxy)-2,3-dinitrotoluene (6) (R′ = H). A mixture of 4-methyl-2,3-dinitrophenol[20] (12 g, 0.04 mol), anhydrous K_2CO_3 (15 g, 0.11 mol), potassium iodide (0.1 g), and benzyl chloride (25 g, 0.2 mol) in acetone (150 mL) was stirred under reflux for 18 h. The cooled mixture was filtered and the filtrate evaporated to dryness in vacuo. The residue was taken up in $CHCl_3$ (400 mL), washed with H_2O, and dried (Na_2SO_4). The solution was evaporated to half-volume in vacuo, pentane (150 mL) added, and the title compound (16.2 g, 93%) recovered by filtration: mp 116–118 °C ($CHCl_3$–hexane); ^1H NMR ($CDCl_3$) δ 2.30 (3 H, s, CH_3), 5.10 (2 H, s, CH_2), 7.03 (1 H, d, J = 8 Hz, H-5), 7.20 (5 H, m, benzyl aromatics), 7.26 (1 H, d, J = 8 Hz, H-6). Anal. ($C_{14}H_{12}N_2O_5$) C, H, N.

3-(Benzyloxy)-6-methyl-1,2-phenylenediamine (7) (R′ = H). A solution of **6** (R′ = H, 7 g, 0.03 mol) in EtOH (300 mL) was hydrogenated over Raney nickel (3 g) at room temperature and pressure until hydrogen uptake ceased. The catalyst was removed by filtration through Celite and the filtrate evaporated to dryness in vacuo to afford the title compound as an oil (5.07 g, 91%): ^1H NMR ($CDCl_3$) δ 2.10 (3 H, s, CH_3), 3.36 (4 H, br, exchangeable NH_2), 4.96 (2 H, s, CH_2), 6.38 (1 H, d, J = 8 Hz, H-5), 6.50 (1 H, d, J = 8 Hz, H-6), 7.30 (5 H, m, benzyl aromatics).

2,3-Dinitrophenol Benzyl Ether. The title compound was prepared from 2,3-dinitrophenol[21] in 90% yield by a procedure identical with that used for the preparation of **6** and crystallized from $CHCl_3$–hexane: mp 100–101 °C. Anal. ($C_{13}H_{10}N_2O_5$) C, H, N.

2,3-Diaminophenol Benzyl Ether. The title compound was prepared in quantitative yield by catalytic hydrogenation of the dinitro analogue over Raney nickel, in a way similar to that used for the preparation of **7**, and used without purification.

4-(Benzyloxy)-1*H*-benzimidazoles (Table I). Method A. The diamine **7** (R′ = H) and an appropriately substituted carboxylic acid (3–6 equiv) were heated together at 120–130 °C for 1–6 h with rapid stirring. After cooling, the mixture was taken up into CH_2Cl_2 and washed successively with 10% aqueous Na_2CO_3 (twice) and H_2O. The organic phase was dried ($MgSO_4$) and evaporated to dryness in vacuo to afford the crude 4-(benzyloxy)-1*H*-benzimidazole, which was purified by recrystallization: ^1H NMR (DMSO-d_6) δ 2.36–2.50 (3 H, s, CH_3), 5.12–5.32 (2 H, s, OCH_2), 6.63–6.74 (1 H, d, J = 8 Hz, H-5), 6.80–6.91 (1 H, d, J = 8 Hz, H-6), 7.31–7.51 (5 H, m, benzyl aromatics), 12.33–13.50 (1 H, br, exchangeable NH).

Method B. A mixture of the diamine **7** (R′ = H) and an appropriately substituted benzaldehyde (2–3 equiv) in xylene was heated under reflux for 3–6 h and allowed to cool overnight. The resulting solid was collected by filtration, washed with Et_2O, and recrystallized from a suitable solvent to afford the 4-(benzyloxy)-1*H*-benzimidazole in moderate yield.

1*H*-Benzimidazol-4-ols (Table II). Method C. A solution of the 4-(benzyloxy)-1*H*-benzimidazole in EtOH was hydrogenated over 10% palladium on charcoal at room temperature and pressure until hydrogen uptake ceased. The catalyst was removed by filtration through Kieselguhr and washed with EtOH and the filtrate evaporated to dryness in vacuo. Crystallization of the resulting solid afforded the expected product in high yield.

Alternatively the crude product could be converted into its hydrochloride salt with ethanolic hydrogen chloride and this salt recrystallized from a suitable solvent: ^1H NMR (DMSO-d_6) δ 2.47–2.61 (3 H, s, CH_3), 6.84–6.92 (1 H, d, J = 8 Hz, H-5), 7.06–7.12 (1 H, d, J = 8 Hz, H-6), 10.72–10.80 (1 H, br s, exchangeable OH), 13.00–16.50 (2 H, br s, exchangeable NH_2^+).

Method D. The 4-(benzyloxy)-1*H*-benzimidazole was heated under reflux in 5 M aqueous HCl for 24 h, the solution allowed to cool, and the resulting precipitate collected by filtration. The solid was washed with H_2O and dried in vacuo to give the essentially pure 1*H*-benzimidazol-4-ol as its hydrochloride salt in high yield. Recrystallization where necessary afforded material of analytical purity.

5-*tert*-Butyl-1*H*-benzimidazol-4-ols (Table II). Method E. The dinitrophenol **5** [R′ = $C(CH_3)_3$] in EtOH was hydrogenated over 10% palladium on charcoal at room temperature and pressure to afford the corresponding diamino compound, which was used without purification. This diamine and a suitable carboxylic acid (4 equiv) were heated together at 120–130 °C under nitrogen for 1–2 h, and the mixture was cooled and extracted with $CHCl_3$. The organic solution was washed with 10% aqueous Na_2CO_3 and H_2O and dried (Na_2SO_4). Evaporation of the solvent in vacuo afforded the expected benzimidazol-4-ol, which was purified by recrystallization.

2-(4-Chlorobenzyl)-7-methyl-1*H*-benzimidazol-4-ol Hydrobromide (34). A suspension of **53** (4.0 g, 14 mmol) in 48% aqueous HBr (400 mL) under nitrogen was heated at 130 °C for 5 h, cooled, and evaporated in vacuo. The residue was crystallized from EtOH–Et_2O to give 3.95 g (80%) of **34** as a white solid: mp 275–277 °C; ^1H NMR (DMSO-d_6) δ 2.45 (3 H, s, CH_3), 4.50 (2 H, s, CH_2), 6.85 (1 H, d, J = 8 Hz, H-5), 7.10 (1 H, d, J = 8 Hz, H-6), 7.50 (4 H, s, aromatic H), 10.60 (1 H, br, exchangeable OH). Anal. ($C_{15}H_{13}ClN_2O$·HBr) C, H, N.

2-(Phenylmethyl)-1*H*-benzimidazol-4-ol (51). The title compound was prepared in two steps from 3-(benzyloxy)-1,2-phenylenediamine and phenylacetic acid by using methods A and C: mp 172–174 °C (EtOH–Et_2O). Anal. ($C_{14}H_{12}N_2O$) C, H, N.

2-[(4-Methoxyphenyl)methyl]-1*H*-benzimidazol-4-ol Hydrochloride (52). The title compound was prepared in two steps from 3-(benzyloxy)-1,2-phenylenediamine and 4-methoxyphenylacetic acid by using methods A and C: mp 194–196 °C (MeOH–Et_2O). Anal. ($C_{15}H_{14}N_2O_2$·HCl) C, H, N.

2-[(4-Chlorophenyl)methyl]-4-methoxy-7-methyl-1*H*-benzimidazole (53). The title compound was prepared in 67% yield according to method A from 3-methoxy-6-methyl-1,2-phenylenediamine[20] and 4-chlorophenylacetic acid: mp 195–197 °C ($CHCl_3$–pentane); ^1H NMR (DMSO-d_6) δ 2.35 (3 H, s, CH_3), 3.85 (3 H, s, OCH_3), 4.12 (2 H, s, CH_2), 6.55 (1 H, d, J = 8 Hz, H-5), 6.85 (1 H, d, J = 8 Hz, H-6), 7.35 (4 H, s, aromatic H), 12.35 (1 H, br, exchangeable NH). Anal. ($C_{16}H_{15}ClN_2O$) C, H, N, Cl.

4-(Benzyloxy)-7-methyl-2-*n*-propyl-1*H*-benzimidazole (55). The title compound was prepared in 46% yield by the reaction of **7** (R′ = H) with butyric acid according to method A: mp 192–194 °C (EtOH–EtOAc); ^1H NMR ($CHCl_3$ + DMSO-d_6) δ 0.91 (3 H, t, J = 8 Hz, CH_2CH_3), 1.75 (2 H, m, $CH_2CH_2CH_3$), 2.45 (3 H, s, Ar CH_3), 2.80 (2 H, t, J = 8 Hz, $CH_2CH_2CH_3$), 5.15 (2 H, s, OCH_2), 6.60 (1 H, d, J = 8 Hz, H-5), 6.85 (1 H, d, J = 8 Hz, H-6), 7.30 (5 H, m, benzyl aromatics). Anal. ($C_{18}H_{20}N_2O$) C, H, N.

7-Methyl-2-*n*-propyl-1*H*-benzimidazol-4-ol Hydrochloride (54). The title compound was prepared from **55** in 64% yield by using method C: mp 204–206 °C (EtOH–EtOAc–Et_2O); ^1H NMR (DMSO-d_6) δ 0.95 (3 H, t, J = 8 Hz, CH_2CH_3), 1.90 (2 H, m, $CH_2CH_2CH_3$), 2.50 (3 H, s, Ar CH_3), 3.10 (2 H, t, J = 8 Hz, $CH_2CH_2CH_3$), 6.85 (1 H, d, J = 8 Hz, H-5), 7.05 (1 H, d, J = 8 Hz, H-6), 10.80 (1 H, br, exchangeable OH). Anal. ($C_{11}N_{14}N_2O$·HCl) C, H, N, Cl.

7-Methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-ol Hydrochloride (56). The diamine **7** (R′ = H) (3.88 g, 17 mmol) was heated under reflux in trifluoroacetic acid (25 mL) for 4 h, allowed to cool, and evaporated to dryness in vacuo. The residue was partitioned between saturated aqueous $NaHCO_3$ solution (100 mL) and CH_2Cl_2 (100 mL), the phases were separated, and the organic phase was washed with H_2O. The dried ($MgSO_4$) organic phase was evaporated to dryness in vacuo to afford the *O*-benzyl derivative of **56**, which was used without purification. This intermediate was dissolved in EtOH (150 mL) and hydrogenated over 10% palladium on carbon at room temperature and atmospheric pressure until hydrogen uptake ceased. The catalyst was removed by filtration and washed with EtOH, and the combined filtrates were acidified with hydrogen chloride gas. Removal of the solvent in vacuo afforded 1.52 g (41%) of **56**: mp 193–4 °C dec (EtOH). Anal. ($C_9H_7F_3N_2O$·HCl·0.25H_2O). C, H, N.

5-*tert*-Butyl-7-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-ol (57). The title compound was prepared in 47% yield according to method E by using trifluoroacetic acid: mp 140–141 °C (Et_2O–pentane). Anal. ($C_{13}H_{15}F_3N_2O$) C, H, N.

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RBL-1 5-Lipoxygenase Assay. 5-Lipoxygenase enzyme was prepared as a 10000*g* supernatant from RBL-1 cells by the method of Jakschik et al.[22] The supernatant was diluted with homogenization buffer to the equivalent of 1.5–2.5 × 10^7 cells/mL and made 2 mM with respect to $CaCl_2$. Aliquots of 0.5 mL were then dispensed into tubes and incubated at 29 °C with 5 μL of dimethyl sulfoxide or the test compound in dimethyl sulfoxide at the desired concentration for 2 min. Then [1-^{14}C]arachidonic acid was added in buffer to give a final concentration of 6.3 μM and 0.2 μCi per incubation, and the reaction was continued at 29 °C for 2 min. The reaction was terminated by the addition of 1 mL of acetone and cooling on ice. Ice-cold saline (0.5 mL) and 100 μL of 2 M formic acid were added, and the mixture was extracted with 2 × 2 mL of $CHCl_3$. The resulting extract was stored under nitrogen at −20 °C until analysis by TLC on silica gel G using the solvent system toluene–dioxane–acetic acid (65:35:1, v/v). Activity was measured as the percentage of the total radioactivity found in 5-HETE and 5,12-diHETE, and inhibition was calculated as $(1 - D/C) \times 100$, where D is the activity in the presence of compound and C is the control activity. IC_{50} values were computed by linear regression analysis using two or more concentrations (each a minimum of three determinations) causing inhibition significantly different from controls ($p < 0.05$) and spanning the 50% value.

Rat Peritoneal Anaphylaxis. Passive peritoneal anaphylaxis (PPA) was carried out in the rat as described previously.[14] Antiserum to ovalbumin (Sigma grade V) was raised in rats by using either Bordetella pertussis vaccine (Burroughs Wellcome, London) or pertussis vaccine adsorbed on alum (Lister Institute, Elstree) as an adjuvant. Rats were given intraperitoneal injections of 2 mL of a 1:5 dilution of the rat antiserum in isotonic saline. Two hours later, 0.3 mL of a 5% solution of Pontamine Sky Blue (G. T. Gurr, London) in isotonic saline was injected intravenously, followed immediately by an intraperitoneal injection of 1 mL of a solution of test compound in saline or in saline containing 1–2% dimethyl sulfoxide. Control rats received 1 mL of saline or saline containing 1–2% dimethyl sulfoxide. Each dose of a compound or of control was given to groups of five to seven rats, and treatments were randomized. The concentrations of drug administered were adjusted so that the required concentration was that in the 6 mL of fluid injected into the peritoneal cavity. After 2.5 min the rats were given an intraperitoneal injection of 5 mL of a Tyrode solution containing 0.4 mL of ovalbumin and 6 units/mL heparin. Exactly 5 min after challenge, the rats were stunned and bled, and the peritoneal fluids were collected and assayed for SRS-A as previously described.[14]

Rat Pleural Exudate Model. The method used was based on that of Ackerman et al.[15] Female rats (Charles River Wistar, 175–225 g) were lightly anesthetized (Hypnorm) and received injections of 0.2 mL of a 2% solution of λ carrageenan into the pleural cavity. Seventy-two hours later the animals were killed by an overdose of pentobarbitone, the pleural cavity was exposed, and 4 mL of a solution containing 0.375% phenol red and EDTA in saline was flushed into the cavity. After gentle massage, the fluid was harvested and the inflammatory exudate was measured by dye dilution using a spectrophotometer. The leukocyte concentration was estimated by the fluorimetric assay of DNA.[23] Compounds were administered orally 1 h before carrageenan, and 24 and 48 h subsequently, suspended in 0.7% methylcellulose solution.

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Registry No. **1**, 2409-55-4; **2**, 103059-07-0; **3**, 103059-06-9; **4**, 103059-08-1; **5**, 103059-09-2; **5**-diamine, 103059-10-5; **6**, 103080-29-1; **7**, 103080-30-4; **10**, 103059-03-6; **11**, 103059-01-4; **12**, 103059-02-5; **13**, 110191-32-7; **14**, 110191-33-8; **15**, 103059-05-8; **16**, 110191-34-9; **17**, 110191-35-0; **18**, 110191-36-1; **19**, 110191-37-2; **20**, 110191-38-3; **21**, 110191-39-4; **22**, 110191-40-7; **23**, 110191-41-8; **24**, 110191-42-9; **25**, 110191-43-0; **26**, 110191-44-1; **27**, 110191-45-2; **28**, 110191-46-3; **29**, 103059-40-1; **29**·HCl, 103059-14-9; **30**, 103059-11-6; **31**, 110191-47-4; **32**, 103059-39-8; **32**-HCl, 103059-13-8; **33**, 103059-19-4; **34**·HBr, 103080-32-6; **35**, 103406-47-9; **35**·HCl, 103059-35-4; **36**, 103059-43-4; **36**·HCl, 103059-21-8; **37**, 110191-48-5; **37**·HCl, 110191-49-6; **38**, 103059-56-9; **38**·HCl, 103059-34-3; **39**, 103059-57-0; **39**·HCl, 103059-36-5; **40**, 103059-53-6; **40**·HCl, 103059-32-1; **41**, 103059-58-1; **41**·HCl, 103059-37-6; **42**, 103059-55-8; **42**·HCl, 103080-35-9; **43**, 103059-54-7; **43**-HCl, 103059-33-2; **44**, 110191-50-9; **44**·HCl, 110191-51-0; **45**, 110191-52-1; **45**·HCl, 110191-53-2; **46**, 103059-46-7; **46**·HCl, 103059-24-1; **47**, 103059-41-2; **47**·HCl, 103059-16-1; **48**, 103059-51-4; **48**·HCl, 103080-34-8; **49**·HCl, 103059-31-0; **50**, 103059-15-0; **51**, 103059-25-2; **52**, 103059-26-3; **53**, 103059-12-7; **54**, 103059-20-7; **55**, 103059-04-7; **56**, 108381-55-1; **56** (*o*-benzyl deriv.), 110191-54-3; **57**, 108381-56-2; $C_6H_5CH_2COOH$, 103-82-2; $C_6H_5(CH_2)_2COOH$, 501-52-0; 4-$CH_3C_6H_4CH_2COOH$, 622-47-9; 4-$CH_3OC_6H_4COOH$, 100-09-4; 4-$CH_3OC_6H_4CH_2COOH$, 104-01-8; 3-$CH_3OC_6H_4CH_2COOH$, 1798-09-0; 2-$CH_3OC_6H_4CH_2COOH$, 93-25-4; 4-$C_2H_5OC_6H_4CH_2COOH$, 4919-33-9; 4-$HOC_6H_4CH_2COOH$, 156-38-7; 3,4-$(CH_3O)_2C_6H_3CH_2COOH$, 93-40-3; 3,4,5-$(CH_3O)_3C_6H_2CH_2COOH$, 951-82-6; 1-$C_{10}H_7CH_2COOH$, 86-87-3; 2-$C_{10}H_7CH_2COOH$, 581-96-4; C_6H_5CHO, 100-52-7; 3-$CF_3C_6H_4$, 454-89-7; 4-$N(CH_3)_2C_6H_4CHO$, 100-10-7; 4-methyl-2,3-dinitrophenol, 68191-07-1; 2,3-dinitrophenol benzyl ether, 103080-31-5; 2,3-dinitrophenol, 66-56-8; 2,3-diaminophenol benzyl ether, 89521-55-1; 3-methoxy-6-methyl-1,2-phenylenediamine, 101251-28-9; 4-chlorophenylacetic acid, 1878-66-6; butyric acid, 107-92-6; trifluoroacetic acid, 76-05-1; 5-lipoxygenase, 80619-02-9; 2-thiopheneacetic acid, 1918-77-0; 3-pyridineacetic acid, 501-81-5; 2-furancarboxaldehyde, 98-01-1.

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